Population genetics of Risso's dolphins (Grampus griseus), Fraser's dolphins (Lagenodelphis hosei) and bottlenose dolphins (Tursiops spp.) in the North Pacific Ocean

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Material Abstract

Thesis title: Population genetics of Risso's dolphins (*Grampus griseus*), Fraser's dolphins (*Lagenodelphis hosei*) and bottlenose dolphins (*Tursiops spp.*) in the North Pacific Ocean

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Abstract: Cetaceans are highly mobile mammals, but many species still exhibit degrees of population structure while inhabiting seemingly boundary-free open waters. Resource specialisation is hypothesized as one of the main drivers of population structure. Using multiple diploid and haploid genetic markers, this study reveals, for the first time, the population genetic structure of Risso's dolphins, Fraser's dolphins and common bottlenose dolphins in the tropical-temperate regions of the western North Pacific Ocean. For the Risso's dolphins, the results showed that there are at least three populations in the North Pacific Ocean, by-and-large parallel to the existing biogeographic provinces; and the direction of gene flow corresponds with the direction of the mainstream currents. Mitochondrial DNA (mtDNA) data showed that the Pacific populations are genetically different from the three populations in the eastern North Atlantic Ocean and the Mediterranean Sea. For the Fraser's dolphins, the genetic differentiation between Japanese and Philippine waters is consistent with the differentiation suggested in an earlier skull morphometric study. For the common bottlenose dolphins, the results suggested that there are at least four populations in the western and central North Pacific Ocean, and the differentiation appears to correspond to habitat types, resembling the scenario of inshore-offshore differentiation seen in other populations of the same species in other regions. The analysis also confirmed that there is no evident gene flow between the two "sister species", the common bottlenose dolphin and the Indo-Pacific bottlenose dolphin (*T. aduncus*), occurring sympatrically in the region. The mtDNA data suggested that the Risso's and Fraser's dolphin populations in the western North Pacific experienced an episode of expansion in the last 10,000 years. Genetic diversity is high in most of the population examined in this study; however, a relatively low effective population size is found in some populations and that may require further conservation attention.

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Declaration

All data presented in this thesis represents the author's own original work, save the individual contributions expressed in the acknowledgements, or sources quoted throughout the text.

Statement of Copyright

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Chapter 1. Thesis Introduction

Population structure plays an important role in maintaining a species' genetic diversity as it buffers selective pressures and prevents large-scale diversity reduction, and facilitates the development of *de novo* alleles through local adaptation (Ralph & Coop 2010; Elmer & Meyer 2011). Identifying a species' population structure is essential for studying the process of local adaptation and evolution (Kawecki & Ebert 2004), in addition for developing conservation strategies in natural resource management (Palsbøll *et al.* 2007). The strength of genetic interchange, or gene flow, is one of the key factors determining the significance of population structure (Hey & Pinho 2012). The presence of geographic barriers is perhaps the most common and obvious factor that prevents gene flow and allows genetic differences to accumulate, resulting in allopatric population structure. In the marine environment, however, such geographic barriers are usually absent, or at least not well defined. The population structure for marine species is therefore often attributed to other mechanisms, such as 'invisible barriers' (*e.g.*, the structure of water masses), physical limits for active and/or passive dispersal, historical vicariant events, and adaptive selection pressure (Palumbi 1994).

Allopatric differentiation plays a role in developing population structure (and speciation) for cetaceans. Cetacean populations inhabiting different ocean basins, different hemispheres for species with 'anti-tropical' distribution, or different river

systems for fresh water species, are generally differentiated (Davies 1963, Rice 1998). However, in a given ocean basin, hemisphere, or river system, population structure can still be detected, even though cetacean species are considered highly mobile (*e.g.*, Hoelzel *et al.* 1998a, b; Escorza-Treviño *et al*. 2005; Adams & Rosel 2006; Fontaine *et al.* 2007; Hollatz *et al*. 2011). Perrin (1984) suggests there are two major patterns for small cetacean population divergence: one is between enclosed seas and the open ocean, and the other is between inshore and offshore waters. Hoelzel (2009) suggests divergence can be attributed to a reunion of allopatric populations, or a process that results in assortative mating, such as resource specialisation, or utilising different breeding grounds. Recent studies further suggest cultural or behavioural differentiation could also promote population differentiation (Rosel *et al.* 2009; Rendell *et al.* 2012; Cantor & Whitehead 2013).

Determining the cause of population structure can be difficult, because the mechanisms are not always mutually exclusive. For instance, the evolution of the sympatric population structure for the transient and resident populations of killer whales (*Orcinus Orca*) in the eastern North Pacific Ocean is still under debate. Some studies suggest that it was the result of a reunion of two allopatrically differentiated populations (Foote *et al*. 2011; Morin *et al*. 2015), while some argue it was due to the high level of *in situ* resource specialisation, intense selection and gene drift pressure on the small populations (Hoelzel *et al.* 2007; Moura *et al.* 2014a; Moura *et al.* 2015). For other

species, multiple integrated factors including diet specialisation, behaviour differentiation, and habitat adaptation have been proposed as determining the genetic divergence of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in southwestern Australian waters (Möller *et al*. 2007), spinner dolphins (*Stenella longirostris*) around Hawaiian archipelagos (Andrews *et al.* 2010), and Franciscana dolphins (*Pontoporia blainvillei*) in the Rio de la Plata estuary, South America (Costa-Urrutia *et al.* 2012).

Identifying population structure, as well as possible mechanisms that drive population differentiation, is important for conservation management. For instance, the impact of climate change, *e.g.*, the rise of sea surface temperature, loss of arctic sea ice, alternation of ocean circulation, and intensification of El Niño/Southern Oscillation events can intensify or remove the barriers, and trigger further threats if the population is already endangered (Whitehead & Rendell 2004; Fontaine *et al.* 2007, Gambaiani *et al*. 2009; Scheinin *et al.* 2011). Population range shifts in recent decades have been reported for cetacean species off northwest Scotland (MacLeod *et al.* 2005), and for the Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) in southwest Gulf of California (Salvadeo *et al.* 2010). Model simulation studies predict that the changes in water temperature may affect the distribution ranges of 88% of cetaceans, and marine mammal richness at lower latitudes will decrease in future decades due to climate change (MacLeod 2009; Kaschner *et al.* 2011). A better understanding of current

population structure will certainly boost the evaluation of climate change impacts on cetaceans.

Population structure used to be determined according to morphological differences or distribution gaps (*e.g.*, Perrin 1984; Rice 1998). However, inference based on genotype and phenotype, or genotype and distribution, can be discordant. Such discrepancies have been reported, for example, between the morphological characters and mitochondrial DNA (mtDNA) variation in spinner dolphins in the Eastern Tropical Pacific (Dizon *et al.* 1991), between the colour patterns and genotypes in Dall's porpoises (*Phocoenoides dalli*) in the western North Pacific Ocean (Hayano *et al.* 2003), and between the genotypes and temporal aggregations in short-beaked common dolphins (*Delphinus delphis*) in the North Atlantic Ocean (Mirimin *et al.* 2009; Moura *et al.* 2013a). This may occur when phenotypic traits are plastic, leading to a weak correlation between genetic and phenotypic variation (Mousseau & Roff 1987; Reed & Frankham 2001; McKay & Latta 2002), or when the phenotypic traits are under strong selective pressure, resulting in little phenotypic variation among populations (Merila & Crnokrak 2001; Moritz 2002; Allendorf & Luikart 2006). The discordance between genotypes and geography can be attributed to seasonal or annual migration between habitats occupied by the same/different population (*e.g.*, Carvalho *et al.* 2014), or a relatively recent segregation event resulting in the lack of sufficient time for lineage sorting (Avise 1992). Nevertheless, Merila & Crnokrak (2001) examined the data from

18 independent studies of plants and animals and found that F_{ST} (the degree of differentiation in neutral marker loci) and Q_{ST} (the degree of differentiation in genes coding quantitative traits, the genetic basis of phenotypic traits) are highly correlated. Although using a set of multiple neutral genetic markers is now a favoured method in assessing population structure (see Moritz 2002; Manel *et al.* 2003; Palsbøll *et al.* 2007; Palstra *et al.* 2008; Allendorf *et al*. 2010), morphological characteristics and distribution breaks may assist the identification of population structure, even though they cannot fully account for the direction and intensity of gene flow, the key component in determining population structure.

There are 50 species of cetaceans that can be found in the North Pacific Ocean (Escorza-Treviño 2009), and population structure has been identified in many of those species (Table 1.1). However, some of the inferences are derived from limited genetic data (*e.g*., solely from the matrilineal inherited mtDNA markers), small sample size, and/or restricted sampling range and therefore warrants further examination. Moreover, most research efforts were spent on the cetaceans in the central and eastern North Pacific, particularly around the Hawaiian Islands and the western coasts of the North American Continent, or along the northern limit of the North Pacific. Knowledge about the population genetic structure for the species inhabiting pantropical western North Pacific Ocean is limited; such a sampling gap is seen in a number of studies attempting

to resolve the global phylogeography for some small cetacean species (*e.g.*, Natoli *et al.* 2006; Amaral *et al*. 2012; Moura *et al*. 2013b; Martien *et al.* 2014).

Population structure of the cetaceans in the coastal regions of the western North Pacific Ocean, however, deserves particular attention. Morphological studies suggest that some globally distributed species may have developed a degree of endemism with distinctive features. For example, the series of distinct morphological features for the "southern form" short-finned pilot whales (*Globicephala macrorhynchus*) in Japanese waters (Kasuya *et al.* 1988), the distinct colour patterns for the *truei* type of Dall's porpoises found in the coastal waters of western North Pacific (Rice 1998), the "dwarfism" found in spinner dolphins in Thai waters (Perrin *et al.* 1999), and a shorter body length characterizing Risso's dolphin (*Grampus griseus*) in the western North Pacific Ocean (Amano & Miyazaki 2004; Chen *et al*. 2011). Distribution gaps have also been observed in a number of small cetacean species (Miyashita 1993; Morisaka *et al.* 2005; Shirakihara *et al.* 2007). However, except that the pattern of distribution clusters in harbour porpoises (*Phocoena phocoena*) and Dall's porpoises is found in agreement with their population genetic structures (Escorza-Treviño *et al*. 2004), and the "southern form" of short-finned pilot whales has been suggested an evolutionary significant unit in a global mtDNA data analysis (Oremus *et al.* 2009), it is unclear whether such morphological or distribution significances for other species are also genetically significant.

Table 1.1. Summary of current knowledge about the population structure of extant cetaceans inhabiting the North Pacific Ocean. The species list is constituted based on the table published in Escorza-Treviño (2009), excluding two ambiguous beaked whale species (*Mesoplodon* sp. A and sp. B) and further including the newly recognized baleen whale species, Omura's whale. The asterisk (*) indicates the species is endemic to the North Pacific region.

Category	Species	Genetic method used1	Sampling coverage	References
No population structure recognized	Sei whale *Balaenoptera borealis*	nuDNA, MS	Full	Wada & Numachi 1991 Kanda *et al.* 2006
	Blue whale *Balaenoptera musculus*	MS	Partial	Costa-Urrutia *et al.* 2013
	Bryde's whale *Balaenoptera brydei*	mtDNA, nuDNA, MS	Partial	Wada & Numachi 1991 Kanda *et al.* 2007
	Indo-Pacific humpback dolphin *Sousa chinensis*	mtDNA, MS	Partial	Chen *et al.* 2010a Lin *et al.* 2012
	Northern right-whale dolphin* *Lissodelphis borealis*	mtDNA	Partial	Dizon *et al.* 1994
	Vaquita* *Phocoena sinus*	mtDNA	Full	Rosel & Rojas-Bracho 1999
Structure among geographic regions: among discrete breeding areas	Bowhead whale *Balaena mysticetus*	mtDNA, MS, SNPs	Full	LeDuc *et al.* 2008 Givens *et al.* 2010 Alter *et al.* 2012 Morin *et al.* 2012a
	Gray whale* *Eschrichtius robustus*	mtDNA, MS	Full	LeDuc *et al.* 2002 Alter *et al.* 2009 Frasier *et al.* 2011 D'Intino *et al.* 2013 Lang *et al.* 2014
	Humpback whale *Megaptera novaeangliae*	mtDNA, nuDNA, MS	Full	Baker *et al.* 1998, 2008
	Sperm whale *Physeter macrocephalus*	mtDNA, MS, SNPs	Partial	Lyrholm & Gyllensten 1998 Mensick *et al.* 2011
	Beluga *Delphinapterus leucas*	mtDNA, MS	Full	Meschersky *et al.* 2013
	Dall's porpoise* *Phocoenoides dalli*	mtDNA, nuDNA, MS	Full	Escorza-Treviño & Dizon 2000 Hayano *et al.* 2003
Structure among geographic regions: among discrete suitable habitats	Indo-Pacific bottlenose dolphin *Tursiops aduncus*	mtDNA	Partial	Kakuda *et al.* 2002 Hayano 2013
	Common bottlenose dolphin (Hawaiian Islands) *Tursiops truncatus*	mtDNA, MS	Full	Martien *et al.* 2012
	Pantropical spotted dolphin (Hawaiian Islands) *Stenella attenuata*	mtDNA, MS	Full	Courbis *et al.* 2014
	Spinner dolphin (Hawaiian Islands) *Stenella longirostris*	mtDNA, MS	Full	Andrews *et al.* 2010
	Killer whale (resident/transient ecotype) *Orcinus orca*	mtDNA, MS, SNPs	Full	Hoelzel *et al.* 2007 Parsons *et al.* 2013 Moura *et al.* 2014b

Category	Species	Genetic method used1	Sampling coverage	References
	Harbour porpoise *Phocoena phocoena*	mtDNA, MS	Full	Chivers *et al.* 2002 Taguchi *et al.* 2010 Crossman *et al.* 2014
	Yangtze finless porpoise* *Neophocaena phocaenoides asiaeorientalis*	mtDNA	Full	Zheng *et al.* 2005
	Finless porpoise* (Yellow Sea populations) *Neophocaena phocaenoides*	mtDNA, MS	Full	Li *et al.* 2011
Structure among geographic regions: semi-closed vs. open waters	Minke whale *Balaenoptera acutorostrata*	mtDNA, nuDNA	Partial	Wada & Numachi 1991 Pastene *et al.* 2007
	Fin whale *Balaenoptera physalus*	mtDNA, nuDNA, MS	Full-range	Wada & Numachi 1991 Bérubé *et al.* 2002 Goto 2007
Structure among geographic regions: nearshore vs. pelagic waters	Common bottlenose dolphin *Tursiops truncatus*	mtDNA, MS	Partial	Segura *et al*. 2006 Martien *et al.* 2012 Lowther-Thieleking *et al.* 2015
	Spinner dolphin *Stenella longirostris*	mtDNA, MS	Partial	Dizon *et al.* 1991 Andrews *et al.* 2010
	Pacific white-sided dolphin* *Lagenorhynchus obliquidens*	mtDNA, MS	Partial	Hayano *et al.* 2004
	Pantropical spotted dolphin *Stenella attenuata*	mtDNA, MS	Partial	Yao *et al.* 2004 Escorza-Treviño *et al.* 2005 Courbis *et al.* 2014
	False killer whale *Pseudorca crassidens*	mtDNA, MS	Partial	Chivers *et al.* 2007 Martien *et al.* 2014
Structure among cultural clans	Sperm whale *Physeter macrocephalus*	mtDNA	Partial	Rendell *et al.* 2012
Structure among sympatric or parapatric morphotypes or ecotypes	Bryde's whale & pygmy Bryde's whale *Balaenoptera brydei* & *B. edeni*	mtDNA, nuDNA, MS	Partial	Wada & Numachi 1991 Kanda *et al.* 2007 Kershaw *et al*. 2013
	North Pacific bottlenose whale* (black & slater-gray forms) *Berardius bairdii*	mtDNA, nuDNA	Partial	Kitamura *et al.* 2013
	Ginkgo-toothed whale (tropical &temperate forms) *Mesoplodon ginkgodens* & *M. hotaula*	mtDNA, nuDNA, Y-cms	Partial	Dalebout *et al*. 2007 Dalebout *et al.* 2014
	Short-finned pilot whale (northern & southern forms) *Globicephala macrorhynchus*	mtDNA	Partial	Oremus *et al*. 2009 Chen *et al*. 2014 Van Cise *et al*. 2016
	Short-beaked common dolphin & long-beaked common dolphin* *Delphinus delphis* & *D. capensis*	mtDNA, nuDNA	Partial	Rosel *et al.* 1994 Amaral *et al*. 2012
	Finless porpoise* *Neophocaena phocaenoides*	mtDNA, MS, SNPs	Partial	Yang *et al*. 2002, 2008 Wang *et al*. 2008 Chen *et al*. 2010b Ju *et al*. 2012 Li *et al.* 2013 Jia *et al.* 2014

Category	Species	Genetic method used1	Sampling coverage	References
	Killer whale (resident, transient and offshore ecotypes) *Orcinus orca*	mtDNA, MS, SNPs	Full	Hoelzel *et al.* 1998b, 2007 Morin *et al*. 2010 Pilot *et al*. 2010 Parsons *et al.* 2013 Moura *et al.* 2014b
Lack of sufficient data to conclude its population structure	North Pacific right whale* *Eubalaena japonica*	mtDNA, nuDNA	Partial	Rosenbaum *et al.* 2000 Gaines *et al.* 2005
	Omura's whale *Balaenoptera omurai*	mtDNA	Partial	Sasaki *et al.* 2006
	Pygmy sperm whale *Kogia breviceps*	mtDNA	Partial	Chivers *et al.* 2005
	Dwarf sperm whale *Kogia sima*	mtDNA	Partial	Chivers *et al.* 2005
	Cuvier's beaked whale *Ziphius cavirostris*	mtDNA	Partial	Dalebout *et al*. 2005
	Longman's beaked whale *Indopacetus pacificus*			
	Perrin's beaked whale* *Mesoplodon perrini*	mtDNA	Partial	Dalebout *et al*. 2002
	Pygmy beaked whale* *Mesoplodon peruvianus*	mtDNA	Partial	Dalebout *et al.* 2007
	Hubbs' beaked whale* *Mesoplodon carlhubbsi*	mtDNA	Partial	Dalebout *et al.* 2007
	Saber-toothed whale* *Mesoplodon stejnegeri*	mtDNA	Partial	Dalebout *et al.* 2007
	Blainville's beaked whale *Mesoplodon densirostris*	mtDNA	Partial	Dalebout *et al.* 2007 Morin *et al.* 2012b
	Rough-toothed dolphin *Steno bredanensis*			
	Striped dolphin *Stenella coeruleoalba*			
	Fraser's dolphin *Lagenodelphis hosei*			
	Risso's dolphin *Grampus griseus*			
	Melon-headed whale *Peponocephala electra*			
	Pygmy killer whale *Feresa attenuata*			
	Irrawaddy dolphin *Orcaella brevirostris*			

[1]: mtDNA, mitochondrial DNA; nuDNA, nuclear DNA intron; Y-cms, Y-chromosome; MS, microsatellites; SNPs, single nucleotide polymorphisms.

On the other hand, there is a growing concern for cetacean conservation in the western North Pacific. Due to the rapid economic development and intensified human demands on aquatic resources, multiple anthropogenic threats, such as small-scale

whaling, incidental catches from fisheries, habitat loss/degradation, contaminant accumulation, acoustic disturbances and recreation abuse, have been proposed as potential risks to local cetacean fauna in this region (see review in Perrin *et al*. 2005; Kasuya 2007, 2011; Robards & Reeves 2011). More than 10,000 individuals, comprising seven dolphin and one porpoise species, have perished in Japanese waters every year (Kasuya 2011). A rough estimate of annual cetacean incidental catching rate in Taiwanese waters is 2,770 dolphins, with about 70% comprised of Risso's dolphins and Fraser's dolphins (*Lagenodelphis hosei*) (Chou 2006). It is estimated that 2,000 dolphins are bycaught in Philippine fisheries every year and the primary composition is spinner dolphin, pantropical spotted dolphin, Fraser's dolphin, bottlenose dolphin, Risso's dolphin, and Irrawaddy dolphin (*Orcaella brevirostris*) (Perrin *et al*. 2005; Young & Iudicello 2007). Estimates suggest that there are about 1,700 bottlenose dolphins and 1,000 spinner dolphins incidentally killed in human fisheries in the central western Pacific (Young & Iudicello 2007). Moreover, while the popularity of the whale watching industry grew rapidly in the past few decades, negative interaction with recreational or transportation vessels also started to emerge (Ng & Leung 2003; Matsuda *et al.* 2011; Parsons 2012). These human impacts cannot be properly evaluated and an effective conservation plan cannot be made without the knowledge of the cetacean's population structure, stability and sustainability. However, such information is still lacking for most of the species in the region (Table 1.1).

One of the aims of this study was to increase the knowledge of cetacean population structure and dynamics to enhance the efficiency of conservation management, and the other was to interpret the significance of biological and environmental factors in shaping cetacean population structure. This research investigated the population genetic structure of Risso's dolphins, Fraser's dolphins and bottlenose dolphins in the North Pacific Ocean, with a focus on their population structure in the western region. Where possible, comparisons were made with populations of the same species worldwide. These species were chosen because they are vulnerable to anthropogenic impact in this region (Perrin *et al.* 2005; Chou 2006; Kasuya 2011), but their population structure, as well as population size, genetic diversity, and social structure, were poorly known (Table 1.1; for details, see Chapters 2—4). Moreover, these dolphin species are highly mobile, globally cosmopolitan, and live sympatrically (or at least parapatrically) in this region, and it was anticipated that studying these species would ultimately provide further inference about the evolutionary mechanisms for inter-/intra population structuring in delphinid species.

As earlier studies based on sighting records suggest that some regional distribution gaps are present for Risso's and bottlenose dolphins in the North Pacific Ocean (Leatherwood *et al*. 1980; Miyashita 1993; Jefferson *et al*. 2014), and morphological differentiation is detected in Fraser's and Risso's dolphins in the western part of the Pacific ocean (Perrin *et al*. 2003; Chen *et al*. 2011), it would be expected to

find some degree of population structure for these species, and the differentiation to be due to oceanographic or resource barriers (*i.e.*, scattered habitat distribution) and resource specialization (local adaptation). Furthermore, with the genetic data, estimates were made for the effective population size, migration rate, and the history of population expansion for each population in each species, to evaluate the dynamics of the populations and the potential of being affected by human disturbance.

Objectives

The objectives of this study were:

To assess population structure, genetic diversity, effective population size, and demographic trends of the Risso's dolphins in the North Pacific Ocean and examine the contradictory hypotheses derived from earlier studies on the external morphology and from regional shipboard survey ("there is population structure in the North Pacific"; Leatherwood *et al*. 1980; Miyashita 1993; Chen *et al*. 2011) and long-term sighting records ("there is no population structure in the North Pacific"; Jefferson *et al*. 2014) (Chapter 2);

To reveal the population genetic structure for the Fraser's dolphins in the western North Pacific, particularly to examine the population differentiation between Japan and

the Philippines that has been proposed in an earlier analysis of skull morphometrics (Perrin *et al.* 2003) (Chapter 3);

To study the population structure of bottlenose dolphins in the western North Pacific with a larger sample size, to confirm there is no gene flow between the two sympatric sister species (Wang *et al.* 1999; Yang *et al.* 2005) and to examine the hypothesis that there is no "near-shore" population established along the eastern Asian coasts due to the presence of Indo-Pacific bottlenose dolphins (Tenzano-Pinto *et al.* 2009; Oremus *et al*. 2015) (Chapter 4); and

To review and compare the differences in the pattern of population structure, genetic diversity and effective population size between and within the species, and to draw inferences about possible ecological/evolutionary mechanisms, influence of climate change, and conservation management (Chapter 5).

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Chapter 2. The population structure and dynamics of Risso's dolphins (*Grampus griseus*) in the Northern Hemisphere, with a focus on the populations in the North Pacific Ocean

Abstract

Cetaceans are highly mobile mammals, but even those species inhabiting seemingly boundary-free open waters still exhibit degrees of population structure. Habitat/resource specialisation and fragmented distribution of habitat/resource have been suggested to be the main processes shaping the population structure of species relying on land-associated, coastal habitats. Here, it is demonstrated that these factors could also influence the population structure of species utilising oceanic habitats. By examining the genetic variation among 19 microsatellite loci in 236 Risso's dolphin samples collected from a range of locations in the North Pacific, it was found that there are at least three Risso's dolphin populations in the region (K=3 in Geneland analysis; F_{ST}=0.009—0.044), and the structure is by-and-large parallel to the biogeographic provinces, suggesting habitat/resource specialisation. The Migrate and Geneclass2 analyses showed that the direction of gene flow appears to agree with the direction of the mainstream currents in the North Pacific. Analyses using mitochondrial DNA data showed that these three populations are genetically different from the populations in the

eastern North Atlantic Ocean and Mediterranean Sea (F_{ST}=0.024—0.317). The estimates from mismatch analysis showed, apart from the population occupying the waters around the Azores and Eastern Tropical Pacific, all populations in the Northern Hemisphere experienced a period of demographic and spatial population expansion in the last 10,000 years. An estimation of the effective population size for the three populations in the North Pacific is presented, although some of the estimates might be inaccurate.

Keywords: Risso's dolphin, Population structure, North Pacific, Oceanic biogeography, microsatellite DNA, mitochondrial DNA

Introduction

Cetaceans (whales, dolphins and porpoises) are highly mobile mammals that have fully adapted to live in an aquatic environment. For those species that utilise the open water environment, there appears to be no physical barrier that would prevent dispersal, and so panmixia may be expected. However, cryptic population structure has been reported in a number of species, even when distribution ranges are apparently connected. It has been suggested that such sympatric or parapatric population structure is a result of a reunion of allopatrically differentiated populations, and/or assortative mating driven by

resource specialisation (Hoelzel *et al.* 2002; Hoelzel 2009). Various examples that support these hypotheses can be found in earlier studies of the population genetics of killer whale (*Orcinus orca*) (Hoelzel *et al.* 1998a, 2007; Foote *et al.* 2011; Moura *et al.* 2014, 2015; Morin *et al.* 2015) and common bottlenose dolphin (*Tursiops truncatus*) (Hoelzel *et al.* 1998b; Möller *et al.* 2007; Rosel *et al.* 2009; Louis *et al.* 2014).

Ballance *et al.* (2006) studied the distribution of several pelagic cetacean species in the Eastern Tropical Pacific and concluded that the distribution pattern can be greatly influenced by species-specific 'distribution-habitat relationships'. These relationships are proposed to reflect the species' preference for oceanographic features (such as types of surface currents or water masses), which is usually associated with the distribution of the species' preferred prey, and that in turn is affected by various gradients of physical features and processes. Therefore, the seemingly boundary-free open water inhabited by pelagic cetaceans may be partitioned by the unevenness of resource distribution, as the populations of coastal species are segregated due to the discontinuity of preferred habitat. This idea is echoed by some pioneering seascape genetics studies for marine mammals; for instance, Fontaine *et al.* (2007) found that profound changes in oceanographic features create barriers that consequently prevent gene flow among the populations of harbour porpoise (*Phocoena phocoena*) in European waters; Andrews *et al* (2010) suggest that the segregation of the two communities of spinner dolphin (*Stenella longirostris*) around the Hawaiian Islands is due to limited resting areas; and

Amaral *et al.* (2012) propose that the population structure of short-beaked common dolphins (*Delphinus delphis*) is correlated with marine productivity and sea surface temperature.

The Risso's dolphin (*Grampus griseus*) is a moderately small odontocete species widely distributed in the world's oceans between 64°N and 46°S, with an apparent preference for temperate waters (water temperature >10°C) and steep continental shelf-edge habitats where water depth is about 400—1000m (Baird 2009; Jefferson *et al.* 2014; Fig. 2.1). This habitat preference reflects an exclusive dependence on cephalopod prey, which is typically found in the upwelling regions along continental slopes (Baumgartner 1997; Smith & Whitehead 1999; Olavarría *et al.* 2001; Frantzis & Herzing 2002; Azzellino *et al.* 2008). Several regional populations or stocks have been proposed according to apparent geographic boundaries or morphological differences. For instance, Risso's dolphins in the US waters are assigned to four geographic stocks for management purposes: the US Atlantic, the Gulf of Mexico, the California/Oregon/Washington and the Hawaii stocks (Carretta *et al.* 2014; Waring *et al.* 2014). Risso's dolphins in the waters around Taiwan and Japan have been suggested to represent an independent population characterised by having a shorter body length (Chen *et al.* 2011). However, such classification of populations/stocks may not always indicate a demographically independent population, which is an appropriate management unit for wildlife conservation (Palsbøll *et al.* 2007). To my knowledge, the

only genetically assessed population structure for Risso's dolphins is the populations between the UK and Mediterranean Sea, and the UK population was found to be isolated with notably low genetic diversity (Gaspari *et al.* 2007).

In the North Pacific Ocean, Risso's dolphins are commonly encountered on both sides of the ocean (Leatherwood et al. 1980; Miyashita 1993; Forney & Barlow 1998; Yang *et al.* 1999; Rosales-Nanduca *et al.* 2011; Jefferson *et al.* 2014). Sighting records, which showed a certain level of geographic clustering, suggest the presence of stock structure (Leatherwood *et al*. 1980; Miyashita 1993; Gerrodette *et al.* 2008; Carretta *et al.* 2014). However, Jefferson *et al.* (2014) argue that many regions in the Pacific Ocean have yet to be properly surveyed, and "the number of records from the central portion of the North Pacific Ocean makes it reasonably clear that the species is found continuously across the North Pacific Ocean basin; there is no evidence of separate western and eastern Pacific populations (p. 62)." Even so, it is unknown if any of these putative stocks are demographically independent. Nor is it known if the unified pan-North Pacific stock has population structure, given that sympatric or parapatric population structure has been observed in other cetacean species. Since this species is constantly harvested in a regional dolphin drive fishery (Kasuya 2007), suspected to be negatively impacted by these regional fisheries (Dolar 1994; Vidal *et al.* 1994; Perrin *et al.* 2005; Chou 2007), and possibly harassed by tourism (Visser *et al.* 2011), verifying the

species' population structure in this region emerges as a critical objective for cetacean conservation management.

One of the objectives of this study was to test the correspondence between these apparent demographic stocks and patterns of population genetic structure. The null hypothesis was there is no population structure for Risso's dolphins in the North Pacific Ocean, as Jefferson et al. (2014) suggested. The other objective was to assess the population dynamics of Risso's dolphins both at present and in the past, and thus to provide further key information in support of the effective conservation of this poorly studied species.

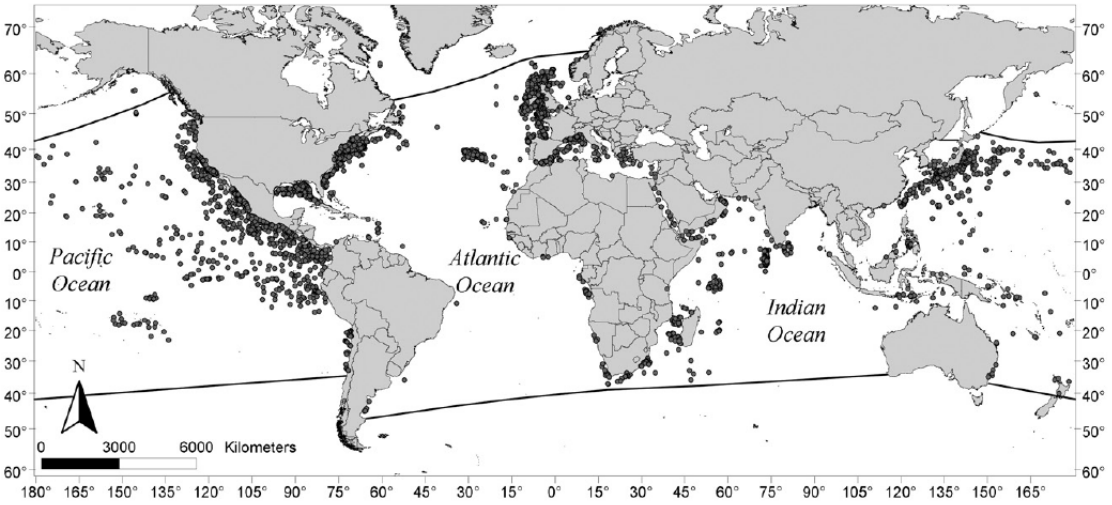

Figure 2.1. The global distribution range of the Risso's dolphin species (inside black lines). The dots indicate the locations of sighting or capture records of the species in 1950—2012. The figure is published as the Figure 1 in Jefferson et al. (2014), and a reuse permit for this thesis has been granted by the publisher John Wiley and Sons under the licence number 3851880562391.

Material and Methods

Sample collection and genomic DNA extraction

Two hundred and ninety six Risso's dolphin tissue samples collected from a range of locations around the North Pacific Ocean were acquired from multiple biological tissue archives in Taiwan (from National Taiwan University), Japan (from National Museum of Natural Science and es-Bank at Ehime University) and the United States (from Southwest Fishery Science Center). The samples were grouped into seven putative populations according to their sampling locations: Taiwan, East Japan, Sea of Japan, the Philippines, Central-Northeast Pacific, Oregon-California Coastal and Eastern Tropical Pacific (Fig. 2.2; Appendix 2.1). Samples from Central-Northeast Pacific, Oregon-California Coastal and Eastern Tropical Pacific were either biopsied from free-ranging dolphins or collected from stranded dolphins and incidental catches in fisheries, whereas those from Taiwan, East Japan and Sea of Japan were chiefly from stranded dolphins, incidental catches in fisheries, or from a group of dolphins targeted in drive fishery (*c.f.* Kim *et al*. 1996; Amano & Miyazaki 2004). Note that the sample sizes from the Central-Northeast Pacific and the Philippines were too small for some analyses.

The identity of species and sex of each sample was derived from the archive records where identification was based on the specimen's external morphological characters and made by knowledgeable researchers. However, when in doubt, species

identity was verified genetically by comparing the sample's mitochondrial DNA (mtDNA) control region sequence against the DNA Surveillance reference database (http://dna-surveillance.fos.auckland.ac.nz; Ross *et al.* 2003). The samples acquired from National Museum of Natural Science and Southwest Fishery Science Center were supplied as titrated DNA reagent; the others were provided as a small portion of skin or muscle tissue preserved in either 99% ethanol or 20% DMSO solution. For all tissue samples, their genomic DNA was isolated and purified following a standard proteinase-K digestion/phenol–chloroform extraction protocol (Sambrook *et al.* 1989). All specimens were transported to and examined at the laboratories in University of Durham (UK) and Kyushu University (Japan), with valid official permits issued by the authorities of Japan, Taiwan, United States and United Kingdom.

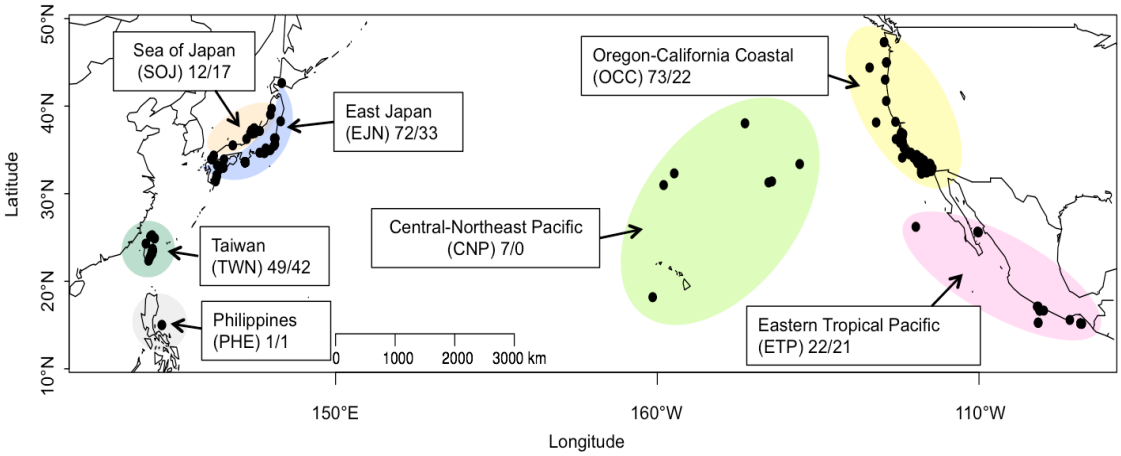

Figure 2.2. A map showing the sampling locations (solid circle) and the range of each defined putative populations (as coloured patches). The sample size (*n*) for each putative population is indicated in the label box as the *n* used in microsatellite data analysis/ the *n* used in mitochondrial data analysis.

Microsatellite DNA fragment amplification and genotyping

For microsatellite analyses, 22 microsatellite loci that have been studied and validated in the same or related species in earlier genetic studies (*e.g.*, Natoli *et al.* 2004; Gaspari *et al.* 2007; Mirimin *et al.* 2011) were chosen (Table 2.1). The microsatellite fragments were amplified using a polymerase chain reaction (PCR) method either individually with GoTaq® Taq DNA polymerase (Promega), or multiplexed with a multiplex PCR preparation kit (Qiagen). The PCR reagents that contained GoTaq® Taq DNA polymerase were prepared in a 20μL scale. The temperature cycle included a denaturation step at 95°C for 120s, followed by 35 cycles of 40s at 94°C, 40s at the best annealing temperature of the locus (Table 2.1), and 70s at 72°C, and a post-extension at 72°C for 10 min. The reagents using the multiplex PCR kit were prepared in a 10μl scale, and the PCR cycle included a denaturation step at 95°C for 15 min, followed by 30 cycles of 40 s at 94°C, 90 s at the annealing temperature for the group of loci, and 60 s at 72°C, and a post-extension at 60°C for 30 min. The fragment analysis was undertaken on an Applied Biosystems 3730 DNA Analyser, and the allele size was determined by an internal standard marker (Genescan-500 ROX, Applied Biosystems) visualised in Peak Scanner v.1 (Applied Biosystems). Every locus in each sample was examined at least twice by the author Ing Chen, and the scores were verified by the author's supervisor Rus Hoelzel.

Table 2.1. The list of used microsatellite markers with optimal annealing temperatures and fragment size range observed for each locus.

Microsatellite locus	Optimal annealing temperature (°C)	Fragment size range	Genbank accession number	Reference
AAT44	58	70-90	AF416501	Caldwell *et al*. 2002.
EV14	60	132-184	G09079	Valsecchi & Amos 1996.
EV37	53	180-206	G09081	
D14	48	106-144		Shinohara *et al*. 1997.
D22	52	114-138		
KWM1b	49	187		Hoelzel *et al.* 1998a.
KWM2b	44	167-181		
KWM9b	58	166-198		
KWM12a	55	158-204		
TexVet7	50	152	AF004907	Rooney *et al*. 1999.
MK3	59	139-159	AF237889	Krützen *et al.* 2001.
MK5	59	198-248	AF237890	
Dde59	52	306-386	AM087093	Coughlan *et al.* 2006.
Dde65	53	184-204	AM087096	
Dde66	52	341-381	AM087097	
Dde69	56	184-220	AM087098	
Dde70	59	105-155	AM087099	
Dde72	58	207-299	AM087100	
Dde84	48	144-164	AM087101	
Sco11	56	187-223	AM087102	Mirimin *et al.* 2006.
Sco28	50	131-149	AM087103	
Sco55	56	216-228	AM087105	

Mitochondrial DNA sequence amplification

The mtDNA sequences of selected Oregon-California Coastal and Eastern Tropical Pacific samples were amplified using GoTaq® protocol with a pair of primers designed to amplify the mtDNA control region sequence in cetaceans, MTCR-F (5'-TTC CCC GGT CTT GTA AAC C-3') and MTCR-R (5'-ATT TTC AGT GTC TTG CTT T-3') (Hoelzel *et al.* 1991). The PCR reactions were prepared according to GoTaq® protocol but converted to a 20µL scale. The PCR cycle included a denaturation step at 95°C for 120s, followed by 35 cycles of 40s at 94°C, 40s at 50°C, and 70s at 72°C, and a post-extension at 72°C for 10min. The amplified mtDNA fragments were purified using

QIAquick® PCR Purification Kit (Qiagen) and then sequenced on an Applied Biosystems 3730 DNA Analyser. The mtDNA sequences for Taiwan, East Japan, Sea of Japan and Philippine samples were amplified and sequenced in the molecular ecology laboratory at Kyushu University, using a set of primers tRpro-F.ceta (5'-ACC ACC AAC ACC CAA AGC TGG AAT-3') and RCR(mod).ceta (5'-CCA TAG CTG AGT CGG TGC AAG CCC-3') (modified by the author's collaborator Shin Nishida from Hoelzel *et al*. 1998a). The PCR reagent was prepared in a 25μL scale, which comprised a dose of PCR buffer, 0.2mM of each dNTP, 0.2mg/mL BSA, 0.2mM of each primer, and 0.625 units TaKaRa®Ex Taq Hot Start Version DNA polymerase (TaKaRa Bio) and 1μL of DNA sample. The temperature cycle included a denaturation at 94°C for 60s, followed by 30 cycles of 10s at 98°C, 45s at 60°C, and 45s at 72°C, and post-extension at 72°C for 60s. The amplified mtDNA fragments were then purified using USB ExoSAP-IT® Kit (Affymetrix), and sequenced on a CEQ2000XL DNA Sequencer (Beckman Coulter Inc.). All sequencing results were visualised in FinchTV (PerkinElmer) and manually corrected using MEGA 5.05 (Tamura *et al*. 2011).

Microsatellite data configuration

Using samples collected from the same school of dolphins may result in non-random sampling of closely related individuals (see examples in Amos *et al.* 1993; Pilot *et al.*

2010; Costa-Urrutia *et al.* 2012; Kita *et al.* 2013). Sampling kin is likely an issue in this sample because some biopsy and drive fishery samples were likely collected from the same school of dolphins. The screening procedure applied in Martien *et al.* (2012) and Lowther-Thieleking *et al*. (2015) was used here to identify and remove closely related kin in the sample. Kingroup v2 (Konovalov *et al.* 2004; Konovalov & Heg 2008) was used to calculate the coefficient of kinship (r) for the sample pairs in the same putative population and conducted a likelihood ratio test to screen possible parent-offspring or full-sibling pairs. If the r value in a pair was over 0.4 (Kita *et al.* 2013) and the likelihood ratio test also indicated the pair was a parent-offspring or full-sibling pair, then one of the samples in the pair would be excluded from further analyses, unless the samples were collected in a different year or location.

The software Micro-Checker was used to screen for null alleles and scoring errors (Van Oosterhout *et al.* 2004). The jack-knife test implemented in the R package *StrataG* was used to screen for samples that are influential to Hardy-Weinberg equilibrium (Morin *et al.* 2009). Arlequin 3.5.1 (Excoffier *et al*. 2005) was used to calculate the observed heterozygosity (Ho) and expected heterozygosity (He) of each locus, and to assess any statistically significant deviation in Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD). Overall deviation, heterozygote deficiency and heterozygote excess were assessed through the Fisher exact test and Markov chain method implemented in the same program (Number of steps in Markov

chain, 1,000,000; number of dememorization steps, 100,000). The significant level for all tests was set as $p<0.05$ after Bonferroni correction. FSTAT 2.9.3.2 (Goudet 1995, 2002) was used to determine the allelic richness and inbreeding coefficient (F_{IS}) for each putative population. Note that the indices associated with Wright's F-Statistics, *i.e.*, H_O, H_E, allelic richness and F_{IS}, were only estimated for putative populations with a sample size larger than 10.

Microsatellite data analysis: population structure

The factorial correspondence analysis (FCA) implemented in Genetix 4.0 (Belkhir *et al*. 2004) was used to demonstrate the similarity among individuals using the microsatellite data. Individuals that have similar series of allelic states (*e.g.*, absence, homozygote or heterozygote) would be clustered in a similar multi-dimensional space. The analysis was conducted with or without using the population information option ('sur population') to generate different plots for comparison. When the 'sur population' option was used, the population information of each individual was referred to the centre for the individual's putative population. The result was presented in a two-dimensional plot using the package *graphic* available in R 3.1.2 (R Core Team 2014, http://www.R-project.org).

STRUCTURE 2.3.4 (Pritchard *et al.* 2000) was used to estimate the most probable number of populations (K). The program uses a Bayesian model-based clustering algorithm to calculate a K that could achieve the minimum HWE and linkage equilibrium between loci within groups, with or without *a priori* knowledge of population information. To estimate the K for the samples, a series of posterior probability likelihood values, LnP(K), was estimated for each value of K (from 1 to 8), using an admixture model with correlated allele frequencies (Falush *et al.* 2003), and the process was repeated in 10 independent runs. All simulations were conducted under 100,000 burn-in and 1,000,000 repeats. The estimation was undertaken with or without using sampling location information (the 'LOCPRIOR' option in the program). When the LOCPRIOR option was used, the identity of the putative population for each individual was taken into account. The best K can be identified as the run with the highest LnP(K); however, the LnP(K) usually continues to increase when K increases in natural populations (Pritchard *et al*. 2000). In this regard, ΔK, the second order rate of change of LnP(K) with respect to K, was suggested a better indicator in determining the highest hierarchical level of K for the samples (Evanno *et al.* 2005). The ΔK was calculated using a web-based software Structure Harvester (http://taylor0.biology.ucla.edu/structureHarvester/; Earl *et al*. 2012), and a graphic result was optimised using accessory software CLUMPP version 1.1.2 (Jakobsson & Rosenberg 2007) and Distruct 1.1 (Rosenberg 2004).

The R package *Geneland* was also used to assess population structure in a spatial context (Guillot *et al*. 2005). This program integrates genotypic (in this case, microsatellite) and spatial coordinate data and simulates all parameters by Bayesian inference and Markov chain Monte Carlo (MCMC) simulation, assuming HWE and linkage equilibrium. The analysis was conducted in two steps (as suggested in Guillot *et al*. 2005): in the first step, the number of clusters (K) was set to vary from 1 to 10 clusters, with 1,000,000 MCMC iterations, 100 thinning, maximum rate of Poisson process fixed to 236 (the number of samples), uncertainty attached to spatial coordinates fixed to 100 km, maximum number of nuclei in the Poisson-Voronoi tessellation fixed to 708. For allelic frequencies setting, the Dirichlet model was used as it has been demonstrated to perform better than the alternative model (Guillot *et al.* 2005). In the second step, the K was fixed to the modal value of K from the 10 runs in the first step, and then conducted the simulation again with 500,000 MCMC iterations, 100 thinning, 100 repeats and the other parameters remaining the same. The top 10 runs with the highest mean logarithm of posterior probability (LPP) in the 100 runs were selected for post-processing. To calculate the posterior probabilities of population membership for each individual and each pixel of the spatial domain, a burn-in of 100 iterations and a spatial domain of 290 pixels along the X-axis and 64 along the Y-axis were used. The consistency of results across these 10 runs was individually checked.

The level of population differentiation among the putative populations was evaluated using Analysis of Molecular Variance (AMOVA) and pairwise comparison of fixation indices, *i.e.*, F_{ST} (Wright 1951) and R_{ST} (Slatkin 1995). The analysis measures the variation of allelic frequencies among putative populations and expects further deviation in more differentiated populations. Since the fixation indices could be less reliably estimated with small sample size (Balloux & Lugon-Moulin 2002), the Philippines and Central-Northeast Pacific populations were excluded from this analysis. In AMOVA, which allows examining the differences for different levels of population hierarchy, the putative populations were arranged into two groups, the Western North Pacific (Taiwan, East Japan and Sea of Japan) and the Eastern North Pacific (Oregon-California Coastal and Eastern Tropical Pacific), to test whether the population differentiation between two sides of North Pacific Ocean was statistically significant. Both AMOVA and pairwise comparison of fixation indices were calculated using the algorithm implemented in Arlequin 3.5.1, with a non-parametric permutation approach with 10,000 permutations.

To examine whether the population differentiation is a result of isolation-by-distance, a redundancy analysis (RDA) was conducted to test the significance of the correlation between genetic distance and geographic distance (Meirmans 2015), using the R package *vegan* (Oksanen *et al.* 2012*)*. The microsatellite data were set as the matrix of dependent variables and the longitude and latitude of the samples were the

independent variables. The statistical significance level for the correlation coefficient was set at $p<0.05$.

Microsatellite data analysis: population dynamics

The effective population size (N_e) and the prevalence of gene flow, *i.e.*, the number of migrants per generation (N_em), were estimated using maximum likelihood coalescent methods implemented in MIGRATE 3.6.6 (Beerli & Felsenstein 1999, 2001). The analysis was conducted using 10 short chains and three long chains, with 20 sampling increments. Recorded genealogies for short chains were 1,000 and for long chains were 10,000. A 10,000 step burn-in and a heating scheme to allow chains to swap between four different temperatures (1, 1.5, 3, and 1,000,000) was set as default. For the first run the start parameters were estimated using an F_{ST}-based measure (Maynard Smith 1970; Nei & Feldman 1972), and in the following run the parameters were updated with the estimates generated from the previous run. The process was repeated five times. The result was shown as estimates for the N_em, the effective population size times the mutation rate ($N_e\mu$) for each population. An approximate N_e was calculated as the $N_e\mu$ divided by a theoretical microsatellite mutation rate, μ=0.01—0.02% (Whittaker *et al*. 2003; Hoelzel *et al.* 2007; Hollatz *et al*. 2011).

To determine whether there was any recent immigration, GeneClass2 was used to search for potential first generation migrants (Piry *et al.* 2004). The program utilizes multilocus genotype data to compute the distribution of genotype likelihoods in a reference population sample with three types of genetic assignment criteria (distance criteria, frequency criteria and Bayesian criteria), and then compares the likelihood computed for the to-be-assigned individual to that distribution. To estimate the probability that an individual was a first generation immigrant, the likelihood was computed using the algorithm described in Paetkau *et al.* (2004), with a frequencies-based method (Paetkau *et al.* 1995). The probability was estimated using MCMC resampling of 1,000 individuals and the type I error was set to 0.01. The sample from the Philippines was excluded from this analysis because it was the only sample for the population and was apparently not sufficient to reflect the genetic structure of the population.

Sex-biased dispersal was assessed using FSTAT 2.9.3.2 (Goudet *et al*. 2002). With the assumption that females are the more philopatric sex, the differences between the sexes were tested for various statistics, including mean and variance of assignment indices, F_{IS}, F_{ST}, relatedness, H_o, and within-group gene diversity (H_S) with two-tailed *t* tests, with 1,000 permutations. Since this analysis is based on fixation indices (*i.e*., F_{ST}), the estimates were calculated for all putative populations except the Philippines

and Central-Northeast Pacific as was applied in the AMOVA and pairwise F-Statistics estimations.

Mitochondrial DNA data configuration

To inspect a broader perspective of Risso's dolphin population structure, the mtDNA control region sequences of the North Pacific samples were compared against the samples collected in the North Atlantic Ocean and Mediterranean Sea. The British and Mediterranean populations were reconstructed according to Gaspari *et al*. (2007), using the 16 mtDNA haplotypes available on GenBank (http://www.ncbi.nlm.nih.gov/genbank/; accession numbers DQ668035-DQ668050). The mtDNA data of 35 dolphins biopsied in the waters around the Azores were also included (Hartman *et al.* unpublished data). Together with the Risso's dolphin sequences obtained in this study, all sequences were aligned using MEGA 5.05 or MEGA 6 to identify the consensus sequence for further analyses.

The software DnaSP version 5.10 (Librado & Rozas 2009) was used to determine the number of variable sites, mtDNA haplotypes, gene diversity (h) and nucleotide diversity (π) for putative populations with a sample size larger than five. To visualize the genealogical distance among the mtDNA haplotypes, a median-joining

network map (Bandelt *et al*. 1999) was constructed using PopART (http://popart.otago.ac.nz).

Mitochondrial DNA data analysis: population structure

As applied in the microsatellite analysis, the level of population differentiation among the putative populations was also evaluated using AMOVA and pairwise comparison of frequency-based and distance-based fixation indices, F_{ST} and Φ_{ST}, using Arlequin 3.5.1. In AMOVA, which allows examining the differences in different level of population hierarchy, the putative populations were classified into two groups, the North Pacific (Taiwan, East Japan, Sea of Japan, Oregon-California Coastal and Eastern Tropical Pacific) and the North Atlantic (British, Mediterranean Sea and Azores), to test whether the population differentiation between the two major ocean basins in the Northern Hemisphere was statistically significant. For Φ_{ST}, the Tamura and Nei model (Tamura & Nei 1993) was used, with a gamma value of 0.326, as it was determined as the best model for the samples, using the Akaike Information Criterion (AIC) implemented in the model comparison program jModelTest 2.1.6 (Darriba *et al.* 2012). The level of differentiation between putative population pairs was estimated with 10,000 non-parametric permutations. The statistical significance level was set at $p<0.05$; Bonferroni correction was applied in pairwise comparison.

Mitochondrial DNA data analysis: population dynamics

To test the neutrality of the mtDNA control region sequences, Arlequin 3.5.1 was used to estimate Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) for each putative population and to test their statistical significance (*i.e.*, different from zero) by simulating 10,000 samples. The statistical significance was set as $p<0.05$ for Tajima's D and $p<0.02$ for Fu's Fs (Fu 1997). The analysis of mismatch distributions implemented in the same program was also conducted to examine if any putative Risso's populations had ever experienced demographic or spatial expansions (Rogers & Harpending 1992; Schneider & Excoffier 1999; Excoffier 2004; Ray *et al*. 2003). The confidence intervals of the estimates were obtained using 10,000 bootstrap simulations of an instantaneous expansion under a coalescent framework. The sum of square deviations (SSD) between the observed and the expected mismatch and the raggedness index (r) of the observed distribution were calculated and tested to evaluate model fitness (Harpending 1994; Schneider & Excoffier 1999).

The time of population expansion (T) was calculated for each putative population using the formula $T=\tau/2u$, where τ is the simulated time of demographic or spatial expansion (derived from the mismatch analysis), and u is the mutation rate per generation for the sequence in use (Rogers 1995). The u can be calculated by u=(length

of the sequence)×(generation time)×(substitution rate; λ). I assumed the generation time of Risso's dolphin to be 22 years, as an average of the age at sexual maturity (8—10 years) and the known age of oldest reproductively-active female (34.5 year-old) (Amano & Miyazaki 2004; Chen *et al.* 2011). For the λ, I used an approximate average of the mtDNA control region λ estimated for multiple animal taxa using ancient DNA samples, which is 1×10^{-7} substitutions/per site/per year (Ho *et al*. 2011a).

Results

Data overview and microsatellite data configuration

Genomic DNA was successfully extracted in 280 of the 296 tissue samples acquired from various sources. For microsatellite analysis, 266 samples were fully genotyped at 22 microsatellite loci, although some samples (n=15) showed a minor level of missing data (ranged from 1 to 4 loci per sample). The genetic assessment showed one sample (ID#4694) was a pilot whale. The Kingroup analysis showed there were 40 potential parent-offspring or full-sibling pairs ($r>0.4$, $p<0.001$). Among them, five pairs were from Oregon-California Coastal, one from Taiwan, one from Sea of Japan and the rest were from East Japan (Table 2.2). The individuals in pairs G1 and G2 were suspected to be replicated samples with mislabeled ID, because they had the same microsatellite profile and mtDNA haplotype. There were a large number of potential parent-offspring or full-sibling pairs from East Japan. Those were samples collected from a single school

of dolphins taken in a drive fishery, and the school was regarded as a nursery school, because it contained a considerable number of females and calves (Amano & Miyazaki 2004). The data of one individual from those putative parent-offspring or full-sibling pairs was discarded to avoid potential sampling bias toward certain kin groups (as the measure applied in Martien *et al.* [2012] and Lowther-Thieleking *et al.* [2015]). For the five pairs from Oregon-California Coastal, except the pair G15, no individual was omitted because they were sampled in different years at different sites, under different occasions. In G15, one of the samples was discarded, because both samples were collected in the same biopsy trip at the same site. In short, 30 individuals were excluded, and there were 236 individuals remained for the following analyses.

In the jack-knife HWE test, 15 samples were identified having a rare allele homozygote (or heterozygote of two rare alleles) that was influential to the estimates of HWE. Most of the alleles were associated with the locus Dde69 or D22 (Table 2.3). Morin *et al*. (2009) suggested poor genomic DNA quality may result in poor microsatellite amplification and consequently promote the likelihood of finding a homozygous rare allele. However, the quality of the genomic DNA appears to be not an issue in these samples, as there was no major difficulty experienced in amplifying the loci or scoring the allele sizes with these samples. Since the presence of these rare allele homozygotes could be natural, these samples were retained for further analyses.

Table 2.2. Potential parent-offspring pairs in the Risso's dolphin samples identified by kinship analysis. The letter following the ID indicates the sex (F, female; M, male; U, unknown), and the ID in bold with asterisk indicates the sample is discarded in further analyses.

Pair no.	ID-1	ID-2	Sample source1	Pop	Sampling year	r	Parent-offspring test, p	Full-sibling test, p
G1	10Gg018(F)*	10Gg100(F)	S	SOJ	2004/2003	1	0	0
G2	10Gg003(M)	10Gg087(U)*	S	EJN	1999/1991	1	0	0
G3	EW01211(F)	EW01229(F)*	DF	EJN	1991	0.658	0	0
G4	EW01207(F)	EW01227(F)*	DF	EJN	1991	0.622	0	0
G5	SW26642(M)	SW88952(M)	S/ BI	OCC	2002/2009	0.615	0	0
G6	EW01223(F)	EW01238(F)*	DF	EJN	1991	0.57	0	0
G7	EW01215(M)	EW01255(F)*	DF	EJN	1991	0.552	1	0
G8	EW01196(F)	EW01218(F)*	DF	EJN	1991	0.539	1	0
G9	EW01233(M)	EW01250(F)*	DF	EJN	1991	0.537	1	0
G10	EW01196(F)*	EW01210(M)	DF	EJN	1991	0.534	1	0
G11	EW01214(M)	EW01216(F)*	DF	EJN	1991	0.523	0	0
G12	EW01219(F)	EW04585(F)*	DF	EJN	1991	0.517	0	0
G13	EW01205(M)	EW01218(F)*	DF	EJN	1991	0.511	1	0
G14	EW01221(M)	EW01251(F)*	DF	EJN	1991	0.505	0	0
G15	SW26306(M)	SW26309(F)*	BI	OCC	2001	0.505	1	0
G16	EW01198(F)	EW01232(F)*	DF	EJN	1991	0.494	1	0
G17	EW01235(F)*	EW01256(F)	DF	EJN	1991	0.475	0	0
G18	EW01197(F)	EW01246(M)*	DF	EJN	1991	0.474	0	0
G19	EW01257(F)	EW01259(M)*	DF	EJN	1991	0.474	1	0
G20	10Gg090(F)*	EW01204(F)	DF	EJN	1991	0.47	1	0
G21	EW01252(F)	EW01253(F)*	DF	EJN	1991	0.467	1	0
G22	EW01218(F)*	EW01243(M)	DF	EJN	1991	0.465	1	0
G23	10Gg090(F)*	EW01217(F)	DF	EJN	1991	0.463	1	0
G24	EW01196(F)	EW01205(M)*	DF	EJN	1991	0.462	1	0
G25	10Gg094(F)*	EW01212(F)	DF	EJN	1991	0.458	1	0
G26	EW01220(F)	EW01237(M)*	DF	EJN	1991	0.455	1	0
G27	EW01195(M)	EW01208(F)*	DF	EJN	1991	0.453	0	0
G28	EW01224(F)	EW05120(M)*	DF	EJN	1991	0.447	1	0
G29	724(M)	726(F)*	FI	TWN	2001	0.445	1	0
G30	10Gg094(F)*	EW01202(F)	DF	EJN	1991	0.442	1	0
G31	EW01199(M)	EW01242(F)*	DF	EJN	1991	0.436	0	0
G32	EW01216(F)*	EW01217(F)	DF	EJN	1991	0.434	1	0
G33	EW01211(F)	EW01245(M)*	DF	EJN	1991	0.42	1	0.001
G34	10Gg091(F)	EW01209(F)*	DF	EJN	1991	0.418	1	0
G35	1291(F)	5001(F)	FI	OCC	1993/1995	0.413	1	0.004
G36	EW01229(F)	EW01245(M)*	DF	EJN	1991	0.404	1	0.002
G37	EW01212(F)	EW01235(F)*	DF	EJN	1991	0.401	1	0.012
G38	EW01217(F)	EW01225(M)*	DF	EJN	1991	0.401	1	0
G39	1291(F)	41842(F)	FI/BI	OCC	1993/2004	0.401	1	0
G40	26642(M)	32940(F)	S	OCC	2002/2003	0.4	1	0

[1] Samplie source: BI, biopsy; DF, drive fishery; FI, fishery interaction; S, stranding.

In the 22 loci microsatellite dataset, the observed heterozygosity ranged from 0.666 to 0.722 for the putative populations (Table 2.4). Two loci, KWM1b and

TexVet7, were monomorphic. The locus EV14 showed both null alleles and deviation from HWE in almost all putative populations (Appendix 2.2). The data of these three loci were therefore discarded. The observed heterozygosity of D22 in the Taiwan population and Dde59 in the East Japan population also significantly deviated from HWE, but it appears to be population specific, therefore the data of these two loci were retained. No locus was eliminated due to significant LD because no pairwise LD was consistently detected in every population. Therefore the following analyses were then conducted using microsatellite data derived from 19 loci (AAT44, D14, D22, Dde59, Dde65, Dde66, Dde69, Dde70, Dde72, Dde84, EV37, KWM12a, KWM2b, KWM9b, MK3, MK5, Sco28, Sco11, Sco55) for a total of 236 individuals.

Table 2.3. The individuals and alleles that are influential to the HWE of the samples.

Sample ID	Pop	Locus	Allele ID (frequency)	Observed/Jack-knife P value	Observed/Jack-knife odds	Odds ratio
11694	ETP	EV37	206 (0.004)	0.000/0.067	0.000/0.072	Inf
1153	TWN	Dde65	190 (0.004)	0.000/0.094	0.000/0.104	518.660
294	TWN	MK5	200 (0.006)	0.034/0.871	0.035/6.734	194.280
738	TWN	Dde66	349 (0.013)	0.013/0.163	0.013/0.194	14.628
38253	ETP	Dde69	196 (0.017)	0.032/0.201	0.033/0.252	7.595
1030	TWN	Dde69	208 (0.049)	0.032/0.075	0.033/0.082	2.459
724	TWN	Dde69	216 (0.094)	0.032/0.061	0.033/0.065	1.969
10Gg023	EJN	Dde69	188 (0.126)	0.032/0.055	0.033/0.058	1.748
EW01240	EJN	Dde69	200/208 (0.239/0.049)	0.032/0.053	0.033/0.056	1.694
39083	OCC	MK5	208 (0.530)	0.034/0.052	0.035/0.055	1.589
EW05119	EJN	D22	130 (0.194)	0.036/0.054	0.038/0.057	1.517
908	TWN	D22	132 (0.105)	0.036/0.052	0.038/0.055	1.458
61944	OCC	D22	132 (0.105)	0.036/0.052	0.038/0.055	1.446
62	OCC	D22	124 (0.188)	0.036/0.050	0.038/0.053	1.399
EW01226	EJN	D22	126 (0.160)	0.036/0.050	0.038/0.053	1.396

Table 2.4. The averages (±SD) of the number of alleles, expected heterozygosity (H_E), observed heterozygosity (H_O), allelic richness and inbreeding coefficient (F_{IS}) across the 22 microsatellite loci within each putative population examined in this study. See Appendix 2.2 for the estimates by locus in each population.

Population	n	No. of alleles	H_E	H_O	Allelic richness	F_{IS}
Taiwan	49	9.842±4.682	0.711±0.222	0.688±0.213	1.653±0.296	0.056
East Japan	72	9.789±4.826	0.705±0.222	0.680±0.219	1.645±0.294	0.057
Sea of Japan	12	6.105±2.208	0.698±0.203	0.697±0.243	1.634±0.278	0.015
Central-Northeast Pacific	7	5.333±2.196	0.743±0.178	0.690±0.221	1.637±0.305	0.089
Eastern Tropical Pacific	22	8.389±3.712	0.739±0.207	0.722±0.210	1.642±0.321	0.052
Oregon-California coastal	73	9.368±4.573	0.691±0.245	0.666±0.238	1.637±0.310	0.062

Microsatellite data analysis: population structure

When sample coordinates were not referenced back to the population centre, the resolution of FCA for Risso's dolphin in the North Pacific was poor: the sum of FC1 and FC2 could only explain about 4% of variances, and no obvious population structure could be found (Fig. 2.3A). However, when using the population centre reference, the power of the analysis increased to 52%, and a pattern of three clusters emerged (Fig. 2.3B). One cluster was composed of individuals from East Japan, Sea of Japan, Taiwan and the Philippines, another cluster consisted of individuals from Oregon-California Coastal and Central Northeast Pacific, and the other cluster consisted of individuals from the Eastern Tropical Pacific. The most informative factor (FC1), which represented 30.8% of the variance in the sample, indicated a difference between the Oregon-California Coastal/Central Northeast Pacific cluster and the other two clusters. The Eastern Tropical Pacific was isolated by the second most informative factor, FC2

(21.1%), and its level of overlapping with the other two major clusters was the most constrained.

A similar pattern of results was found in STRUCTURE analysis. When the LOCPRIOR option was not used, although the Evanno's ΔK suggested the most likely number of populations (K) was 2, the best estimate is K=1 according to the estimate of mean LnP(K) and the graphic output (Table 2.5, Fig. 2.4A). When the LOCPRIOR option was used, on the other hand, the Evanno's ΔK, LnP(K) value indicated K=2, while the graphic result showed meaningful structure for K=2 and K=3 (Fig 2.4B, C). In the K=2 scenario, the individuals from Oregon-California Coastal and Central Northeast Pacific were assigned to one cluster, and the individuals from Eastern Tropical Pacific, East Japan, Sea of Japan, Taiwan and the Philippines were assigned to the other. In the K=3 scenario, the pattern was identical but the individuals from Eastern Tropical Pacific were isolated from the western North Pacific cluster. The partitioning appears to agree with the segregation of FC1 in the FCA result (Fig. 2.4B), but the differentiation at FC2 was only revealed when K=3 (Fig. 2.4C).

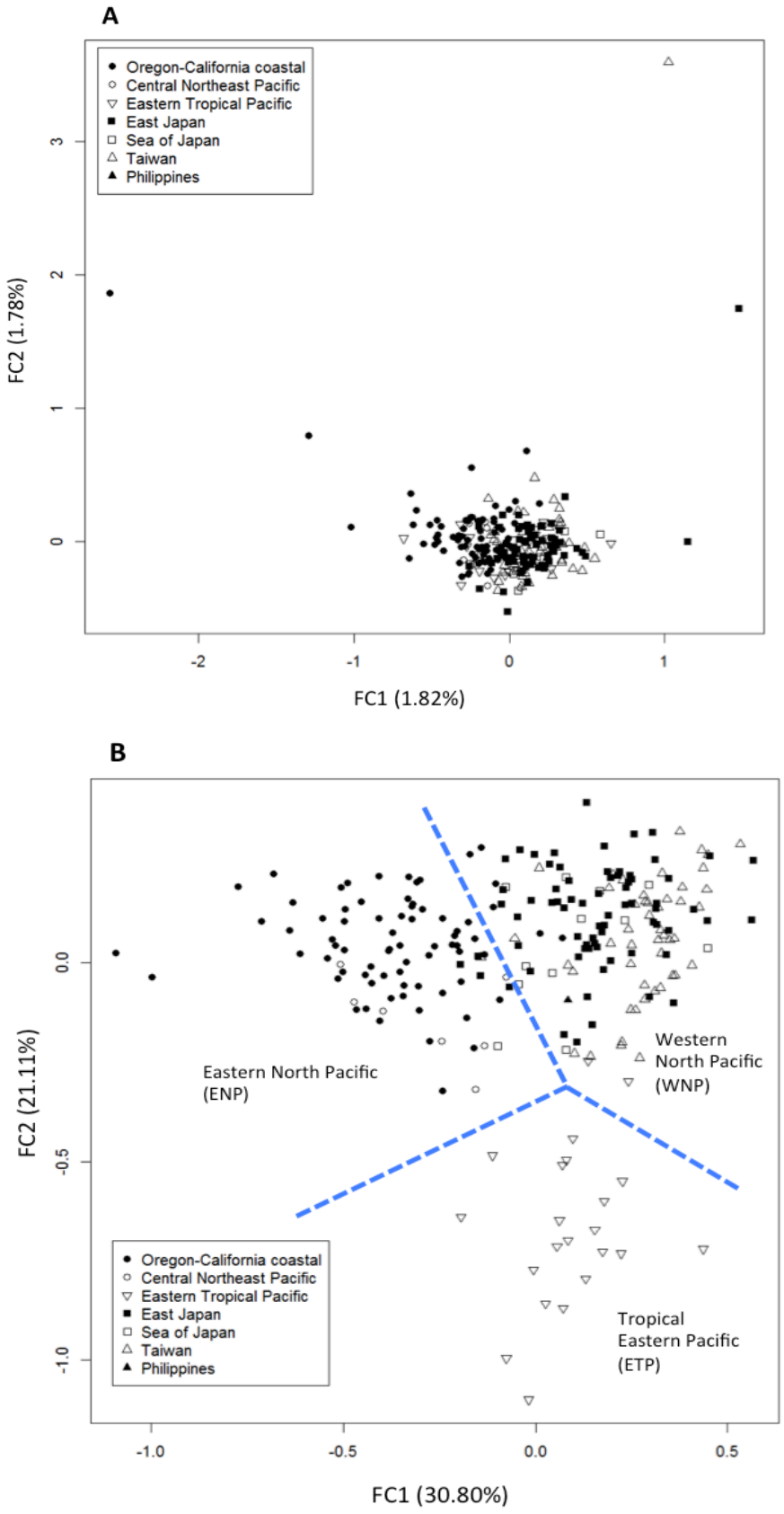

Figure 2.3. Results of the factor correspondence analysis (FCA) A) without using population information; B) using population information. The two most informative factors (FC1 and FC2) are assigned as the X and Y axes in the plots, and the numbers in parentheses in each axis indicate the percentage of the variance explained by the factor.

Table 2.5. The Evanno table generated by Structure Harvester for the STRUCTURE analysis. The asterisk indicates the most likely K (i.e., the largest ΔK value).

LOCPRIOR option	K	Mean LnP(K)	SD of LnP(K)	Ln'(K)	|Ln''(K)|	ΔK
Used	1	-15215.44	0.7792	NA	NA	NA
Used	2*	-15286.11	63.9325	-70.67	1155.79	18.078284
Used	3	-16512.57	193.5957	-1226.46	1611.76	8.325393
Used	4	-16127.27	267.9372	385.3	938.54	3.502835
Used	5	-16680.51	282.8168	-553.24	105.73	0.373846
Used	6	-17339.48	466.5879	-658.97	335.92	0.71995
Used	7	-17662.53	253.8915	-323.05	57.12	0.224978
Used	8	-17928.46	367.4595	-265.93	NA	NA
Not used	1	-15215.45	0.5701	NA	NA	NA
Not used	2*	-15080.89	17.5687	134.56	211.34	12.029356
Not used	3	-15157.67	40.4682	-76.78	45.92	1.134718
Not used	4	-15280.37	141.2912	-122.7	1.93	0.01366
Not used	5	-15401.14	147.3893	-120.77	2.82	0.019133
Not used	6	-15524.73	266.5318	-123.59	100.48	0.376991
Not used	7	-15547.84	429.8739	-23.11	35.38	0.082303
Not used	8	-15606.33	227.1227	-58.49	NA	NA

In the first step of the Geneland analysis testing the candidate K ranging from 1 to 10, most of the results (nine out of the 10 runs) indicated K=3 was the most likely number of populations for the samples, and the only different result indicated was K=4. In the second step of the analysis, by fixing the K to K=3, there were seven different spatial distribution patterns among the results of the 10 runs with the highest LPP. Five runs revealed a true K=3 spatial pattern (Fig. 2.5A—D), two showed a distribution pattern of K=2 (Fig. 2.5E—F), and the remaining three runs failed to reveal any population structure (*i.e.*, K=1). In the results of the five runs that show a true K=3 pattern, two of them were essentially the same as the pattern seen in the FCA result, suggesting there is a population in the Western North Pacific, another in the Eastern North Pacific, and the other in the Eastern Tropical Pacific (Fig. 2.5A). The distribution pattern shown in Fig. 2.5B was substantially the same pattern as Fig. 2.5A; the only

difference was that the sample from Hawaiian waters was assigned to different populations (*i.e.*, Western North Pacific versus Eastern Tropical Pacific). The population membership of Risso's dolphins in the central Tropical Pacific appears to be ambiguous, as it often swung between the Western North Pacific and Eastern Tropical Pacific, and in some cases it was even identified as an independent population (Fig. 2.5C—D).

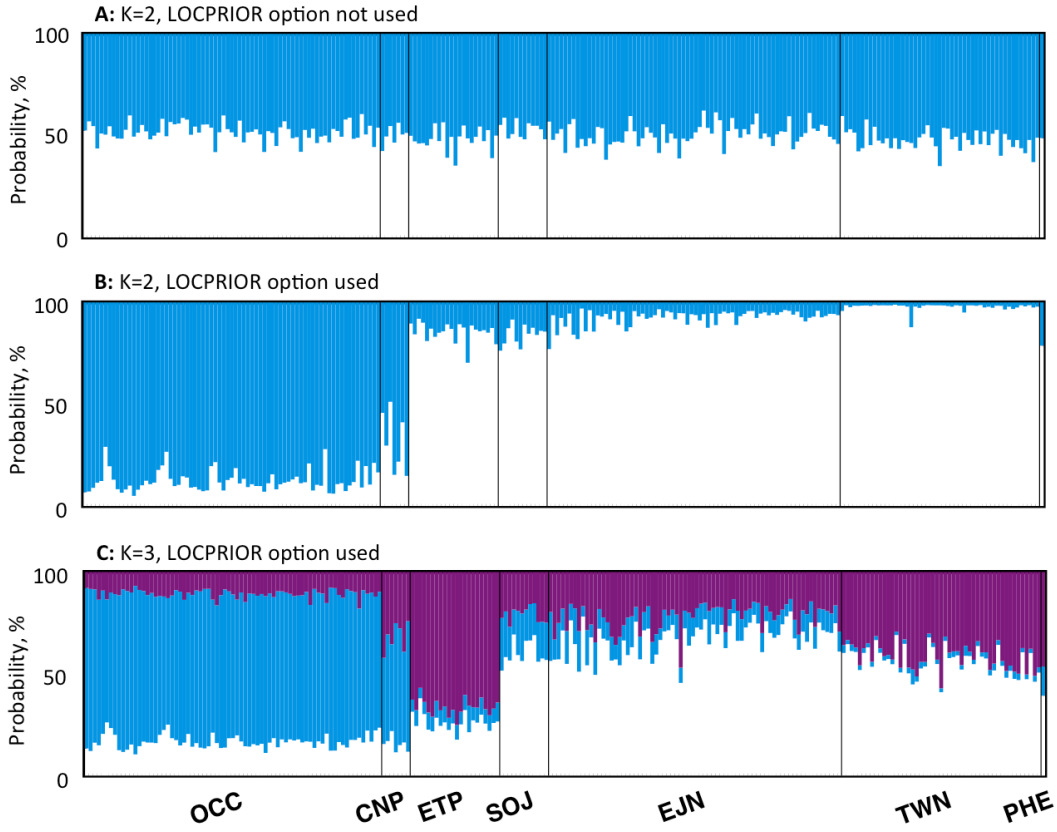

Figure 2.4. Inferred population structure from the STRUCTURE analysis: A) K=2, using no LOCPRIOR option, B) K=2, using LOCPRIOR option, and C) K=3, using LOCPRIOR option. Each vertical column represents an individual and is divided into K coloured segments that indicate the individual's estimated membership probability in K clusters. Individuals are clustered based on their *a priori* population identity, which is labelled at the bottom of the figure. See Fig. 2.2 for population abbreviations.

The result of the two runs that showed a K=2 pattern was shown in Fig. 2.5E and 2.5F. The result shown in Fig. 2.5E suggested an isolated population of Eastern Tropical Pacific, coincided with the differentiation pattern suggested by FC2 in the FCA result (Fig. 2.3B). The population membership shown in Fig. 2.5F, on the other hand, suggested those from Eastern Tropical Pacific and Western North Pacific were from the same population, in agreement with the K=2 result found in the STRUCTURE analysis (Fig. 2.4B).

From this Geneland analysis it appears to be difficult to determine a definite population membership for Risso's dolphins in the North Pacific. Nevertheless, some patterns are consistently seen in all cases, whether it was K=2 or 3. For instance, the samples for Oregon-California Coastal and Eastern Tropical Pacific were always assigned to different populations, and the sample collected in Hokkaido, Japan, was consistently grouped with those samples collected from the Northeast Pacific Ocean (*i.e.*, Oregon-California Coastal), rather with those samples collected in adjacent waters (East Japan and Sea of Japan).

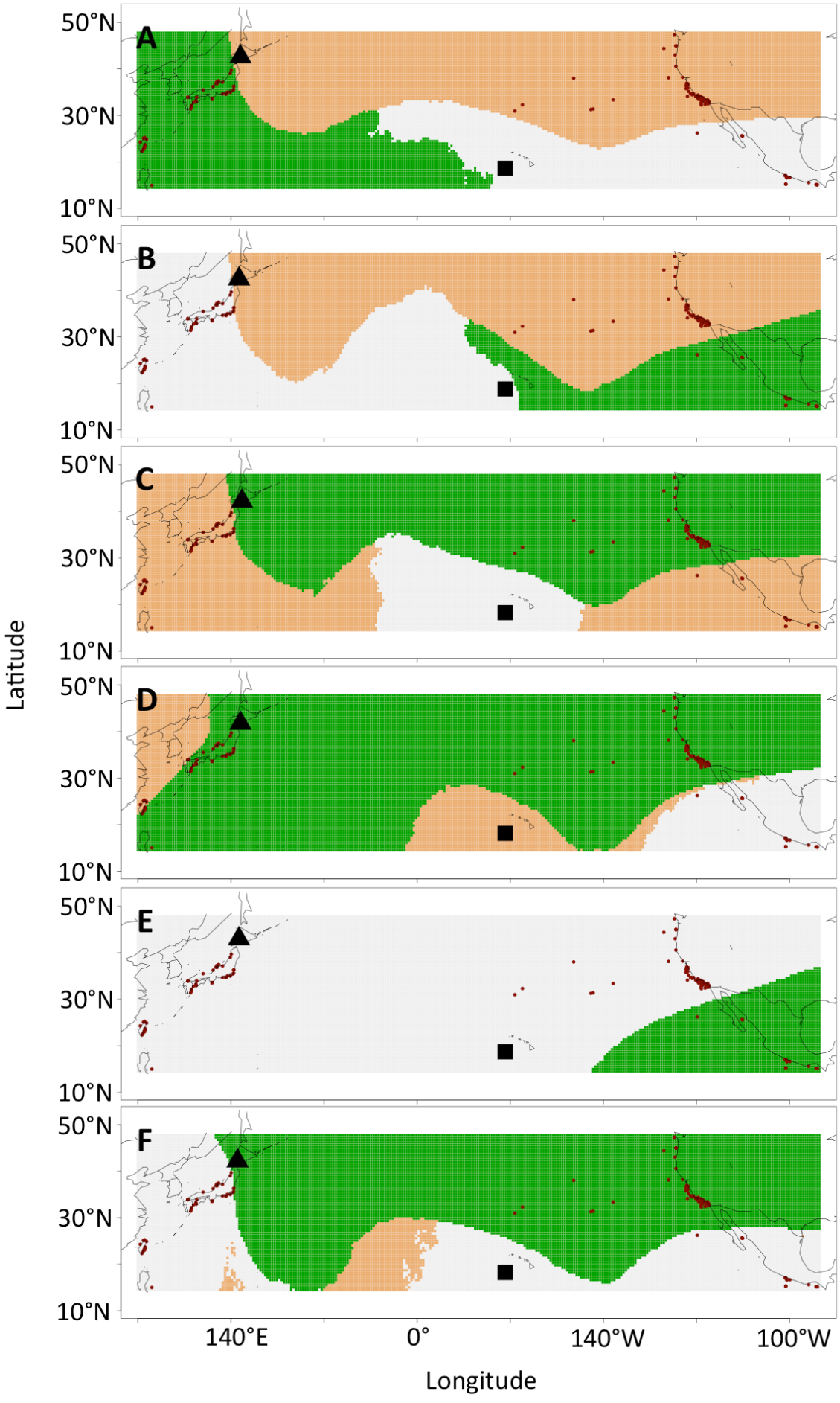
A
B
C
D
E
F
50°N
30°N
10°N
140°E
0°
140°W
100°W
Latitude
Longitude

Figure 2.5. Individual population membership assignment patterns shown in the 10 runs with the highest LPP for K=3 in Geneland analysis. Four different patterns suggesting K=3 are shown in A—D; two patterns suggesting K=2 are shown in E and F. The one pattern suggesting K=1 is not shown here. The red dots represent the samples; the colours indicate the distribution of K clusters based on the mode of simulated posterior probability for each pixel. Solid triangle, the Hokkaido sample; solid square, the Hawaiian sample.

In the F_{ST} analysis, the AMOVA result indicated that the differences between western and eastern North Pacific population groups, and among the six testable putative populations, were both statistically significant (Table 2.6A). Pairwise F_{ST} comparison further showed that the putative population of Oregon-California Coastal was the most differentiated population (F_{ST}=0.011—0.016, p=0—0.002), and the populations in the western North Pacific population group were the least differentiated (F_{ST}=0.001—0.004, p=0.393—0.5; Table 2.7). Differentiation between Sea of Japan and Eastern Tropical Pacific was only marginally significant (F_{ST}=0.0097, p=0.045).

When the level of differentiation was evaluated using R_{ST}, the AMOVA no longer supported any sign of differentiation between western and eastern North Pacific population groups, but the differentiation among the six putative populations persisted, although the statistical power was only marginal (Table 2.6B). Pairwise comparison results showed that significant differentiation was only found for comparisons with the Oregon-California Coastal population (R_{ST}=0.019—0.044, p=0—0.022), which was consistent with the differentiation pattern shown in STRUCTURE analysis.

Differentiation between the Eastern Tropical Pacific and Oregon-California Coastal, and between East Japan and Sea of Japan were ambiguous, as the statistical significance was marginal (Table 2.7).

Table 2.6. Result of AMOVA using different distance method: A) number of different alleles (F_{ST}) and B) Sum of squared size difference (R_{ST}). The five putative populations are grouped into two groups, the western North Pacific group (TWN, SOJ, EJN) and the eastern North Pacific group (OCC, ETP).

	Sum of squares	Variance components	Percentage variation	P	Fixation index
A: No. of different alleles (F_{ST})					
Among groups	20.842	0.043	0.639	0.001**	0.006
Among populations within groups	28.495	0.039	0.582	0.0001***	0.006
Within populations	2992.140	6.658	98.779	0.000***	0.012
Total	3041.478	6.741			
B: Sum of squared size difference (R_{ST})					
Among groups	942.170	2.034	0.974	0.105	0.010
Among populations within groups	1145.583	2.458	1.177	0.009**	0.012
Within populations	91830.620	204.292	97.848	0.000***	0.022
Total	93918.373	208.784			

*: p<0.05, **: p<0.01; ***: p<0.001

Table 2.7. Pairwise estimates of genetic divergence between the five putative populations. Above the diagonal shows the estimates using F_{ST} and below the diagonal shows the estimates using R_{ST}.

			F_{ST}				
		n	OCC	ETP	SOJ	EJN	TWN
	OCC	73	--	0.016***	0.013**	0.011***	0.016***
	ETP	22	0.019*	--	0.010*	0.012***	0.009**
R_{ST}	SOJ	12	0.044**	0.009	--	0.001	0.005
	EJN	72	0.021***	0.000	0.023*	--	0.001
	TWN	49	0.035***	-0.008	0.023	0.001	--

*: p<0.05, **: p<0.01; ***: p<0.001

When all samples were involved, the RDA result showed that the geography (longitude and latitude) explains 2.39% of total genetic variance and the effect was

statistically significant (F=2.848, p<0.001). The partial RDA result showed that more genetic variance was explained by longitude (1.42%) than latitude (0.84%). However, if the RDA was conducted using samples from the eastern populations only, namely those from Oregon-California Coastal, Central-Northeast Pacific and Eastern Tropical Pacific, the effect became insignificant (F=1.435, p=0.061), even though the geography was found to explain 2.82% of the genetic variance. The partial RDA result suggests latitude could be a significant factor for the genetic structure of Risso's dolphin on the eastern side of the North Pacific, although it explains only 1.88% of the genetic variance and the statistical significance was also marginal (F=1.911, p=0.029) (Table 2.8). Although the results seems to suggest that the population structure of Risso's dolphins in the North Pacific Ocean is due to the effect of isolation by distance, the lack of sufficient samples collected uniformly across the study area raises the possibility that the correlation between genetic distance and geographic distance is simply reflecting the difference of two (or more) distant populations which have been segregated due to other effects.

To sum up the results shown in FCA, Geneland, STRUCTURE and pair-wise F-statistics comparisons, it can be concluded that the population structure of Risso's dolphins in the North Pacific Ocean consists of three major populations: one in the Western North Pacific (East Japan, Sea of Japan, Taiwan and the Philippines), another in the Eastern Tropical Pacific, and the third in the Eastern North Pacific (Oregon-

California Coastal and Central Northeast Pacific), with the population membership for the dolphins inhabiting the Central Tropical Pacific (*e.g.,* Hawaiian waters) remaining unsettled.

Table 2.8. Results of the redundancy analysis (RDA). The eastern populations include the samples from OCC, ETP and CNP.

Variable	Variance explained	Proportion of total variance	F	P value
All samples, n=236				
Longitude+Latitude	5.187	0.024	2.848	>0.001***
Longitude only	3.097	0.014	3.400	>0.001***
Latitude only	1.824	0.008	2.003	0.016*
Eastern populations, n=102				
Longitude+Latitude	6.37	0.028	1.435	0.061
Longitude only	1.766	0.008	0.796	0.662
Latitude only	4.241	0.019	1.911	0.029*

*: $p<0.05$, **: $p<0.01$; ***: $p<0.001$

Microsatellite data analysis: population dynamics

To estimate the effective population size and migration rate for the three main populations concluded in the population structure section (*i.e.*, the Western North Pacific, Eastern North Pacific and Eastern Tropical Pacific populations), the microsatellite data of putative populations East Japan, Sea of Japan, Taiwan and the Philippines were combined as a Western North Pacific population, with the data of Oregon-California Coastal and Central Northeast Pacific populations as an Eastern North Pacific population, for Migrate analysis. The Hokkaido sample from East Japan

and the Hawaiian sample from Central Northeast Pacific were excluded from this Migrate analysis due to the uncertainty of their population identity.

The estimates of $N_e\mu$ suggest the N_e was about the same but slightly larger for the Eastern North Pacific population ($N_e\mu$=0.457, 95%CI=0.439—0.476) than for the Western North Pacific population ($N_e\mu$=0.371, 95%CI=0.361—0.382). The $N_e\mu$ estimate for the Eastern Tropical Pacific population was 3.399 (95%CI=3.197—3.619), which is about an order of magnitude larger than the estimates for the other two populations (Table 2.9). The estimates for the number of migrants per generation (N_em) ranged from 0.053 to 0.287 among the three putative populations. Interestingly, despite the geographic adjacency of the two eastern populations and the 'remoteness' of the western population, the estimates suggest that the Eastern North Pacific population received more immigrants from the Western North Pacific (N_em=0.227, 95%CI=0.207—0.249) than from the Tropical Eastern Pacific population (N_em=0.096, 95%CI=0.083—0.110), and dispatched more immigrants to the Western North Pacific (N_em=0.287, 95%CI=0.255—0.321) than to the Tropical Eastern Pacific population (N_em=0.053, 95%CI=0.038—0.072). The lowest rates of N_em were usually associated with the Eastern Tropical Pacific population, implying that emigration to the Eastern Tropical Pacific was the least likely route of long-term gene flow for the other two populations (Table 2.9).

Table 2.9. Estimates of effective population size times mutation rate ($N_e\mu$) and number of migrants per generation (N_em) from the microsatellite data. The host populations are in columns and the source populations are in rows. The 2.5th and 97.5th profile likelihood estimates are given in parentheses. Abbreviations: ENP, Eastern North Pacific; ETP, Eastern Tropical Pacific; WNP, Western North Pacific.

	Source population	Host population		
		ENP	ETP	WNP
$N_e\mu$		0.457 (0.439—0.476)	3.399 (3.197—3.619)	0.371 (0.361—0.382)
N_e (low)		2285 (2197—2378)	16996 (15983—18093)	1855 (1804—1908)
N_e (high)		4570 (4394—4755)	33991 (31966—36186)	3710 (3608—3816)
N_em	ENP		0.053 (0.038—0.072)	0.287 (0.255—0.321)
	ETP	0.096 (0.083—0.110)		0.159 (0.136—0.184)
	WNP	0.227 (0.207—0.249)	0.191 (0.161—0.226)	

Geneclass2 identified five individuals that were potentially first-generation migrants in their home population (Table 2.10). The result agrees with the long-term gene flow estimated by Migrate that the Western North Pacific population is likely playing the role of major immigrant source, while the Eastern North Pacific population is the main immigrant host. Although the fact that four of the five individuals are male may imply the existence of sex-biased dispersal, the two-tailed *t* tests for examining sex-biased dispersal revealed that the difference of dispersal pattern between the sexes was statistically insignificant (Table 2.11).

Table 2.10. Potential first generation migrants identified in GeneClass2. The asterisk indicates the most likely origin for the sample. The letter following the ID indicates the sex (F, female; M, male). Abbreviations: ENP, Eastern North Pacific; ETP, Eastern Tropical Pacific; WNP, Western North Pacific.

ID	Host population	-LOG(L_home/L_max)	p	Potential origin population		
				ENP	ETP	WNP
				-log(L)	-log(L)	-log(L)
10Gg032(M)	WNP	2.78	0.002	25.737	23.682*	26.462
11694(M)	ETP	3.761	0.001	29.108	27.939	24.179*
1564(F)	ENP	2.057	0.005	25.303	25.465	23.247*
23799(M)	ENP	1.592	0.007	24.952	25.48	23.359*
39082(M)	ENP	1.753	0.007	22.833	21.08*	22.388

Table 2.11. Sex-biased dispersal assessments by two-tailed *t* tests. None of the assessments are statistically significant.

	n	F_{IS}	F_{ST}	Relativeness	Ho	Hs	Mean assignment	Var assignment
Female	104	0.028	0.015	0.029	0.681	0.701	0.418	28.853
Male	130	0.041	0.010	0.019	0.674	0.703	-0.334	24.182
Overall	234	0.034	0.013	0.025	0.677	0.702		
P-value		0.413	0.192	0.185	0.525	0.699	0.271	0.553

Mitochondrial DNA analysis: data overview

The mtDNA control region sequences of 140 Risso's dolphin samples from the Eastern Tropical Pacific, Oregon-California Coastal, Japanese, Taiwanese and Philippine waters were successfully amplified. A 473bp consensus sequence was identified after aligning the sequences with the published British and Mediterranean, and unpublished Azorean sequences. Among the total 213 sequences, fifty-six variable sites were found characterizing 85 unique haplotypes. There was no haplotype shared between the North Pacific Ocean and North Atlantic Ocean/Mediterranean Sea, and there was only one haplotype shared between the North Atlantic Ocean (from the Azores population) and

the Mediterranean Sea. Five haplotypes were shared between the eastern (Oregon-California Coastal and Eastern Tropical Pacific) and the western North Pacific Ocean (East Japan, Sea of Japan, Taiwan) (Table 2.12). In the North Pacific Ocean, the Oregon-California Coastal and Eastern Tropical Pacific populations appeared to have higher frequencies of private haplotypes (0.73 and 0.82, respectively), suggesting some level of lineage sorting and consistent with a significant level of differentiation. The median-joining network tree showed a scattered tree with many missing haplotypes, and no clear concordance between geography and haplotype clustering (Fig. 2.6). The genetic and nucleotide diversity was high in all putative populations except for the British population (Table 2.13).

Table 2.12. The frequency of 85 mtDNA haplotypes detected among the nine putative populations in the Northern Hemisphere. See Appendix 2.3 for the definitions of the haplotypes. Abbreviations: ETP, Eastern Tropical Pacific; OCC, Oregon-California Coastal; EJN, East Japan; SOJ, Sea of Japan; TWN, Taiwan; PHE, the Philippines; MED, Mediterranean Sea; UK, British waters; AZR, Azores.

	Putative population								
Haplotype ID	ETP	OCC	EJN	SOJ	TWN	PHE	MED	UK	AZR
n	21	22	33	17	42	1	24	18	35
Hap_1	7	3		1					
Hap_2	2								
Hap_3	1		3	1					
Hap_4	1								
Hap_5	2								
Hap_6	2								
Hap_7	2								
Hap_8	1								
Hap_9	1								
Hap_10	1								
Hap_11	1								
Hap_12		1							
Hap_13		1							
Hap_14		2							
Hap_15		1	1	2					
Hap_16		1							
Hap_17		2							
Hap_18		2	2	2	6				
Hap_19		1		1					
Hap_20		3							
Hap_21		1							
Hap_22		1							
Hap_23		1							
Hap_24		1							
Hap_25		1							
Hap_26			2		1				
Hap_27			1						
Hap_28				1					
Hap_29			1						
Hap_30			2		2				
Hap_31			1						
Hap_32			1						
Hap_33			6	6	8				
Hap_34				2					
Hap_35			2						
Hap_36			1						
Hap_37			2						
Hap_38			1						
Hap_39			1	1	9				
Hap_40			1						
Hap_41					5				
Hap_42					1				
Hap_43					2				
Hap_44					3				
Hap_45					1				
Hap_46					1				
Hap_47					1				
Hap_48					1				
Hap_49					1				

	Putative population								
Haplotype ID	ETP	OCC	EJN	SOJ	TWN	PHE	MED	UK	AZR
n	21	22	33	17	42	1	24	18	35
Hap_50			1						
Hap_51			1						
Hap_52			1						
Hap_53						1			
Hap_54			1						
Hap_55			1						
Hap_56							2		
Hap_57							3		
Hap_58							3		1
Hap_59							1		
Hap_60							3		
Hap_61							4		
Hap_62							1		
Hap_63							2		
Hap_64							1		
Hap_65							1		
Hap_66							1		
Hap_67							1		
Hap_68							1		
Hap_69								1	
Hap_70								5	
Hap_71								12	
Hap_72									6
Hap_73									2
Hap_74									5
Hap_75									1
Hap_76									1
Hap_77									1
Hap_78									3
Hap_79									1
Hap_80									3
Hap_81									5
Hap_82									2
Hap_83									1
Hap_84									2
Hap_85									1

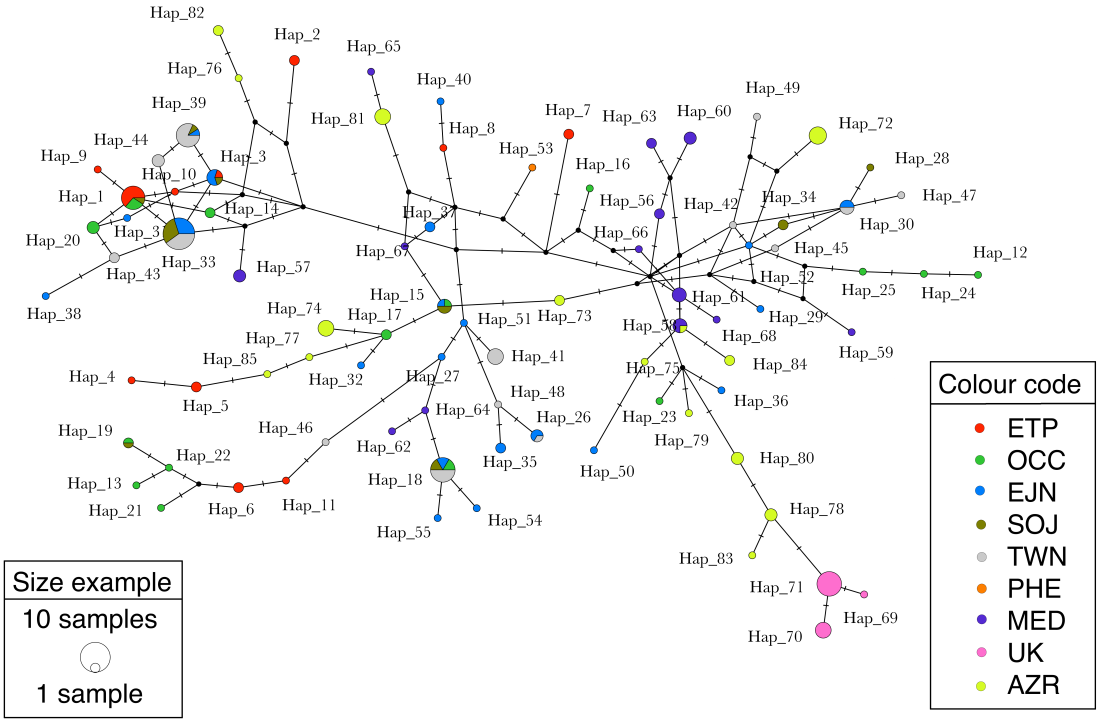

Figure 2.6. The Median-joining network tree for the 85 mtDNA control region haplotypes. Each circle represents a unique haplotype. The size of circle indicates the number of individuals having the haplotype and the colour shade indicates the proportion of each population within the haplotype. The number of hatch marks at the lines indicates the number of mutational steps separating the haplotypes. Solid circles indicate missing intermediate haplotypes.

Table 2.13. The number of mtDNA haplotypes, genetic diversity, nucleotide diversity, and population dynamic indices for the putative populations and all samples. ENP includes OCC and ETP; WNP includes EJN, SOJ and TWN.

Pop	n	No. of variable sites	No. of haplotypes	Gene diversity, h (SD)	Nucleotide diversity, π (SD)	Tajima's D	Fu's Fs
All	213	56	85	0.975 (0.004)	1.72% (0.04%)	-0.416	-24.419***
ENP	43	37	25	0.939 (0.027)	1.82% (0.13%)	0.008	-6.476
WNP	93	40	35	0.924 (0.016)	1.42% (0.07%)	-0.456	-11.580**
EJN	33	31	21	0.956 (0.022)	1.51% (0.10%)	-0.239	-6.601*
SOJ	17	24	9	0.868 (0.068)	1.42% (0.21%)	-0.206	0.292
TWN	42	27	14	0.891 (0.024)	1.33% (0.11%)	0.013	-0.166
OCC	22	31	15	0.96 (0.02)	1.89% (0.14%)	0.187	-2.475
ETP	21	27	11	0.88 (0.06)	1.71% (0.22%)	0.303	0.164
AZR	35	24	15	0.926 (0.021)	1.543% (0.09%)	0.870	-0.802
MED	24	25	13	0.938 (0.025)	1.306% (0.15%)	-0.287	-1.757
UK	18	2	3	0.503 (0.103)	0.113% (0.03%)	-0.191	-0.161

*: p<0.05, **: p<0.01; ***: p<0.001

Mitochondrial DNA data analysis: population structure

The AMOVA results showed significant differentiation between the two major ocean basins (the North Pacific Ocean versus North Atlantic Ocean-Mediterranean Sea), and among the seven putative populations (Table 2.14). In pairwise F_{ST} and Φ_{ST} comparisons, the differences were all statistically significant except for the pairs of putative populations in the Western North Pacific, and those in the Eastern North Pacific (Table 2.15). As for the results of the pairwise F_{ST} and R_{ST} comparisons using microsatellite data, the pairwise F_{ST} and Φ_{ST} among East Japan, Sea of Japan and Taiwan were statistically insignificant, suggesting that the dolphins inhabiting these region are from the same population (*i.e.*, the Western North Pacific population). However, the differentiation between Oregon-California Coastal and Eastern Tropical Pacific was statistically supported in the microsatellite data, but not supported in this mtDNA sequence data.

Nevertheless, the pairwise F_{ST} and R_{ST} results indicate there are at least five Risso's dolphin populations in the Northern Hemisphere: a population in the Western North Pacific, a population in the Eastern North Pacific, a population occurring in Azorean waters, a population in British waters, and a Mediterranean Sea population, and these populations are all well differentiated. Among these populations, the British population is the most distinct population based on the F_{ST} and Φ_{ST} values (Table 2.15).

Table 2.14. Result of AMOVA using different distance method: A) number of different alleles (F_{ST}) and B) Tamura and Nei model (φ_{ST}). The eight putative populations are grouped into two groups, the Pacific group (TWN, SOJ, EJN, OCC, ETP) and the Atlantic-Mediterranean group (MED, UK, AZR).

	Sum of squares	Variance components	Percentage variation	P	Fixation index
A: No. of different alleles (F_{ST})					
Among groups	2.782	0.0103	2.06	0.048*	0.021
Among populations within groups	9.867	0.047	9.36	0.000***	0.096
Within populations	90.219	0.442	88.58	0.000***	0.114
Total	102.868	0.499			
B: Tamura and Nei model (φ_{ST})					
Among groups	99.154	0.813	16.21	0.018*	0.162
Among populations within groups	107.097	0.552	11.01	0.000**	0.131
Within populations	744.977	3.652	72.78	0.000***	0.272
Total	951.228	5.017			

*: p<0.05, **: p<0.01; ***: p<0.001

Table 2.15. Pairwise divergence between the eight putative populations in the Northern Hemisphere estimated using F_{ST} (above the diagonal) and φ_{ST} (below the diagonal) based on the mtDNA data set.

		F_{ST}							
		ETP	OCC	EJ	SOJ	TW	MED	UK	AZR
φ_{ST}	ETP	--	0.035 *	0.076 ***	0.106 ***	0.114 ***	0.090 ***	0.302 ***	0.096 ***
	OCC	0.013	--	0.035 **	0.060 **	0.063 **	0.050 ***	0.260 ***	0.057 ***
	EJ	0.083 **	0.030	--	0.005	0.024 *	0.052 ***	0.249 ***	0.059 ***
	SOJ	0.063 *	0.031	-0.009	--	0.026	0.096 ***	0.317 ***	0.101 ***
	TW	0.112 **	0.078 *	0.010	-0.018	--	0.086 ***	0.276 ***	0.092 ***
	MED	0.265 ***	0.155 ***	0.149 ***	0.209 ***	0.238 ***	--	0.268 ***	0.065 ***
	UK	0.504 ***	0.455 ***	0.536 ***	0.642 ***	0.604 ***	0.571 ***	--	0.263 ***
	AZR	0.196 ***	0.124 ***	0.151 ***	0.203 ***	0.248 ***	0.115 ***	0.356 ***	--

*: p<0.05, **: p<0.01, ***: p<0.001

Mitochondrial DNA data analysis: population dynamics

A Tajima's D or Fu's Fs estimate of zero indicates the locus being examined is a neutral marker for the population, or there is no significant evidence of population size change in the past. A negative Tajima's D was estimated for East Japan, Sea of Japan, Mediterranean Sea and British waters, as well as for the Western North Pacific and all samples, but none of the values was statistically significant to zero. A negative Fu's Fs estimate was made for all putative populations expect for Sea of Japan and Eastern Tropical Pacific, but the value was only significant for the East Japan population, when the samples from western North Pacific Ocean were regarded as a single population (Western North Pacific), or when all samples were regarded as a global Risso's population (Table 2.13). A negative value of Fu's Fs means an excess number of alleles, a phenomenon when a population has experienced a recent population expansion or genetic hitchhiking (Fu 1997). Therefore, it suggests the overall Risso's dolphin population in the Northern Hemisphere, or at least the population in the western North Pacific Ocean, has expanded.

In the mismatch distribution analysis, the individuals from East Japan, Sea of Japan, Taiwan and the Philippines were assigned to a Western North Pacific population, and the individuals from Oregon-California Coastal and Eastern Tropical Pacific were assigned to an Eastern North Pacific population, according to the population structure revealed in the F_{ST} tests in the previous session. However, as microsatellite evidence

suggests that Oregon-California Coastal and Eastern Tropical Pacific could be different populations, an additional set of analyses that treated these two as independent populations was also conducted.

The results show that for most populations the distribution of observed pairwise nucleotide site differences did not differ significantly from the unimodal demographic and/or spatial expansion models, suggesting most of the populations experienced a period of rapid demographic and spatial expansion (Table 2.16; Fig 2.7, 2.8). In the three populations, the fitting to the model was rejected: the demographic expansion model for the Azores population (SSD=0.0211, p=0.0233; r=0.04771, p=0.0118), the spatial model for the British population (SSD=0.02304, p=0.0293), and the demographic expansion model for all samples (SSD=0.00548, p=0.0494). However, the raggedness index in the later two cases was not statistically significant (p=0.237 and p=0.149, respectively). For the Azores population, the distribution of observed pairwise nucleotide site differences appeared to be multimodal (Fig. 2.7, 2.8), and its fitness to the demographic model was statistically rejected by both indices, suggesting the demographic structure of the Azores population has remained stable through time. The mismatch distribution for the Eastern Tropical Pacific and Mediterranean Sea appeared to be multimodal as well. However, the statistics suggested it was not different from the unimodal model.

The estimates showed that, except for the British population, all Risso's dolphin populations experienced demographic expansions at about the same time, about 3,000–6,000 years ago (Table 2.16). The expansion time estimated for the British population is remarkably recent, only dated back to the last 706 years ago. A similar pattern was found for the estimates of the timing of a spatial expansion: except for the British population, all Risso's dolphin populations experienced spatial expansions at about the same time, about 2,000–4,000 years ago, which is somewhat later than the time of demographic expansion (Table 2.16). The expansion time for the British population is again later (73–924 years ago).

Table 2.16. Estimated parameters for the putative Risso's dolphin populations in the Northern Hemisphere under demographic expansion (A) and spatial expansion (B) models. τ is the time since expansion measured in mutational time units, SSD is the sum of squared deviation in goodness-of-fit test, T is the time of expansion estimated based on a substitution rate $\lambda=1x10^{-7}$. The 2.5th and 97.5th profile likelihood estimates are given in parentheses.

	τ (95% CI)	SSD (p value)	Raggedness index (p value)	T (95% CI)
A: Demographic expansion model				
ETP	12.105 (3.732—17.775)	0.033 (0.156)	0.055 (0.155)	5816 (1793—8541)
OCC	10.539 (5.354—14.414)	0.013 (0.326)	0.025 (0.307)	5064 (2573—6926)
ENP	10.973 (4.988—15.943)	0.010 (0.389)	0.019 (0.274)	5272 (2397—7660)
WNP	9.057 (4.518—12.852)	0.019 (0.057)	0.024 (0.082)	4352 (2171—6175)
MED	8.615 (2.814—13.350)	0.008 (0.732)	0.025 (0.585)	4139 (1352—6415)
UK	0.688 (0—1.469)	0.023 (0.134)	0.194 (0.228)	331 (0—706)
AZR	8.436 (4.686—11.104)	0.021 (0.023*)	0.048 (0.012*)	4053 (2252—5335)
All samples	9.195 (6.166—11.023)	0.005 (0.049*)	0.009 (0.143)	4418 (2963—5296)
B: Spatial expansion model				
ETP	8.056 (3.924—17.688)	0.026 (0.480)	0.055 (0.593)	3871 (1885—8499)
OCC	8.223 (5.184—13.412)	0.014 (0.379)	0.025 (0.635)	3951 (2491—6444)
ENP	7.484 (4.397—15.841)	0.011 (0.604)	0.019 (0.799)	3596 (2113—7611)
WNP	8.003 (4.944—11.329)	0.021 (0.178)	0.024 (0.695)	3845 (2376—5443)
MED	4.554 (1.816—13.232)	0.012 (0.542)	0.025 (0.771)	2188 (873—6358)
UK	0.687 (0.152—1.923)	0.023 (0.029*)	0.194 (0.237)	330 (73—924)
AZR	8.017 (4.636—10.826)	0.017 (0.292)	0.048 (0.320)	3852 (2228—5202)
All samples	9.029 (6.328—10.292)	0.006 (0.078)	0.009 (0.460)	4338 (3041—4945)

*: $p<0.05$

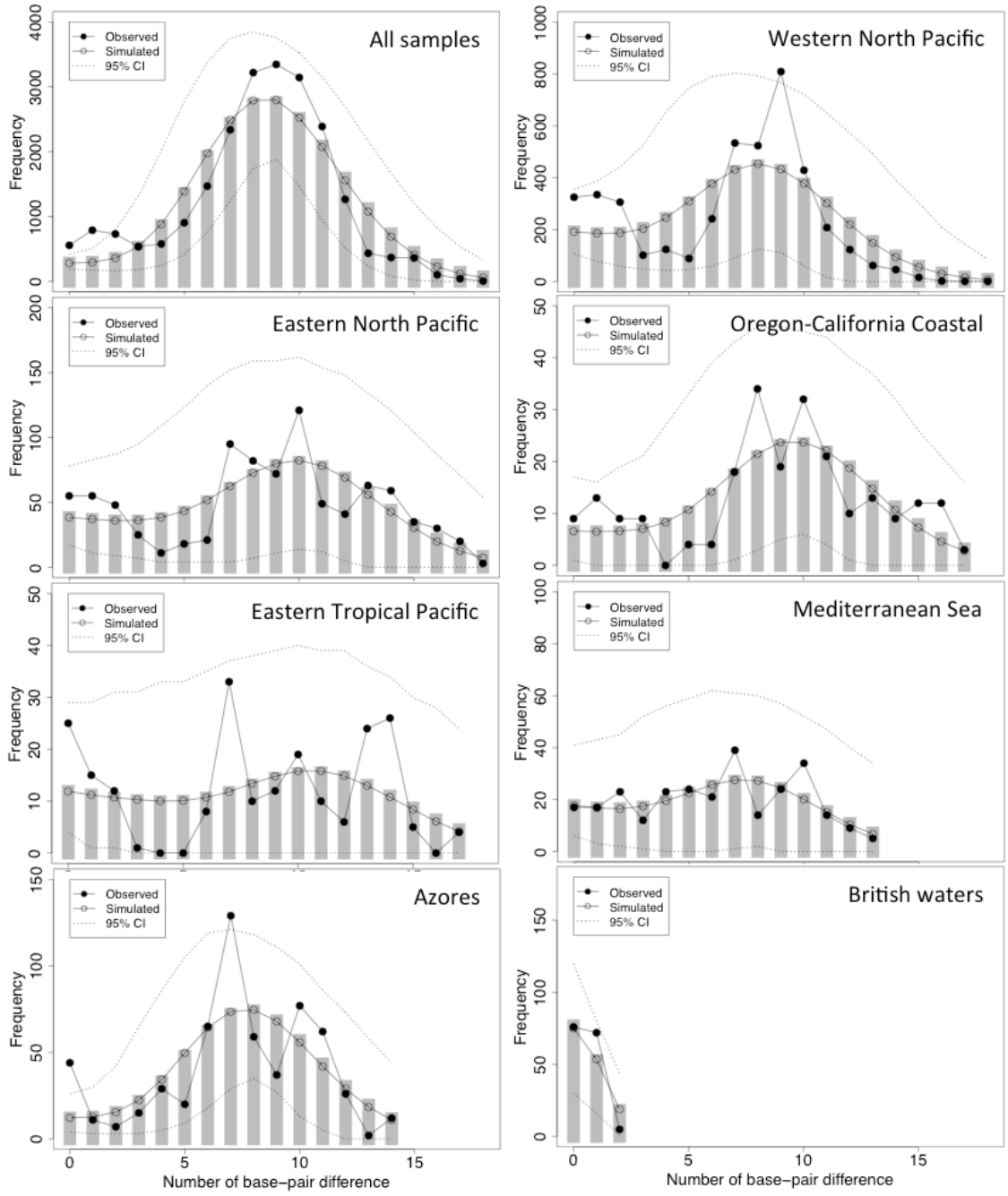

Figure 2.7. Mismatch distribution under a demographic expansion model for all samples (Northern Hemisphere) and for the populations. The x-axis shows the number of pairwise base pair differences and the y axis shows the frequency of the pairwise comparisons. The vertical bars (in grey) indicate the model frequency for the pairwise base-pair differences.

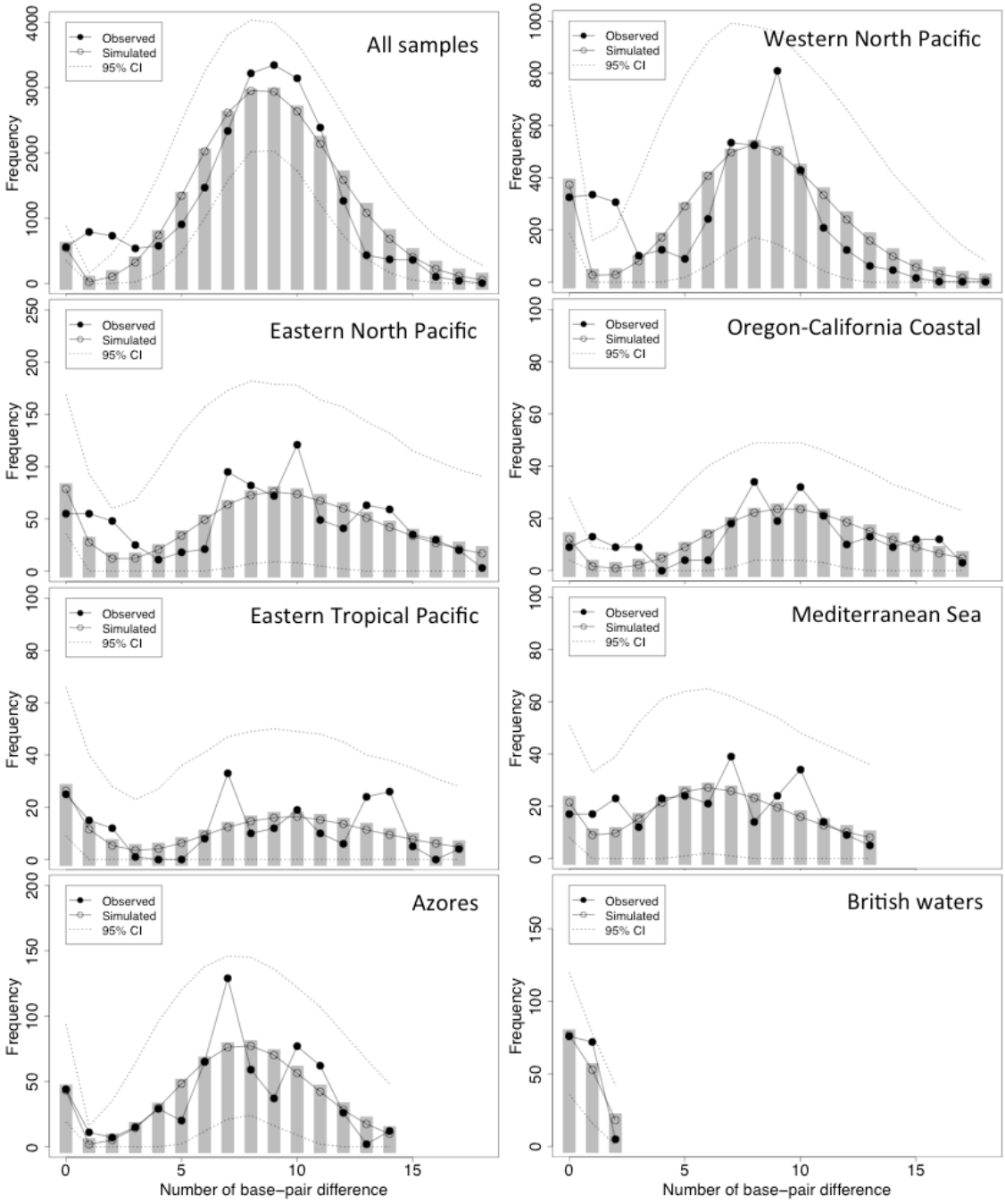

Figure 2.8. Mismatch distribution under a spatial expansion model for all samples (Northern Hemisphere) and for the populations. The x-axis shows the number of pairwise base pair differences and the y axis shows the frequency of the pairwise comparisons. The vertical bars (in grey) indicate the model frequency for the pairwise base-pair differences.

Discussion

Risso's dolphin Population structure in the North Pacific Ocean

The findings reject the hypothesis that there is no population differentiation for Risso's dolphins in the North Pacific Ocean. Jefferson *et al.* (2014) proposed that hypothesis because they found the sighting records of Risso's dolphins across the eastern and western North Pacific were continuous. Indeed, the cluster pattern of sighting records usually reflects the pattern of population aggregation (Frère *et al*. 2011; Chapter 4), but for Risso's dolphins, as their distribution may have changed through time (Leatherwood *et al*. 1980; Kruse *et al*. 1999), using data compiled from 62-year sighting records may falsely merge multiple populations, if different populations have occupied the same site at different times. In fact, several regional distribution gaps for Risso's dolphins in the North Pacific Ocean have been proposed in their original papers (e.g., Leatherwood *et al.* 1980; Miyashita 1993), but they are neglected in the review. Here, the analysis based on genetic evidence shows that some of them might be genuine population boundaries.

In the eastern North Pacific Ocean, Risso's dolphins are distributed from the equator to the southern Gulf of Alaska (around 56°N) (Jefferson *et al*. 2014). The sampling range for this study was from 5°N to 45°N, and two distinct populations are identified. The distribution of the samples of these two populations coincides with the two major aggregation zones of Risso's dolphins found in the coastal waters of the

eastern North Pacific Ocean: one off mid-southern California (30—40°N, 105—125°W) and the other off southwest Mexico (0—15°N, 80—100°W) (Leatherwood *et al*. 1980). The results support an earlier notion suggesting that dolphins found off the US west coast and northern Baja California may be distinct from the dolphins found farther south in tropical waters of the Gulf of California and the eastern Tropical Pacific (Forney & Barlow 1998). In addition, the long-term and on-going gene flow estimates further show that the exchange of migrants between these two populations is limited, confirming that the large distribution gap of Risso's dolphin sightings between 22—29°N is likely a genuine population boundary rather than a result of insufficient survey effort (Leatherwood *et al*. 1980). The mechanism that segregated the two populations is unclear and warrants further investigation, but the lack of gene flows may be due to the disconnection and heterogeneity of the habitats. Risso's dolphins appear to be highly dependent on habitat featuring upwelling regions along continental slopes (Baumgartner 1997; Smith & Whitehead 1999; Olavarría *et al.* 2001; Frantzis & Herzing 2002; Tynan *et al.* 2005; Azzellino *et al.* 2008) and the distribution of such habitat appears to be fragmented between the two regions (Zaytsev *et al.* 2003; Fiedler & Talley 2006). Furthermore, the California Current System and Eastern Tropical Pacific represent two distinct water masses and the lack of direct exchange of surface waters (Sverdrup *et al*. 1942) may passively disadvantage migrations. Even when dispersal does occur, as the two regions have contrasting environmental characteristics and have evolved into

different ecosystems (White 1994; Fiedler & Talley 2006; Spalding *et al.* 2012), the incompatibility resulting from niche specialisation may limit the settlement of immigrants from other populations (*e.g.*, Palumbi 1994, Louis *et al.* 2014).

Leatherwood *et al*. (1980) also proposed an additional aggregation zone north 43°N, off the waters of northwest Oregon and Washington States. However, the authors themselves suspect that this aggregation zone represents a temporary shift of the southern population (*i.e.,* the population off southern Californian waters), or an artefact of insufficient survey effort at the time, because the aggregation was only observed over a limited period, mostly in summertime. It is apparent that Risso's dolphins are less abundant in higher latitudes (Jefferson *et al.* 2014), and as a result, only a few were sampled from the region north of 43°N in this study. This consequently limits this study to explore the population structure of Risso's dolphin in the higher latitudes of the eastern North Pacific. Nevertheless, the STRUCTURE, Geneland and FCA results did not conclude that samples collected from higher latitudes (off Oregon and Washington States) are distinguishable from those collected from lower latitudes (off southern California), which may be consistent with the notion that the existence of this 43°N boundary is equivocal.

In the western part of the North Pacific Ocean, the analysis results indicate that Risso's dolphins occupying the waters around Japan and Taiwan are from the same population. The findings agree with earlier morphological studies, which suggest no

difference in skull morphometry between the dolphins found in the eastern and western coasts of Japan (Mizue & Yoshida 1962) and no difference in body length measurement and the age at sexual maturity between dolphins found off Taiwan and the eastern coast of Japan (Chen *et al.* 2011). By comparing the stable isotope ratios of carbon ($\partial^{13}C$) and nitrogen ($\partial^{15}N$), there seems to be no significant difference for diet preference between the dolphins around Taiwan and around Japan (Endo *et a*l. 2010; Liu *et al*. 2015). This result is not unexpected because the waters around Taiwan and southern Japan are both embedded in the Kuroshio Current System, a unique hydrodynamic system dominant in the western coast of the North Pacific Ocean, characterised by a speedy Kuroshio Current that carries warm, high salinity water flowing from Luzon of the Philippines northeast to the eastern coast of Japan year-round (Sverdrup *et al.* 1942; Barkley 1970) (Fig. 2.9). The northern boundary of this population is likely to be at around 35—40°N, where the mainstream Kuroshio Current leaves the coast of Japan and turns eastwardly, and the dolphins are seldom observed in the waters north of 40°N (Miyashita 1993). In this regard, it seems reasonable that the Geneland analysis indicates that the dolphin beached at the coast of Hokkaido, north Japan (43°N) is unlikely to affiliate with the western North Pacific population. However, it is perplexing that the analysis suggests that this dolphin was from the eastern North Pacific population. Since over 90% of dolphin carcasses would be expected to submerge within 40 days (Peltier *et al.* 2012) and the stranding rate decreases when the distance to shore increases (Williams *et al.*

2011; Wells *et al.* 2015), it is unlikely that the carcass had drifted such an enormous distance from the eastern coasts of North Pacific Ocean to Hokkaido. While it may be a casual migrant from the eastern North Pacific population, or an indication that the range of the eastern North Pacific population may reach the northern part of western North Pacific Ocean, the possibility of mis-assignment by the Geneland analysis cannot be excluded.

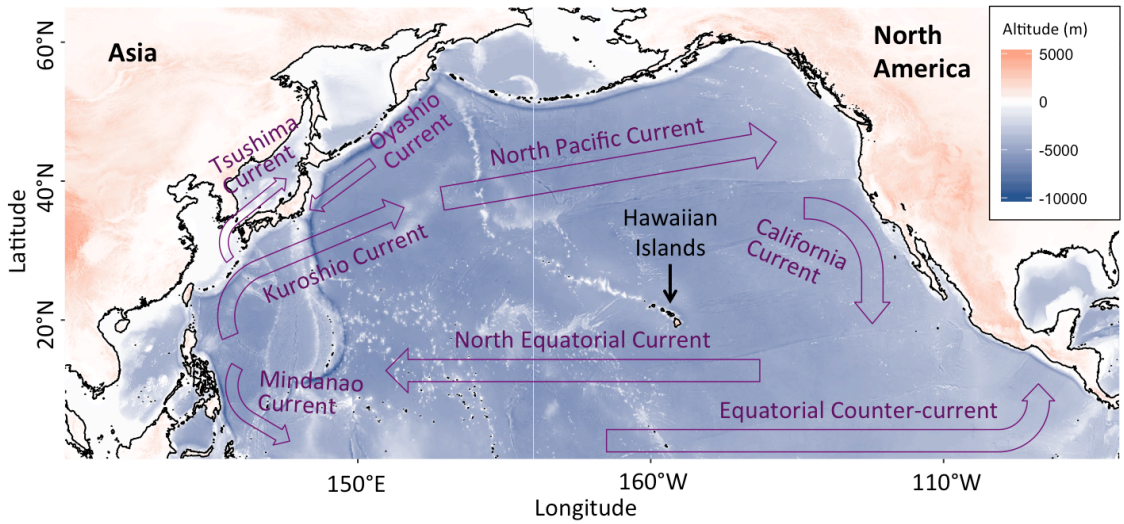

Figure 2.9. A map of North Pacific Ocean showing the abyssal topography and the major surface ocean currents mentioned in this study.

There is no genetic differentiation found between the dolphins in the western and eastern coasts of Japan (*i.e.*, Sea of Japan versus East Japan), even though the habitat may be somewhat different as the two regions are classified into different biogeographic provinces (*i.e.*, the Sea of Japan province and the Kuroshio-Oyashio Current province, respectively; Spalding *et al.* 2012). It is suspected that the Tsushima

Warm Current, a branch of Kuroshio Current flowing northward through the Korean/Tsushima Strait in to the basin of Sea of Japan (Takikawa *et al.* 2005) (Fig. 2.9), that is playing a crucial role connecting these waters and promoting gene flow around the Japanese archipelago. The Tsushima Warm Current transports a maximum volume of water to the Sea of Japan in the spring and autumn, but is at a minimum in winter (Takikawa *et al.* 2005), and this seems to correspond with the seasonal movement of Risso's dolphins off Nagasaki, south to Korean/Tsushima Strait, where the dolphins perform a 'feeding migration' in winter and 'parturient migration' in summer (Mizue & Yoshida 1962). Even though there are clues suggesting Risso's dolphins could freely move through the Korean/Tsushima Strait, given that the samples from the Sea of Japan were all stranded dolphins found on the Japanese coasts, it is possible that a certain portion of the samples was not resident in the Sea of Japan but instead drifted into the region. Therefore, the possibility cannot be excluded by now, that there could be one or more demographically independent Risso's dolphin populations in the Sea of Japan, since the knowledge of their distribution, abundance and residency here has yet been explored.

Miyashita (1993) also suggested there are two major aggregation zones for Risso's dolphins in the high sea western North Pacific Ocean: one in the waters offshore of Japan enclosed by 25–45°N, 148°–157°E, and the other in the high sea region of the same latitude, but east of 162°E. However, due to the lack of genetic samples from

those regions, the population structure for the dolphins in these two aggregation zones cannot be verified in this study. Such structure seems to be plausible though, as there have been a number of studies suggesting that other pelagic cetacean species establish genetically isolated populations in the central North Pacific Ocean (Andrews *et al.* 2010; Courbis *et al*. 2014; Martien *et al.* 2012, 2014), and the Geneland analysis in this study suggests that there might be an isolated Risso's dolphin population around the vicinity of the Hawaiian Islands. Nevertheless, the sample size is too limited to draw an inference, and the population structure in high sea Central Pacific warrants further investigation indeed.

The southern boundary for the Western North Pacific population remains uncertain, although the analyses showed that the Philippine sample is always grouped with samples collected from Taiwan and East Japan, possibly indicating that Philippine waters are in the range of the Western North Pacific population. The Philippines are located at the junction where the westward North Equatorial Current breaks into a northward Kuroshio Current and a southward Mindanao Current (Toole *et al*. 1990; Fine *et al*. 1994) and it is unknown whether the diverged currents could act as physical boundaries segregating dolphin populations within the northern and southern regions of Philippine waters. So far, the sightings of Risso's dolphins have only been reported along the coasts of Sulu Sea, central Philippines (Dolar *et al*. 2006; Jefferson *et al.*

2014). A closer inspection is needed to elucidate the population structure for this species in the Philippine waters.

Risso's dolphin Population structure in the eastern North Atlantic Ocean and Mediterranean Sea

Gaspari *et al.* (2007) reported clear population differentiation between the Risso's dolphins found in British waters and the Mediterranean Sea. In this study, further mtDNA data for the dolphins from the Azores, a sub-tropical region of the eastern North Atlantic Ocean, are added up to the analysis. The result shows that the Azores population is neither related to the British population, nor the Mediterranean population. As for the North Pacific Ocean, the population structure of Risso's dolphins in the eastern North Atlantic Ocean seems to be compatible with the structure of regional ocean biogeography; that is, an Azores population for the North Central Atlantic province, a Mediterranean population for the Mediterranean Sea province, and a British population for the Northern European Seas (Spalding *et al*. 2007, 2012).

The Mediterranean Sea is a semi-enclosed water mass connected with the North Atlantic Ocean only via a narrow channel, the Strait of Gibraltar. Bearzi *et al.* (2011) suggest that Risso's dolphins are rarely sighted in the Strait of Gibraltar and adjacent waters because the waters in the Strait are too shallow to be a preferred habitat for

Risso's dolphins, and that the presence of abundant long-finned pilot whales (*Globicephala melas*) in the strait creates inter-species competitive exclusion. The presence of a physical geographic barrier is certainly a main factor that isolates the Mediterranean population from other populations. However, it remains untested whether this Mediterranean population is, like the population of other dolphin species, further differentiated within the Mediterranean Sea (*e.g.,* Natoli *et al.* 2005, 2008).

On the other hand, between the Azores and British populations, oceanographic segregation and habitat specialisation are probably the two main triggers reinforcing population differentiation. The Azores are located in the northeast edge of the North Atlantic Gyre, which circulates high salinity waters clock-wise in the centre of the North Atlantic Ocean, whereas the British Isles are surrounded by a series of coastal seas in the northeast North Atlantic Ocean, influenced by the North Atlantic Drift, an eastern extension of the Gulf Stream that flows across the North Atlantic Ocean without passing the Azores and adjacent waters (Sverdrup *et al.* 1942) (Fig. 2.10). Furthermore, the habitat characteristics for Risso's dolphins around the Azores and Britain are remarkably dissimilar: the dolphins off the Azores prefer the continental slope area where the water depth is between 500–1200m (Pereira 2008; Silva *et al.* 2014), while those found in British waters are usually encountered within the continental shelf area at 50—100m depth (Evans 2013) (Fig. 2.10). In addition, Risso's dolphins found in the Azores and British waters both exhibit some degree of site fidelity (de Boer *et al.* 2013;

Hartman *et al.* 2008, 2015; Silva *et al*. 2014), which may have also promoted the population's endemicity (Schmidt 2004; Rosel *et al.* 2009). Even though population differentiation between the Azores and the British waters can be apparent, the population structure is assessed using data from one mtDNA locus only. Further investigations using multiple diploid markers (either microsatellite or single nucleotide polymorphisms, SNPs) will certainly provide more insights into the population structure, the measures of inbreeding depression, and the estimates for effective population size or gene flow.

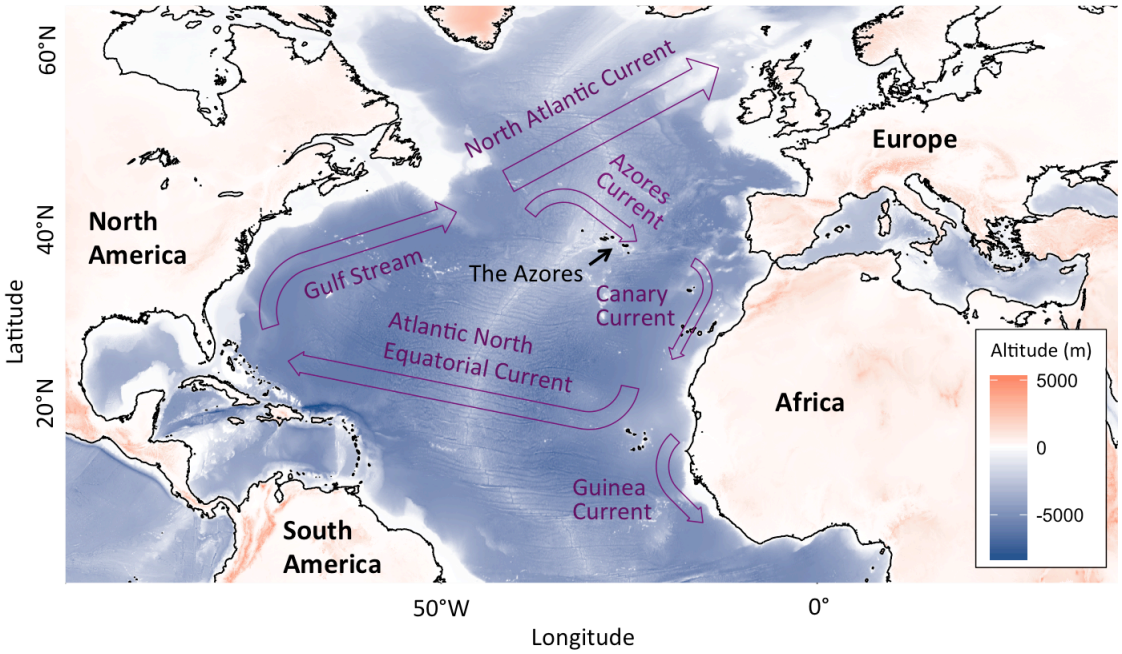

Figure 2.10. A map of North Atlantic Ocean showing the abyssal topography and the major surface ocean currents mentioned in this study.

Population dynamics and climate change

The analysis shows, overall, the Risso's dolphin species experienced both demographic and spatial expansion in the past, but the signal is not very strong or fully consistent among measures and populations. Exceptions are the population inhabiting the western North Pacific Ocean, which has a stronger expansion signal, and the populations inhabiting the Azores waters and the Eastern Tropical Pacific, which exhibit a sign of a stable population.

The rapid expansion detected for the western North Pacific population could be a result of successfully adapting to specialise on *Enoploteuthis chunii*, a species of squid presumably endemic and abundant in the western North Pacific Ocean (Jereb & Roper 2011; Clarke & Young 1998; Isoda 2000; Wang *et al.* 2012). This could be associated with gradually seizing the habitats formally occupied by their potential resource competitor, the long-finned pilot whale, which have gone extinct in the North Pacific Ocean during the last hundreds to thousands of years (Kasuya 2011). Perhaps more decisively, the dramatic shift of the mainstream Kuroshio Current at the East Taiwan Channel during the last glacial cycle (Gallagher *et al.* 2015) may have caused Risso's dolphins to explore further potential habitats around the Japanese archipelago. The short body length of Risso's dolphins in the western North Pacific Ocean (adult size about 250–270cm, rarely exceeded 300cm; Amano & Miyazaki 2004; Chen *et al.* 2011) could

be a by-product of rapid life-history trait evolution driven by population expansion (Phillips *et al.* 2010).

In contrast, the mtDNA signal for the Azores population suggests a stable population, possibly indicating that the population has dominated those waters for an extended period of time. Similar interpretation may be applicable to the Eastern Tropical Pacific population, as the Tajima's D and Fu's Fs for the Eastern Tropical Pacific population are both positive, and the mismatch distribution appears to be multimodal, even though these indices are not statistically significant. Earlier analyses have revealed that the waters around the Azores were not strongly affected by the Last Glacial Maximum, and represent one of the glacial refugia for marine organisms in the North Atlantic Ocean (Rogerson *et al*. 2004; Maggs *et al*. 2008). The sea surface temperature in the Eastern Tropical Pacific is always above 22°C (Zhang *et al.* 2014) and the upwelling system is consistently present through times (Toth *et al*. 2015). This suggests that a suitable habitat could have been maintained through the cold period for Risso's dolphins.

The estimated timing of population expansion depends on an accurate estimate of the mtDNA control region substitution rate. The universal rate used in this study was calibrated using ancient DNA samples ($\lambda=1\times10^{-7}$) (Ho *et al.* 2011a), which is an order of magnitude or more faster than rates for dolphins calibrated with fossil records or the time of divergence for close-related species ($\lambda=5\times10^{-9}$—3×10^{-8}; Hoelzel *et al*. 1991;

Baker *et al*. 1993; Harlin *et al.* 2003; Hayano *et al*. 2004). Such discrepancy is likely due to the fact that the mtDNA substitution rate is time-dependent, declining exponentially when the depth of time increases (Ho *et al.* 2011b). For population level inference, using a substitution rate estimated based on ancient DNA data, which reflects approximately the evolutionary history within the recent 50,000 (or less) years, is likely more appropriate than using a rate calibrated by fossil records or phylogenetic divergence scaled in millions of years (Ho *et al*. 2011b; Foote *et al.* 2012). However, the true rate likely varies among taxa (Ho *et al.* 2011a), and so estimates made here are approximate.

The onset of Risso's dolphin population expansion is suggested taking place at 3,000—6,000 years ago. Using the published phylogenetic rate of 7×10^{-8} (Harlin *et al*. 2003), this range would be about 4,000—7,500 years ago. In either case the time of population expansion would be within the period of deglaciation following the last glacial maximum (19,000—20,000 years ago; Clark *et al*. 2009), and most likely during the beginning of the Holocene (about 11,500 years ago, Mayewski *et a*l. 2004). The physical environment during the deglaciation featuring an increase of sea surface water temperature and sea level (Clark *et al*. 2009) appears to be favoured by Risso's dolphin, and the shift of ocean current course (*e.g*., Pak *et al.* 2012; Gallagher *et al.* 2015) could have promoted the accessibility of higher latitude habitats after the glacial retreat. In the western North Pacific Ocean, the extinction of long-finned pilot whales, which might be

dated back hundreds of years ago (Kasuya 2011), might have created Risso's dolphins a competitor-free niche to fit in even more rapidly.

Relatively low genetic diversity likely indicates that the British population is a founder population, and the estimates indicate that the time of population expansion for this population is later, within the last 1,000 years. Unlike the Azores and the Mediterranean populations of dolphins being sheltered in ice-free regions at all times, the waters around the British Isles were frozen during the Last Glacial Maximum (Clark *et al.* 2012). The significant delay of population expansion of this British population is possibly not only because of the late availability of accessible habitats in higher latitudes, but also time associated with adapting to the novel habitat. Risso's dolphins in British waters inhabit shallow continental shelf waters and consume octopuses *Eledone cirrhosa* as are of their main prey items (Evans 2013; MacLeod *et al.* 2014). This is remarkably different from 'typical' Risso's dolphin populations found in other regions, which occupy steep continental slope waters and mainly feed on squids (Kruse *et al.* 1999; Baird 2009). The population expansion detected here may therefore be associated with a successful colonization by a founder population, further involving a rapid niche shift.

Effective population size for the North Pacific populations

The estimates suggest the $N_e\mu$ for the western North Pacific population and for the eastern North Pacific population are similar (0.371 and 0.457) but smaller than the estimates for other oceanic small cetacean populations (See Chapter 5). However, the $N_e\mu$ reported in this study can be problematic, as the demographic feature of the populations appears to be violating the model assumption in Migrate analysis, which assumes the population size is consistent through times. The program tends to underestimate the $N_e\mu$ if the population size was increasing (Beerli 2009). The mtDNA analysis results suggest, at least for the western North Pacific population, the population size of the Risso's dolphin populations has increased greatly. The underestimation is evident when calculating the N_e/N ratio (Frankham 1995) using the N_e estimate for the western North Pacific population (Ne=1,804—3,816) and the census population size (N) estimated for the Risso's dolphins in Japanese waters (N=83,289). The N_e/N ratio would be 0.022—0.046, which appears to be unrealistically small for mammals (Frankham 1995). Considering the standard N_e/N ratio for Risso's dolphin is likely to be around 0.1—0.4 (see below), the contemporary N_e for the western North Pacific population is likely to be about an order of magnitude larger than the long-term N_e estimated in the Migrate analysis.

On the other hand, the N_e/N ratio for the eastern North Pacific population appears to be more realistic. It could be calculated as 0.184—0.399 when using the N estimated for the Risso's dolphins in the California Current System (N=11,910; Barlow

& Forney 2007). The calculation for the N_e/N ratio for the Eastern Tropical Pacific population is about the same magnitude, which is 0.145—0.328 (N=110,457, Gerrodette *et al.* 2008; Taylor *et al.* 2012). Since the ratio is above the estimate from a meta-analysis of N_e/N for wildlife populations (0.1—0.11) (Frankham 1995), the seemingly low N_e estimate for the eastern North Pacific population can be a reflection of low census population size (Hare *et al*. 2011), rather than other factors such as biased reproductive success, biased sex ratio, highly age-structured populations, and/or a recent population bottleneck (Nunney 1993; Hedrick 2005; Charlesworth 2009).

Synthesis, conservation implication and future study possibilities

The results show there are at least six populations of Risso's dolphins in the Northern Hemisphere, and the structure seemingly agrees with the structure of ocean biogeography (*viz.* Sverdrup *et al*. 1942; White 1994; Spalding *et al.* 2012). Such correlation is reasonable regarding the facts that the distribution of Risso's dolphins is restricted by habitat availability (Baumgartner 1997; Praca & Gannier 2008), and since the environmental/ecological characteristics of such habitats in different biogeographic regions is supposed to be dissimilar, a certain degree of local habitat/resource specialisation appears to be necessary to establish and maintain a stable population. Further studies integrating quantified environmental data and genetic data from more

advanced molecular techniques, *e.g*., next generation sequencing, would be able to verify this hypothesis, and possibly identify candidate genes associated with adaptation (Stapley *et al.* 2010; Ekblom & Galindo 2011). Other factors, such as isolation-by-distance, inter-species competitive exclusion (Shane 1995; Bearzi *et al.* 2011), and cultural or habitat unfamiliarity (Rosel *et al*. 2009; Cantor & Whitehead 2013), might also play some roles in preventing gene flows, and consequently reinforce the population structure. To present a comprehensive view of Risso's dolphin population structure in the world, it is needed to include further samples from the Central Pacific Ocean, as well as other regions where Risso's dolphins are found, *i.e*., the South Pacific, North Atlantic, South Atlantic, Mediterranean Sea and Indian Oceans.

The estimates suggest that some Risso's dolphin populations experienced a level of demographic and spatial expansion, possibly within the past 10,000 years. The timing cannot be accurately estimated due to the lack of accurate information for the species-specific parameters, *i.e*., the mtDNA control region substitution rate and the time length of a generation. Hence, it cannot be concluded that the expansion of the British population in the recent 1,000 years may be associated with anthropogenic global warming (Doney *et al*. 2012). Nevertheless, in general, the time frame for Risso's dolphin population expansion is in agreement with the early Holocene population expansion, which has also been suggested for a number of cetacean species (Banguera-Hinestroza *et al.* 2014; Louis *et al*. 2014; Moura *et al*. 2014; Chapter 3).

Year-round occurrence of Risso's dolphins detected by earlier sighting, stranding and acoustic records suggests the dolphins may be resident in the waters around Taiwan, Santa Catalina Island (southern California), Eastern Tropical Pacific, the Azores, British Isles and the northwest Mediterranean Sea (Bompar 1997; Ballance *et al*. 2006; Hartman *et al.* 2008, 2015; Soldevilla *et al.* 2010; Chen *et al.* 2011; Silva *et al*. 2014). The genetic analyses using the samples collected from these regions further suggest these are different populations, and for conservation purposes these populations should be managed as independent units. This study highlights the necessity of genetic approaches in determining population units and population dynamics for the conservation of highly mobile marine species such as cetaceans. However, the N_e estimates reported in this study, at least for the western North Pacific population, reflect the long-term N_e, which is least useful in real-time conservation management. Instead, the contemporary N_e should be assessed if the purpose is to monitor any on-going impact of human activities on wildlife populations. To assess the contemporary N_e, the genetic samples need to be collected systematically from individuals of known ages and across multiple generations (Hare *et al*. 2011). This is particularly challenging for long-lived animals such as cetaceans, but highlights the importance of establishing and maintaining long-term population monitoring and periodic sampling projects to achieve successful conservation management (*e.g*., Wells 2014).

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Appendices

Appendix 2.1. List of the samples acquired for this study. Note not all sample in this list were used in the study. The samples analysed are indicated as 'Y' in 'Used in Analyses?' column, as well as noted in 'MS' (microsatellite genotyping) and 'mtDNA' (mtDNA haplotype) columns. Abbreviations for the Contributors: Es-Bank, the Center for Enviornmental Studies at Ehime University (Japan); NMST, National Museum of Science, Tokyo; NTU, National Taiwan University; SWFSC, Southwest Fisheries Science Center (USA).

Appendix 2.2. Presence of null alleles, number of alleles, allelic richness, inbreeding coefficient (F_{IS}), observed heterozyogsity (H_O) and expected heterozygosity (H_E) for the 22 microsatellite loci examined in this study. The loci marked by asterisk are discarded from further analyses.

Appendix 2.3. Polymorphic sites in Risso's dolphin mtDNA control region haplotypes. The dot indicates identical site to the top sequence and the dash indicates an insertion–deletion event. The number in the top row indicates the position of the variable site in the 473bp sequence.

Appendix 2.1.

Longitude	Latitude	ID	Location	Population	Sample source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
-118.916666	34.033333	72	Leo Carillo Beach	Oregon-California Coastal	Stranding	SWFSC	1981	M	N/A	N/A	N
-121.166666	35.183333	141	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1991	U	N/A	N/A	N
-121.066666	34.433333	776	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1992	M	N/A	N/A	N
-118.966666	32.716666	1293	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1993	F	N/A	N/A	N
-118.033333	32.4	2165	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1993	U	N/A	N/A	N
-122.116666	35.616666	4695	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1992	U	N/A	N/A	N
-117.5	32.85	8759	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1997	M	N/A	N/A	N
-122.216666	36.416666	9336	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1997	U	N/A	N/A	N
-117.316666	33.083333	23942	San Diego	Oregon-California Coastal	Stranding	SWFSC	2001	U	N/A	N/A	N
-117.616666	33.416666	26643	San Clemente City Beach	Oregon-California Coastal	Stranding	SWFSC	2002	U	N/A	N/A	N
N/A	N/A	74703	San Diego	Oregon-California Coastal	Stranding	SWFSC	2008	U	N/A	N/A	N
-117.283333	32.833333	77617	San Diego	Oregon-California Coastal	Stranding	SWFSC	2008	M	N/A	N/A	N
N/A	N/A	94471	San Miguel Island	Oregon-California Coastal	Stranding	SWFSC	2010	U	N/A	N/A	N
-120.133333	34.133333	101144	Channel Islands	Oregon-California Coastal	Biopsy	SWFSC	2010	F	N/A	Hap_14	Y
-118.483333	33.366666	124011	Cottonwood Beach, Los Angeles	Oregon-California Coastal	Stranding	SWFSC	2010	U	N/A	N/A	N
-117.25	32.916666	62	Torrey Pines State Beach	Oregon-California Coastal	Stranding	SWFSC	1990	F	Y	N/A	Y
-121.283333	34.7	144	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1991	F	Y	N/A	Y
-118.95	32.716666	1291	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1993	F	Y	N/A	Y
-118.966666	32.716666	1294	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1993	M	Y	N/A	Y
-121.666666	35.2	1301	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1992	F	Y	N/A	Y
-125.983333	38.133333	1564	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1992	F	Y	N/A	Y
-121.933333	34.116666	1875	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1993	M	Y	N/A	Y

Longitude	Latitude	ID	Location	Population	Sample source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
-127	44.4	1877	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1993	F	Y	N/A	Y
-118.133333	32.416666	2167	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1993	M	Y	N/A	Y
-122.9	36.233333	4694	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1993	F	Y	Sequenced	N
-119	32.4	4771	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1995	F	Y	N/A	Y
-120.9	34.55	5001	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1995	F	Y	N/A	Y
-120.9	34.55	5004	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1995	M	Y	N/A	Y
-120.866666	34.533333	5007	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1995	F	Y	N/A	Y
-120.866666	34.533333	5008	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1995	M	Y	N/A	Y
-118.966389	33.348889	6157	N/A	Oregon-California Coastal	Biopsy	SWFSC	1996	F	Y	N/A	Y
-122.083333	36.966666	6963	Santa Cruz	Oregon-California Coastal	Stranding	SWFSC	1997	M	Y	N/A	Y
-122.216666	36.416666	9335	US west offshore	Oregon-California Coastal	Fishery	SWFSC	1997	M	Y	N/A	Y
-119.666666	34.416666	11204	Santa Barbara	Oregon-California Coastal	Stranding	SWFSC	1998	F	Y	N/A	Y
-118.933333	32.283333	23155	US west offshore	Oregon-California Coastal	Fishery	SWFSC	2000	F	Y	N/A	Y
-122.233333	35.8	23187	US west offshore	Oregon-California Coastal	Fishery	SWFSC	2000	F	Y	N/A	Y
-117.35	32.833333	23799	San Diego	Oregon-California Coastal	Biopsy	SWFSC	2001	M	Y	N/A	Y
-117.35	32.9	23800	San Diego	Oregon-California Coastal	Biopsy	SWFSC	2001	M	Y	N/A	Y
-124.583333	43.016666	25435	Coastal	Oregon-California Coastal	Biopsy	SWFSC	2001	M	Y	Hap_16	Y
-119.366666	34.016666	26306	US west coast	Oregon-California Coastal	Biopsy	SWFSC	2001	M	Y	Hap_17	Y
-119.366666	34.016666	26307	US west coast	Oregon-California Coastal	Biopsy	SWFSC	2001	F	Y	Hap_17	Y
-119.366666	34.016666	26308	US west coast	Oregon-California Coastal	Biopsy	SWFSC	2001	M	Y	Hap_18	Y
-119.366666	34.016666	26309	US west coast	Oregon-California Coastal	Biopsy	SWFSC	2001	F	Y	Hap_18	N
-117.566666	33.366666	26642	San Onofre State Beach	Oregon-California Coastal	Stranding	SWFSC	2002	M	Y	N/A	Y
-117.4	32.883333	28480	San Diego	Oregon-California Coastal	Biopsy	SWFSC	2002	M	Y	N/A	Y
-121.9	36.45	32931	Monterey	Oregon-California Coastal	Stranding	SWFSC	1997	M	Y	Hap_1	Y
-121.8	36.816666	32940	Monterey	Oregon-California Coastal	Stranding	SWFSC	2003	F	Y	Hap_18	Y
-121.933333	35.966666	39080	US west offshore	Oregon-California Coastal	Fishery	SWFSC	2003	M	Y	N/A	Y

Longitude	Latitude	ID	Location	Population	Sample source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
-121.933333	35.966666	39082	US west offshore	Oregon-California Coastal	Fishery	SWFSC	2003	M	Y	N/A	Y
-121.933333	35.966666	39083	US west offshore	Oregon-California Coastal	Fishery	SWFSC	2003	F	Y	N/A	Y
-121.933333	35.966666	39084	US west offshore	Oregon-California Coastal	Fishery	SWFSC	2003	M	Y	N/A	Y
-119	34	39556	Ventura	Oregon-California Coastal	Stranding	SWFSC	2003	F	Y	N/A	Y
-121.983333	36.783333	41842	Monterey	Oregon-California Coastal	Biopsy	SWFSC	2004	F	Y	N/A	Y
-122	36.8	41843	Monterey	Oregon-California Coastal	Biopsy	SWFSC	2004	F	Y	Hap_19	Y
-122	36.8	41844	Monterey	Oregon-California Coastal	Biopsy	SWFSC	2004	F	Y	Hap_20	Y
-121.983333	36.8	41845	Monterey	Oregon-California Coastal	Biopsy	SWFSC	2004	F	Y	Hap_21	Y
-121.983333	36.8	41846	Monterey	Oregon-California Coastal	Biopsy	SWFSC	2004	F	Y	Hap_22	Y
-121.983333	36.8	41847	Monterey	Oregon-California Coastal	Biopsy	SWFSC	2004	F	Y	Hap_23	Y
-122.133333	36.766666	41850	Monterey	Oregon-California Coastal	Biopsy	SWFSC	2004	M	Y	Hap_20	Y
-117.383333	32.6	48586	San Diego	Oregon-California Coastal	Biopsy	SWFSC	2005	F	Y	N/A	Y
-124.383333	44.983333	51127	Coastal	Oregon-California Coastal	Biopsy	SWFSC	2005	M	Y	N/A	Y
-124.383333	44.983333	51128	Coastal	Oregon-California Coastal	Biopsy	SWFSC	2005	F	Y	N/A	Y
-124.383333	44.983333	51129	Coastal	Oregon-California Coastal	Biopsy	SWFSC	2005	M	Y	N/A	Y
-124.383333	44.983333	51130	Coastal	Oregon-California Coastal	Biopsy	SWFSC	2005	M	Y	N/A	Y
-117.366666	32.95	52456	San Diego	Oregon-California Coastal	Biopsy	SWFSC	2006	M	Y	N/A	Y
-120.6	34.683333	53165	Santa Barbara	Oregon-California Coastal	Stranding	SWFSC	2004	F	Y	N/A	Y
-121.95	36.583333	57814	Monterey	Oregon-California Coastal	Stranding	SWFSC	2005	M	Y	Hap_20	Y
-124.75	47.3	61944	Coastal	Oregon-California Coastal	Biopsy	SWFSC	2006	F	Y	Hap_1	Y
-124.75	47.3	61945	Coastal	Oregon-California Coastal	Biopsy	SWFSC	2006	M	Y	Hap_24	Y
-117.633333	33.416666	66557	Orange	Oregon-California Coastal	Stranding	SWFSC	2007	F	Y	N/A	Y
-121.969123	36.58246	73404	Monterey	Oregon-California Coastal	Stranding	SWFSC	2006	M	Y	Hap_25	Y
-119.1	34.1	76436	Ventura	Oregon-California Coastal	Stranding	SWFSC	2008	F	Y	N/A	Y
-118.016666	32.966666	76971	US west coast	Oregon-California Coastal	Biopsy	SWFSC	2008	M	Y	Hap_1	Y
-117.866666	32.566666	79772	Dana Point, Long Beach, Catalina	Oregon-California Coastal	Biopsy	SWFSC	2009	M	Y	N/A	Y

Longitude	Latitude	ID	Location	Population	Sample source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
-118.933333	32.916666	79774	San Clemente Island	Oregon-California Coastal	Biopsy	SWFSC	2009	M	Y	N/A	Y
-121.05	34.7	79937	US west offshore	Oregon-California Coastal	Fishery	SWFSC	2008	M	Y	N/A	Y
-119.483333	33.483333	87478	NW off Santa Catalina Island	Oregon-California Coastal	Biopsy	SWFSC	2009	U	Y	N/A	Y
-118.716666	33.166666	87480	NW off Santa Catalina Island	Oregon-California Coastal	Biopsy	SWFSC	2009	M	Y	N/A	Y
-119.483333	33.483333	87483	NW off Santa Catalina Island	Oregon-California Coastal	Biopsy	SWFSC	2009	M	Y	N/A	Y
-118.566666	33.816666	88952	Marina del Rey	Oregon-California Coastal	Biopsy	SWFSC	2009	M	Y	N/A	Y
-119.7	33.733333	88975	Oxnard	Oregon-California Coastal	Biopsy	SWFSC	2009	M	Y	N/A	Y
-118.65	33.083333	94804	Southern California offshore	Oregon-California Coastal	Biopsy	SWFSC	2010	M	Y	N/A	Y
-120.133333	34.133333	101142	Channel Islands	Oregon-California Coastal	Biopsy	SWFSC	2010	F	Y	Hap_12	Y
-120.133333	34.133333	101143	Channel Islands	Oregon-California Coastal	Biopsy	SWFSC	2010	M	Y	Hap_13	Y
-118.333333	33.45	101159	Channel Islands	Oregon-California Coastal	Biopsy	SWFSC	2010	F	Y	Hap_14	Y
-118.333333	33.45	101160	Channel Islands	Oregon-California Coastal	Biopsy	SWFSC	2010	F	Y	N/A	Y
-122.97714	38.207738	101851	Sonoma	Oregon-California Coastal	Stranding	SWFSC	2010	M	Y	N/A	Y
-118.466666	33.383333	102563	Santa Catalina Island	Oregon-California Coastal	Stranding	SWFSC	2010	M	Y	N/A	Y
-124.416666	40.583333	124025	Humboldt	Oregon-California Coastal	Stranding	SWFSC	2005	M	Y	Hap_15	Y
-117.516666	32.6	125882	San Diego South	Oregon-California Coastal	Biopsy	SWFSC	2011	M	Y	N/A	Y
-160.716666	18.183333	73679	Southwest of the Hawaiian Islands	Central-Northeast Pacific	Fishery	SWFSC	2007	F	Y	N/A	Y
-157.366666	32.316666	53476	North of the Hawaiian Islands	Central-Northeast Pacific	Fishery	SWFSC	2006	F	Y	N/A	Y
-159.016666	30.983333	62830	North of the Hawaiian Islands	Central-Northeast Pacific	Fishery	SWFSC	2007	F	Y	N/A	Y
-146.366666	38.033333	78761	North of the Hawaiian Islands	Central-Northeast Pacific	Fishery	SWFSC	2008	M	Y	N/A	Y
-142.2	31.4	93900	Northeastern Pacific Ocean	Central-Northeast Pacific	Fishery	SWFSC	2010	F	Y	N/A	Y

Longitude	Latitude	ID	Location	Population	Sample source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
-142.683333	31.283333	125652	Northeastern Pacific Ocean	Central-Northeast Pacific	Fishery	SWFSC	2011	F	Y	N/A	Y
-137.866666	33.383333	125653	Northeastern Pacific Ocean	Central-Northeast Pacific	Fishery	SWFSC	2011	M	Y	N/A	Y
-109.366667	24.183333	37967	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	2003	U	N/A	N/A	N
-100.566667	16.65	11692	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	1998	M	Y	Hap_1	Y
-100.566667	16.65	11693	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	1998	F	Y	N/A	Y
-100.566667	16.65	11694	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	1998	M	Y	Hap_2	Y
-110.066667	25.583333	15899	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	1999	M	Y	Hap_3	Y
-110.216667	25.65	15900	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	1999	M	Y	Hap_1	Y
-110.216667	25.65	15901	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	1999	M	Y	Hap_4	Y
-99.933333	16.65	15997	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	1999	F	Y	Hap_1	Y
-119.816667	26.216667	37971	US west offshore	Eastern Tropical Pacific	Biopsy	SWFSC	2003	M	Y	Hap_1	Y
-100.8	17.1	38113	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	2003	M	Y	Hap_1	Y
-100.966667	17.1	38114	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	2003	M	Y	Hap_5	Y
-94.05	15.15	38251	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	2003	F	Y	Hap_5	Y
-94.05	15.15	38252	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	2003	M	Y	Hap_6	Y
-94.05	15.15	38253	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	2003	M	Y	Hap_7	Y
-94.05	15.15	38254	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	2003	M	Y	Hap_1	Y
-94.216667	15.183333	38255	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	2003	M	Y	Hap_8	Y
-94.216667	15.183333	38256	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	2003	F	Y	Hap_6	Y
-94.216667	15.183333	38257	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	2003	M	Y	Hap_9	Y
-94.216667	15.183333	38258	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	2003	M	Y	Hap_1	Y
-95.883333	15.583333	38266	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	2003	M	Y	Hap_7	Y
-100.8	15.266667	38275	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	2003	M	Y	Hap_2	Y
-100.8	15.266667	38276	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	2003	M	Y	Hap_10	Y
-100.8	15.266667	38277	Mexico	Eastern Tropical Pacific	Biopsy	SWFSC	2003	M	Y	Hap_11	Y

Longitude	Latitude	ID	Location	Population	Sample source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
121.53223	23.483738	579	Hualien	Taiwan	Fishery	NTU	2000	F	N/A	Hap_18	Y
121.53223	23.483738	623	Hualien	Taiwan	Fishery	NTU	2000	F	N/A	Hap_43	Y
121.87	24.9	629	Ilan	Taiwan	Fishery	NTU	2000	F	N/A	Hap_26	Y
139.1412727	35.16213712	10Gg005	Kanakawa	East Japan	Stranding	NMST	2001	F	N/A	Hap_27	Y
131.5933782	33.24748452	10Gg012	Oita	East Japan	Stranding	NMST	2003	U	N/A	Hap_3	Y
139.7762148	34.97545194	10Gg013	Chiba	East Japan	Stranding	NMST	2003	F	N/A	Hap_31	Y
141.4669212	38.26669879	10Gg101	Miyagi	East Japan	Stranding	NMST	M34066	U	N/A	Hap_33	Y
135.94924	33.593924	EW01248	Taiji	East Japan	Whaling	Es-Bank	1991	M	N/A	N/A	Y
121.53223	23.483738	284	Hualien	Taiwan	Fishery	NTU	2000	M	Y	Hap_18	Y
121.53223	23.483738	287	Hualien	Taiwan	Fishery	NTU	2000	M	Y	Hap_41	Y
121.53223	23.483738	290	Hualien	Taiwan	Fishery	NTU	2004	M	Y	Hap_39	Y
121.53223	23.483738	292	Hualien	Taiwan	Fishery	NTU	2004	M	Y	Hap_44	Y
121.53223	23.483738	294	Hualien	Taiwan	Fishery	NTU	2004	M	Y	Hap_39	Y
121.53223	23.483738	451	Hualien	Taiwan	Fishery	NTU	2000	F	Y	Hap_39	Y
121.53223	23.483738	500	Hualien	Taiwan	Fishery	NTU	2004	F	Y	Hap_33	Y
121.53223	23.483738	503	Hualien	Taiwan	Fishery	NTU	2004	F	Y	Hap_39	Y
120.511139	24.292417	573	Taichung	Taiwan	Stranding	NTU	2000	M	Y	Hap_39	Y
121.53223	23.483738	576	Hualien	Taiwan	Fishery	NTU	2001	M	Y	Hap_46	Y
121.53223	23.483738	582	Hualien	Taiwan	Fishery	NTU	2000	F	Y	Hap_33	Y
121.53223	23.483738	586	Hualien	Taiwan	Fishery	NTU	2000	F	Y	Hap_33	Y
121.53223	23.483738	590	Hualien	Taiwan	Fishery	NTU	2001	M	Y	Hap_30	Y
121.53223	23.483738	592	Hualien	Taiwan	Fishery	NTU	2000	F	Y	Hap_42	Y
121.53223	23.483738	594	Hualien	Taiwan	Fishery	NTU	2000	F	Y	Hap_33	Y
121.53223	23.483738	626	Hualien	Taiwan	Fishery	NTU	2000	M	Y	Hap_41	Y
121.53223	23.483738	724	Hualien	Taiwan	Fishery	NTU	2001	M	Y	Hap_30	Y
121.53223	23.483738	726	Hualien	Taiwan	Fishery	NTU	2001	F	Y	Hap_30	N

Longitude	Latitude	ID	Location	Population	Sample source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
121.53223	23.483738	733	Hualien	Taiwan	Fishery	NTU	2001	M	Y	N/A	Y
121.53223	23.483738	738	Hualien	Taiwan	Fishery	NTU	2005	M	Y	Hap_33	Y
121.53223	23.483738	742	Hualien	Taiwan	Fishery	NTU	2004	F	Y	Hap_41	Y
121.396274	23.088597	871	Taitung	Taiwan	Fishery	NTU	2005	F	Y	Hap_39	Y
121.396274	23.088597	874	Taitung	Taiwan	Fishery	NTU	2005	M	Y	Hap_39	Y
121.396274	23.088597	877	Taitung	Taiwan	Fishery	NTU	2005	M	Y	Hap_41	Y
121.871178	24.905306	884	Ilan	Taiwan	Stranding	NTU	2006	F	Y	Hap_39	Y
121.53223	23.483738	893	Hualien	Taiwan	Fishery	NTU	2004	M	Y	Hap_44	Y
121.53223	23.483738	897	Hualien	Taiwan	Fishery	NTU	2004	M	Y	Hap_18	Y
121.53223	23.483738	901	Hualien	Taiwan	Fishery	NTU	2005	M	Y	Hap_45	Y
121.53223	23.483738	908	Hualien	Taiwan	Fishery	NTU	2006	M	Y	N/A	Y
121.396274	23.088597	1030	Taitung	Taiwan	Fishery	NTU	2006	F	Y	Hap_18	Y
121.396274	23.088597	1035	Taitung	Taiwan	Fishery	NTU	2006	F	Y	Hap_33	Y
121.396274	23.088597	1040	Taitung	Taiwan	Fishery	NTU	2006	F	Y	Hap_33	Y
121.414471	23.115755	1045	Taitung	Taiwan	Stranding	NTU	2006	F	Y	Hap_47	Y
121.396274	23.088597	1053	Taitung	Taiwan	Fishery	NTU	2006	M	Y	Hap_33	Y
121.53223	23.483738	1061	Hualien	Taiwan	Fishery	NTU	2005	M	Y	Hap_39	Y
121.266597	25.120128	1074	Taoyuan	Taiwan	Stranding	NTU	2007	M	Y	Hap_18	Y
121.266597	25.120128	1075	Taoyuan	Taiwan	Stranding	NTU	2007	M	Y	Hap_43	Y
121.266597	25.120128	1078	Taoyuan	Taiwan	Stranding	NTU	2007	M	Y	Hap_44	Y
121.266597	25.120128	1081	Taoyuan	Taiwan	Stranding	NTU	2007	M	Y	Hap_49	Y
121.419472	23.227056	1120	Taitung	Taiwan	Stranding	NTU	2004	F	Y	Hap_18	Y
121.53223	23.483738	1153	Hualien	Taiwan	Fishery	NTU	2005	F	Y	Hap_41	Y
121.471711	25.254478	1254	Taipei	Taiwan	Stranding	NTU	2008	M	Y	Hap_48	Y
141.6307816	42.6337174	10Gg001	Hokkaido	East Japan	Stranding	NMST	1999	F	Y	Hap_15	Y
138.9196639	34.65338702	10Gg002	Shizuoka	East Japan	Stranding	NMST	1999	F	Y	Hap_26	Y

Longitude	Latitude	ID	Location	Population	Sample source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
140.5623418	36.29416226	10Gg003	Ibaraki	East Japan	Stranding	NMST	1999	M	Y	Hap_3	Y
131.4724559	31.80350844	10Gg008	Miyazaki	East Japan	Stranding	NMST	2002	M	Y	Hap_18	Y
139.8581756	35.00031174	10Gg009	Chiba	East Japan	Stranding	NMST	2002	M	Y	Hap_26	Y
138.9204402	34.65393181	10Gg010	Shizuoka	East Japan	Stranding	NMST	2003	M	Y	Hap_29	Y
132.5495873	32.9635973	10Gg011	Ehime	East Japan	Stranding	NMST	2003	F	Y	Hap_30	Y
140.5770456	35.63564914	10Gg014	Chiba	East Japan	Stranding	NMST	2003	M	Y	Hap_32	Y
132.5277516	32.96279867	10Gg021	Ehime	East Japan	Stranding	NMST	2006	F	Y	Hap_33	Y
131.4566001	31.84211287	10Gg022	Miyazaki	East Japan	Stranding	NMST	2007	F	Y	Hap_35	Y
138.2018081	34.6767493	10Gg023	Shizuoka	East Japan	Stranding	NMST	2007	F	Y	Hap_33	Y
131.5354732	32.1182585	10Gg026	Miyazaki	East Japan	Stranding	NMST	2008	M	Y	Hap_36	Y
131.5312528	32.11012651	10Gg028	Miyazaki	East Japan	Stranding	NMST	2008	M	Y	Hap_18	Y
140.4120953	35.45976398	10Gg029	Chiba	East Japan	Fishery	NMST	2008	F	Y	Hap_35	Y
131.3434538	31.41060628	10Gg030	Miyazaki	East Japan	Stranding	NMST	2009	M	Y	Hap_37	Y
131.9656307	32.8021531	10Gg032	Oita	East Japan	Stranding	NMST	2009	M	Y	Hap_38	Y
138.947033	34.662309	10Gg036	Shizuoka	East Japan	Stranding	NMST	2010	F	Y	Hap_40	Y
139.223175	35.036692	10Gg085	Shizuoka	East Japan	Stranding	NMST	1994	F	Y	Hap_37	Y
132.659167	33.914722	10Gg086	Ehime	East Japan	Stranding	NMST	1986	F	Y	Hap_33	Y
140.470556	36.316944	10Gg087	Ibaraki	East Japan	Stranding	NMST	1991	U	Y	Hap_3	N
140.637222	36.316944	10Gg088	Ibaraki	East Japan	Stranding	NMST	2002	M	Y	Hap_50	Y
135.94924	33.593924	10Gg090	Taiji	East Japan	Whaling	NMST	1991	F	Y	Hap_3	Y (mtDNA)
135.94924	33.593924	10Gg091	Taiji	East Japan	Whaling	NMST	1991	F	Y	Hap_30	Y
135.94924	33.593924	10Gg092	Taiji	East Japan	Whaling	NMST	1991	F	Y	Hap_33	Y
135.94924	33.593924	10Gg093	Taiji	East Japan	Whaling	NMST	1991	F	Y	Hap_51	Y
135.94924	33.593924	10Gg094	Taiji	East Japan	Whaling	NMST	1991	F	Y	Hap_39	Y (mtDNA)
132.470278	33.2425	10Gg095	Ehime	East Japan	Stranding	NMST	2005	M	Y	Hap_52	Y

Longitude	Latitude	ID	Location	Population	Sample source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
135.94924	33.593924	10Gg102	Taiji	East Japan	Whaling	NMST	NA	F	Y	Hap_54	Y
135.94924	33.593924	10Gg103	Taiji	East Japan	Whaling	NMST	NA	F	Y	Hap_33	Y
131.533	32.115	10Gg104	Miyazaki	East Japan	Stranding	NMST	2010		Y	Hap_55	Y
135.94924	33.593924	EW01194	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01195	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01196	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW01197	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01198	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01199	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01200	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01201	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01202	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01204	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01205	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y	N/A	N
135.94924	33.593924	EW01206	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01207	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01208	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW01209	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW01210	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01211	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01212	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01213	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01214	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01215	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01216	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW01217	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y

Longitude	Latitude	ID	Location	Population	Sample source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
135.94924	33.593924	EW01218	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW01219	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01220	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01221	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01222	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01223	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01224	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01225	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y	N/A	N
135.94924	33.593924	EW01226	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01227	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW01228	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01229	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW01231	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01232	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW01233	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01235	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW01237	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y	N/A	N
135.94924	33.593924	EW01238	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW01239	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01240	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01241	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01242	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW01243	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01244	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01245	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y	N/A	N
135.94924	33.593924	EW01246	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y	N/A	N

Longitude	Latitude	ID	Location	Population	Sample source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
135.94924	33.593924	EW01247	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01249	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01250	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW01251	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW01252	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01253	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW01254	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01255	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW01256	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01257	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01258	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01259	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y	N/A	N
135.94924	33.593924	EW01260	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW01261	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01262	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y		Y
135.94924	33.593924	EW01264	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW04585	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y	N/A	N
135.94924	33.593924	EW05119	Taiji	East Japan	Whaling	Es-Bank	1991	F	Y		Y
135.94924	33.593924	EW05120	Taiji	East Japan	Whaling	Es-Bank	1991	M	Y	N/A	N
121.128044	22.719825	GGDU02	Taitung	Taiwan	Stranding	NTU	2012	M	Y	N/A	Y
120.924892	22.350892	GGDU03	Pingdong	Taiwan	Stranding	NTU	2012	M	Y	N/A	Y
121.53223	23.483738	GGDU04	Hualien	Taiwan	Fishery	NTU	2000	M	Y	N/A	Y
121.53223	23.483738	GGDU05	Hualien	Taiwan	Fishery	NTU	2000	M	Y	N/A	Y
121.53223	23.483738	GGDU06	Hualien	Taiwan	Fishery	NTU	1998	M	Y	N/A	Y
121.550911	23.699333	GGDU07	Hualien	Taiwan	Stranding	NTU	2013	F	Y	N/A	Y
121.53223	23.483738	GGDU08	Hualien	Taiwan	Fishery	NTU	2012	F	Y	N/A	Y

Longitude	Latitude	ID	Location	Population	Sample source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
121.53223	23.483738	GGDU09	Hualien	Taiwan	Fishery	NTU	1998	M	Y	N/A	Y
137.0543661	37.20941127	10Gg016	Ishikawa	Sea of Japan	Stranding	NMST	2004	M	N/A	Hap_33	Y
137.4180917	36.91797894	10Gg017	Toyama	Sea of Japan	Stranding	NMST	2004	M	N/A	Hap_34	Y
137.1943032	37.29797056	10Gg033	Ishikawa	Sea of Japan	Stranding	NMST	2009	M	N/A	Hap_39	Y
137.115853	37.241361	10Gg084	Ishikawa	Sea of Japan	Stranding	NMST	2003	U	N/A	Hap_33	Y
131.061389	34.356389	10Gg089	Yamaguchi	Sea of Japan	Stranding	NMST	2003	U	N/A	Hap_33	Y
136.9166853	37.22689669	10Gg004	Ishikawa	Sea of Japan	Stranding	NMST	2001	F	Y	Hap_1	Y
130.692624	33.93715414	10Gg006	Fukuoka	Sea of Japan	Stranding	NMST	2001	F	Y	Hap_28	Y
137.357679	37.4645313	10Gg007	Ishikawa	Sea of Japan	Stranding	NMST	2004	F	Y	Hap_18	Y
130.9067934	34.06370436	10Gg018	Yamaguchi	Sea of Japan	Stranding	NMST	2004	F	Y	Hap_33	N
139.8541862	39.03329647	10Gg019	Yamagata	Sea of Japan	Stranding	NMST	2004	M	Y	Hap_34	Y
140.060244	39.70010499	10Gg020	Akita	Sea of Japan	Stranding	NMST	2006	M	Y	Hap_15	Y
136.9966007	36.84738986	10Gg024	Toyama	Sea of Japan	Stranding	NMST	2007	M	Y	Hap_33	Y
138.1962892	37.17030215	10Gg025	Niigata	Sea of Japan	Stranding	NMST	2008	F	Y	Hap_33	Y
137.2972602	37.44207359	10Gg027	Ishikawa	Sea of Japan	Stranding	NMST	2008	M	Y	Hap_18	Y
136.1622669	36.25055217	10Gg031	Fukui	Sea of Japan	Stranding	NMST	2009	U	Y	Hap_3	Y
136.7609877	36.92165495	10Gg034	Ishikawa	Sea of Japan	Stranding	NMST	2009	M	Y	Hap_15	Y
134.0215109	35.52069508	10Gg035	Tottori	Sea of Japan	Stranding	NMST	2010	M	Y	Hap_19	Y
130.7599564	33.92361871	10Gg100	Fukuoka	Sea of Japan	Stranding	NMST	2003	F	Y	Hap_33	Y
123	15	10Gg096	Philippines	Philippines	Unknown	Es-Bank	1996	F	N/A	Hap_53	Y
123	15	PH2668	Philippines	Philippines	Fishery	SWFSC	1992	M	Y	N/A	Y

Appendix 2.2.

Locus	Null alleles	No. alleles	Allele richness	F_{IS}	H_O	H_E	P-value	s.d.	Null alleles	No. alleles	Allele richness	F_{IS}	H_O	H_E	P-value	s.d.
Pop	Taiwan								East Japan							
n	49								72							
AAT44		4	1.242	-0.096	0.265	0.242	1	0		4	1.242	-0.093	0.264	0.242	1	0
D14		13	1.839	0.076	0.776	0.839	0.078	0		12	1.862	-0.064	0.917	0.862	0.82	0
D22		10	1.854	0.117	0.755	0.854	0.006	0		12	1.865	0.086	0.792	0.865	0.137	0
Dde59		14	1.911	-0.008	0.918	0.911	0.296	0	Y	13	1.873	0.174	0.722	0.873	0.002	0
Dde65		7	1.726	0.045	0.694	0.726	0.292	0		6	1.738	0.041	0.708	0.738	0.224	0
Dde66		12	1.716	0.088	0.653	0.716	0.196	0		11	1.745	-0.025	0.764	0.745	0.293	0
Dde69		15	1.882	0.006	0.878	0.882	0.016	0		9	1.834	0.017	0.819	0.834	0.331	0
Dde70		12	1.815	0.024	0.796	0.815	0.643	0		13	1.753	0.078	0.694	0.753	0.324	0
Dde72		20	1.934	0.017	0.918	0.934	0.2	0		23	1.905	-0.012	0.917	0.906	0.136	0
Dde84		8	1.7	-0.012	0.708	0.7	0.892	0		9	1.684	0.087	0.625	0.684	0.491	0
EV14*	Y	16	1.858	0.42	0.5	0.858	0	0	Y	12	1.802	0.43	0.458	0.802	0	0
EV37		10	1.801	0.007	0.796	0.801	0.378	0		9	1.745	0.055	0.704	0.745	0.685	0
KWM12a		17	1.885	-0.036	0.917	0.885	0.178	0		17	1.9	0.044	0.861	0.9	0.273	0
KWM1b*		1	1	NA	NA	NA	NA	NA		1	1	NA	NA	NA	NA	NA
KWM2b		6	1.702	0.099	0.633	0.702	0.351	0.001		6	1.687	0.132	0.597	0.687	0.567	0
KWM9b		7	1.703	0.101	0.633	0.703	0.642	0		11	1.788	0.048	0.75	0.788	0.021	0
MK3		6	1.804	0.01	0.796	0.804	0.436	0.001		7	1.799	-0.008	0.806	0.799	0.718	0
MK5		12	1.647	0.118	0.571	0.647	0.074	0		10	1.653	-0.064	0.694	0.653	0.442	0.001
Sco28		3	1.117	-0.045	0.122	0.117	1	0		3	1.094	0.263	0.069	0.094	0.143	0
TexVet7*		1	1	NA	NA	NA	NA	NA		1	1	NA	NA	NA	NA	NA
Sco11		7	1.812	0.046	0.776	0.812	0.586	0.001		7	1.812	-0.009	0.819	0.812	0.925	0
Sco55		4	1.423	-0.11	0.469	0.423	0.963	0		4	1.408	0.013	0.403	0.408	0.24	0
Mean		9.842		0.056	0.688	0.711					9.789	0.057	0.68	0.705		
S.D.		4.682			0.213	0.222					4.826		0.219	0.222		

(Continues)

Locus	Null alleles	No. alleles	Allele richness	F_{IS}	H_O	HE	P-value	s.d.	Null alleles	No. alleles	Allele richness	F_{IS}	H_O	H_E	P-value	s.d.
Pop	Sea of Japan								Central North Pacific							
n	12								7							
AAT44		4	1.308	-0.086	0.333	0.308	1	0		2	1.264	-0.091	0.286	0.264	1	0
D14		7	1.841	-0.2	1	0.841	0.568	0		8	1.868	0.014	0.857	0.868	0.856	0
D22		7	1.822	-0.12	0.917	0.822	0.521	0		4	1.747	0.25	0.571	0.747	0.228	0
Dde59		10	1.88	-0.043	0.917	0.88	0.337	0		7	1.846	-0.014	0.857	0.846	0.552	0
Dde65		5	1.659	0.379	0.417	0.659	0.14	0		3	1.714	0.213	0.571	0.714	1	0
Dde66		8	1.862	-0.168	1	0.862	0.97	0		5	1.802	0.304	0.571	0.802	0.204	0
Dde69		7	1.844	-0.09	0.917	0.844	0.78	0		5	1.769	0.077	0.714	0.769	0.712	0
Dde70		6	1.703	0.054	0.667	0.703	0.525	0.001		5	1.659	-0.091	0.714	0.659	0.851	0
Dde72		9	1.899	-0.021	0.917	0.899	0.779	0		9	1.934	0.089	0.857	0.934	0.505	0
Dde84		4	1.533	-0.1	0.583	0.533	1	0		6	1.747	0.048	0.714	0.747	0.294	0
EV14*		6	1.699	0.294	0.5	0.699	0.078	0		4	1.648	0.357	0.429	0.648	0.067	0
EV37		7	1.761	0.015	0.75	0.761	0.855	0		3	1.67	0.158	0.571	0.67	0.778	0
KWM12a		11	1.899	0.076	0.833	0.899	0.153	0		9	1.912	-0.105	1	0.912	1	0
KWM1b*		1	1	NA	NA	NA	NA	NA		1	1	NA	NA	NA	NA	NA
KWM2b		4	1.63	0.349	0.417	0.63	0.225	0		3	1.67	-0.071	0.714	0.67	0.776	0
KWM9b		5	1.757	0.01	0.75	0.757	0.818	0		6	1.857	0	0.857	0.857	0.935	0
MK3		5	1.754	-0.111	0.833	0.754	0.97	0		7	1.89	-0.135	1	0.89	1	0
MK5		5	1.54	0.077	0.5	0.54	0.137	0		6	1.802	0.118	0.714	0.802	0.424	0
Sco28		3	1.163	-0.023	0.167	0.163	1	0		1	1	NA	NA	NA	NA	NA
TexVet7*		1	1	NA	NA	NA	NA	NA		1	1	NA	NA	NA	NA	NA
Sco11		5	1.83	0.1	0.75	0.83	0.7	0		6	1.857	0.178	0.714	0.857	0.201	0
Sco55		4	1.572	-0.02	0.583	0.572	0.84	0		2	1.363	0.625	0.143	0.363	0.23	0
Mean			6.105	0.015	0.697	0.698				5.333		0.089	0.69	0.743		
S.D.			2.208		0.243	0.203				2.196			0.221	0.178		

(Continues)

Locus	Null alleles	No. alleles	Allele richness	F_{IS}	H_O	H_E	P-value	s.d.	Null alleles	No. alleles	Allele richness	F_{IS}	H_O	H_E	P-value	s.d.
Pop	Eastern Tropical Pacific								Oregon-California Coastal							
n	22								73							
AAT44		3	1.21	-0.082	0.227	0.21	1	0		3	1.344	-0.076	0.37	0.344	0.122	0
D14		9	1.851	-0.07	0.909	0.851	0.85	0		12	1.824	0.022	0.806	0.824	0.285	0
D22		7	1.837	0.134	0.727	0.837	0.602	0		10	1.833	0.104	0.746	0.833	0.035	0
Dde59		11	1.873	0.17	0.727	0.873	0.032	0		16	1.842	0.09	0.767	0.842	0.22	0
Dde65		5	1.667	0.186	0.545	0.667	0.018	0		6	1.755	0.039	0.726	0.755	0.498	0.001
Dde66		10	1.834	0.075	0.773	0.834	0.146	0		9	1.785	0.111	0.699	0.785	0.586	0
Dde69		9	1.841	0.083	0.773	0.841	0.374	0		9	1.809	0.007	0.803	0.809	0.806	0
Dde70		11	1.823	0.063	0.773	0.823	0.292	0		13	1.742	0.04	0.712	0.742	0.214	0
Dde72		18	1.933	-0.023	0.955	0.933	0.981	0		20	1.9	-0.035	0.932	0.9	0.835	0
Dde84		6	1.742	-0.232	0.909	0.742	0.651	0		8	1.721	0.108	0.644	0.721	0.057	0
EV14*	Y	9	1.815	0.516	0.4	0.815	0	0	Y	14	1.896	0.452	0.493	0.896	0	0
EV37		10	1.813	0.164	0.682	0.813	0.229	0		8	1.755	-0.049	0.792	0.755	0.065	0
KWM12a		14	1.904	-0.006	0.909	0.904	0.664	0		15	1.895	0.059	0.843	0.895	0.284	0
KWM1b*		1	1	NA	NA	NA	NA	NA		1	1	NA	NA	NA	NA	NA
KWM2b		5	1.7	0.093	0.636	0.7	0.812	0		5	1.587	0.184	0.479	0.587	0.019	0
KWM9b		8	1.83	0.014	0.818	0.83	0.665	0		12	1.823	0.001	0.822	0.823	0.605	0
MK3		8	1.82	-0.054	0.864	0.82	0.785	0		9	1.828	-0.006	0.833	0.828	0.54	0.001
MK5		8	1.633	-0.079	0.682	0.633	0.912	0		9	1.693	-0.028	0.712	0.693	0.869	0
Sco28		1	1	NA	NA	NA	NA	NA		2	1.014	0	0.014	0.014	1	0
TexVet7*		1	1	NA	NA	NA	NA	NA		1	1	NA	NA	NA	NA	NA
Sco11		5	1.78	-0.11	0.864	0.78	0.568	0		8	1.795	0.035	0.767	0.795	0.053	0
Sco55		4	1.215	-0.061	0.227	0.215	1	0		4	1.18	-0.067	0.192	0.18	1	0
Mean		8.389		0.052	0.722	0.739				9.368		0.062	0.666	0.691		
S.D.		3.712			0.21	0.207				4.573			0.238	0.245		

Appendix 2.3.

	Variation site																											
Haplotype ID	1	21	25	32	34	37	52	58	69	70	95	107	117	136	147	150	151	153	166	167	171	176	185	190	192	195	196	197
Hap_1	G	A	T	T	C	T	G	G	T	T	A	C	T	T	C	G	C	C	C	A	T	C	T	T	G	T	C	C
Hap_2	.	.	.	.	T	C	.	A	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	C	T	.
Hap_3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	.	A	.	.	.
Hap_5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.
Hap_6	.	.	.	.	.	.	.	.	.	.	G	T	C	.	.	.	.	.	T	.	C	T	.	.	A	.	.	.
Hap_7	.	.	.	.	.	.	.	.	.	.	.	T	C	.	T	.	.	.	T	.	C	.	.	.	A	.	T	.
Hap_8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.	.	.	.	A	.	T	.
Hap_9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_11	.	.	.	.	.	.	.	.	.	.	G	T	C	.	.	.	.	.	T	.	C	.	.	.	A	.	.	.
Hap_12	.	.	.	.	.	.	.	.	C	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.
Hap_13	.	.	.	C	.	.	.	.	.	.	G	T	C	.	.	.	.	.	T	.	C	T	.	.	A	.	T	.
Hap_14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
Hap_15	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	.	C	.	.	.	A	.	T	.
Hap_17	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_19	.	.	.	C	.	.	.	.	.	.	G	T	C	.	.	.	.	.	T	.	.	T	.	.	A	.	T	.
Hap_20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
Hap_21	.	.	.	.	.	.	.	.	.	.	G	T	C	.	.	.	.	.	T	.	C	T	.	.	A	.	T	.
Hap_22	.	.	.	C	.	.	.	.	.	.	G	T	C	.	.	.	.	.	T	.	C	T	.	.	A	.	T	.
Hap_23	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	T	G	C	.	C	.	A	.	T	.

	Variation site																											
Haplotype ID	1	21	25	32	34	37	52	58	69	70	95	107	117	136	147	150	151	153	166	167	171	176	185	190	192	195	196	197
Hap_24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.
Hap_25	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	G	C	.	.	.	A	.	.	.
Hap_26	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_27	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_28	.	.	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	.	T	G	C	.	.	.	A	.	T	.
Hap_29	.	.	.	.	.	.	.	.	.	.	.	.	.	C	T	.	.	.	T	G	C	.	.	.	A	.	T	.
Hap_30	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	G	C	.	.	.	A	.	T	.
Hap_31	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
Hap_32	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_33	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_34	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	G	C	.	.	.	A	.	T	.
Hap_35	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.
Hap_36	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	T	G	C	.	.	.	A	.	T	.
Hap_37	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_38	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G	C	T	.	.	.	.	T	.
Hap_39	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T	.
Hap_40	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	C	.	.	.	A	.	T	.
Hap_41	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_42	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	G	C	.	.	.	A	.	T	.
Hap_43	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.
Hap_44	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T	.
Hap_45	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	G	C	.	.	.	A	.	T	.
Hap_46	.	.	.	.	.	.	.	.	.	.	G	.	C	.	.	.	.	.	T	.	.	.	.	.	A	.	.	.
Hap_47	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	G	C	.	.	.	A	.	T	.
Hap_48	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	A	.	T	.

	Variation site																												
Haplotype ID	1	21	25	32	34	37	52	58	69	70	95	107	117	136	147	150	151	153	166	167	171	176	185	190	192	195	196	197
Hap_49	.	.	.	.	.	.	.	.	.	.	.	.	.	C	T	.	T	.	T	G	C	.	.	.	A	.	.	.
Hap_50	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	C	T	.
Hap_51	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_52	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	G	C	.	.	.	A	.	T	.
Hap_53	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	.	C	.	.	.	A	.	T	.
Hap_54	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_55	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_56	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	T	G	C	.	.	.	A	.	T	.
Hap_57	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.
Hap_58	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	G	C	.	.	.	A	C	T	.
Hap_59	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	.	.	T	G	C	.	.	.	A	.	.	.
Hap_60	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	A	.	.	T	G	C	.	.	.	A	.	T	.
Hap_61	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	G	C	.	.	.	A	C	T	.
Hap_62	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_63	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	A	.	.	T	G	C	.	.	.	A	.	T	.
Hap_64	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_65	.	.	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_66	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	G	C	.	.	.	A	C	T	.
Hap_67	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_68	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	G	C	.	.	.	A	C	T	.
Hap_69	.	.	.	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	T	G	.	.	.	.	A	.	.	.
Hap_70	.	.	.	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	T	G	C	.	.	.	A	.	.	.
Hap_71	.	.	.	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	T	G	.	.	.	.	A	.	.	.
Hap_72	.	G	.	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.	T	G	C	.	.	.	A	.	T	.
Hap_73	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	.	C	.	.	.	A	.	T	.

	Variation site																												
Haplotype ID	1	21	25	32	34	37	52	58	69	70	95	107	117	136	147	150	151	153	166	167	171	176	185	190	192	195	196	197
Hap_74	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_75	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	G	C	.	.	.	A	C	T	.
Hap_76	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
Hap_77	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	A	.	.	T
Hap_78	.	.	.	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	T	G	.	.	.	.	A	.	T	.
Hap_79	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	T	G	C	.	.	.	A	.	.	.
Hap_80	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	T	G	.	.	.	.	A	.	T	.
Hap_81	.	.	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	.	T	.	.	.	.	.	A	.	T	.
Hap_82	A	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
Hap_83	.	.	.	.	.	.	A	.	.	.	.	T	.	C	.	.	.	.	T	G	.	.	.	.	A	.	T	.
Hap_84	.	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.	.	T	G	C	.	.	.	A	C	.	.
Hap_85	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	.	.	.	.	.	A	.	.	T

(Continues)

	Variable site																												
Haplotype ID	202	209	212	214	218	220	228	229	261	294	295	296	297	313	314	315	356	357	359	360	361	397	399	406	408	420	429	442
Hap_1	G	A	A	C	T	A	A	G	C	A	C	C	C	T	A	G	C	T	G	T	C	C	C	C	C	G	C	G
Hap_2	.	.	.	T	.	.	.	A	.	.	T	.	T	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.
Hap_3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_4	.	.	.	T	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_5	.	.	.	T	.	.	.	.	T	.	T	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.
Hap_6	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	.	T	.	A	.	T	.	.	.	.	.	T	.
Hap_7	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_8	.	.	.	T	.	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.
Hap_9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
Hap_10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_11	.	.	.	T	.	.	.	.	T	.	T	.	T	.	.	.	T	.	A	.	T	.	.	.	.	.	T	.
Hap_12	.	.	.	T	.	.	G	A	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_13	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	.	T	.	A	C	T	.	.	.	.	.	T	.
Hap_14	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_15	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.
Hap_16	.	.	.	T	.	.	.	.	T	.	.	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	.
Hap_17	.	.	.	T	.	.	.	.	T	.	T	.	T	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.
Hap_18	.	.	.	T	.	.	.	.	T	.	.	T	.	C	.	.	T	.	A	.	.	.	.	.	.	.	T	.
Hap_19	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	.	T	.	A	.	T	.	.	.	.	.	T	.
Hap_20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_21	.	.	G	T	.	.	.	.	T	.	.	.	T	.	.	.	T	.	A	.	T	.	.	.	.	.	T	.
Hap_22	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	.	T	.	A	.	T	.	.	.	.	.	T	.
Hap_23	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_24	.	.	.	T	.	.	G	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_25	.	.	.	T	.	.	G	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_26	.	G	.	T	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	T	.

	Variable site																												
Haplotype ID	202	209	212	214	218	220	228	229	261	294	295	296	297	313	314	315	356	357	359	360	361	397	399	406	408	420	429	442
Hap_27	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	T	.	A	.	.	.	.	.	.	.	T	.
Hap_28	.	.	.	T	.	.	.	A	T	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_29	.	.	.	T	.	.	.	A	T	.	.	.	T	.	.	.	.	.	.	.	.	.	.	T	.	.	T	.
Hap_30	.	.	.	T	.	.	.	A	T	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_31	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_32	.	.	.	T	.	.	.	.	T	.	T	.	T	.	.	.	T	C	.	.	.	.	.	.	.	.	T	.
Hap_33	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_34	.	.	.	T	.	.	.	A	T	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_35	.	G	.	T	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.
Hap_36	.	.	.	T	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_37	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	T	.	T	.
Hap_38	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.
Hap_39	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_40	.	.	.	T	.	.	.	.	T	.	.	T	T	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.
Hap_41	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.
Hap_42	.	.	.	T	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_43	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_44	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_45	.	.	.	T	.	.	.	A	T	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_46	.	.	.	T	.	.	.	.	T	.	T	.	T	.	.	.	T	.	A	.	T	.	.	.	.	.	T	.
Hap_47	.	.	.	T	.	.	.	A	T	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	T	.
Hap_48	.	G	.	T	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.
Hap_49	.	.	.	T	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_50	.	.	.	T	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_51	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	.
Hap_52	.	.	.	T	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.

	Variable site																												
Haplotype ID	202	209	212	214	218	220	228	229	261	294	295	296	297	313	314	315	356	357	359	360	361	397	399	406	408	420	429	442
Hap_53	.	.	.	T	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.
Hap_54	.	.	.	T	.	.	.	.	T	.	.	T	.	C	.	.	T	.	A	.	.	.	.	.	.	.	T	A
Hap_55	.	.	.	T	.	.	.	.	T	.	.	T	.	C	G	.	T	.	A	.	.	.	.	.	.	.	T	.
Hap_56	.	.	.	T	.	G	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_57	.	.	.	T	.	.	.	.	.	.	.	.	T	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap_58	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_59	.	.	.	T	.	.	.	A	T	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_60	.	.	.	T	.	G	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_61	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_62	.	.	.	T	.	.	.	.	T	.	.	.	.	C	.	.	T	.	A	.	.	.	T	.	.	.	T	.
Hap_63	.	.	.	T	.	G	.	.	T	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_64	.	.	.	T	.	.	.	.	T	.	.	.	.	C	.	.	T	.	A	.	.	.	.	.	.	.	T	.
Hap_65	A	.	.	T	.	.	.	.	T	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
Hap_66	.	.	.	T	.	.	.	.	T	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_67	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	.	.	C	.	.	.	.	.	.	T	.	T	.
Hap_68	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.
Hap_69	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	.	.	C	.	.	.	T	.	.	.	.	T	.
Hap_70	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.
Hap_71	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.
Hap_72	.	.	.	T	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_73	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_74	C	.	.	T	.	.	.	.	T	.	T	.	T	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.
Hap_75	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_76	.	.	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.
Hap_77	.	.	.	T	.	.	.	.	T	.	T	.	T	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.
Hap_78	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.

	Variable site																												
Haplotype ID	202	209	212	214	218	220	228	229	261	294	295	296	297	313	314	315	356	357	359	360	361	397	399	406	408	420	429	442
Hap_79	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_80	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_81	.	.	.	T	.	.	.	.	T	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
Hap_82	.	.	.	T	.	.	.	.	.	.	.	T	.	C	.	.	.	C	.	.	.	.	.	.	.	.	T	.
Hap_83	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	T	.
Hap_84	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
Hap_85	.	.	.	T	.	.	.	.	T	.	T	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.

Chapter 3. The population structure of Fraser's dolphins (*Lagenodelphis hosei*) in the North Pacific Ocean, Gulf of Mexico and Caribbean Sea

Abstract

The existence of any living Fraser's dolphin (*Lagenodelphis hosei*) was uncertain until the early 1970s. Its late discovery means that this tropical species is one of the least well-studied delphinids in the world. The increasing acquaintance of this tropical species in the waters of higher latitude in recent years suggests the species can potentially be regarded as a marine bio-indicator of climate change. Little is known about the population structure of Fraser's dolphin in the world, although earlier morphological studies have identified geographic variation in this species. Here the study presents the first population genetic study for the Fraser's dolphin species. In this study, the genetic data of 18 diploid microsatellite loci and one haploid mitochondrial DNA (mtDNA) locus from 112 Fraser's dolphin samples were examined. The results of the factorial correspondence analysis (FCA), pairwise comparison of fixation indices (F-Statistics) and Geneland analysis suggested that there is subtle population structure in the western North Pacific Ocean. Small sample sizes from other regions provided preliminary data suggesting a need for further studies. For example, samples from the

Caribbean Sea formed a distinct cluster compared to all other samples in the dataset. The results of neutrality tests of Tajima's D and Fu's Fs and mismatch analysis based on mtDNA data indicated that the populations, in terms of the one found in Japanese waters, may have expanded have expanded spatially and demographically in the past, possibly during the last global deglaciation when the sea level and global temperature started to rise. The effective population size for the demographically stable population, the Philippine population, is at the same magnitude to the estimates of other coastal or riverine dolphin populations, suggesting that Fraser's dolphin populations can be fragile under unregulated human disturbances. The unexpectedly high numbers of null alleles and inbreeding coefficient in the Taiwan population could be indicating a mixing population but warrants further research efforts.

Keywords: Fraser's dolphin, *Lagenodelphis hosei*, population genetics, microsatellite analysis, mtDNA control region analysis, North Pacific, conservation

Introduction

Investigating population structure is fundamental to understanding the ecology and evolution of a species. It is equally vital for stakeholders to evaluate the species' sustainability and vulnerability under anthropogenic disturbances in a given area.

Assessing the population structure of cetaceans (whales, dolphins and porpoises) can be particularly challenging, because cetaceans are in general less accessible than most terrestrial animals. As a result, of the 87 cetacean species on the IUCN Red List, the species status of 45 (51.7%) is still classified as 'Data Deficient', and the population trends of 72 species (82.8%) remains unknown (IUCN 2014).

The Fraser's dolphin (*Lagenodelphis hosei*) is one of the least studied dolphin species in the world. The species was not recognized until it was described by Fraser (1956) based on a skull specimen collected from Sarawak, Borneo in 1895. But it was not until the early 1970s that further reports of new specimens from the Eastern Tropical Pacific, South Africa, Australia, Taiwan and Japan, as well as sighting records of living individuals in the Eastern Tropical Pacific and Central Pacific, started to emerge (Perrin *et al*. 1973; Tobayama *et al.* 1973). In the succeeding decades further sighting, stranding and bycatch records from the regions of North and South Atlantic Ocean were reported (Caldwell *et al*. 1976; Hersh & Odell 1986; Leatherwood *et al.* 1993; Bones *et al.* 1998; Moreno *et al.* 2003; Weir *et al.* 2008; Gomes-Pereira *et al*. 2013), and today this small cetacean species is known to be widespread in pan-tropical regions of the Pacific, Atlantic and Indian Oceans (Jefferson *et al*. 2011; Hammond *et al.* 2012) (Fig. 3.1).

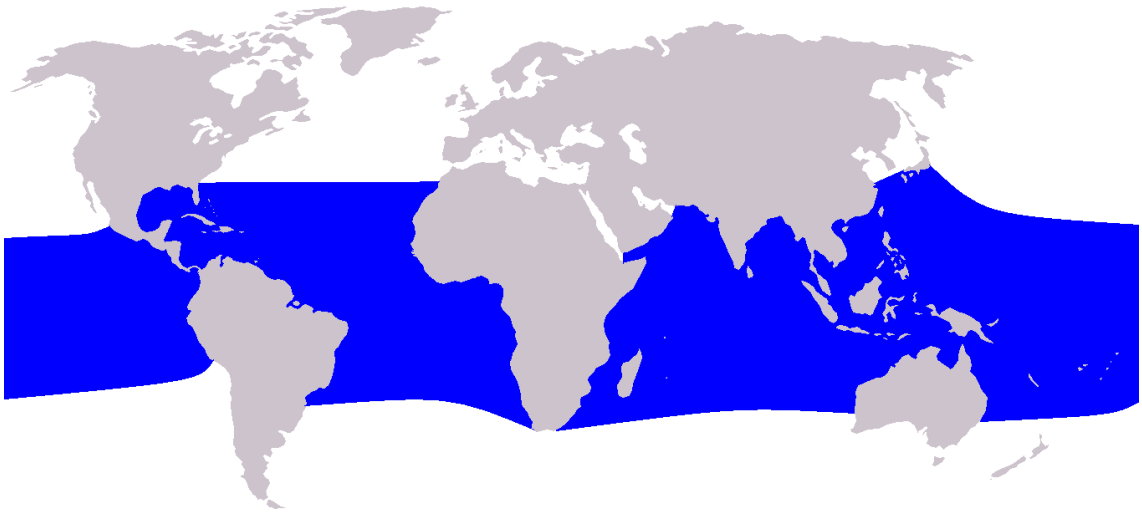

Figure 3.1. A map showing the distribution range of the Fraser's dolphin in the world (in blue). By Alessio Marrucci en:User:Pcb21 (en:) [GFDL or CC-BY-SA-3.0], accessed via Wikimedia Commons.

The occurrence of Fraser's dolphin is usually associated with tropical or subtropical climate and deeper waters (Jefferson *et al*. 2011). In the Tropical-North Pacific Ocean, the abundance of Fraser's dolphin was estimated as 289,300 in the Eastern Tropical Pacific (Wade & Gerrodette 1993), about 13,500 in the eastern Sulu Sea, the Philippines (Dolar *et al*. 2006), and about 10,200 in Hawaiian waters (Barlow 2006). This species is commonly encountered in Taiwanese waters (Yang *et al.* 1999; Chou 2006; Chen *et al.* 2011) where no reliable abundance estimate is available. Fraser's dolphin is uncommon in Japanese waters; only a few had stranded along the southern or southeastern coasts of Japan, and a school of over 100 Fraser's dolphins had been caught from Taiji in 1991 (Tobayama *et al.* 1973; Amano *et al.* 1996; Perrin *et al*. 2003). In the North Atlantic Ocean, on the other hand, the records of Fraser's dolphin (either from stranding or sighting) are rare and scattered. Sighting and stranding records

are mostly reported from the Gulf of Mexico and Caribbean Sea, but constant, yearly observation of this species has only been reported in the waters around the Lesser Antilles in the eastern edge of the Caribbean Sea (Gomes-Pereira *et al.* 2013). Würsig *et al.* (2000) suggest that Fraser's dolphins are more common in the Gulf of Mexico than any other region in the North Atlantic Ocean, although the abundance might be as few as only 1,000 individuals. Mullin & Fulling (2004) used line-transect abundance survey data collected in 1996—1997 and 1999—2001 and estimated there were 726 Fraser's dolphins in the northern Gulf of Mexico.

The awareness of geographic variation in Fraser's dolphin emerges as further specimens from different regions are revealed and compared. Pigmentation is found to be different between the specimens collected from South Africa and the Eastern Tropical Pacific Ocean (Perrin *et al*. 1973). The dolphins inhabiting the Atlantic Ocean are suspected of having a larger body size than those living in the Pacific Ocean (Van Bree *et al.* 1986), although Amano *et al.* (1996) argued that the body size was comparable in the dolphins found in French and Japanese waters. Significant differences in skull morphometric measurements are found between specimens collected from the Philippines and Japan, and between specimens collected in the Pacific and Atlantic Oceans (Perrin *et al.* 2003). Furthermore, an analysis of social behaviour suggests that the pod size of Fraser's dolphin in the Atlantic Ocean is in general smaller than those sighted in the Pacific Ocean (Gomes-Pereira *et al*. 2013).

However, poor sample availability limits those studies to conclude the dolphin's population structure, and pigmentation, morphological differences and social behaviours can be plastic and may not always reflect patterns of gene flow (West-Eberhard 1989; Crispo 2008; Prada *et al.* 2008).

Fraser's dolphins are suffering impacts from anthropogenic activities, namely incidental catches from fisheries and small-scale whaling. Dolar (1994) reported that Fraser's dolphin is frequently involved in the purse seine and driftnet fisheries in Palawan, central Visayas and northern Mindanao in the Philippines. Chou (2006) proposed Fraser's dolphin to be one of the most incidentally caught cetacean species in Taiwanese fisheries. Jefferson & Leatherwood (1994) reported that there were 773 dolphins killed in the tuna purse seines in the Eastern Tropical Pacific between 1971 and 1977, and 125 between 1986 and 1989. Legal and illegal direct catches used to be carried out in the Philippines, Taiwan, Japan, Sri Lanka, the Lesser Antilles, Indonesia and South Africa occasionally (Jefferson & Leatherwood 1994; Perrin *et al.* 2005). Unfortunately, the severity of such human impacts remains undetermined, because the knowledge of the species' population structure, population size, genetic connectivity, and ecological status is still insufficient.

The objective of this study was to use genetic techniques to investigate the population structure of Fraser's dolphins in the North Pacific Ocean, Gulf of Mexico and Caribbean Sea with a focus on the regions around the Philippines, Taiwan and

Japan, where the conflict between the dolphins and human fisheries is perhaps the most intense in the world. Based on the finding of an earlier morphological study (Perrin *et al.* 2003), the hypothesis to test in this study was that Fraser's dolphin populations are genetically differentiated between the Pacific and Atlantic Oceans, with the potential for further regional differentiation between Japanese and Philippine waters.

Material and Methods

Sample collection

The samples used in this study were subsamples from the tissue archives in the Cetacean Laboratory at National Taiwan University (Taiwan), es-Bank at Ehime University (Japan), National Museum of Natural Science (Japan), and Southwest Fishery Science Center, National Oceanic and Atmospheric Administration (USA). A total of 143 samples were acquired, representing dolphins inhabiting a range of localities in the North Pacific Ocean, Gulf of Mexico and Caribbean Sea. These were categorized into seven geographic groups, namely, Japan, Taiwan, the Philippines, Central North Pacific, Eastern Tropical Pacific, Gulf of Mexico and Caribbean Sea (Fig. 3.2, Appendix 3.1). All samples were collected from dead dolphins, either stranded or perished in fishery interactions. Except the samples from the Central North Pacific, those were biopsied from free-ranging dolphins. The species and sex identity was

acquired from the archive records where identification was based on the external morphological characters of the specimens and made by presumably knowledgeable researchers. When in doubt this was verified by the genetic assessments. Samples supplied by the Southwest Fishery Science Center were titrated DNA solutions; the others were provided as a small portion of skin or muscle tissue samples preserved in either 99% ethanol or 20% DMSO solution saturated with sodium chloride.

All specimens, except a set of 15 Philippine specimens archived in es-Bank, were transported to and examined in the Molecular Ecology Group laboratory in University of Durham, with valid official permits issued by the authorities of Japan, Taiwan, United States and United Kingdom. The 15 Philippine specimens archived in es-Bank were examined in the laboratory at Kyushu University, and due to the difficulty of calibrating microsatellite data generated from different laboratories (Delmotte et al. 2001), these 15 samples were not included in the microsatellite analyses.

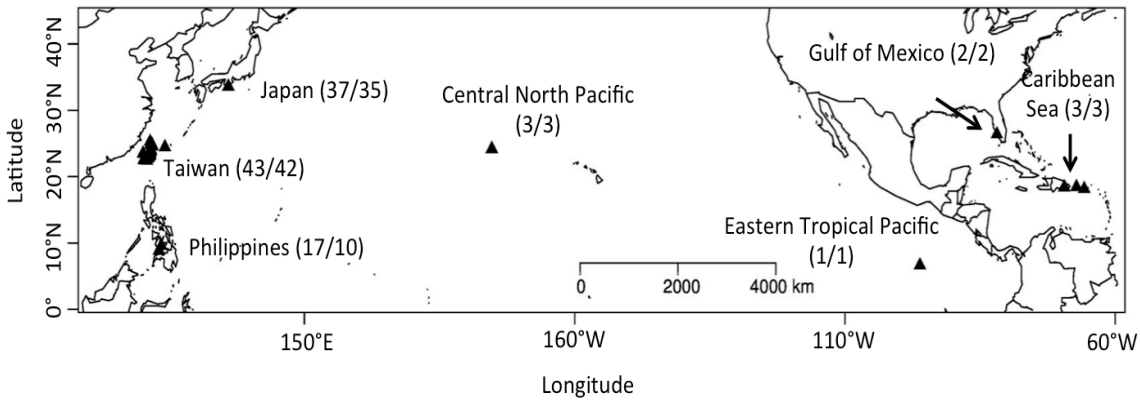

Figure 3.2. Map of the sampling locations. The numbers in the parentheses indicate the sample number of microsatellite/mitochondrial DNA analysis.

DNA extraction, fragment amplification and genotyping

Genomic DNA of each sample (except those acquired from Southwest Fishery Science Center and the set of Philippine samples examined in Kyushu University) was isolated and purified by a standard proteinase-K digestion/phenol–chloroform extraction protocol (Sambrook et al. 1989). Samples acquired from Southwest Fishery Science Center, as well as those es-Bank Philippine samples examined in Kyushu University, were prepared using conventional commercialized DNA extraction kit (QIAGEN).

Twenty-four microsatellite and one mitochondrial DNA (mtDNA) markers were chosen for examination, as those loci have been used in earlier genetic studies for other delphinid species (e.g., Natoli et al. 2004; Gaspari et al. 2007; Mirimin et al. 2011). The procedure of amplifying and genotyping the microsatellite and mtDNA fragments through polymerase chain reaction (PCR) and Sanger sequencing method was as described in Chapter 2, and the details for the microsatellite DNA loci, including their optimal annealing temperatures and allele size ranges for this sample set, are provided in Table 3.1.

Table 3.1. List of used microsatellite markers with optimal annealing temperatures and fragment size range observed for each locus in Fraser's dolphin samples.

Microsatellite locus	Optimal annealing temperature (°C)	Fragment size range	Genbank accession number	Reference
AAT44	58	70-106	AF416501	Caldwell et al. 2002.
EV14	60	134-166	G09079	Valsecchi and Amos. 1996.
EV37	54	179-265	G09081	
D14	48	113-137		Shinohara et al. 1997.
D22	52	109-117		
KWM1b	46	179-195		Hoelzel et al. 1998.
KWM2a	50	136-164		
KWM2b	44	157-179		
KWM9b	58	157-193		
KWM12a	54	146-194		
TexVet5	58	182-224	AF004905	Rooney et al. 1999.
TexVet7	57	153-181	AF004907	
MK3	56	136-172	AF237889	Krützen et al. 2001.
MK5	53	201-247	AF237890	
Dde59	56	232-440	AM087093	Coughlan et al. 2006.
Dde65	56	172-208	AM087096	
Dde66	50	338-378	AM087097	
Dde69	54	196-220	AM087098	
Dde70	56	117-135	AM087099	
Dde72	58	241-271	AM087100	
Dde84	49	136-158	AM087101	
Sco11	56	175-203	AM087102	Mirimin et al. 2006.
Sco28	58	135-139	AM087103	
Sco55	56	216-220	AM087105	

Microsatellite data analysis: data configuration

The microsatellite data checking software Micro-Checker 2.2.3 was used to screen for null alleles and potential scoring errors, where expected homozygote and heterozygote allele size difference frequencies were generated using a Markov Chain Monte Carlo (MCMC) simulation method (Van Oosterhout et al. 2004). Low levels of microsatellite genotyping errors, which usually result from using poor quality genome extractions, may yield a false homozygote signal for rare alleles and consequent deviation from Hardy-Weinberg equilibrium (HWE) (Morin et al. 2009). To test for this effect, the

jackknife analysis described in Morin et al. (2009) was used to screen for individuals influential to the HWE estimates. The test was undertaken using an R package *strataG* with R 3.1.2 (http://www.r-project.org/).

The observed heterozygosity (H_O), expected heterozygosity (H_E) and the significance of any deviation from HWE were estimated for each locus using Arlequin 3.5.1 (Excoffier & Lischer 2010). The overall deviation, heterozygote deficiency and heterozygote excess were assessed through the Fisher exact test and MCMC method implemented in the same program (Number of steps in Markov chain, 1,000,000; number of dememorization steps, 100,000). FSTAT 2.9.3.2 was used to determine the allelic richness and inbreeding coefficient (F_{IS}) in each geographic group (Goudet 1995, 2002).

Microsatellite data analysis: population structure

The factorial correspondence analysis (FCA) implemented in Genetix 4.0 was used to assess the genetic similarity among individuals (Belkhir et al. 2004). The program projects individuals on a multi-dimensional space according to their allelic states (absence, homozygote or heterozygote), and individuals with similar series of allelic states would be clustered together. Both with and without using population information

('sur population') options were used to generate different plots for comparison, and the figures were reconstructed using the R package *graphics*.

The software STRUCTURE 2.3.4 was used to assess the most likely number of populations (K) by estimating the population membership of each individual using a Bayesian inference assignment method (Pritchard et al. 2000). The parameter setting for the analysis was as described in Chapter 2, with the testing K ranged from 1 to 8. When the LOCPRIOR option was used, each individual was assigned to a predefined population according to its geographical group identity. The highest hierarchical K was then determined by calculating the delta K (Evanno et al. 2005) using the web-based software Structure Harvester (http://taylor0.biology.ucla.edu/structureHarvester/; Earl et al. 2012) and the graphic result was optimised using accessory software CLUMPP version 1.1.2 (Jakobsson & Rosenberg 2007) and Distruct 1.1 (Rosenberg 2004).

The R package *Geneland* was also used to assess population structure in a spatial context (Guillot et al. 2005). The procedure of conducting this analysis was as described in Chapter 2, with setting the maximum rate of Poisson process fixed to 106 (the number of samples), and maximum number of nuclei in the Poisson-Voronoi tessellation fixed to 318, and the clusters (K) to vary from 1 to 8 in the first step. To calculate the posterior probabilities of population membership for each individual and each pixel of the spatial domain, a burn-in of 100 iterations and a spatial domain of 174

pixels along the X-axis and 27 along the Y-axis were used. The consistency of results across these 10 runs was individually checked.

The degree of population differentiation among the geographic groups was evaluated by the fixation indices, F_{ST} (Wright 1951) and R_{ST} (Slakin 1995), using the algorithm implemented in Arlequin. A non-parametric permutation approach with 10,000 permutations was used to assess the statistical significance of the fixation statistics between each pair of geographic groups, with a significance level set at $p<0.05$ (for all tests). As the F-Statistics is less reliable with small sample size (Balloux & Lugon-Moulin 2002), the estimates were only calculated for geographic groups with larger sample size (i.e., sample size >10).

Microsatellite data analysis: effective population size, gene flow, potential migrants and sex-biased dispersal

The indicators of long-term effective population size, the effective population size times mutation rate ($N_e\mu$), and long-term gene flows, the number of migrants per generation (N_em), were estimated using maximum likelihood coalescent methods implemented in MIGRATE version 3.6.6 (Beerli & Felsenstein 1999, 2001). To determine whether there was any recent immigration, GeneClass2 was used to search for potential first generation migrants (Piry et al. 2004). To assess the presence of sex-biased dispersal in

the samples, the program for assessing sex-biased dispersal implemented in FSTAT 2.9.3.2 (Goudet et al. 2002). The setting of these three analyses was as described in Chapter 2. Note the estimates were only calculated for the geographic groups with sufficient sample size (i.e., n>10), that is, for the Japan, Taiwan and the Philippines groups.

Mitochondrial DNA analysis

The software PopART (http://popart.otago.ac.nz) was used to construct a median-joining network map (Bandelt et al. 1999) to visualize the genealogical distance among the mtDNA haplotypes. DnaSP version 5.10 was used to estimate gene diversity (h) and nucleotide diversity (π), and to conduct Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) neutrality tests. The analysis of mismatch distributions implemented in Arlequin was conducted in order to examine whether these populations ever experienced a demographic or a spatial expansion (Rogers & Harpending 1992; Schneider & Excoffier 1999; Excoffier 2004; Ray et al. 2003). The confidence interval of the estimates was obtained under 10,000 bootstrap simulations of an instantaneous expansion under a coalescent framework. The sum of square deviations (SSD) between the observed and the expected mismatch and the raggedness index (r) of the observed distribution were calculated and tested to evaluate model fitness (Harpending 1994; Schneider &

Excoffier 1999). An approximate time of expansion for each geographic group (*T*) was calculated by the formula $T=\tau/2u$, where τ is the simulated time of demographic or spatial expansion, and u is the mutation rate for the sequence in use (Rogers 1995). The u can be calculated by u=(length of the sequence)×(generation time)×(substitution rate; λ).

The generation time was calculated as 12.5 years, by averaging the age reaching sexual maturity (5—10 years old) and the oldest age of the Fraser's dolphins that have been aged (17.5 year-old; Amano et al. 1996). For the λ, two rates were used: one is an approximate average rate estimated using multiple ancient DNA samples of a number of animal taxa (1×10^{-7} substitutions/per site/per year; Ho et al. 2011), and the other is a rate estimated using fossil-phylogenetic distance calibration (7×10^{-8} substitutions/per site/per year; Harlin et al. 2003).

The mtDNA dataset was also used to assess the level of population differentiation among the geographic groups. The frequency-based and distance-based F-statistics, F_{ST} and Φ_{ST}, were estimated using Arlequin. For Φ_{ST}, the Tamura and Nei model was used (Tamura & Nei 1993), because it was the closest model available to the TVM+I model, which was determined as the best model for the samples using the Akaike Information Criterion (AIC) implemented in the model comparison program jModelTest 2.1.6 (Darriba et al. 2012). The level of differentiation between sample

group pairs was estimated with 10,000 permutations. The estimates were only calculated for the Japan, Taiwan and the Philippines groups.

Results

Sample screening

Useful genetic information was successfully amplified in 120 samples: 96 samples for mtDNA and 115 samples for microsatellite loci analyses (Appendix 3.1). Seven samples that were probably not Fraser's dolphin according to their genotypes: six were from the Philippines, and one was from Taiwan. By comparing their mtDNA control region sequence data against the DNA Surveillance reference database (http://dna-surveillance.fos.auckland.ac.nz; Ross et al. 2003), two samples (#2660 and #EW590) were identified as spinner dolphin (*Stenella longirostris*), two (#5560 and #718) as common bottlenose dolphin (*Tursiops truncatus*), and one (#EW581) as Indo-Pacific bottlenose dolphin (*T. aduncus*). The FCA of 24 microsatellite loci data also confirmed that #2660 and #5560 from the Philippines were apparently different from other Fraser's dolphin samples, but the Taiwan specimen (#718), was not as distinct although it possessed a common bottlenose dolphin mtDNA haplotype (Fig. 3.3). Two more Philippine samples (#2646 and #5558) showed a microsatellite genetic profile apparently different from other 'typical' Fraser's dolphins (Fig. 3.3), although their

mtDNA sequence could not be amplified for further confirmation. Since the species identity of these samples might be problematic and in need of further investigation, these seven samples were excluded from further analyses.

In addition, a putative male Philippine sample (#2602) was evidently mislabeled, because 1) the DNA sexing test indicated it was a female, and 2) it shared exactly the same microsatellite profile as another female sample (#2601) also collected in the Philippines in 1994. There were two samples (one female and the other male) collected in Taiwan at the same time (#887 and #892, respectively) sharing the same mtDNA haplotype and one allele at every microsatellite locus, indicating that they were highly likely a mother-calf pair. The Philippine sample (#2602) and the male Taiwanese sample (#892) were therefore excluded from further analyses.

With the jackknife HWE test, three Japanese samples and one Taiwanese sample were found that each had a rare allele homozygote that was influential to the estimates of HWE (Table 3.2). Morin et al. (2009) suggested poor genomic DNA quality might result in poor microsatellite amplification and consequently promote the likelihood of finding a homozygous rare allele. The difficulty of amplifying the mtDNA sequence of #EW546 might be indicative of poor DNA extraction quality. However, the other three samples were not of poor quality. A trail analysis excluding these four samples showed their influence on the results to be negligible. Therefore, these samples were retained in the dataset for further analysis.

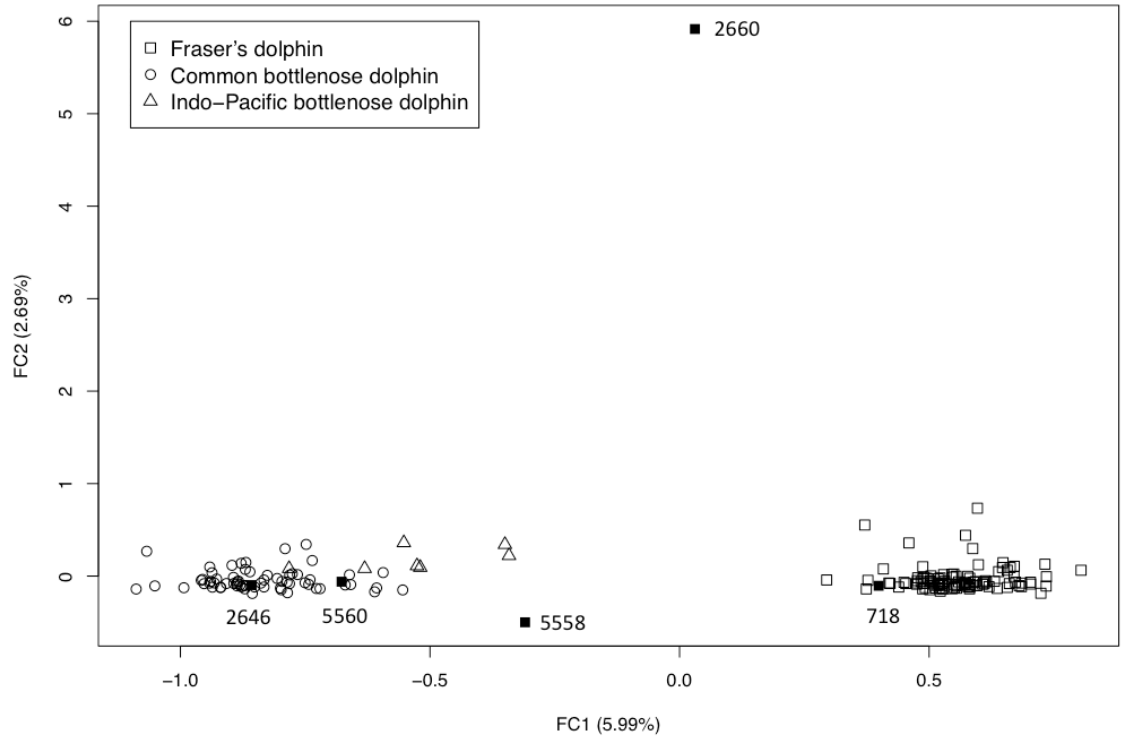

Figure 3.3. Factor correspondence analysis (FCA) result for the 24 microsatellite loci data for all acquired Fraser's and bottlenose dolphin samples (see Chapter 4). Numbers in parentheses indicates the percentage of the variance explained by the factor/axis. The solid squares are the questionable Fraser's dolphin samples, which either has a non-Fraser's dolphin mtDNA haplotype or a microsatellite profile unlikely to be a Fraser's dolphin.

Table 3.2. The result from the jackknife test showing the individuals and alleles that are influential to HWE in the samples.

ID	Group	Locus	Allele (frequency)	Observed/Jack-knife P value	Observed/Jack-knife odds	Odds ratio
EW00562	Japan	TexVet7	181 (0.01)	0.0018/0.2795	0.0018/0.388	215.126
821	Taiwan	AAT44	106 (0.014)	0.0045/0.0688	0.0045/0.074	16.345
EW00546	Japan	TexVet5	190 (0.029)	0.0144/0.0968	0.0146/0.107	7.336
EW00545	Japan	KWM9b	185 (0.038)	0.0117/0.0563	0.0118/0.06	5.039

Microsatellite data analysis: data configuration

Microsatellite data from 106 samples were used, and less than 5% of the samples had any missing data in one to four loci. All 24 microsatellite loci showed different degrees of polymorphism (Table 3.3). Null alleles and significant deviation from HWE were detected for a number of loci and geographic groups, and they were most common in the Taiwan group. Even after excluding the five 'outlier' Taiwan samples identified in FCA, it was not possible to alleviate the issue of having high null alleles and HWE deviation rates in the Taiwan group (Fig. 3.4; see below). The Taiwan group also had the highest average inbreeding coefficient (F_{IS}=0.176, see Table 3.3). Significant LD was rarely detected in the three major sample groups. To avoid the unpredictable influence of the presence of null alleles and HWE deviated loci (Carlsson 2008), subsequent analyses were conducted excluding the six loci that showed signs of null allele presence or significant deviation of HWE in at least two geographic groups. This resulted in retaining 18 loci for the following analyses (i.e., AAT44, D14, D22, Dde65, Dde69, Dde70, Dde72, Dde84, KWM1b, KWM2b, KWM9b, MK3, MK5, Sco11, Sco28, Sco55, TexVet5 and TexVet7).

Table 3.3. Averages (±SD) of number of alleles, observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), and allelic richness of the 24 microsatellite loci in the Fraser's dolphin samples, grouped by geographic groups. The results for geographic groups of Central North Pacific, Eastern Tropical Pacific, Gulf of Mexico and Caribbean Sea were not shown as their sample size was small and provided little insights into HWE deviation and allelic heterozygosity. See Appendix 3.2 for the estimates by locus in each population.

Geographic group	n	No. of alleles	H_E	H_O	Allelic richness	F_{IS}
Japan	37	7.25±3.404	0.637±0.229	0.596±0.230	1.634±0.231	0.049
Taiwan	43	9±4.16	0.702±0.192	0.573±0.182	1.675±0.235	0.176
The Philippines	17	5.375±2.318	0.653±0.240	0.609±0.271	1.626±0.270	0.068

Microsatellite data analysis: population structure

The FCA analysis grouped most individuals into the same cluster when the 'sur population' option was not used (Fig. 3.4A). This option identifies the centre of a cluster for an identified population, and recalculates individual positions relative to their cluster centre (Belkhir et al. 2004). There were five individuals from the Taiwan group scattered peripherally around the main cluster of samples (the 'outliers' in Fig. 3.4A). These samples do not share the same mtDNA haplotype or a common mtDNA lineage.

When the population information was used, the percentage of variance explained by the factors was notably increased (Fig. 3.4B). The most informative factor (FC1) explained 27.1% of variance and segregated the Taiwanese samples from the remaining samples; the second most informative factor (FC2) explained 20.79% of variance and separated the samples from the Central North Pacific from the remaining samples; and the third factor (FC3) which explained 16.22% of variance separated the Philippine samples and the Caribbean samples from the Japanese samples. The pattern of

clustering was not always consistent with the geographic groups; for instance, some Taiwanese samples remained in the 'main' cluster while many were segregated as a distinguishable cluster by FC1.

Whether the LOCPRIOR option was used in STRUCTURE or not, the most likely number of populations was K=1 in all cases based on LnP(K) values (Table 3.4). When the result was evaluated based on delta K, the most likely number of populations identified by STRUCTURE was K=2 (Table 3.4). A result giving high support for K=2 and K=5 (LOCPRIOR applied; Table 3.4) is consistent with K=1 since delta K can never identify K=1, and instead suggests support for multiple values of K (Evanno et al. 2005). In other words, the STRUCTURE analysis failed to reveal population structure detected by other analyses (i.e., FCA, Geneland and pairwise F-Statistics comparison), probably due to low power (Latch et al. 2006). However, among the uneven assignments to Taiwan when K=2, all five of the FCA outliers (see above) show high assignments to the second cluster (p=0.77—0.82; Fig. 3.5).

In the Geneland analysis, the five runs with the highest LPP values in the initial 10 runs suggested K=4 as the most likely number of populations, whereas the other five runs supported K=2. By fixing the K to K=4 in the second step of the analysis, it revealed six different population structure patterns in the 10 runs with the highest LPP values (Fig. 3.6). The patterns shown in five of the 10 runs were in fact suggesting three slightly different patterns of K=3, as the fourth population identified in the map contains

no sample (the 'ghost population'; Guillot et al. 2005). The only consistency found across all six patterns of Fraser's dolphin population distribution was that there was always a population that consisted of Caribbean Sea samples only.

By comparing the six population distribution patterns, it was shown that the samples from Japan and Taiwan were always assigned to the same population (Fig. 3.6A—E), while there was one run suggesting some of the samples from Taiwan could be from a different population (Fig. 3.6F). The samples from the Philippines and Central North Pacific were always assigned to the same population (Fig. 3.6A, C—F), except two of the 10 runs that suggested the Central North Pacific samples were from a unique population (Fig. 3.6B). The population identity for the samples from the Eastern Tropical Pacific and Gulf of Mexico was less certain, although it seems the sample from the Eastern Tropical Pacific was more often grouped with the samples from Japan and Taiwan (six of the 10 runs; Fig. 3.6B—D, F) and the samples from the Gulf of Mexico were more often grouped with the samples from the Philippines (six of the 10 runs; Fig. 3.6B, C, E, F).

In the pairwise F-Statistic comparisons among samples from three geographic groups (Japan, Taiwan and the Philippines), the F_{ST} for all pairs was significantly different from zero and ranged from 0.009 to 0.012, but none of the pairwise R_{ST} values were significant (Table 3.5A).

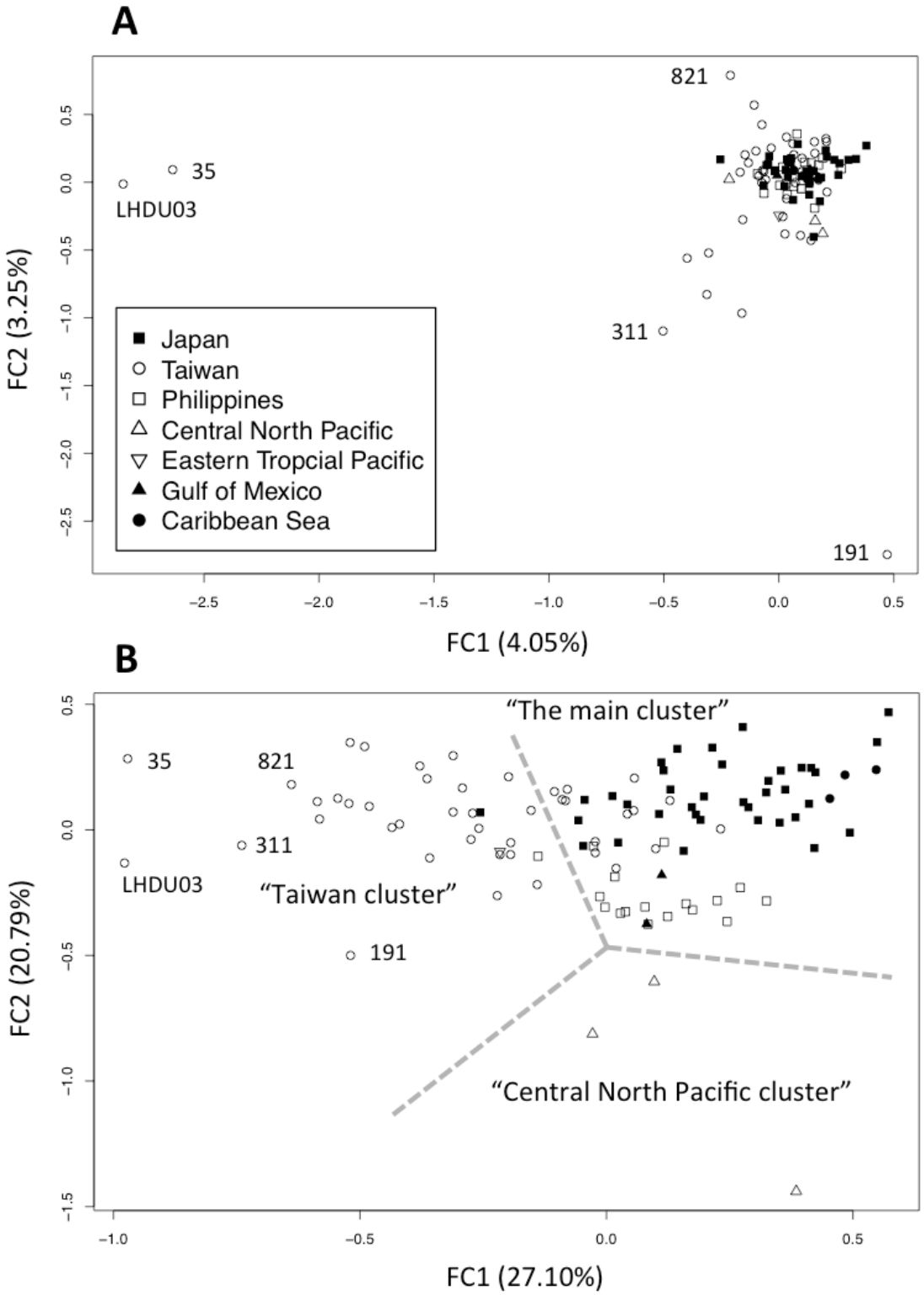

Figure 3.4. Factor correspondence analysis (FCA) results: A) without using population information; (B) using population information. The two most informative factors (FC1 and FC2) were assigned as the X and Y axes in the figure, and the numbers in parentheses in each axis indicates the percentage of the variance explained by the factor. The specimen ID of the five outliers was attached to the data point.

Table 3.4. The Evanno tables generated by Structure Harvester based on the results from STRUCTURE analysis.

LOCPRIOR option	K	Mean LnP(K)	SD LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
Used	1	-5216.05	0.4552	NA	NA	NA
Used	2	-5294.59	61.9034	-78.54	101.24	1.635452
Used	3	-5474.37	114.5322	-179.78	17.01	0.148517
Used	4	-5671.16	192.3951	-196.79	71.56	0.371943
Used	5	-5939.51	221.3678	-268.35	481.82	2.176558
Used	6	-5726.04	247.4806	213.47	128.49	0.519192
Used	7	-5641.06	256.9341	84.98	55.92	0.217643
Used	8	-5612	232.0099	29.06	NA	NA
Not used	1	-5216.27	0.4762	NA	NA	NA
Not used	2	-5296.25	37.4779	-79.98	170.97	4.561883
Not used	3	-5547.2	79.9775	-250.95	68.57	0.857366
Not used	4	-5866.72	83.8923	-319.52	247.43	2.949376
Not used	5	-5938.81	180.2097	-72.09	133.87	0.742857
Not used	6	-6144.77	152.1594	-205.96	330.82	2.174167
Not used	7	-6681.55	262.6884	-536.78	408	1.553171
Not used	8	-6810.33	375.4795	-128.78	NA	NA

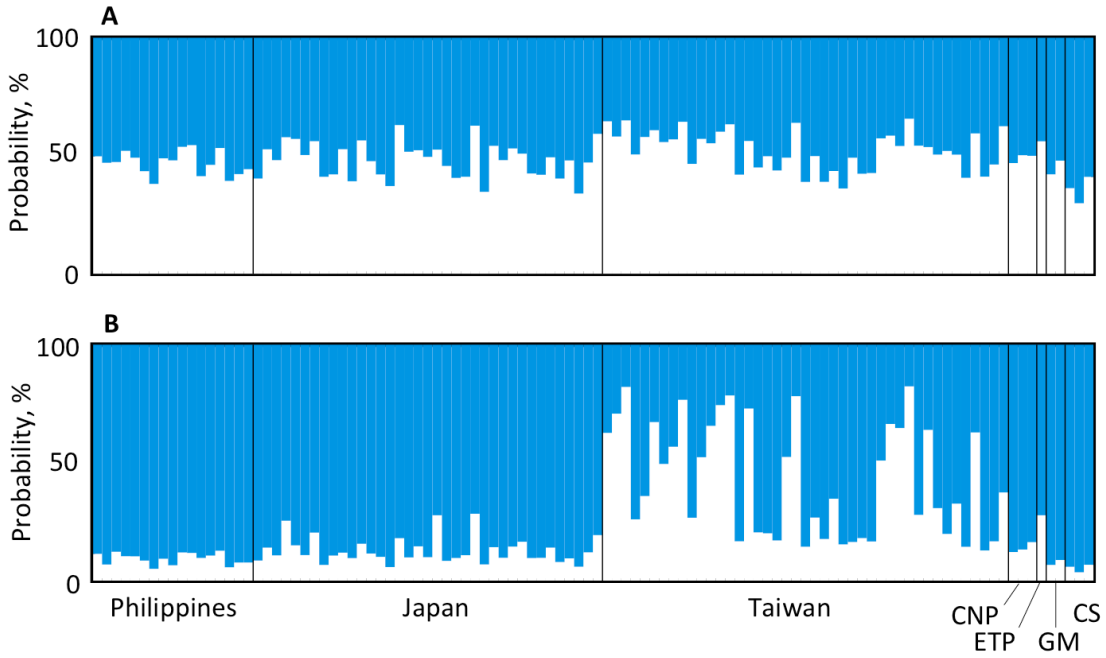

Figure 3.5. Individual's population membership under the best K scenario predicated by STRUCTURE analysis using 18 microsatellite loci data, (A) not using or (B) using the LOCPRIOR option. Each column represents one individual, and the light grey/ dark grey portion in each column indicates the probability of the individual being assigned to a population. In (B) the specimen ID of the five outliers denoted in the FCA was attached to data column. Abbreviations: CNP, Central North Pacific; ETP, Eastern Tropical Pacific; GM, Gulf of Mexico; CS, Caribbean Sea.

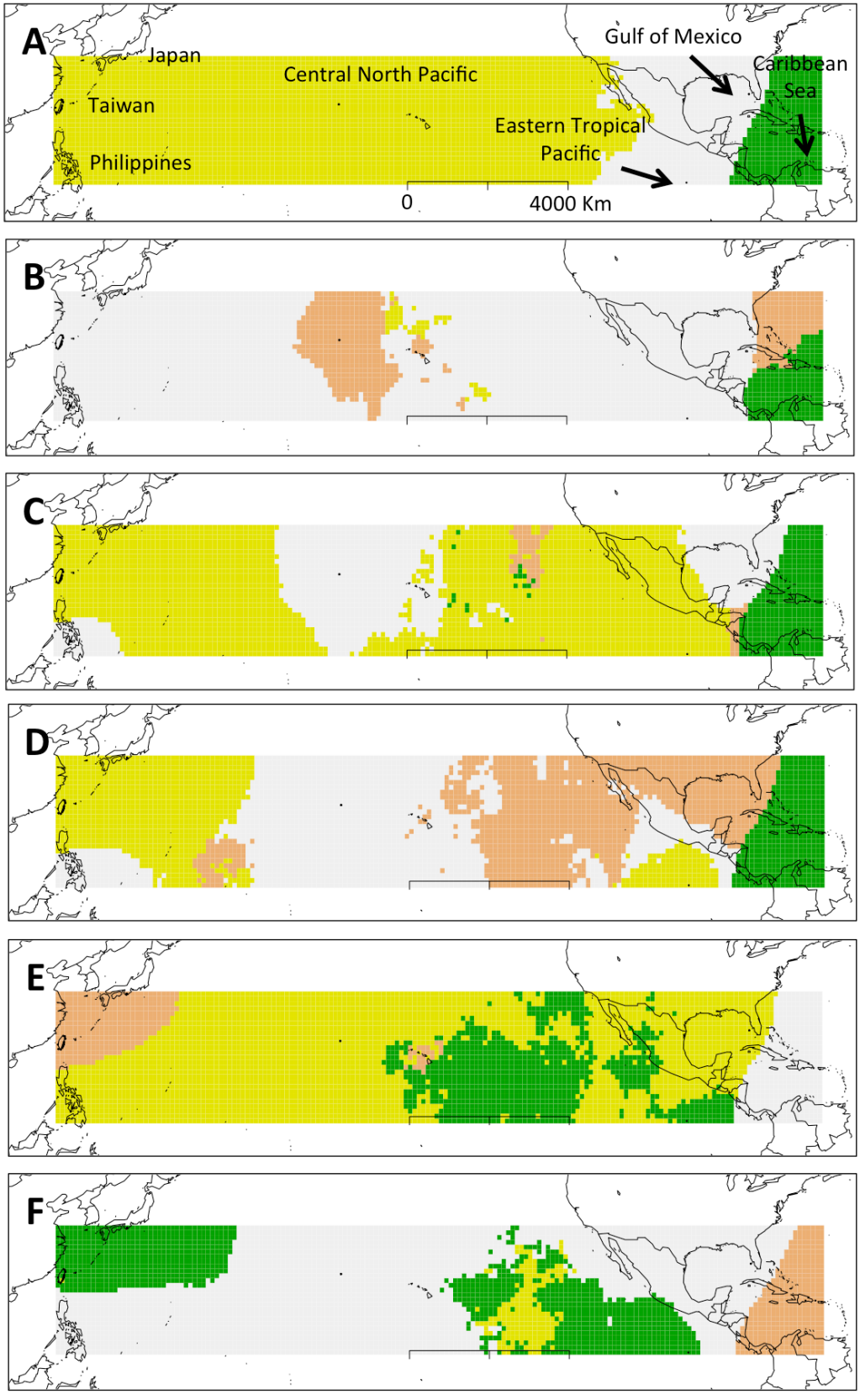

Figure 3.6. The six variations of the individual population membership assignment patterns shown in the 10 run with the highest LPP for K=4 in Geneland analysis. The dots represent the samples and the colours indicate the distribution of K clusters based on the mode of simulated posterior probability for each pixel.

Table 3.5. Pairwise divergence between the three main geographic groups for A) microsatellite data and B) for mtDNA data. The divergence was estimated using F_{ST} (above the diagonal), R_{ST} for microsatellite data (below the diagonal in panel A) and Φ_{ST} for mtDNA data (below the diagonal in panel B).

			F_{ST}		
		n	Japan	Taiwan	Philippines
A	Microsatellite				
	Japan	37		0.009**	0.012**
R_{ST}	Taiwan	43	0		0.011*
	Philippines	17	0.017	0.015	
B	mtDNA				
	Japan	35		0.01	0.029*
Φ_{ST}	Taiwan	42	0.009		0.034*
	Philippines	10	0.031	-0.017	

*: P<0.05; **: P<0.01

Microsatellite data analysis: effective population size, gene flow and sex-biased dispersal

Samples from Japan, Taiwan and the Philippines were treated as distinct populations due to the interest of conservation management when estimating the indicators of effective population size ($N_e\mu$) and migration rate (N_em), even though the population boundaries among these regions appear to be ambiguous. The estimates revealed the Philippine group had the largest effective population size ($N_e\mu$=0.992) while Japan had the smallest ($N_e\mu$=0.337; Table 3.6). Assuming a mutation rate for microsatellite loci of 0.01—0.02% (Whittaker et al., 2003; Hoelzel et al. 2007; Hollatz et al. 2011), the range of effective population size for each group was between 1,500 to 10,000 individuals (Table 3.6). The gene flow estimates showed there was less than one migrant per generation among the three putative populations (Table 3.6). The most prevailing

migration routes were from the south (Taiwan and the Philippines) to the north (Japan), concurring with the flow of the Kuroshio Current, the main ocean current in the region through times (See Fig. 2.9). Interestingly, there was almost no southbound gene flow detected (i.e., from Japan to Taiwan or the Philippines).

Two potential first generation migrants were identified among the three major sampling groups (Japan, Taiwan and the Philippines); both of them were female (Table 3.7). There was no strong indication of sex-biased dispersal, although the difference in genetic diversity with group (Hs) was statistically significant between the sexes (Table 3.8).

Table 3.6. The estimates of effective population size times mutation rate ($N_e\mu$) and number of migrants per generation (N_em) for the three Fraser's dolphin geographic groups. The N_e is calculated assuming the average microsatellite mutation rate (μ) is 0.01% for N_e (high) and 0.02% for N_e (low) (Whittaker *et al.* 2003; Hoelzel *et al.* 2007; Hollatz *et al.* 2011). The 2.5th and 97.5th profile likelihood estimates are given in parentheses.

	Source group	Host group		
		Japan	Taiwan	Philippines
$N_e\mu$		0.337 (0.313—0.364)	0.542 (0.516—0.571)	0.992 (0.926—1.065)
N_e (low)		1686 (1564—1821)	2711 (2578—2854)	4962 (4631—5324)
N_e (high)		3372 (3128—3641)	5423 (5155—5708)	9924 (9261—10647)
N_em	Japan		0.001 (0—0.002)	0 (0—0.001)
	Taiwan	0.607 (0.536—0.685)		0.003 (0.002—0.005)
	Philippines	0.541 (0.473—0.616)	0.008 (0.006—0.01)	

Table 3.7. Potential first generation migrants identified in GeneClass2.

ID	Sex	Geographic group	Potential source group	-LOG(L_home / L_max)	p
LHDU09	F	Taiwan	Japan	3.307	0.003
EW566	F	Japan	Taiwan	3.061	0.001

Table 3.8. Sex-biased dispersal assessments for the dolphins in western North Pacific by two-tailed t tests.

	n	F_{IS}	F_{ST}	Relativeness	Ho	Hs	Mean assignment	Var assignment
Female	40	0.056	0.010	0.019	0.561	0.595	0.539	20.916
Male	57	0.056	0.009	0.018	0.593	0.628	-0.378	17.523
Overall	97	0.056	0.010	0.018	0.580	0.614		
P-value		1.000	0.950	0.955	0.261	0.016*	0.306	0.573

*: P<0.05

Mitochondrial data analysis

A 779 bp mtDNA control region sequence was amplified in 96 samples. The 64 variable sites characterized 48 unique haplotypes (Appendix 3.3). The median-joining network tree showed a scattered tree with many missing haplotypes (Fig. 3.7). Little geographic concordance could be recognized in the clustering of the geographic groups. However, it showed that there were more haplotypes shared between Taiwan and Japan than between Taiwan and the Philippines, or between the Philippines and Japan (Fig. 3.7, Table 3.9). The genetic and nucleotide diversity was high in most geographic groups, although this could not be usefully assessed for the Caribbean Sea or Gulf of Mexico due to small sample size (Table 3.10).

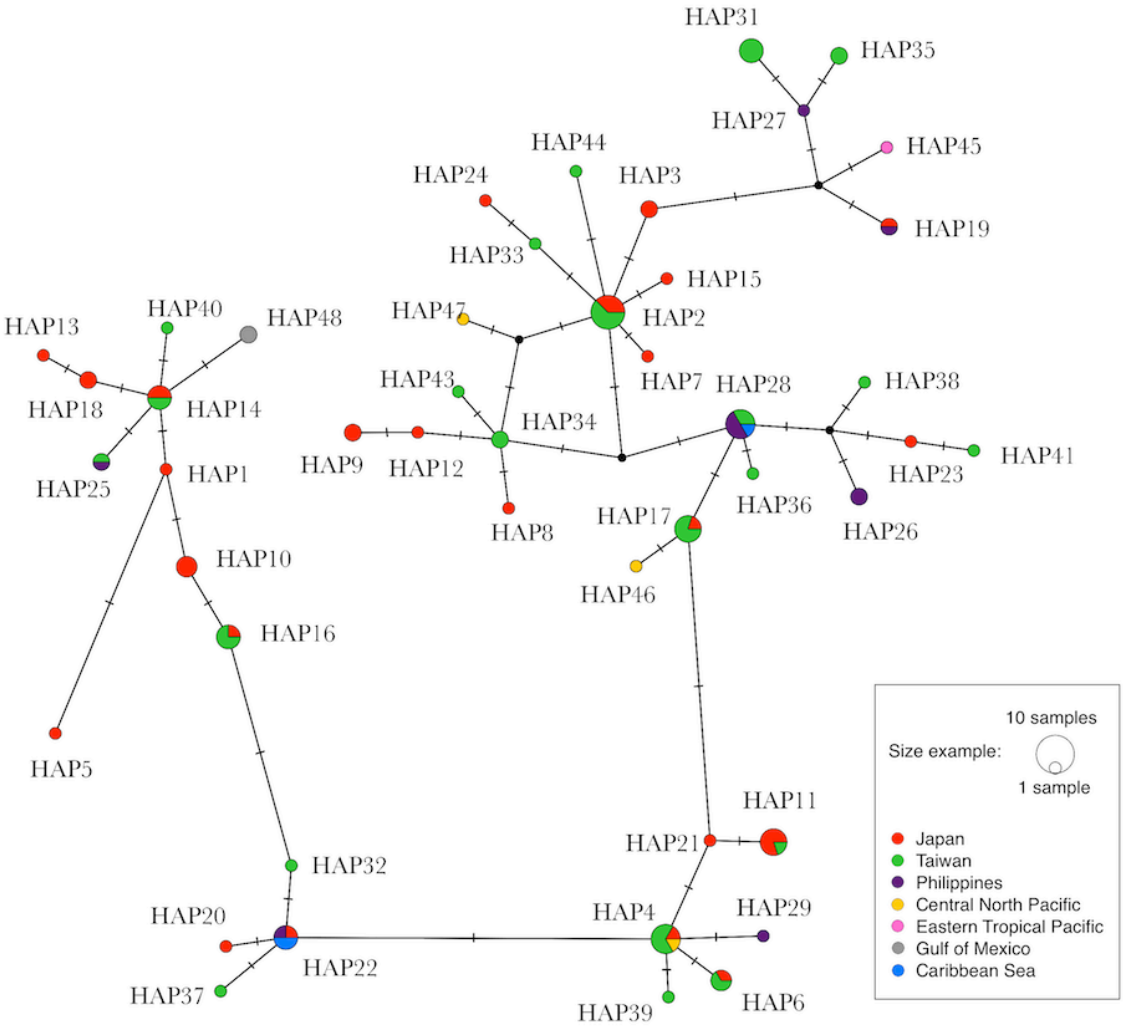

Figure 3.7. The Median-joining network tree for Fraser's dolphin mtDNA control region haplotypes. Each circle represents a unique haplotype. The size of circle indicates the number of individuals having the haplotype and the colour shade indicates the proportion of each population within the haplotype. The number of hatch marks at the lines indicates the number of mutational steps separating the haplotypes. Solid circles indicate missing intermediate haplotypes. Abbreviations: CNP, Central North Pacific; ETP, Eastern Tropical Pacific.

Table 3.9. Frequency of 48 mtDNA haplotypes detected among the seven geographic groups. See Appendix 3.3 for the definitions of the haplotypes.

	Geographic group						
Haplotype ID	Japan	Taiwan	Philippines	Central North Pacific	Eastern Tropical Pacific	Gulf of Mexico	Caribbean Sea
Hap 1	1						
Hap 2	3	4					
Hap 3	2						
Hap 4	1	4		1			
Hap 5	1						
Hap 6	1	2					
Hap 7	1						
Hap 8	1						
Hap 9	2						
Hap 10	3						
Hap 11	4	1					
Hap 12	1						
Hap 13	1						
Hap 14	2	2					
Hap 15	1						
Hap 16	1	3					
Hap 17	1	4					
Hap 18	2						
Hap 19	1		1				
Hap 20	1						
Hap 21	1						
Hap 22	1		1				2
Hap 23	1						
Hap 24	1						
Hap 25		1	1				
Hap 26			2				
Hap 27			1				
Hap 28			2				
Hap 29			1				
Hap 30		2	1				1
Hap 31		4					
Hap 32		1					
Hap 33		1					
Hap 34		2					
Hap 35		2					
Hap 36		1					
Hap 37		1					
Hap 38		1					
Hap 39		1					
Hap 40		1					
Hap 41		1					
Hap 42		1					
Hap 43		1					
Hap 44		1					
Hap 45					1		
Hap 46				1			
Hap 47				1			
Hap 48						2	

Table 3.10. Number of haplotypes, genetic diversity, nucleotide diversity, and population dynamic indices derived from the 779bp mtDNA control region sequence for the three geographic groups in Western North Pacific, the Western North Pacific, and All sequences including samples from central North Pacific, Eastern Tropical Pacific, Gulf of Mexico and Caribbean Sea.

Geographic group	n	No. variable sites	No. haplotypes	Haplotype diversity, h (SD)	Nucleotide diversity, π, (SD)	Tajima's D	Fu's Fs
Japan	35	44	24	0.973 (0.014)	0.012 (0.10%)	-0.41	-6.834**
Taiwan	42	40	22	0.958 0.013)	0.012 (0.07%)	-0.041	-3.197*
Philippines	10	26	7	0.911 (0.077)	0.012 (0.21%)	-0.076	0.64
Western North Pacific	87	61	42	0.973 (0.006)	0.012 (0.06%)	-0.777	-14.233***
All sequences	96	64	46	0.974 (0.005)	0.012 (0.05%)	-0.824	-17.243***

*** $p<0.001$, ** $p<0.01$, * $p<0.05$

A negative Tajima's D was estimated for all three testable sample groups (Japan, Taiwan, and the Philippines), although none of the values were significantly different from zero. All Fu's Fs estimates were also negative, and the value was significant for Japan and Taiwan (Table 3.10), indicating an excess of low-frequency haplotypes in these two populations, indicative of an expansion or selective sweep. The statistical significance of Fu's Fs for all samples suggests that Fraser's dolphins in the western North Pacific may have experienced a period of rapid population growth. The mismatch distribution for all three geographic groups did not appear to be unimodal (Fig. 3.8); however, the SSD value and the raggedness index (*r*) were small and statistically

insignificant in all groups (Table 3.11), suggesting the distribution did not differ significantly from the population expansion and/or spatial expansion models.

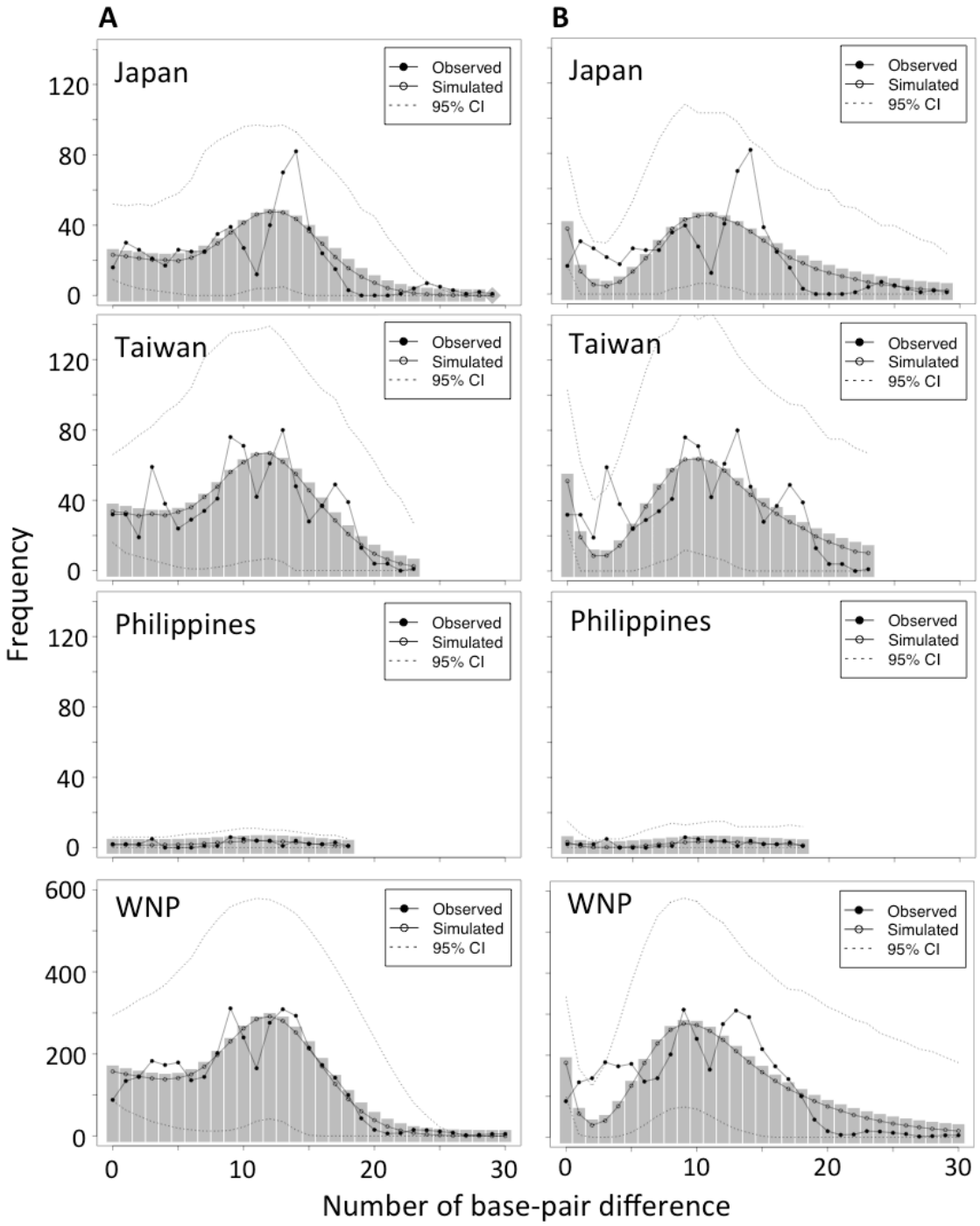

Figure 3.8. Observed and expected mismatch distributions under (A) demographic and (B) spatial expansion models. The vertical bars (in grey) indicate the model frequency in each scenario. Note the scale of frequency for Western North Pacific (WNP) is different from the other panels.

The τ of demographic and spatial expansion estimated for the Fraser's dolphin populations in the western North Pacific Ocean, as well as the estimated time of expansion, is shown in Table 3.11. The expansion time for each geographic group (Japan, Taiwan and the Philippines) was similar. The estimate shows the time of spatial expansion was likely later than the time of demographic expansion, although the estimates of both models shared a similar range of 95% CI. Based on a mutation rate u calculated as 9.7375×10^{-4} (using λ=1x10^{-7}) or 6.8162×10^{-4} (using λ=7x10^{-8}), the chronological time for the expansion to take place was estimated to be around 2,000—11,000 years ago (Table 3.11).

The pairwise F-statistics comparison showed the difference in any paired F_{ST} with the Philippines was statistically significant (with Japan and Taiwan, F_{ST}=0.033 and 0.029, p=0.022 and 0.026, respectively; Table 3.5B). In the Φ_{ST} comparison, however, none of the estimates was statistically significant. On the other hand, the exact tests based on both haplotype frequencies and the Tamura and Nei model suggest the three geographic groups were well differentiated (the exact p value for the global test of differentiation based on haplotype frequencies, p=0±0; based on Tamura and Nei's distance model, p=0.001±0.001; Table 3.12).

Table 3.11. The estimates of parameters under the A) demographic expansion and B) spatial expansion models for the three major geographic groups and the Western North Pacific as one group. τ is the time since expansion measured in mutational time units, SSD is the sum of squared deviation in goodness-of-fit test. T_1 and T_2 are the time of demographic/spatial changes for each geographic group calculated using substitution rates (λ) of $1x10^{-7}$ (Ho et al. 2010) and $7x10^{-8}$ (Harlin et al. 2003), respectively. The 95% profile likelihood for the estimates is given in parentheses.

Geographic group	τ (95% CI)	SSD	Raggedness index	T_1 (95% CI)	T_2 (95% CI)
A) Demographic expansion model					
Japan	13.4 (7.254—17.988)	0.012	0.014	6881 (3725—9236)	7021 (3801—9425)
Philippines	12.6 (4.996—17.707)	0.023	0.044	6470 (2565—9092)	6602 (2618—9278)
Taiwan	11.5 (5.68—19.568)	0.005	0.011	5905 (2917—10048)	6026 (2976—10253)
Western North Pacific	13.1 (6.051—18.041)	0.003	0.004	6727 (3107—9264)	6864 (3170—9453)
B) Spatial expansion model					
Japan	8.396 (4.8—20.161)	0.021	0.014	4311 (2465—10352)	4399 (2515—10564)
Philippines	9.042 (5.105—18.239)	0.026	0.044	4643 (2621—9365)	4738 (2675—9556)
Taiwan	7.551 (4.547—19.242)	0.01	0.011	3877 (2335—9880)	3956 (2382—10082)
Western North Pacific	7.091 (4.265—20.619)	0.009	0.004	3641 (2190—10587)	3715 (2235—10803)

Table 3.12. Pairwise non-differentiation exact P values estimated based on mtDNA haplotype frequencies (above the diagonal) or stepwise mutation model (Tamura and Nei's distance model; below the diagonal).

	Japan (n =35)	Taiwan (n=42)	Philippines (n=10)
Japan		0.022±0.005*	0.022±0.002*
Taiwan	0.019±0.004*		0.004±0.001**
Philippines	0.024±0.003*	0.003±0.001**	

** p<0.01, * p<0.05

Discussion

The results support the earlier suggestion based on skull morphometrics that Fraser's dolphin populations are differentiated within the North Pacific and between the Pacific

and the Atlantic Oceans (Perrin *et al*. 2003). The results also show that Fraser's dolphin in the western North Pacific Ocean experienced a period of population expansion in the past. Further details are discussed below, as well as other issues regarding potential technical limitations and conservation implications.

Population structure of Fraser's dolphin

Perrin *et al*. (2003) propose that Fraser's dolphins found in Japanese and Philippine waters are morphologically different: the skulls of Japanese samples were broader and the rostrums were wider, with a larger orbit and internal nares, and a longer braincase. The genetic data presented in this study, which were derived from the same stock of samples examined by Perrin *et al*. (2003), provide further support for differentiation among these populations, although the magnitude of the difference was small and not fully supported by all analyses. For example, the population differentiation identified by FCA and F_{ST} is not always supported by STRUCTURE and R_{ST}. However, the inference of STRUCTRE and R_{ST} may be ignorable because 1) the population structure revealed in STRUCTURE analysis can be less reliable (<97% assignment accuracy) when the F_{ST} is less than 0.05 (Latch *et al*. 2006) and when the sample size is uneven (Kalinowski 2011), and 2) false R_{ST} estimations are likely to be generated when the step-wise mutation model assumed for calculating R_{ST} is not applicable in one or more

of the microsatellite loci examined in this study, on top of the homoplastic nature of microsatellite loci (Selkoe & Toonen 2006). Nevertheless, it is acknowledged that the F_{ST} estimates, particularly for those paired with the Taiwan group, could have been overestimated due to the presence of null alleles in the Taiwan group (Chapuis & Estoup 2007). Inconsistent results and low F_{ST} estimates indicate the population structure is indeed ambiguous, and it might be due to the presence of ongoing gene flow among the populations, or the lack of sufficient time for the genetic markers to reveal the recent population divergence (Neigel 2002).

It is difficult to interpret the pattern of population structure for Fraser's dolphins in the western coasts of the North Pacific Ocean when the knowledge for their exact distribution range, fidelity of natal habitat, and migration behaviour is virtually lacking. However, the disconnection of oceanographic processes between Japan and the Philippines may cause the differentiation. The Philippine samples were collected from the eastern coasts of the Sulu Sea, which is a semi-enclosed deep-sea body of water. The waters at the edge of the basin used to be shallow, less than 420 m during glaciation epochs (Wang 1999; Voris 2000), and this may be an environmental barrier isolating the Fraser's dolphins in the Sulu Sea from other offshore populations considering Fraser's dolphins' preference for deep waters (Jefferson *et al.* 2011). Moreover, the Sulu Sea accepts surface currents from the Celebes Sea in the south in summertime and forms an anti-clockwise gyre in its own basin in wintertime (Global Ocean Associates

2004), and the circulation in the Celebes Sea is mostly driven by the input flow from the Mindanao Current, a strong, southward branch of the North Equatorial Current (Hogan 2013). But the Japanese coasts are mostly influenced by the Kuroshio Current, the northward branch of the North Equatorial Current, flowing in an opposite direction to the Mindanao Current (Toole *et al*. 1990; Fine *et al*. 1994) (Fig. 2.9). Therefore even in the shallow-water barrier has no longer existed to-date, the prevailing sea currents appear to obstruct direct migrations between the Sulu Sea and the Japanese coasts. The analyses, which show that neither southward gene flow nor recent gene exchange is present between the Philippine and Japanese populations, appear to support this scenario.

Even so, the Japanese samples studied here were probably from a group of vagrants that travelled from another region, potentially more southern and tropical, considering the facts that Fraser's dolphins prefer pantropical, deep offshore waters (Jefferson *et al*. 2011) and are indeed seldom found in the temperate waters around Japan (Amano *et al*. 1996). Similar events may be represented by a group of Fraser's dolphins that stranded in France (van Bree *et al.* 1986), and unusual sightings off the Azores and Madeira archipelagos (Gomes-Pereira *et al*. 2013). As reports of Fraser's dolphin in the high sea region of the western North Pacific Ocean are scarce, the range of this 'Japanese' population of Fraser's dolphins is still uncertain. The Geneland analysis suggests that the Taiwanese and Japanese samples are mostly assigned to the

same population, and in the FCA a number of Taiwanese samples are clustered with the Japanese samples, possibly indicating some level of connectivity. Connectivity between Japanese and Taiwanese waters may be expected given that the waters off Taiwan and southeastern Japan are well connected by the Kuroshio Current and share identical ocean biogeography (Barkley 1970; Spalding *et al*. 2012), and is evident by the GeneClass analysis that detected two potential first-generation migrants between the two regions.

On the other hand, the data show greater deviation from HWE and a high F_{IS} in the Taiwanese sample, suggesting the possibility of an unaccounted factor affecting the interpretations. Waples (2015) suggests positive assortative mating, self-fertilization, the Wahlund effect (Wahlund 1928), presence of null alleles, nonrandom sampling and selection favouring homozygotes (underdominance) are all possible causes that would result in a departure from HWE with a positive F_{IS}. In the case, the presence of null alleles is likely to be a factor influencing HWE estimates for the Taiwanese sample, although it not clear why only this population should have been affected across multiple loci. The Wahlund effect—a mixing of two genetically distinct populations of individuals in one population— could also be the cause of HWE deviation in the Taiwan group, as possible mixing is suggested in the FCA and perhaps in the STRUCTURE analysis. Even so, Zhivotovsky (2015) suggests that null alleles or allelic dropout are more likely the main causes for HWE deviation than the Wahlund effect in

most cases. An exception would be if one of the populations was depauperate of variation compared to the other (Zhivotovsky 2015), but that was not evidently the case for the samples. Confounding the analysis, however, is the fact that the Taiwan sample could be representing both the potential mixing population, and the reference source population.

The speculation of multiple Fraser's dolphin populations off Taiwan is based solely on genetic data. Further examinations from morphological, ecological or physiological perspectives are inevitably needed to validate this hypothesis. Parapatric distributions of "inshore" and "offshore" populations have been reported for a number of small delphinids phylogenetically close to the Fraser's dolphin (*e.g.*, Hoelzel *et al.* 1998; Escorza-Treviño *et al.* 2005; Amaral *et al*. 2007; Courbis *et al.* 2014; Lowther-Thieleking *et al*. 2015; see Table 1.1), which makes it a credible hypothesis for this species worth testing in future. This is further supported by what seems to be a divergence of diet preference in the Fraser's dolphins found in Taiwanese waters. A systematic examination of the stomach content data obtained from 27 adult dolphins incidentally caught in Taiwanese fisheries (Wang 2003) showed that 20 dolphins consumed diversified prey items (*i.e.,* both fish and cephalopods) while seven consumed cephalopods only. Interestingly, a similar divergence of prey preferences in Fraser's dolphin has been reported elsewhere in the world: fish and cephalopods are almost equally predominant components in the diet of Fraser's dolphins in the eastern

Tropical Pacific and in the Sulu Sea (Robison & Craddock 1983; Dolar *et al*. 2003), while cephalopods are predominant and fish only account for 4% of the diet of Fraser's dolphins in South African waters (Sekiguchi *et al.* 1992).

Very small sample sizes mean that only limited inference can be drawn for population comparisons outside the western North Pacific coasts. In some cases the inference was contradictory, for instance the Geneland result suggests connectivity between the Central North Pacific and the Philippines, but the FCA result suggests possible differentiation. In this case the FCA analysis may be more informative since it is based on individual genotypes, but still may not reflect a representative sample from the Central North Pacific. Moreover, it seems potentially problematic to find that the dolphins in the Gulf of Mexico are genetically more similar to those in the North Pacific Ocean than those in the Caribbean Sea, though there may be a similar pattern in the Bryde's whale (*Balaenoptera edeni)*. The Bryde's whale population in the Gulf of Mexico, which belongs to a *unique* phylogenetic lineage of the species, was more related to those in the North Pacific (*B. edeni edeni*) than those in the western North Atlantic (*B. edeni brydei*) (Rosel & Wilcox 2014). To confirm the status of the Fraser's dolphin population in these regions, further examination with appropriate sample sizes is crucially needed.

Fraser's dolphin population expansion in the past

The data suggest that Fraser's dolphin populations in the western North Pacific Ocean have been expanding in both population size and distribution range; the expansion is most pronounced for the Japanese population and least for the Philippine population. Amano *et al*. (1996) suggest that the Fraser's dolphins found in Japanese waters have an earlier age at sexual maturity, shorter calving interval and shorter longevity than the striped dolphins (*Stenella coeruleoalba*) found in the same region. Even though it is unknown whether these are common life history traits for all Fraser's dolphin populations, they are life history traits featuring fast reproductive cycles and useful for a species' to colonize new habitat rapidly, indicating this could be a population at the front of an expansion (Philips *et al*. 2010). On the other hand, the Philippine population has a larger effective population size, a positive Fu's Fs estimate (although insignificant), and a mismatch distribution profile revealing a relatively stable population history through time. This may suggest a core population, or at least a population being less impacted during the glacial period. As a species' demographic profile may reflect its latitudinal distribution shift in response to climate change (Walther *et al.* 2002; Perry *et al.* 2005), further studies are needed to better assess the distribution and population structure of Fraser's dolphins in tropical regions, the "rear edge" of the species' range (Hampe & Petit 2005).

The estimated timing of these events depends on an accurate estimate of the mtDNA control region substitution rate, and this issue has been discussed in detail in Chapter 2. The two substitution rates used here to calculate the time of expansion both suggest Fraser's dolphin populations in the western North Pacific expanded 2,000—11,000 years ago. This estimate of population expansion time is within the period of deglaciation following the last glacial maximum (19,000—20,000 years ago; Clark *et al*. 2009), and most likely during the beginning of the Holocene (about 11,500 years ago; Mayewski *et al.* 2004). At this time sea surface temperatures and sea level were significantly higher than during the earlier glacial period (Clark *et al*. 2009) and that could have expanded the tropical-subtropical climate and deep-water environment favoured by Fraser's dolphins (Jefferson & Leatherwood 1994; Jefferson *et al.* 2011). The Migrate analysis suggests prevalence of northbound long-term gene flow for Fraser's dolphins in the western North Pacific Ocean, and could be indicating the tendency of the southern populations to explore further suitable habitat in the north during the warm period. Early Holocene population expansion has also been proposed for other cetacean species (Banguera-Hinestroza *et al.* 2014; Louis *et al.* 2014; Moura *et al*. 2014; Chapter 2); see Chapter 5 for further discussion.

The demographic or spatial expansion of Fraser's dolphin populations might have been witnessed even in the modern age. Increasingly frequent encounters of Fraser's dolphins around the Lesser Antilles, Caribbean Sea (Watkins *et al*. 1994;

Rinaldi & Rinaldi 2011) may be a result of demographic expansion of a regional endemic population, or a spatial invasion by other Atlantic population. A recent field study reported an association between the presence of Fraser's dolphins and extreme sea-surface parameters in the temperate waters around the Azores, suggesting the species could be a potential bio-indicator of global climate change (Gomes-Pereira *et al.* 2013).

Effective population size, conservation implication and future perspectives

In general the effective population sizes for presumably abundant oceanic delphinids are large. For instance, the $N_e\mu$ values range between 1.435 and 5.894 for short-beaked common dolphin (*Delphinus delphis*) populations along the southeastern coasts of Australia (Möller *et al.* 2011) and between 1.52 and 3.45 for the common bottlenose dolphin populations near the Hawaiian Archipelago (Martien *et al.* 2012). Although Fraser's dolphins are usually regarded as one of those abundant oceanic delphinids (Jefferson & Leatherwood 1994; Jefferson *et al.* 2011), the $N_e\mu$ estimates for this species in the western North Pacific are seemingly more comparable to coastal or riverine dolphin populations, such as the common bottlenose dolphin in the western North Atlantic, including the Gulf of Mexico ($N_e\mu$ =0.44—0.98; Rosel *et al.* 2009), and the Amazon river dolphin (*Inia geoffrensis*) in the Brazilian Amazon ($N_e\mu$ = 0.2375 and

0.3050; Hollatz *et al.* 2011). One possibility is that the $N_e\mu$ in the populations was underestimated, since the program Migrate would suggest an underestimated $N_e\mu$ if the population size was not consistent but instead increasing though times (Beerli 2009), and it is likely the case for the Japan and Taiwan populations, as the mtDNA analyses suggest these populations expanded in the past. However, there is no sufficient evidence showing strong population fluctuation for the Philippine population, and the $N_e\mu$ for this population (0.926—1.065) is still less than the estimates for those oceanic populations. In addition, given the census population size for the dolphins in the eastern Sulu Sea has been estimated to be N=13,500 (Dolar *et al*. 2006), the ratio of effective population size to the census population size (Ne/N) (Frankham 1995) for the Philippine population can be calculated as ranging from 0.34 to 0.79, which is not particularly low in mammals (Frankham 1995). Therefore, the relatively low estimates of $N_e\mu$ for Fraser's dolphin populations, as least for the Philippine population, may simply reflect a smaller census population size (Hare *et al*. 2011). This would imply that the Fraser's dolphin populations, even a stable one, could be as much at risk as vulnerable coastal or riverine populations of dolphins. Therefore the conservation management of Fraser's dolphin populations may require reconsideration, as this species is currently considered as an offshore, oceanic delphinid with least conservation concern (Jefferson *et al*. 2011, Hammond *et al*. 2012). In particular, the impact of frequent Fraser's dolphin bycatches in the fisheries around the Philippines, Taiwan and eastern Tropical Pacific (Dolar 1994,

Jefferson & Leatherwood 1994, Perrin *et al.* 2005, Chou 2006) now warrants a reassessment as new information about their population structure, effective population size and the intensity of gene flow has emerged.

Finally, further samples from the extensive distribution range of Fraser's dolphins, namely in the eastern Tropical Pacific, South Pacific, high-seas Atlantic Ocean and Indian Ocean, should be included in future studies to reveal the species' global population structure and expansion history. Perrin *et al.* (2003) conducted a discriminant analysis using morphometric measurements from Fraser's dolphin skulls collected in the western North Pacific (n=71), North Atlantic (n=22), southwestern Indian Ocean (n=12) and western South Pacific (n=7). They found that the first canonical axis distinguished the North Atlantic samples from the other samples, and the second axis separated northern hemisphere samples (western North Pacific and North Atlantic) from southern hemisphere samples (southwestern Indian Ocean and western South Pacific). If the differentiation in skull morphometric measurement does reflect population genetic structure, then future studies may find the North Atlantic population the most distinctive among the other populations, and possibly identify further differentiated populations in the Southern Hemisphere. It is anticipated that, by examining more Fraser's dolphin samples from a broader range, further light would be shed on the effect of global climate change on the dynamics of the world's tropical dolphin populations.

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Appendices

Appendix 3.1. List of the samples acquired for this study. Note not all sample in this list were used in the study. The samples analysed are indicated as 'Y' in 'Used in Analyses?' column, as well as noted in 'MS' (microsatellite genotyping) and 'mtDNA' (mtDNA haplotype) columns. Abbreviations for the Contributors: Es-Bank, the Center for Enviornmental Studies at Ehime University (Japan); NTU, National Taiwan University; SWFSC, Southwest Fisheries Science Center (USA).

Appendix 3.2. Presence of null alleles, number of alleles, allelic richness, inbreeding coefficient (F_{IS}), observed heterozyogsity (H_O) and expected heterozygosity (H_E) for the 24 microsatellite loci examined in this study. The loci marked by asterisk are discarded from further analyses.

Appendix 3.3. Polymorphic sites in the 48 Fraser's dolphin mtDNA control region haplotypes. The dot indicates identical site to the top sequence and the dash indicates an insertion–deletion event. The number in the top row indicates the position of the variable site in the 779bp sequence.

Appendix 3.1.

Longitude	Latitude	ID	Location	Population	Sample Source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
-69.416666	18.416666	48119	San Pedro Macoris	Caribbean Sea	Stranding	SWFSC	2001	M	Y	Hap_22	Y
135.950142	33.593888	EW00542	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	Hap_5	Y
135.950142	33.593888	EW00543	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_6	Y
135.950142	33.593888	EW00544	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	Hap_7	Y
135.950142	33.593888	EW00545	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	Hap_8	Y
135.950142	33.593888	EW00546	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	(unamplified)	Y
135.950142	33.593888	EW00547	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	Hap_9	Y
135.950142	33.593888	EW00548	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	Hap_10	Y
135.950142	33.593888	EW00549	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_11	Y
135.950142	33.593888	EW00550	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	Hap_12	Y
135.950142	33.593888	EW00551	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	Hap_1	Y
135.950142	33.593888	EW00552	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	Hap_13	Y
135.950142	33.593888	EW00554	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_14	Y
135.950142	33.593888	EW00555	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_15	Y
135.950142	33.593888	EW00556	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	(poor seq quality)	Y
135.950142	33.593888	EW00557	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_16	Y
135.950142	33.593888	EW00558	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_11	Y
135.950142	33.593888	EW00559	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_17	Y
135.950142	33.593888	EW00560	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_18	Y
135.950142	33.593888	EW00561	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_19	Y
135.950142	33.593888	EW00562	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_11	Y
135.950142	33.593888	EW00563	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	Hap_20	Y
135.950142	33.593888	EW00564	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_21	Y
135.950142	33.593888	EW00565	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	Hap_22	Y
135.950142	33.593888	EW00566	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_10	Y

Longitude	Latitude	ID	Location	Population	Sample Source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
135.950142	33.593888	EW00567	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_3	Y
135.950142	33.593888	EW00568	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	Hap_2	Y
135.950142	33.593888	EW00569	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	Hap_10	Y
135.950142	33.593888	EW00570	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_11	Y
135.950142	33.593888	EW00571	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_2	Y
135.950142	33.593888	EW00572	Taiji	Japan	Drive fishery	Es-Bank	1991	M	(poor quality)	Hap_3	Y
135.950142	33.593888	EW00573	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	(poor seq quality)	Y
135.950142	33.593888	EW00574	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_9	Y
135.950142	33.593888	EW00575	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	Hap_2	Y
135.950142	33.593888	EW00576	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_23	Y
135.950142	33.593888	EW00577	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_18	Y
135.950142	33.593888	EW00578	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_14	Y
135.950142	33.593888	EW00579	Taiji	Japan	Drive fishery	Es-Bank	1991	F	Y	Hap_24	Y
124.219722	24.454444	EW04872	Okinawa	Taiwan	Stranding	Es-Bank	2007	M	Y	Hap_17	Y
135.950142	33.593888	EW1265	Taiji	Japan	Drive fishery	Es-Bank	1991	M	Y	Hap_4	Y
123.549135	9.568629	2597	Siaton?	Philippines	Bycatch	SWFSC	1994	F	(poor quality)	(unamplified)	N
N/A	N/A	2602	Philippines	Philippines	Bycatch	SWFSC	1994	M	Y	N/A	N
N/A	N/A	2648	Philippines	Philippines	Bycatch	SWFSC	1993	M	(poor quality)	(unamplified)	N
N/A	N/A	2651	Philippines	Philippines	Bycatch?	SWFSC	1993	F	(poor quality)	(unamplified)	N
N/A	N/A	2654	Philippines	Philippines	Bycatch	SWFSC	1993	F	(poor quality)	(unamplified)	N
N/A	N/A	2660	Philippines	Philippines	N/A	SWFSC	1993	F	Y	S. longirostris	N
123.549135	9.568629	5554	Siaton	Philippines	N/A	SWFSC	1994	M	(poor quality)	(unamplified)	N
123.549135	9.568629	5555	Siaton	Philippines	N/A	SWFSC	1994	U	(poor quality)	(unamplified)	N
123.549135	9.568629	5557	Siaton	Philippines	N/A	SWFSC	1994	F	(poor quality)	(unamplified)	N
123.549135	9.568629	5560	Siaton	Philippines	N/A	SWFSC	1994	F	Y	T. truncatus	N
123.549135	9.568629	5561	Siaton	Philippines	N/A	SWFSC	1994	F	(poor quality)	(unamplified)	N

Longitude	Latitude	ID	Location	Population	Sample Source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
N/A	N/A	KUL1	Philippines	Philippines	N/A	Es-Bank	1996	M	N/A	(poor seq quality)	N
N/A	N/A	KUL10	Philippines	Philippines	N/A	Es-Bank	1996	M	N/A	(poor seq quality)	N
N/A	N/A	KUL12	Philippines	Philippines	N/A	Es-Bank	1996	M	N/A	(poor seq quality)	N
N/A	N/A	KUL14	Philippines	Philippines	N/A	Es-Bank	1996	M	N/A	(poor seq quality)	N
N/A	N/A	KUL15	Philippines	Philippines	N/A	Es-Bank	1996	M	N/A	(poor seq quality)	N
N/A	N/A	KUL2	Philippines	Philippines	N/A	Es-Bank	1996	U	N/A	(poor seq quality)	N
N/A	N/A	KUL3	Philippines	Philippines	N/A	Es-Bank	1996	F	N/A	S. longirostris	N
N/A	N/A	KUL4	Philippines	Philippines	N/A	Es-Bank	1996	F	N/A	(poor seq quality)	N
N/A	N/A	KUL5	Philippines	Philippines	N/A	Es-Bank	1996	F	N/A	(poor seq quality)	N
N/A	N/A	KUL6	Philippines	Philippines	N/A	Es-Bank	1996	M	N/A	T. aduncus	N
N/A	N/A	KUL7	Philippines	Philippines	N/A	Es-Bank	1996	F	N/A	(poor seq quality)	N
N/A	N/A	KUL8	Philippines	Philippines	N/A	Es-Bank	1996	M	N/A	(poor seq quality)	N
123.116666	8.85	394	Siaton	Philippines	Bycatch	SWFSC	1991	M	Y	Hap_25	Y
123.116666	8.85	395	Siaton	Philippines	Bycatch	SWFSC	1991	F	Y	(unamplified)	Y
123.116666	8.85	399	Siaton	Philippines	Bycatch	SWFSC	1991	F	Y	Hap_26	Y
123.549135	9.568629	2594	Siaton?	Philippines	Bycatch	SWFSC	1994	M	Y	(poor seq quality)	Y
N/A	N/A	2595	Philippines	Philippines	Bycatch	SWFSC	1994	F	Y	(unamplified)	Y
123.549135	9.568629	2596	Siaton?	Philippines	Bycatch	SWFSC	1994	F	Y	(poor seq quality)	Y
123.549135	9.568629	2598	Siaton?	Philippines	Bycatch	SWFSC	1994	M	Y	(poor seq quality)	Y
123.549135	9.568629	2599	Siaton?	Philippines	Bycatch	SWFSC	1994	M	Y	Hap_27	Y
123.549135	9.568629	2600	Siaton?	Philippines	Bycatch	SWFSC	1994	M	Y	(unamplified)	Y
N/A	N/A	2601	Philippines	Philippines	Bycatch	SWFSC	1994	F	Y	(poor seq quality)	Y
123.549135	9.568629	2604	Siaton?	Philippines	Bycatch	SWFSC	1994	F	Y	(unamplified)	Y
123.549135	9.568629	2606	Siaton?	Philippines	Bycatch	SWFSC	1994	M	Y	Hap_29	Y
N/A	N/A	2607	Philippines	Philippines	Bycatch	SWFSC	1994?	M	Y	(unamplified)	Y
N/A	N/A	2646	Philippines	Philippines	Bycatch	SWFSC	1993	F	Y	(unamplified)	Y

Longitude	Latitude	ID	Location	Population	Sample Source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
N/A	N/A	2653	Philippines	Philippines	Bycatch	SWFSC	1993	F	Y	Hap_28	Y
N/A	N/A	2671	Philippines	Philippines	Bycatch	SWFSC	1992	F	Y	Hap_19	Y
123.549135	9.568629	5553	Siaton	Philippines	Bycatch	SWFSC	1994	M	Y	(unamplified)	Y
123.549135	9.568629	5556	Siaton	Philippines	Bycatch	SWFSC	1994	M	Y	(unamplified)	Y
123.549135	9.568629	5558	Siaton	Philippines		SWFSC	1994	F	Y	(unamplified)	Y
123.549135	9.568629	5559	Siaton	Philippines	Bycatch	SWFSC	1994	F	Y	(poor seq quality)	Y
N/A	N/A	7452	Philippines	Philippines	Bycatch	SWFSC	1997	M	Y	Hap_28	Y
N/A	N/A	KUL11	Philippines	Philippines	N/A	Es-Bank	1996	F	N/A	Hap_30	Y
N/A	N/A	KUL13	Philippines	Philippines	N/A	Es-Bank	1996	M	N/A	Hap_22	Y
N/A	N/A	KUL9	Philippines	Philippines	N/A	Es-Bank	1996	F	N/A	Hap_26	Y
N/A	N/A	48083	Ponce	Caribbean Sea	Stranding	SWFSC	1997	F	(poor quality)	(unamplified)	N
-67.166666	18.45	48101	Aguadilla	Caribbean Sea	Stranding	SWFSC	1999	M	Y	Hap_22	Y
-65.733333	18.166666	48133	Humacao	Caribbean Sea	Stranding	SWFSC	2002	M	Y	Hap_30	Y
121.507673	23.494786	718	Hualien	Taiwan	Bycatch	NTU	2001	M	Y	T. truncatus	N
121.507673	23.494786	892	Hualien	Taiwan	Bycatch	NTU	2005	M	Y	Hap_39	N
120.312417	22.561889	8	Kaohsiung	Taiwan	Stranding	NTU	2000	F	Y	Hap_4	Y
121.507673	23.494786	35	Hualien	Taiwan	Bycatch?	NTU	1998	M	Y	Hap_6	Y
120.919194	22.417944	51	Taitung	Taiwan	Stranding?	NTU	2000	M	Y	Hap_33	Y
121.507673	23.494786	59	Hualien	Taiwan	Bycatch	NTU	2000	M	Y	Hap_11	Y
121.507673	23.494786	60	Hualien	Taiwan	Bycatch	NTU	2000	M	Y	Hap_30	Y
121.507673	23.494786	66	Hualien	Taiwan	Bycatch	NTU	2000	M	Y	Hap_4	Y
120.168281	23.485762	191	Chiayi	Taiwan	Seizure	NTU	2001	M	Y	Hap_2	Y
121.507673	23.494786	299	Hualien	Taiwan	Bycatch	NTU	2000	M	Y	Hap_34	Y
121.507673	23.494786	303	Hualien	Taiwan	Bycatch	NTU	2004	M	Y	Hap_30	Y
121.507673	23.494786	304	Hualien	Taiwan	Bycatch	NTU	2004	M	Y	Hap_4	Y
121.507673	23.494786	309	Hualien	Taiwan	Bycatch	NTU	2004	M	Y	Hap_35	Y

Longitude	Latitude	ID	Location	Population	Sample Source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
121.507673	23.494786	311	Hualien	Taiwan	Bycatch	NTU	2004	F	Y	Hap_35	Y
121.507673	23.494786	441	Hualien	Taiwan	Bycatch	NTU	2000	M	Y	Hap_16	Y
N/A	N/A	479	Hualien	Taiwan	Bycatch?	NTU	2000	F	(poor quality)	Hap_25	Y
121.507673	23.494786	599	Hualien	Taiwan	Bycatch	NTU	2000	M	Y	Hap_31	Y
121.507673	23.494786	602	Hualien	Taiwan	Bycatch	NTU	2000	M	Y	Hap_36	Y
121.507673	23.494786	606	Hualien	Taiwan	Bycatch	NTU	2001	M	Y	Hap_37	Y
N/A	N/A	665	Pingdong	Taiwan	Stranding?	NTU	2001	F	(poor quality)	Hap_31	Y
121.507673	23.494786	715	Hualien	Taiwan	Bycatch	NTU	2001	F	Y	Hap_38	Y
N/A	N/A	721	Hualien	Taiwan	Bycatch	NTU	2001		Y	Hap_2	Y
121.750357	25.199706	770	Unknown	Taiwan	N/A	NTU	2005	M	Y	Hap_17	Y
121.822972	24.821972	821	Ilan	Taiwan	Stranding	NTU	2004	M	Y	Hap_14	Y
121.507673	23.494786	887	Hualien	Taiwan	Bycatch	NTU	2005	F	Y	Hap_39	Y
121.507673	23.494786	911	Hualien	Taiwan	Bycatch	NTU	2005	M	Y	Hap_16	Y
121.825406	24.832894	1090	Ilan	Taiwan	Stranding	NTU	2007	F	Y	Hap_40	Y
121.507673	23.494786	1126	Hualien	Taiwan	Bycatch	NTU	2005	F	Y	Hap_34	Y
121.507673	23.494786	1150	Hualien	Taiwan	Bycatch	NTU	2005	M	Y	Hap_2	Y
121.185977	22.7744	1155	Taitung	Taiwan	Bycatch?	NTU	2006	M	Y	Hap_41	Y
121.587056	23.829978	1201	Hualien	Taiwan	Stranding	NTU	2008	M	Y	Hap_16	Y
121.194842	22.790447	1323	Taitung	Taiwan	Stranding	NTU	2009	M	Y	Hap_42	Y
121.570108	23.780314	1349	Hualien	Taiwan	Stranding	NTU	2009	M	Y	Hap_17	Y
121.380286	23.094267	9553	Taitung	Taiwan	Bycatch	NTU	1997	F	Y	(unamplified)	Y
121.380286	23.094267	9554	Taitung	Taiwan	Bycatch	NTU	1997	M	Y	Hap_17	Y
121.873346	24.59563	9555	Ilan	Taiwan	Bycatch	NTU	1994	M	Y	Hap_32	Y
121.608394	23.946056	LHDU01	Hualien	Taiwan	Stranding	NTU	2010	M	Y	Hap_14	Y
121.468631	25.251217	LHDU02	Taipei	Taiwan	Stranding	NTU	2011	M	Y	(poor seq quality)	Y
121.429139	23.283908	LHDU03	Taitung	Taiwan	Stranding	NTU	2010	F	Y	Hap_2	Y

Longitude	Latitude	ID	Location	Population	Sample Source	Contributor	Year	Sex	MS	mtDNA	Used in analyses?
121.849505	24.708223	LHDU04	Ilan	Taiwan	Seizure?	NTU	2004	M	Y	Hap_6	Y
121.507673	23.494786	LHDU06	Hualien	Taiwan	Bycatch?	NTU	1998	M	Y	Hap_31	Y
121.614897	23.972028	LHDU07	Hualien	Taiwan	Stranding	NTU	2013	F	Y	Hap_31	Y
121.412617	23.123489	LHDU08	Taitung	Taiwan	Stranding	NTU	2012	M	Y	Hap_43	Y
121.630533	24.030253	LHDU09	Hualien	Taiwan	Stranding	NTU	2012	F	Y	(poor seq quality)	Y
121.427019	23.270394	LHDU10	Taitung	Taiwan	Stranding	NTU	2013	M	Y	Hap_4	Y
120.359556	22.507319	LHDU11	Kaoshiung	Taiwan	Stranding	NTU	2012	F	Y	Hap_44	Y
-76.166666	34.916666	2809	North Cove Banks, NC	West Atlantic	Stranding	SWFSC	1993	F	(poor quality)	(unamplified)	N
-81.883333	26.4	2507	Fort Myers Beach, FL	Gulf of Mexico	Stranding	SWFSC	1994	M	Y	Hap_48	Y
-81.883333	26.4	2509	Fort Myers Beach, FL	Gulf of Mexico	Stranding	SWFSC	1994	F	Y	Hap_48	Y
-96.13333	6.63333	15529	Eastern Tropical Pacific	Eastern Tropical Pacific	Bycatch	SWFSC	1975	F	Y	Hap_45	Y
-175.316666	24.2	30468	Hawaii	Central North Pacific	Biopsy	SWFSC	2002	M	Y	Hap_46	Y
-175.316666	24.2	30469	Hawaii	Central North Pacific	Biopsy	SWFSC	2002	F	Y	Hap_4	Y
-175.316666	24.2	30470	Hawaii	Central North Pacific	Biopsy	SWFSC	2002	M	Y	Hap_47	Y

Appendix 3.2.

Locus	Null alleles	No. of alleles	Allelic richness	F_{IS}	H_O	H_E	p	SD
Pop	Japan							
n	37							
AAT44		8	1.719	0.152	0.622	0.719	0.008	0.01%
D14		10	1.777	-0.008	0.789	0.787	0.73	0.03%
D22		3	1.385	0.089	0.368	0.395	0.35	0.05%
Dde59*	Y	8	1.662	0.555	0.316	0.661	0	0.00%
Dde65		7	1.715	-0.02	0.737	0.714	0.69	0.04%
Dde66*		10	1.748	0.024	0.737	0.746	0.655	0.03%
Dde69		5	1.639	0.07	0.605	0.645	0.033	0.02%
Dde70		4	1.443	-0.224	0.526	0.434	0.707	0.04%
Dde72		11	1.879	0.047	0.816	0.877	0.021	0.01%
Dde84		6	1.775	-0.012	0.789	0.775	0.868	0.03%
EV14*		12	1.886	0.116	0.763	0.884	0.047	0.02%
EV37*	Y	7	1.398	0.392	0.237	0.424	0	0.00%
KWM12a*	Y	13	1.827	0.152	0.711	0.831	0.125	0.02%
KWM1b		4	1.637	-0.234	0.763	0.633	0.311	0.05%
KWM2a*	Y	9	1.799	0.224	0.605	0.79	0	0.00%
KWM2b		4	1.439	0.077	0.395	0.456	0.422	0.04%
KWM9b		10	1.781	-0.003	0.789	0.789	0.124	0.03%
MK3		10	1.801	-0.047	0.842	0.808	0.711	0.04%
MK5		12	1.845	0.04	0.816	0.843	0.513	0.05%
Sco11		2	1.053	-0.014	0.053	0.052	1	0.00%
Sco28		2	1.387	-0.333	0.526	0.393	0.04	0.02%
Sco55		2	1.128	-0.059	0.132	0.125	1	0.00%
TexVet5		8	1.742	0.091	0.684	0.745	0.672	0.05%
TexVet7		7	1.751	0.102	0.684	0.753	0.088	0.03%
Mean			1.634	0.049	0.596	0.637		
SD			0.231	0.184	0.23	0.229		

Locus	Null alleles	No. of alleles	Allelic richness	F_{IS}	H_O	H_E	p	SD
Pop	Taiwan							
n	43							
AAT44		8	1.769	0.154	0.643	0.769	0.032	0.02%
D14		8	1.751	0.041	0.714	0.741	0.427	0.03%
D22		3	1.482	0.134	0.405	0.476	0.289	0.05%
Dde59*	Y	11	1.843	0.313	0.571	0.843	0	0.00%
Dde65		10	1.775	0.101	0.69	0.777	0.036	0.01%
Dde66*	Y	15	1.888	0.251	0.659	0.888	0	0.00%
Dde69	Y	8	1.752	0.198	0.595	0.752	0.002	0.01%
Dde70	Y	7	1.586	0.271	0.439	0.596	0.128	0.03%
Dde72		10	1.788	-0.004	0.81	0.785	0.88	0.03%
Dde84	Y	9	1.798	0.166	0.659	0.799	0.003	0.01%
EV14*	Y	13	1.847	0.316	0.595	0.852	0	0.00%
EV37*	Y	14	1.587	0.409	0.357	0.573	0	0.00%
KWM12a*	Y	14	1.827	0.215	0.643	0.827	0.008	0.01%
KWM1b		8	1.633	0.119	0.571	0.628	0.058	0.02%
KWM2a*	Y	12	1.857	0.262	0.65	0.865	0.003	0.01%
KWM2b	Y	7	1.661	0.581	0.286	0.653	0	0.00%
KWM9b	Y	8	1.79	0.267	0.571	0.788	0.013	0.01%
MK3		16	1.877	0.073	0.81	0.874	0.02	0.01%
MK5		13	1.836	0.026	0.81	0.837	0.183	0.04%
Sco11		1	1	NA	NA	NA	NA	NA
Sco28		2	1.373	0.067	0.333	0.367	0.67	0.05%
Sco55		2	1.068	-0.024	0.071	0.07	1	0.00%
TexVet5		10	1.756	0.108	0.667	0.755	0.603	0.05%
TexVet7		7	1.645	0.004	0.634	0.643	0.508	0.05%
Mean			1.675	0.176	0.573	0.702	0.211	
SD			0.235	0.146	0.182	0.192	0.312	

Locus	Null alleles	No. of alleles	Allelic richness	F_{IS}	H_O	H_E	p	SD
Pop					Philippines			
n					17			
AAT44		7	1.823	0.013	0.813	0.823	0.727	0.04%
D14		6	1.758	-0.01	0.765	0.758	0.532	0.05%
D22		3	1.169	-0.043	0.176	0.169	1	0.00%
Dde59*		5	1.775	0.092	0.706	0.775	0.856	0.03%
Dde65		7	1.813	-0.088	0.882	0.813	0.496	0.05%
Dde66*	Y	6	1.818	0.356	0.533	0.818	0.115	0.03%
Dde69		4	1.62	0.15	0.529	0.62	0.254	0.05%
Dde70		4	1.597	-0.189	0.706	0.597	0.536	0.05%
Dde72		8	1.799	-0.262	1	0.799	0.34	0.04%
Dde84		6	1.856	0.109	0.765	0.856	0.652	0.05%
EV14*	Y	8	1.863	0.325	0.588	0.863	0.033	0.02%
EV37*	Y	3	1.399	0.712	0.118	0.399	0.001	0.00%
KWM12a*		6	1.72	0.313	0.5	0.72	0.007	0.01%
KWM1b		4	1.579	-0.309	0.75	0.579	0.106	0.03%
KWM2a*	Y	9	1.829	0.48	0.438	0.829	0	0.00%
KWM2b		3	1.221	0.207	0.176	0.221	0.179	0.04%
KWM9b		8	1.792	0.055	0.75	0.792	0.545	0.04%
MK3		8	1.841	-0.05	0.882	0.841	0.639	0.05%
MK5		8	1.863	-0.165	1	0.863	0.547	0.04%
Sco11		1	1	NA	NA	NA	NA	NA
Sco28		2	1.428	-0.103	0.471	0.428	1	0.00%
Sco55		2	1.059	0	0.059	0.059	1	0.00%
TexVet5		7	1.772	0.113	0.688	0.772	0.871	0.03%
TexVet7		4	1.626	-0.133	0.706	0.626	0.519	0.04%
Mean		5.375	1.626	0.068	0.609	0.653		
SD		2.318	0.27	0.246	0.271	0.24		

Appendix 3.3.

	Variable site																																
Haplotype ID	6	65	114	140	142	143	164	167	168	173	174	195	206	207	216	257	262	263	265	303	306	308	309	330	331	332	337	338	339	340	341	342	351
Hap 1	A	A	A	T	T	A	A	T	A	T	T	T	A	C	T	T	C	T	G	T	A	T	T	A	T	C	T	T	A	T	A	T	C
Hap 2	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	T	C	.	.	.	.	C	.	.	T	C	C	.	.	.	.	.
Hap 3	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	T	C	.	.	.	.	C	.	.	T	.	C	.	.	.	.	.
Hap 4	.	.	.	.	.	G	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	G	.	.
Hap 5	C	.	.	G	G	.	.	.	.	.	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap 6	.	.	.	.	.	G	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	G	.	.	.	C	.	.	G	.	.
Hap 7	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	T	C	.	.	.	.	C	.	.	T	C	C	.	.	.	.	.
Hap 8	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	T	C	.	.	.	.	.	.
Hap 9	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	T	C	C	.	.	.	.	.
Hap 10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap 11	.	.	.	.	.	G	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	C	.	.	.	.	C	.	.	G	.	.
Hap 12	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	T	C	C	.	.	.	.	.
Hap 13	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap 14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap 15	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	T	C	A	.	.	.	C	.	.	T	C	C	.	.	.	.	.
Hap 16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap 17	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	C	.	.	.	.	.
Hap 18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap 19	.	-	G	.	.	.	.	C	G	C	.	.	.	.	.	.	.	C	.	C	.	.	C	.	.	T	.	C	.	.	.	C	.
Hap 20	.	.	.	.	.	G	G	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.
Hap 21	.	.	.	.	.	G	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	C	.	.	.	.	C	.	.	G	.	.
Hap 22	.	.	.	.	.	G	G	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.
Hap 23	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T	C	C	.	.	.	.	T
Hap 24	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	T	C	.	.	.	.	C	.	.	.	C	C	.	.	.	.	.

	Variable site																																		
Haplotype ID	6	65	114	140	142	143	164	167	168	173	174	195	206	207	216	257	262	263	265	303	306	308	309	330	331	332	337	338	339	340	341	342	351
Hap 25	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap 26	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T	C	C	.	.	.	.	.
Hap 27	.	-	G	.	.	.	.	C	G	C	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	T	.	C	.	.	.	C	.
Hap 28	.	.	G	.	.	.	.	.	G	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T	C	C	.	.	.	.	.
Hap 29	.	.	.	.	.	G	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	G	.	.
Hap 30	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T	C	C	.	.	.	.	.
Hap 31	.	-	G	.	.	.	.	C	G	C	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	T	.	C	.	.	G	C	.
Hap 32	.	.	.	.	.	G	G	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.
Hap 33	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	T	C	.	.	.	.	C	.	.	.	C	C	.	.	.	.	.
Hap 34	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	T	C	C	.	.	.	.	.
Hap 35	.	-	G	.	.	.	.	C	G	C	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	T	.	C	.	.	.	C	.
Hap 36	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	.	.	.	.	G	.	C	.	.	T	C	C	.	.	.	.	.
Hap 37	.	.	.	.	.	G	G	.	.	C	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	C	.	.	.	.	.
Hap 38	.	-	G	.	.	.	.	C	G	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T	C	C	.	.	.	.	.
Hap 39	.	.	.	.	.	G	.	.	.	C	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	C	.	.	G	.	.
Hap 40	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap 41	.	-	G	.	.	.	.	.	G	C	.	.	G	.	.	.	.	.	.	.	.	.	C	.	.	T	C	C	.	.	.	.	T
Hap 42	.	.	G	.	.	.	.	.	G	C	.	.	.	.	.	.	T	C	.	.	.	.	C	.	.	T	C	C	.	.	.	.	.
Hap 43	.	-	G	.	.	.	.	.	G	C	.	.	.	.	G	.	.	C	.	.	.	.	C	.	G	T	C	C	G	.	.	.	.
Hap 44	.	-	G	.	.	.	.	C	G	C	.	.	.	.	G	.	T	C	.	.	.	.	C	.	.	T	C	C	.	.	.	.	T
Hap 45	.	-	G	.	.	.	.	C	G	C	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	T	.	C	.	.	.	C	T
Hap 46	.	-	G	.	.	.	.	.	G	C	.	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	C	.	.	.	.	.
Hap 47	.	-	G	.	.	.	.	.	G	C	.	.	.	.	.	.	T	C	.	.	.	.	C	.	.	T	C	C	.	C	.	.	.
Hap 48	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T

(Table continues)

	Variable site																															
Haplotype ID	352	353	361	368	385	387	400	405	407	428	449	467	483	503	512	515	519	537	547	584	597	600	620	651	655	664	676	705	725	735	761	768	779
Hap 1	C	C	G	T	C	C	A	G	A	T	C	A	G	G	T	C	A	C	G	T	G	T	T	G	T	G	A	G	G	C	G	G	T
Hap 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 4	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 5	.	.	.	.	.	.	G	.	G	.	.	.	A	A	.	.	T	.	T	C	.	.	-	T	.	.	.	.	C	.	C	.	.
Hap 6	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 7	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 8	.	.	A	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	A	.	.	.	.	.
Hap 9	.	.	.	C	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	C	.	.	-	.	.	A	.	A	.	.	.	.	.
Hap 10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.
Hap 11	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 12	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	C	.	.	-	.	.	A	.	A	.	.	.	.	.
Hap 13	.	.	A	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.
Hap 14	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.
Hap 15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.
Hap 17	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 18	.	.	A	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.
Hap 19	.	T	A	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 20	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	-	.	.	.	.	A	.	.	.	.	.
Hap 21	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 22	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.
Hap 23	.	.	A	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 25	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.

	Variable site																																
Haplotype ID	352	353	361	368	385	387	400	405	407	428	449	467	483	503	512	515	519	537	547	584	597	600	620	651	655	664	676	705	725	735	761	768	779
Hap 26	.	.	A	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	-	.	C	A	.	.	.	.	.	.	.
Hap 27	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	A	.	-	.	.	A	.	.	.	.	.	.	.
Hap 28	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 29	.	.	.	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	.	.	A	.	.	.	.	.	.	A
Hap 30	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 31	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	A	C	-	.	.	A	.	.	.	.	.	.	.
Hap 32	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.
Hap 33	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 34	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	A	.	.	.	.	.
Hap 35	.	T	.	.	.	.	.	.	.	.	.	G	.	.	.	T	.	.	.	C	A	.	-	.	.	A	.	.	.	.	.	.	.
Hap 36	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 37	T	.	.	.	.	.	.	.	.	C	T	.	.	.	.	T	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.
Hap 38	.	.	A	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 39	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 40	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.
Hap 41	.	.	A	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 42	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 43	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	A	.	.	.	.	.
Hap 44	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	T	.	.	.	.	C	.
Hap 45	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	C	.	.	-	.	.	A	.	A	.	.	.	.	.
Hap 46	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	.	.	.	.	.	.
Hap 47	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	-	.	.	A	.	A	.	.	.	.	.
Hap 48	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	T	.	.	.

Chapter 4. The population structure of bottlenose dolphins (*Tursiops spp.*) in the western North Pacific Ocean

Abstract

Bottlenose dolphins (*Tursiops* spp.) are widely distributed in the world's tropical to temperate waters, exhibiting remarkable geographical variation in morphology, life history and genetic diversity, and such variation has made the taxonomy of the genus controversial. Significant population structure has been reported for the most widely distributed species, the common bottlenose dolphin (*T. truncatus*), in almost all ocean basins except the study region, the western North Pacific Ocean. This is the first study documenting an extensive range of genetic variation for the common bottlenose dolphins in the western North Pacific Ocean, based on genetic data derived from 20 microsatellite and one mitochondrial DNA (mtDNA) markers in 75 bottlenose dolphin samples collected from Taiwanese, Japanese and Philippine waters. Together with 344 published mtDNA control region sequences of the same species from the same or adjacent regions, the study reveals the presence of at least four populations of common bottlenose dolphins in the western and central North Pacific Ocean (F_{ST}=0.041—0.135). The results from Factorial correspondence analysis (FCA), Structure analysis and Geneland analysis showed a differentiation pattern that corresponds to habitat types,

resembling the scenario of inshore-offshore differentiation seen in other populations of the same species in other regions. The analysis also confirmed that there is no evident gene flow between the two 'sister species' common bottlenose dolphins and Indo-Pacific bottlenose dolphins (*T. aduncus*) occurring sympatrically in the study region. The data suggested there may be population structure for the Indo-Pacific bottlenose dolphins as well, although more samples and analyses are needed for strong inference.

Key words: *Tursiops truncatus*, *Tursiops aduncus*, Japan, Taiwan, population structure, genetic diversity, Northwest Pacific Ocean, microsatellite, mitochondrial DNA

Introduction

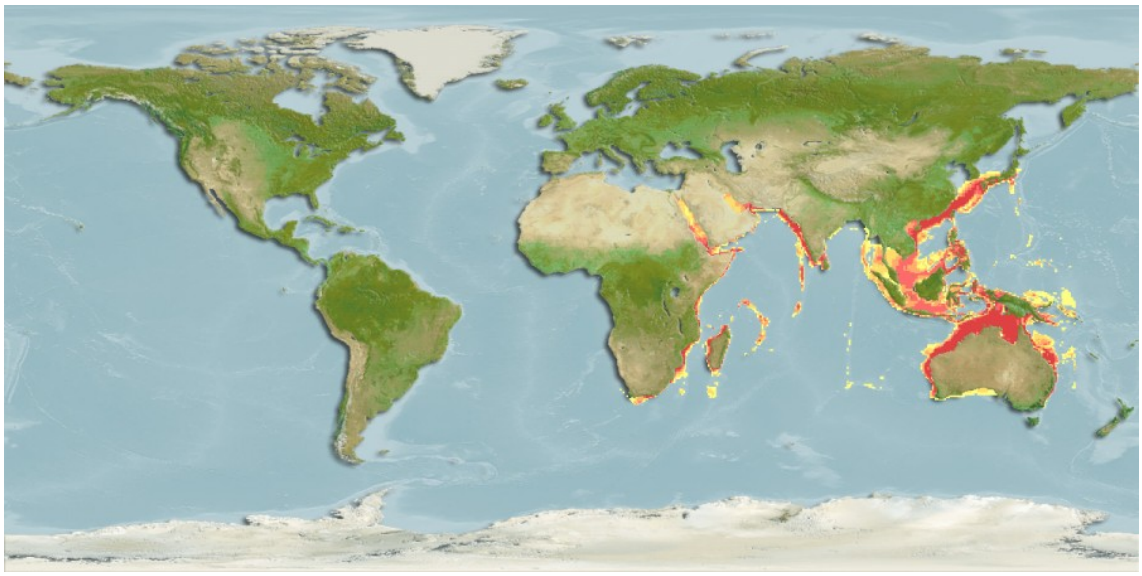
A wildlife management unit is usually defined by the significance of morphologic, genetic or demographic distinctiveness of a population, which is often, but not necessarily, associated with the presence of geographical barriers (Allendorf and Luikart 2006). Identifying such management units is imperative in wildlife conservation, as it assists the preservation of intra-species diversity and the species' future adaptive potential. Oceanic dolphin species usually show an unexpected level of division into differentiated populations, given their capacity for extensive dispersal and the lack of obvious geographic barriers (Hoelzel 2009). The species studied in this study, the

bottlenose dolphin (*Tursiops* spp.), has provided a number of classic examples regarding the parapatric or sympatric distribution of differentiated populations or species (see below).

Bottlenose dolphins are widely distributed in the world's tropical to temperate marine environment, including along the coasts of all major continents and many oceanic islands, over shallow offshore banks or sandbars, and in pelagic open waters (Rice 1998) (Fig. 4.1). There is a remarkable degree of geographical variation in bottlenose dolphin skeletal morphology, life history and genetic diversity, and such variation makes the taxonomy of the genus controversial (Rice 1998, Wells and Scott 2009). In the study region in the western North Pacific Ocean, two species of dolphins in the genus *Tursiops* have been recognised: the Indo-Pacific bottlenose dolphin (*T. aduncus*; hereinafter IPBD) and the common bottlenose dolphin (*T. truncatus*; hereinafter CBD). These two species are distributed parapatrically, or even sympatrically in particular areas. The distribution of IPBD is chiefly in the coastal waters of warm-temperate to tropical Indo-Pacific regions from southern Japan to western South Africa and southeast Australia, where the water depth is always less than 200 m (Wang and Yang 2009) (Fig. 4.1A). The distribution of CBD, on the other hand, ranges from the southern Okhotsk Sea to the South China Sea and the Hawaiian waters in the western North Pacific region, in both coastal and pelagic habitats (Miyashita 1993, Rice 1998, Wells and Scott 2009) (Fig. 4.1B). The distribution range of these two

species overlaps from the East China Sea and Taiwan Strait to the South China Sea (Zhou and Qian 1985; Wang et al. 1999, 2000; Yang et al. 2005).

A



B

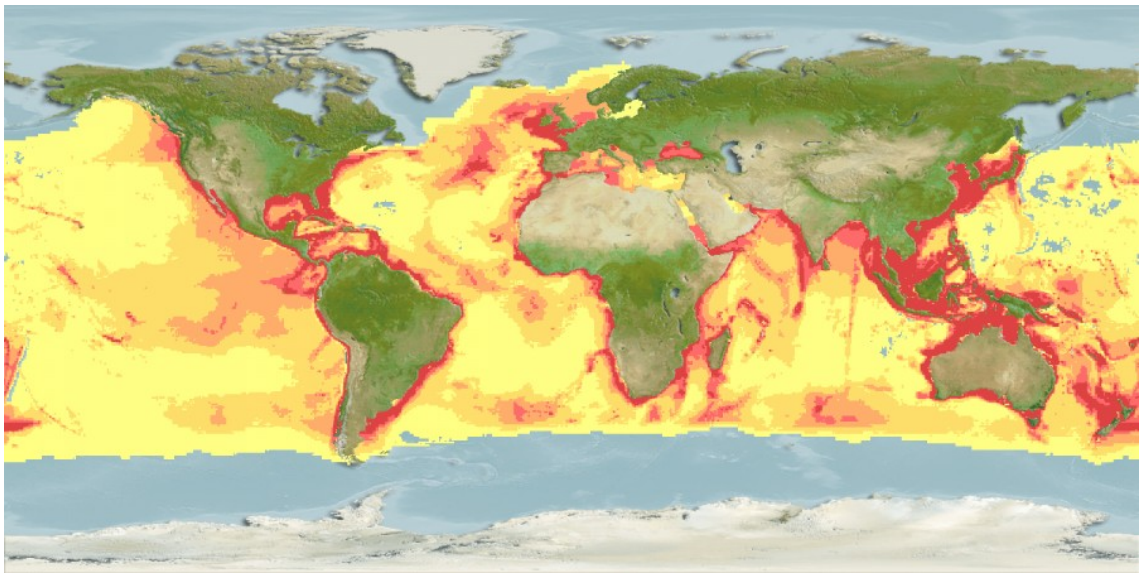


Figure 4.1. Reviewed global distribution maps for A) Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) and B) common bottlenose dolphin (*Tursiops truncatus*). The colour indicates the relative probabilities of occurrence, from high (100%, in red) to low (1%, in yellow). The maps were extracted from AquaMaps (www.aquamaps.org), version of Aug. 2013. Web. Accessed 20 Apr. 2016.

Earlier studies have provided morphologic and genetic evidence showing that IPBD and CBD are two distinct species (LeDuc et al. 1999; Wang et al. 1999, 2000; Hale et al. 2000; Kemper 2004; Natoli et al. 2004; Yang et al. 2005; Kurihara and Oda 2007; Moura et al. 2013). Although it is claimed that there was no genetic or morphologic intermediates found between the two species in eastern Asian waters (Wang et al. 1999, 2000; Yang et al. 2005), the use of a single mitochondrial DNA (mtDNA) marker and limited sampling means this remains an open question. In fact, the two species can interbreed freely and produce reproductively viable female hybrids in a captive environment (Hale et al. 2000). Potential descendants of hybrids between the two species are found in the CBD populations in Hawaiian and Japanese waters (Martien et al. 2012, Hayano 2013). Natural hybrids among other dolphin species can be common (Sylvestre and Tasaka 1985, Herzing and Johnson 1997, Yazdi 2002, Amaral et al. 2014). It is therefore worthwhile to re-examine the hybridization issue between these two species.

Even within the CBD species, significant differentiation between coastal and offshore populations has been reported from various locations, including the western North Atlantic Ocean (Hoelzel et al. 1998, Kingston and Rosel 2004), the eastern North Atlantic Ocean (Louis et al. 2014a), and the eastern North Pacific Ocean (Lowther-Thieleking et al. 2015). The population structure of CBD can be defined at an even finer regional scale, such as within the Gulf of Mexico (Sellas et al. 2005), northern Bahamas

(Parsons et al. 2006), the waters around New Zealand (Tezanos-Pinto et al. 2009), Ireland (Mirimin et al. 2011), Hawaiian archipelagos (Martien et al. 2012) and the Adriatic Sea (Gaspari et al. 2015a). In contrast, this species' population structure in the western North Pacific Ocean is little known. Two recent papers analysed mtDNA control region sequence data for CBD and IPBD with samples from the western South Pacific Ocean and hypothesised that 1) the coastal ecotype of CBD is lacking in the Indo-western Pacific Ocean and 2) this is because the coastal habitat has been occupied by IPBD (Tezanos-Pinto et al. 2009, Oremus et al. 2015a). However, using only mtDNA data to determine the distribution of CBD ecotypes can be problematic, given that the coastal and pelagic CBD lineages in the world are not reciprocally monophyletic (Moura et al. 2013), and a lack of lineage sorting appears to be a common phenomenon for the coastal and offshore CBD populations in the North Pacific Ocean (Segura et al. 2006, Lowther-Thieleking et al. 2015).

Miyashita (1993) proposed a pattern of three-stock structure for CBD in the western North Pacific Ocean (for the waters off eastern Japan) based on eight-year transect line survey data: a Japanese coastal population (from the east coasts of Japan to the west of 142°E), a Japanese offshore population (between 30°N and 42°N and from the east of 145°E to the antimeridian), and a southern offshore population (between 23°N and 30°N, and between 127°E and the antimeridian). However, the three-stock hypothesis has yet been validated using molecular markers. Kita et al. (2013) sequenced

a group of 165 CBD culled in a drive fishery hunt in Japan for a 402bp mtDNA control region sequence and compared against published sequences worldwide (using 290bp). They report that those dolphins were "related more closely to oceanic types from Chinese waters than other geographic regions" (p. 476). The study was unfortunately unable to provide further insights into the population structure of CBD in the western North Pacific Ocean, because there were too few CBD mtDNA sequences from Asian and adjacent waters available for comparison at the time, and the sample set was spatially and temporally invariable.

Both CBD and IPBD are affected by multiple anthropogenic threats, such as small-scale whaling and negative fishery interactions in this western North Pacific study region (Perrin *et al.* 2005; Kasuya 2007; Young and Iudicello 2007; Robards and Reeves 2011). There were more than 26,000 bottlenose dolphins caught in Japanese waters during 1972–2008 (Kasuya 2011), and ~1,700 bottlenose dolphins are incidentally killed in human fisheries in the western-central Pacific Ocean every year (Young and Iudicello 2007). The aim of this study is to investigate the population structure of the bottlenose dolphin in the western North Pacific Ocean to help assess the impact of human disturbance, with an emphasis on the CBD since this species is a common target in the dolphin drive fishery (Kasuya 2007, Oremus et al. 2015b). This study is based on genetic data derived from 20 microsatellite DNA and one mtDNA markers in 75 bottlenose dolphins collected from Japanese, Taiwanese and Philippine

waters over three decades, together with 344 published mtDNA control region sequences from the same or adjacent waters. This work reveals population structure of at least two coastal populations for CBD in the western North Pacific Ocean, and confirms that the gene flow is restricted between CBD and IPBD.

Material and Methods

Tissue sample collection and genomic DNA preparation

Sixty-eight CBD and seven IPBD from the archives in the Cetacean Laboratory at National Taiwan University (Taiwan), the es-Bank at Ehime University (Japan), and the Southwest Fishery Science Center (SWFSC), National Oceanic and Atmospheric Administration (United States) were included in this study (Fig. 4.2; Appendix 4.1). The two samples supplied by SWFSC (collected from the Philippine waters) were initially identified as Fraser's dolphin (*Lagenodelphis hosei*), but since both of them were assigned to CBD based on microsatellite genotypes, those two samples were included in this study (See Fig. 3.3 in Chapter 3).

Specimens were collected from various locations in Japan, Taiwan and the Philippines (Fig. 4.2; Appendix 4.1). It is assumed that the Taiwanese and Philippine samples collected from fishery interactions, including incidental catches or illegal trade in fish markets, were from dolphins inhabiting local waters (as assumed by Wang et al.

1999). Captive dolphins in Japanese aquaria were understood to either have been captured in Japanese coastal waters during 1988—2004, or born in captivity with both parents originating from Japanese coastal waters. Species identity was acquired from the archives, and verified by the genetic assessments. For CBD samples, each was assigned to one of the four putative populations based on its sampling location (i.e., West Japan, East Japan, Taiwan and the Philippines; Fig. 4.2A). All captive dolphin samples were assigned to the East Japan population, because their genotypes were more similar to those from East than West Japan (see Results).

For the samples acquired from the archives in Taiwan and Japan, a small portion of skin or muscle tissue was subsampled and preserved in either 99% ethanol or 20% DMSO solution saturated with Sodium Chloride, stored frozen until use. Genomic DNA was isolated and purified by a standard proteinase-K digestion/phenol–chloroform extraction protocol (Sambrook et al. 1989), and preserved in TE buffer (10mM Tris-HCl, 0,1mM EDTA, pH7.4). The Philippine samples were provided as extracted genomic DNA by the SWFSC. All specimens were transported to and examined at the Molecular Ecology Group laboratory in University of Durham, with valid official permits issued by the authorities of Japan, Taiwan, United States and United Kingdom.

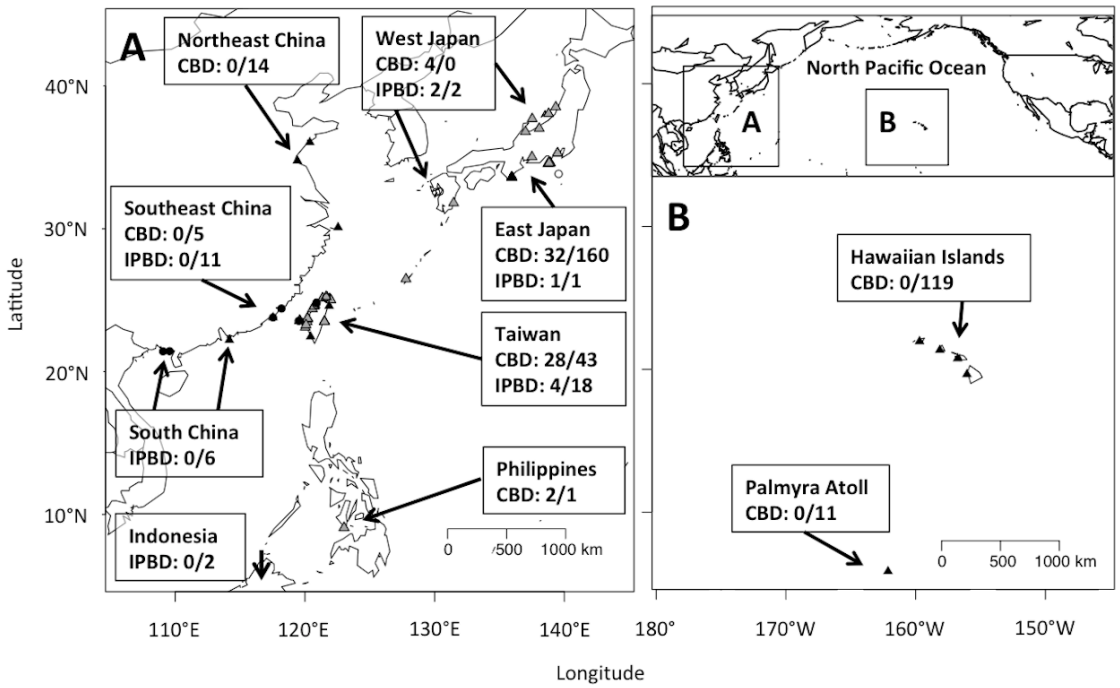


Figure 4.2. The sampling locations for the Indo-Pacific bottlenose dolphins (IPBD, *Tursiops aduncus*; open circle) and common bottlenose dolphins (CBD, *T. truncatus*; grey triangle) examined in this study, and the sampling locations of extra IPBD (solid circle) and CBD (solid triangle) mitochondrial DNA sequences acquired from the Genbank. Note the sampling location(s) of two IPBD from Indonesia were not indicated, because such information is deficient (Wang et al. 1999).

DNA fragment amplification and genotyping

Twenty-four microsatellite loci and a 388 bp mtDNA control region sequence were chosen as they have been conventionally used and validated in earlier dolphin genetic studies, including bottlenose dolphin studies (Shinohara et al. 1997, Krützen et al. 2001, Natoli et al. 2004, Mirimin et al. 2011). The procedure of amplifying microsatellite and mtDNA fragments through polymerase chain reaction (PCR) method was as described

in Chapter 2, and the details for the microsatellite loci, including their optimal annealing temperatures and allele size ranges for this sample set, are provided in Table 4.1.

Table 4.1. Information for the 24 microsatellite loci examined in this study.

Microsatellite locus	Optimal annealing temperature (°C)	Fragment size range		Genbank accession number	Reference
		CBD	IPBD		
AAT44	60	82-100	85-88	AF416501	Caldwell et al. 2002.
EV14	60	130-166	130-160	G09079	Valsecchi and Amos. 1996.
EV37	57	182-228	202-208	G09081	
D14	48	114-136	126-132		Shinohara et al. 1997.
D22	52	110-130	114-118		
KWM1b	45	187-191	185-187		Hoelzel et al. 1998.
KWM2a	43	136-156	136-152		
KWM2b	44	171-179	175		
KWM9b	55	168-180	182-186		
KWM12a	46	164-180	170-182		
TexVet7	60	153-167	157-161	AF004907	Rooney et al. 1999.
MK3	60	135-165	161-175	AF237889	Krützen et al. 2001.
MK5	58	209-237	211-219	AF237890	
Dde59	52	233-397	251-401	AM087093	Coughlan et al. 2006.
Dde65	48	180-200	184-200	AM087096	
Dde66	48	341-361	351-359	AM087097	
Dde69	55	200-216	204-212	AM087098	
Dde70	55	117-157	133-139	AM087099	
Dde72	52	235-275	249-259	AM087100	
Dde84	48	135-159	141-151	AM087101	
Sco11	55	203-231	203-227	AM087102	Mirimin et al. 2006.
Sco28	55	127-147	127-139	AM087103	
Sco55	55	216-220	216-220	AM087105	

Microsatellite data configuration

Using samples collected from individuals from the same school of dolphins that perished together in a drive fishery, or biopsied/stranded at the same time/same site may result in non-random sampling of closely related pedigrees. It is known that in some cases bottlenose dolphins tend to travel together with their relatives, i.e., as parent-

offspring pairs (Kita et al. 2013, Wells 2014). In the sample set, the 17 CBD samples collected from the drive fishery may be subject to such concern, even though the collection should have been a result of random sampling from the 98 dolphins killed at the time (Kasuya 2011). As a precautionary measure, the kinship within the sample was assessed using Kingroup v2 (Konovalov et al. 2004, Konovalov and Heg 2008) when the sampling context may artificially bias towards sampling close kin. In those cases one of the samples in a pair that had a coefficient of kinship $r > 0.5$ would be excluded, unless the samples were collected in a different year or location. The same measure was applied in earlier CBD studies using biopsy samples (Martien et al. 2012, Lowther-Thieleking et al. 2015).

Arlequin 3.5.1 (Excoffier et al. 2010) was used to examine linkage disequilibrium (LD) among loci within putative populations, to estimate the observed heterozygosity (H_O) and expected heterozygosity (H_E) of each locus in each population, and to assess the significance of any deviation from Hardy-Weinberg equilibrium (HWE) in each population. Overall deviation, heterozygote deficiency and heterozygote excess were assessed through the Fisher exact test and Markov chain method implemented in the program (Number of steps in Markov chain, 1,000,000; number of dememorization steps, 100,000). The level of statistic at significance for the test was set at $p<0.05$ after Bonferroni correction (i.e., for 24 loci, $p<0.002$). A locus that showed 1) a presence of null alleles in any population, 2) significant LD to any locus in every

population, or 3) a significant deviation from HWE in more than two populations, was discarded in subsequent analyses. The allelic richness and inbreeding coefficient (F_{IS}) for each locus in each putative population were estimated using FSTAT 2.9.3.2 (Goudet 1995, 2002).

Tests for genetic differentiation and possible hybridization between IPBD and CBD

The genetic differentiation between IPBD and CBD was investigated by factorial correspondence analysis (FCA) implemented in Genetix 4.0 (Belkhir et al. 2004), which generates a graphic result, plotting the two or three most informative factors based on individual genotypes in a two or three-dimension space. Differentiation was also assessed using STRUCTURE (Pritchard et al. 2000), which is based on individual genotypes using Bayesian inference assignment methods. The likelihood values associated with putative numbers of populations (in this case, species) (K) were estimated by six independent runs for each value of K (from 1 to 3) assuming admixture applying a burn-in length of 100,000 and a length of simulation of 1,000,000 repeats. The delta K (ΔK) that reflects the highest hierarchical level was determined by the Evanno method implemented in Structure Harvester (Earl et al. 2012). The graphic result was optimized using CLUMPP (Jakobsson and Rosenberg 2007) and DISTRUCT (Rosenberg 2004). Runs were undertaken without using the 'LOCPRIOR' option,

which means assuming there was no species structure in the sample set. Once the K was determined, another set of analysis using the USEPOPINFO option was conducted to search for potential hybrids or descendants of hybrids (up to two generations) between the two species, following the method described in Martien et al. (2012).

Population structure analyses for CBD in the western North Pacific Ocean

The FCA function in Genetix was also used here in searching for clusters of individual genotypes associated with the putative populations. Both with and without using population centre information ('sur population') options were used to generate different plots for comparison, and the figures were reconstructed using an R package *graphics*. The population structure was assessed using STRUCTURE with the same settings as described above, while here the K was set from 1 to 6 and 10 independent runs were conducted. The analysis was undertaken with and without using the 'LOCPRIOR' function as two independent assessments.

The R package *Geneland* (Guillot et al. 2005) was used to assess the population structure in a spatial context. Because the program requires information of precise spatial coordinates for each genotyped individual, those CBD samples with ambiguous sampling locations were excluded for this analysis. In particular, the Japanese samples collected from the aquaria and Taiwanese samples confiscated in the fish markets were

excluded. The setting for the analysis was as described in Chapter 2, with the number of clusters (K) set to vary from 1 to 6 in the first step, a maximum rate of Poisson process fixed to 41 (the number of samples), and maximum number of nuclei in the Poisson-Voronoi tessellation fixed to 123. To calculate the posterior probabilities of population membership for each individual and each pixel of the spatial domain, a burn-in of 100 iterations and a spatial domain of 151 pixels along the X-axis and 250 along the Y-axis were used.

The degree of population differentiation among the geographic groups was evaluated by the fixation indices, F_{ST} (Wright 1951) and R_{ST} (Slatkin 1995), using the algorithm implemented in Arlequin 3.5.1. Because the F-Statistics is less reliable with small sample size (Balloux and Lugon-Moulin 2002), the F_{ST} and R_{ST} were only estimated for the putative populations with sufficient samples, i.e., the East Japan (n=32) and Taiwan (n=28) populations. A non-parametric permutation approach with 10,000 permutations was used to assess the statistical significance of the fixation statistics, with a significance level set at $p<0.05$.

Population dynamics of CBD in the western North Pacific Ocean

The effective population size (N_e) and long-term gene flow, i.e., the number of migrants per generation (N_em), were estimated using maximum likelihood coalescent methods

implemented in MIGRATE version 3.6.6 (Beerli and Felsenstein 1999, 2001). To infer the presence of recent gene interchange, GeneClass2 was used to search for potential first generation migrants (Piry et al. 2004). To assess if sex-biased dispersal occurs in the CBD samples, the sex-biased dispersal tests implemented in FSTAT (Goudet et al. 2002) were used. The program settings for these analyses were as described in Chapter 2.

Mitochondrial DNA data analyses

Published mtDNA control region sequences for both species from the same or adjacent regions, i.e., Taiwan and southeast China (Wang et al. 1999, Yang et al. 2005), Japan (Kita et al. 2013), northeast China (Yang et al. 2005), and Hawaii and Palmyra Atoll/Kingman Reef (Martien et al. 2012), were acquired from the GenBank database (http://www.ncbi.nlm.nih.gov/genbank). Associated information such as sampling year, haplotype frequency and pedigree relationship, if applicable, were referenced to their original publications (Appendix 4.2). The sequences were then aligned together with the sequences generated for this study in MEGA5 (Tamura et al. 2011) for further analyses.

To minimise the effect of parent-offspring pairs (from sampling events that could be biased towards close kin) to the overall population genetic structure, one of the individuals from all recognized parent-offspring pairs was discarded (See description in

previous section *Microsatellite data configuration*). In the published sequences, the pedigree relationship among individuals for Japanese, Hawaiian and Palmyra samples was well documented (Martien et al. 2012, Kita et al. 2013), but the kinship information for Chinese samples was not available (Wang et al. 1999, Yang et al. 2005). It is assumed that there was no parent-offspring pair sampled in Wang et al. (1999) and Yang et al. (2005), because 1) their samples were collected in independent stranding or occasional fishery interaction events, 2) only a few individuals shared the same haplotype, and more importantly, 3) those samples sharing the same haplotype were not collected at the same time or location. The published and newly sequenced mtDNA sequences were assigned to one of six putative populations based on their sampling geography; that is, Japan, Northeast China (including Zhoushan, Qingdao, and Lianyunggang), Southeast China (including Dongshan, Taiwan, Hong Kong, the Philippines), South China (Beihai), and Indonesia, Hawaii and Palmyra (Fig. 4.2; Appendix 4.2).

DnaSP v5 (Librado and Rozas 2009) was used to identify unique haplotype(s) and estimate the nucleotide diversity (π) and gene diversity (h) for each putative population, as well as for the overall species. Indices for evaluating selective neutrality, i.e., Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997), were also estimated using DnaSP. Mismatch distributions implemented in Arlequin were also conducted to test for population expansion signals (Rogers and Harpending 1992, Schneider and Excoffier

1999, Excoffier 2004, Ray et al. 2003). The confidence intervals of the estimates were obtained under 10,000 bootstrap simulations of an instantaneous expansion under a coalescent framework. The sum of square deviations (SSD) between the observed and the expected mismatch and the raggedness index (r) of the observed distribution were calculated and tested to evaluate the fitness of the models (Harpending 1994, Schneider and Excoffier 1999).

Global tests of genetic differentiation among samples, as well as a differentiation test between all pairs of putative populations, were assessed using a Fisher's exact test (Raymond and Rousset 1995) implemented in Arlequin, with a 10,000-permutation setting. Pairwise F_{ST} and Φ_{ST} between all pairs of putative populations were calculated and tested for significance using Arelquin. The significance level was set as $p<0.05$.

To study whether the population structure can be inferred by the presence of any evolutionary significant unit in the sample set, MrBayes 3.2 (Ronquist et al. 2012) was used to reconstruct the phylogeny of all haplotypes using a Bayesian Markov Chain Monte Carlo (MCMC) analysis. The evolutionary model for the test was determined by jModelTest 2.1.5 (Darriba et al. 2012); the sampling increment was set at 100 and diagnostics at every 1,000 generations; at least 900,000 generations were simulated to generate the consensus tree. The final consensus tree was visualized and edited for optimal display in FigTree v.1.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Microsatellite data editing

All samples (n=75) were successfully genotyped using at least 23 microsatellite loci, and the missing data rate for all loci (n=24) was less than 5%, except for locus TexVet5, which had 8.8% missing data. The H_O across the 24 loci ranged from 0.553 to 0.721, and the average coefficient of kinship (*r)* ranged from -0.422 to 0.089 among the populations (Table 4.2). A close pedigree relationship (r>0.5) was observed in three CBD pairs, all from the East Japan population (Table 4.3). The Pair T2 was sampled from the same drive fishery stock, and therefore it was reasonable to suspect it was a parent-offspring pair. The other two pairs (T1 and T3) were samples collected from the same aquarium; although they were sampled in different years (which means the dolphins died in different years), these dolphins may have been caught from the same drive fishery stock, or be close kin through breeding in captivity. The data of the two individuals (EW1299 and EW1344) were therefore excluded from subsequent analyses to avoid potential sampling bias.

Null alleles were detected in three loci (Dde72, EV37 and KWM12a) in the CBD East Japan population. Significant deviation from HWE was also detected in the same loci in the East Japan and Taiwan populations (Appendix 4.3). A significant sign of LD was only found between EV37 and KWM2a in IPBD samples. The loci that had

a >5% missing data rate (TexVet5) and showed null alleles and/or deviated from HWE (Dde72, EV37 and KWM12a) were discarded, and thus it resulted a set of microsatellite genotypic data from 20 loci (AAT44, D14, D22, Dde59, Dde65, Dde66, Dde69, Dde70, Dde84, EV14, KWM1b, KWM2a, KWM2b, KWM9b, MK3, MK5, Sco11, Sco28, Sco55 and TexVet7) in 73 dolphins being used in following analyses.

Table 4.2. The averages (SD) of the number of alleles, expected heterozygosity (H_E), observed heterozygosity (H_O), allelic richness, inbreeding coefficient (F_{IS}) and coefficient of kinship (r) across the 24 microsatellite loci within each putative population examined in this study. Number in parentheses is the SD of the estimate. See Appendix 4.3 for the estimates by locus in each population.

Species	Population	n	No. of alleles	H_E	H_O	Allelic richness	F_{IS}	r
CBD	Taiwan	28	7.833 (3.384)	0.738 (0.184)	0.721 (0.184)	1.738 (0.184)	0.024	-0.036
	East Japan	32	8.125 (3.971)	0.739 (0.167)	0.692 (0.184)	1.739 (0.167)	0.064	0.006
	West Japan	4	3.875 (1.314)	0.719 (0.212)	0.685 (0.241)	1.689 (0.254)	0.055	0.089
	Philippines	2						-0.422
IPBD	All samples	7	3.609 (0.988)	0.650 (0.126)	0.553 (0.179)	3.5 (1.103)	0.055	0.16

Table 4.3. The information for the three potential parent-offspring pairs in the CBD samples. The letter following the ID indicates the sex (F, female; M, male) and the ID marked by asterisk is the sample being discarded from further analyses.

Pair no.	ID-1	ID-2	Sample source	Sampling location	Sampling year	r
T1	EW1342(M)	EW1344(F)*	Captivity	Shimoda Aquarium (ECJ)	1995/1998	0.64
T2	EW1294(M)	EW1299(F)*	Drive fishery	Taiji (ECJ)	1986	0.58
T3	EW1344(F)*	EW1351(F)	Captivity	Shimoda Aquarium (ECJ)	1998/2000	0.51

Genetic distances between CBD and IPBD

Both FCA and STRUCTURE analysis results showed a clear genetic difference between CBD and IPBD (Fig. 4.3, 4.4). Both ΔK and LnP(K) values supported K=2 as the best estimation from the STRUCTURE analysis, suggesting these two species are genetically well differentiated. No individual possessed an intermediate genotype, or any inter-species pair exhibiting a close pedigree relationship (i.e., r >0.5). The ancestry assignment test showed three CBD individuals, from Japan, Taiwan, and the Philippines respectively (Fig. 4.4), that may have had an IPBD grandparent, although the probability was only between 13-37% (Table 4.4). The Philippine sample with the highest hybrid probability was initially identified as a Fraser's dolphin. If this was not a result of mislabeling in the sample archive, it may suggest that this individual has a confusing external appearance that resulted in misidentification. The Japanese sample was from a captive dolphin; however it is unknown whether the dolphin was a descendant of hybrids in the wild, or born in the aquarium with a hybrid pedigree. The Taiwanese sample was from a dolphin incidentally caught in the fisheries in the east coast of Taiwan, where the reports of IPBD sightings are still absent despite intense survey effort (Yang et al. 1999, Chou 2007). The FCA result also showed that there was a considerable genetic distance among the IPBD samples. The variation constituted by the seven IPBD in the most important factor (Factor 1; the X-axis) was apparently greater than the variation in 66 CBD (0.91 vs. 0.55).

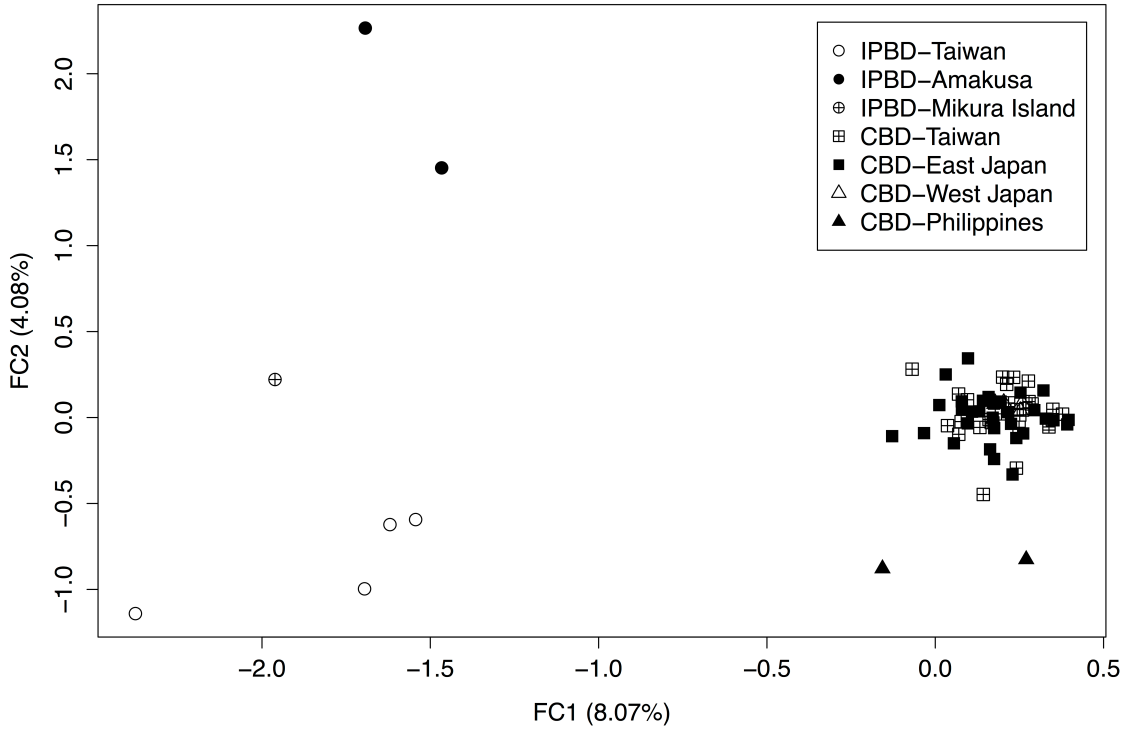


Figure 4.3. The result of a factor correspondence analysis (FCA) of 20 microsatellite loci data for all acquired bottlenose dolphin samples, without using the 'sur population' option. Numbers in parentheses indicates the percentage of the variance explained by the factor/axis.

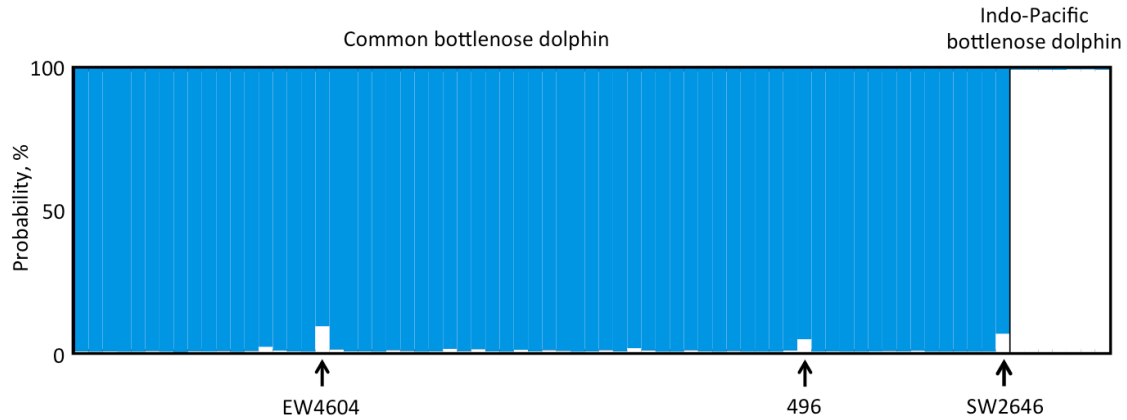


Figure 4.4. The best population model (K=2) predicated by STRUCTURE analysis for CBD and IPBD without using LOCPRIOR option. Each column represented one individual, and the colour portion in each column indicated the probability of the individual being assigned to a population. The arrows indicate the three potential descendants of hybrids between the two species.

Table 4.4. The ancestry assignment result for the three CBD individuals having the least probability being a 'pure' CBD.

Species	ID (Sex)	Sampling location	Probability of being a exclusive CBD descendant	Probability of being a exclusive IPBD descendant	Probability of having a IPBD parent	Probability of having a IPBD grandparent
CBD	EW04604 (M)	Japanese aquarium	0.777	0	0.005	0.218
CBD	496 (M)	Taiwan	0.863	0	0.001	0.136
CBD	SW2646 (F)	Philippines	0.596	0	0.035	0.369

Population structure for CBD inferred from microsatellite analyses

The FCA result showed that when the 'sur population' option was not used, the Philippine samples had the most distinct genotypes, and the samples from Japan and Taiwan grouped together in a central cluster (Fig. 4.5A). FC1 and FC2 together explained 8.73% of the variance. On the other hand, when the 'sur population' option referencing individuals to a population centre was used, population-specific clusters could be identified and the power of FC1 and FC2 rose to 82.95%. The genotypes of the Philippine samples remained highly distinct from the other samples, but East Japan and Taiwan-West Japan formed two overlapping clusters defined by FC2 (Fig. 4.5B). The 14 captive dolphin samples provided by Japanese aquaria grouped with samples from East Japan rather than West Japan. It is noteworthy that an East Japan sample, EW4842, was clustered among the Taiwan-West Japan samples, and the same clustering pattern can also be found in the Geneland analysis (see below). This young

male dolphin was stranded at the coast of Miyazaki, which was the most southerly sampling site for the putative East Japan population. This 'mis-grouping' could reflect limitations to the resolution of the analysis, evidence of direct migration between populations, or the result of a carcass drifting between regions (Bilgmann et al. 2011). The West Japan samples were segregated from the Taiwanese samples and became an independent cluster by the third factor, FC3. This factor explained the remaining 17.05% of the variance.

In the first step of Geneland analysis, the 10 simulations all indicated that the most likely number of populations for the sample set was K=3. With the K fixed to K=3 in the second step, the analysis suggested eight variations of population distribution patterns for CBD among the 10 runs with the highest LPP in 100 simulations. These eight variations all showed approximately the same clustering pattern, with a few samples in each panel being grouped to different clusters (Fig. 4.6). The basic pattern was a cluster grouping samples from the west coast of Japan, western and northern coasts of Taiwan, and the sample collected in Miyazaki, Japan ("the West Coast Cluster"); a cluster for the samples from the eastern coast of Taiwan and from Taiji, Japan ("the East Coast Cluster"); and a cluster for the samples from the Philippines ("the South Tropical Cluster"). The samples collected from Tainan (southwest Taiwan) and Shizuoka (east Japan) swung among the three clusters, but usually grouped with the South Tropical Cluster (Fig. 4.6A, B, F, H). The sample collected from Aichi, eastern

Japan oscillated between the East Coast Cluster (Fig. 4.6A—E, G) and the West Coast Cluster (Fig. 4.6F, H). In short, the analysis suggests that the population membership of CBD in the western North Pacific Ocean corresponds with regional oceanographic features. This is reflected in a cluster of samples from the vicinity of the offshore North Pacific Ocean (the East Coast Cluster), a cluster from the coastal waters of the northeast Asian continent (the West Coast Cluster), and a cluster of samples from Philippine waters (the South Tropical Cluster).

The STRUCTURE analysis, on the other hand, suggested different numbers of K when different criteria were applied. According to the Evanno's ΔK estimates, the most likely K would be K=2 (when the LOCPIROR option was not used) or K=5 (when the LOCPIROR option was used) for the sample; while if the result was evaluated by the conventional LnP(K) values, the most likely K should be K=1 for both cases (Table 4.5). However, the graphic output for K=2 with LOCPIROR option revealed subtle differentiation among the putative populations, while K=3 and K=5 provided no further resolution (Fig. 4.7). When K=2, STRUCTURE clustered the East Japan and Philippine populations in one group, the Taiwan and West Japan populations in the other. The F_{ST} estimated between East Japan and Taiwan was as little as 0.013, but it was statistically significant from zero (p=0); while the R_{ST} was 0.055, which was not statistically significant from zero (p=0.068±0.002). This implies that genetic differentiation between the two regions could exist, but may be difficult to detect.

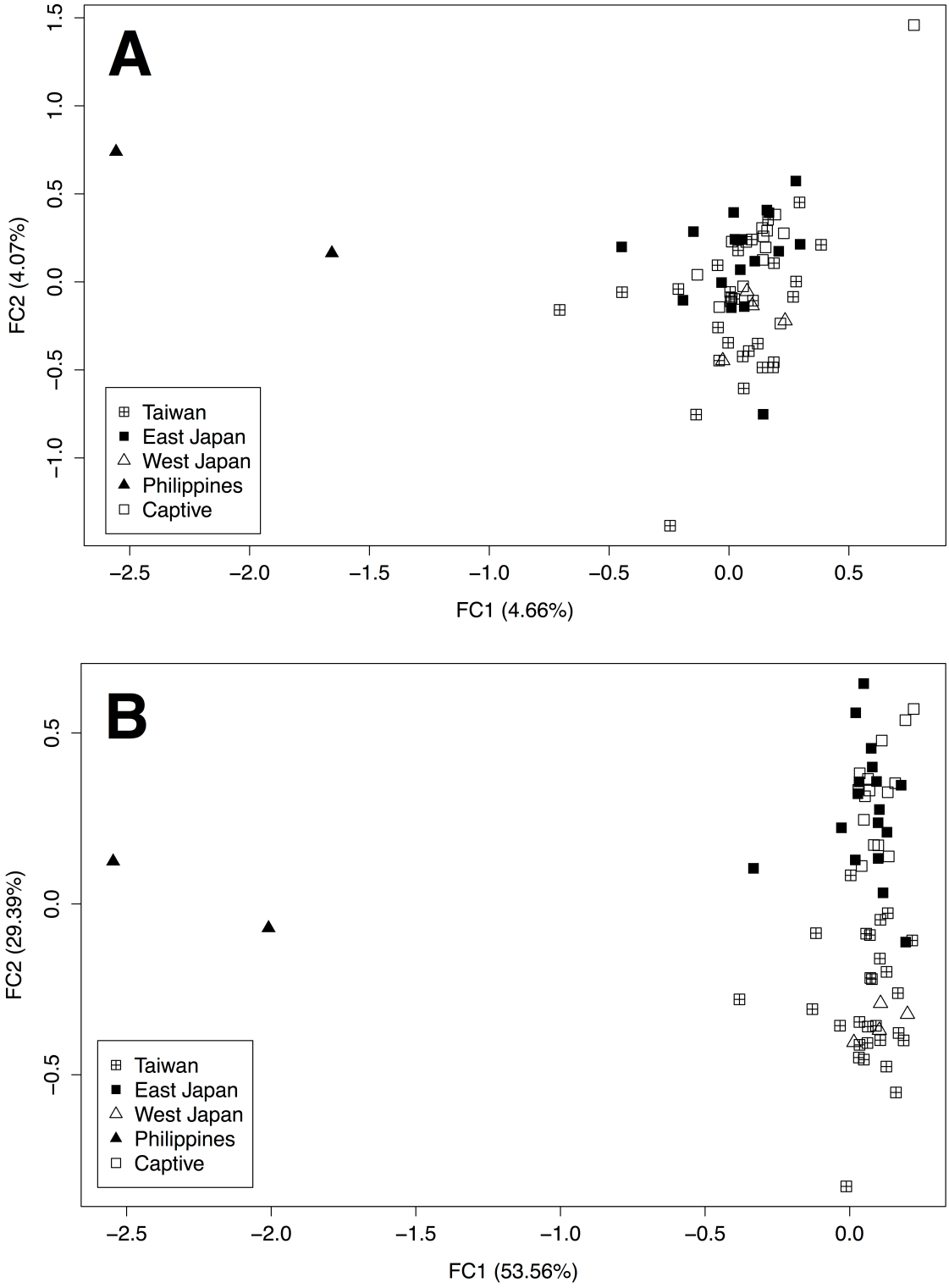


Figure 4.5. The results of the FCA for the CBD without using LOCPRIOR option (A) or using LOCPRIOR option (B). The two most informative factors (FC1 and FC2) were assigned as X and Y axes in the plot, and the numbers in parentheses in each axis indicates the percentage of the variance explained by the factor.

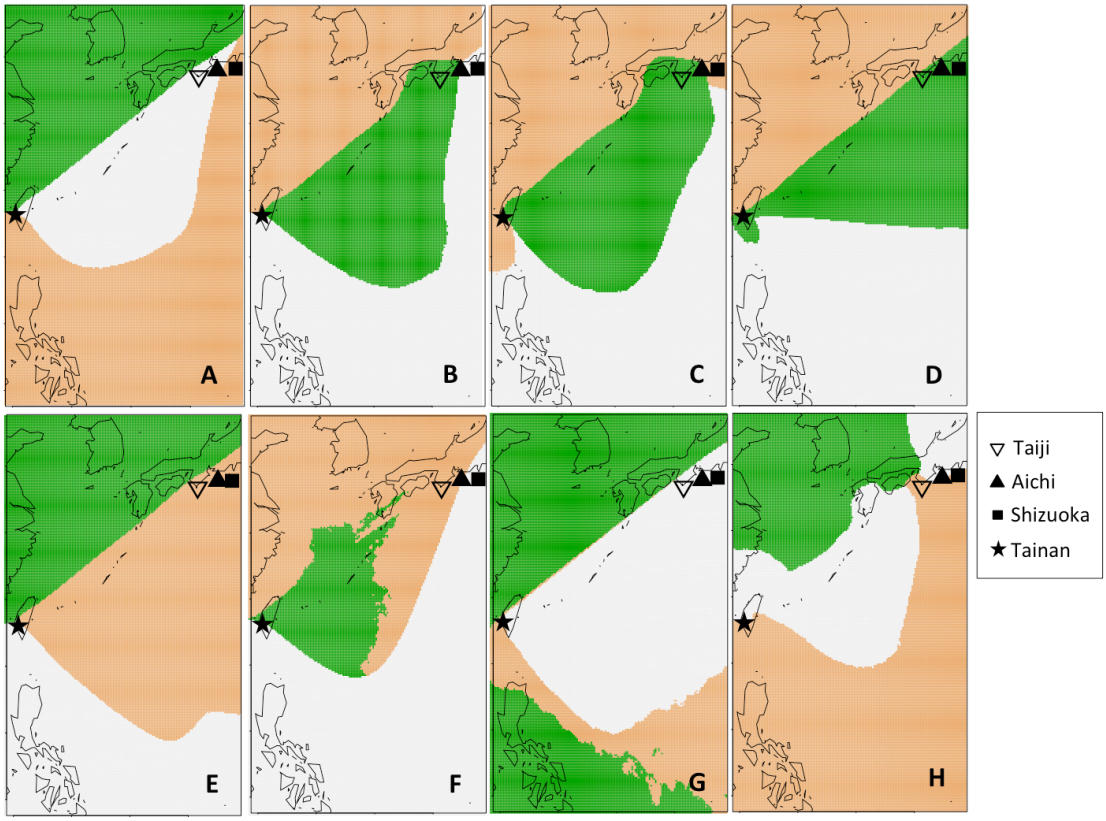


Figure 4.6. The six variations of the individual population membership assignment patterns shown in the 10 runs with the highest LPP for K=3 in Geneland analysis. The colours indicate the distribution of K clusters based on the mode of simulated posterior probability for each pixel. Some landmarks mentioned in the text are labelled.

Table 4.5. The Evanno table generated by the Structure Harvester based on the results of STRUCTURE analysis using the CBD data of 20 microsatellite loci.

LOCPRIOR option	K	Mean LnP(K)	SD of LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
Not used	1	-4311.47	0.4398	NA	NA	NA
Not used	2	-4361.22	22.0843	-49.75	376.91	17.066896
Not used	3	-4787.88	186.7699	-426.66	271.74	1.454945
Not used	4	-4942.8	357.0556	-154.92	7.18	0.020109
Not used	5	-5104.9	413.923	-162.1	80.02	0.193321
Not used	6	-5186.98	416.6069	-82.08	NA	NA
Used	1	-4311.39	0.2846	NA	NA	NA
Used	2	-4477.45	140.7378	-166.06	19.24	0.136708
Used	3	-4662.75	251.0307	-185.3	200.32	0.79799
Used	4	-4647.73	156.1957	15.02	98.26	0.629083
Used	5	-4534.45	42.2544	113.28	96.22	2.27716
Used	6	-4517.39	124.921	17.06	NA	NA

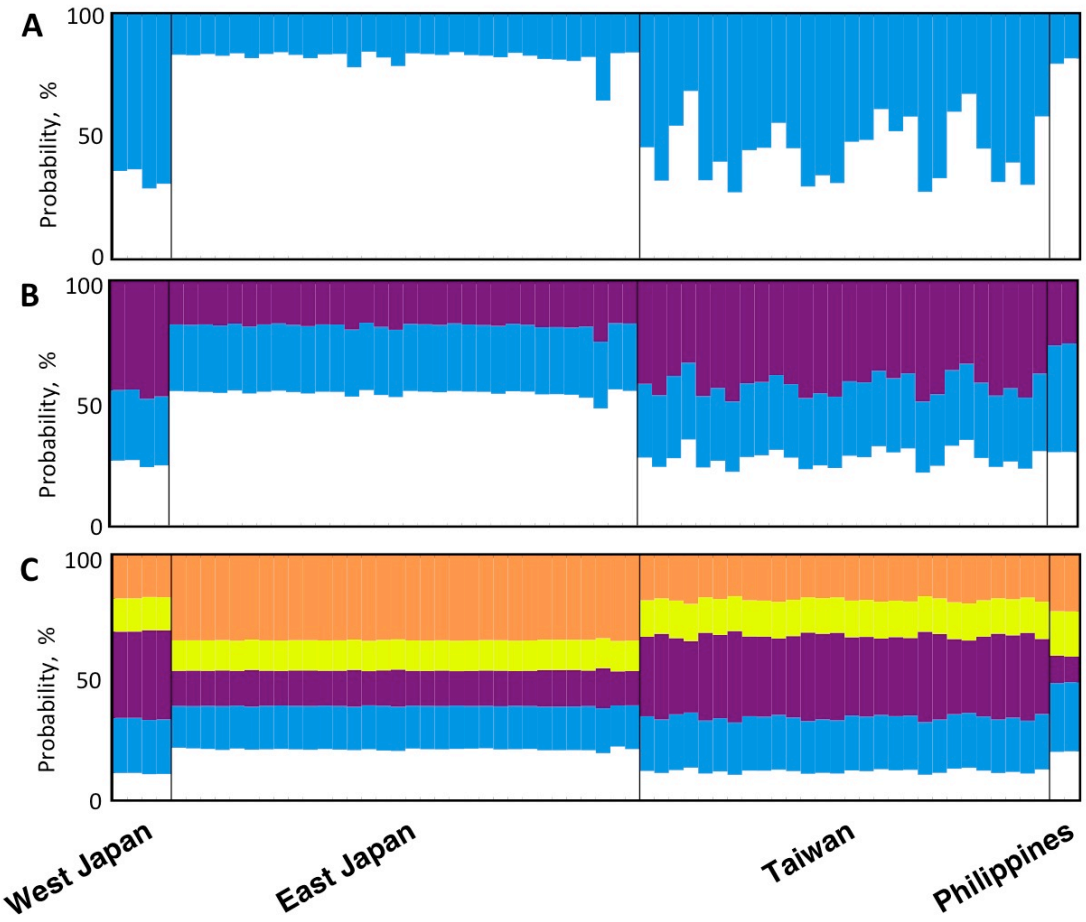


Figure 4.7. Individual's population membership under a series of K predicated by STRUCTURE analysis using 20 microsatellite loci data and the LOCPRIOR option: (A) K=2, (B) K=3, and (C) K=5. Each column represents one individual, and the colour portion in each column indicated the probability of the individual being assigned to a population.

Population dynamics for CBD inferred from microsatellite analyses

To evaluate the population dynamics for CBD populations, the samples were reorganised into three clusters based on the result of Geneland analysis: a West Coast Cluster (the samples from western coasts of Taiwan and Japan, and from Miyazaki), an East Coast Cluster (the samples from eastern coast of Taiwan and Taiji), and a South Tropical cluster (the samples from the Philippines). However, the South Tropical

Cluster was discarded because the sample size of this cluster was apparently insufficient to provide useful insights. The samples from Tainan, Aichi, and Shizuoka were excluded from the analyses of this section due to the uncertainty of their population identity.

The Migrate analysis estimated that the $N_e\mu$ values for the East Coast Cluster were slightly larger than the West Coast Cluster (Table 4.6). With the N_e estimates, the ratio of effective to census population size (N_e/N) can be calculated to evaluate the level of biased reproductive success in each population (Frankham 1995). The census population size (N) estimated for the 'Japanese Coastal' population (N=37,000; Miyashita 1993) was used to calculate the N_e/N for East Coast Cluster, and the N estimated for the CBD in southwest Japanese waters (N=35,000, Kasuya 2011) was used to calculate the N_e/N for West Coast Cluster. The calculation showed the N_e/N for both populations to be similar in magnitude, ranging from 0.042 to 0.059, or from 0.084 to 0.118, depending on what microsatellite mutation rate was used (Table 4.6).

The estimate of the number of migrants per generation (N_em) from the West Coast Cluster to East Coast Cluster was about double to the N_em *vice versa* (Table 4.6). Both estimates were small, suggesting there was less than one immigrant per generation between the two regions in roughly the last $4N_e$ generations (Kingman 1982, Wilson and Rannala 2003). The GeneClass analysis, on the other hand, suggested there were

three potential first-generation immigrants (Table 4.7), suggesting a recent presence of gene flow. There was no sign of sex-biased dispersal (Table 4.8).

Table 4.6. The estimates of effective population size times mutation rate ($N_e\mu$) and number of migrants per generation (N_em) from the two CBD populations recognized in Geneland analysis. The N_e is calculated assuming the average microsatellite mutation rate (μ) is 0.01% for N_e (high) and 0.02% for N_e (low) (Whittaker et al., 2003, Hoelzel et al. 2007, Hollatz et al. 2011). The ratio of effective to census population size (N_e/N, Frankham 1995) is calculated using the census population size (N) estimated for the 'Japanese Coastal' population (N=37,000; Miyashita 1993) for East Coast Cluster, and the N for the CBD in the southwest Japanese waters (N=35,000; Kasuya 2011) for West Coast Cluster. The 2.5th and 97.5th profile likelihood estimates are given in parentheses.

	Source population	Host population			
		East Coast		West Coast	
$N_e\mu$		0.400	(0.367—0.437)	0.321	(0.293—0.353)
N_e (low)		2000	(1836—2186)	1605	(1465—1766)
N_e (high)		4001	(3671—4371)	3211	(2930—3532)
N_e (low)/N		0.054	(0.05—0.059)	0.046	(0.042—0.05)
N_e (high)/N		0.108	(0.099—0.118)	0.092	(0.084—0.101)
N_em	East Coast			0.057	(0.046—0.070)
	West Coast	0.106	(0.089—0.125)		

Table 4.7. The potential first-generation immigrants in the East and West Coast Clusters of CBD.

ID	Sex	Current population	Potential source population	-LOG(L_home / L_max)	p
870	Male	West Coast	East Coast	1.766	0.009
1014	Male	West Coast	East Coast	3.827	0.002
EW1340	Female	East Coast	West Coast	1.492	0.006

Table 4.8. The sex-biased dispersal test results for the CBD samples from the East and West Coast Clusters.

	n	Mean of assignment indices	Variation of assignment indices	F_{IS}	F_{ST}	Ho	Hs	Relativeness
Female	18	0.280	12.605	0.038	0.033	0.661	0.6873	0.0608
Male	16	-0.315	12.716	0.009	0.034	0.684	0.6903	0.065
Overall	34			0.036	0.018	0.672	0.6969	0.0342
p		0.602	0.986	0.503	0.94	0.5	0.863	0.908

MtDNA genetic diversity of CBD and IPBD in the western and central North Pacific Ocean

This study revealed 42 novel CBD mtDNA control region sequences for dolphins from Taiwan, East Japan and the Philippines, and seven for IPBD from Taiwan and Japan. Together with the published sequences acquired from GenBank (n=311), a 388bp consensus mtDNA sequence from a total of 393 sequences was gathered and reconstructed as five putative CBD populations (East Japan, Northeast China, Southeast China, Hawaii and Palmyra) and four IPBD populations (Japan, Southeast China, South China and Indonesia) (Fig. 4.2, Appendix 4.2). According to the AIC and BIC indices calculated by jModelTest, the best model for reconstructing a phylogenetic tree for the genus using the mtDNA sequence samples was HKY+I+G.

For the CBD data set, 64 haplotypes were identified among the 353 sequences, defined by 82 variable sites, including two deletion gaps (Appendix 4.4). The overall genetic diversity (h) was 0.935 and nucleotide diversity (π) was 0.0197. The h and π for each population was similarly high, ranging from 0.824 to 0.909 and 1.368% to 2.193%, respectively (Table 4.9). Hap_2 was the most widespread haplotype; it was found in all populations except Palmyra (Fig. 4.8, 4.9). The Hap_2 was also the most dominant haplotype in the western North Pacific Ocean (28.3% of all samples). Within that region, it was most common in Northeast China (42.9%, accession number AF459509-15), and in the school of dolphins culled in the drive fishery in 2005 (30.4%,

previously published as Haplotype Ttr06, GenBank accession number AB303159). Hap_16 was the only haplotype shared between the western North Pacific (in Southeast China) and tropical Central Pacific (in Palmyra Atoll). It is noteworthy that in the phylogenetic tree, Hap_17, 25 and 33 were isolated from the major CBD-IPBD lineage, potentially indicating a lineage sorting process is still ongoing in CBD in the western North Pacific populations (Fig 4.7).

Table 4.9. Summary of the DNA haplotype diversity, nucleotide diversity, and indices for testing locus neutrality for the CBD and IPBD populations sampled in this study. Number in parentheses is the SD of the estimate.

	Sample size	No. of haplotypes	Haplotype diversity	Nucleotide diversity	Tajima's D	Fu's Fs
CBD						
East Japan	160	23	0.870 (0.019)	1.368% (0.103%)	-0.81835	-1.788
SE China	49	20	0.908 (0.025)	2.193% (0.314%)	-0.74485	-1.607
NE China	14	8	0.824 (0.098)	1.638% (0.427%)	-1.09647	0.216
Hawaii	119	20	0.868 (0.016)	2.124% (0.088%)	-0.09236	1.449
Palmyra	11	7	0.909 (0.066)	1.851% (0.423%)	-0.42215	0.526
Overall	353	63	0.935 (0.008)	1.966% (0.079%)	-1.25991	-22.17**
IPBD						
Japan	3	2	0.667 (0.314)	0.346% (0.163%)	NA	1.061
SE China	29	14	0.899 (0.036)	1.365% (0.110%)	0.99207	-2.389
S China	6	3	0.733 (0.155)	1.195% (0.351%)	0.99488	2.76
Indonesia	2	2	1 (0.5)	1.039% (0.519%)	NA	1.386
Overall	40	18	0.924 (0.022)	1.395% (0.084%)	0.66414	-4.005*

*: P<0.05; **: P<0.01

For IPBD, 19 variable sites were found defining18 haplotypes in a total of 40 sequences from the coastal waters around Japan, Southeast China and South China. The overall *h* was 0.924 and π was 1.395%; the h and π for IPBD populations were lower than CBD populations in general (Table 4.9). The high genetic diversity for the

Indonesian population was likely an artefact due to sample size insufficiency. The seven IPBD samples were assigned to five haplotypes (Hap_66, 71, 72, 74 and 78) and none of those was a novel haplotype (Fig. 4.8, 4.9). The two Japanese samples from the same region (Amakusa) shared the same haplotype. Hap_59 was an IPBD haplotype from a putative CBD specimen collected from Hawaiian waters (M34066), and the introduction of this alien haplotype to the CBD population was regarded as a result of introgression in the distant past (Martien et al. 2012).

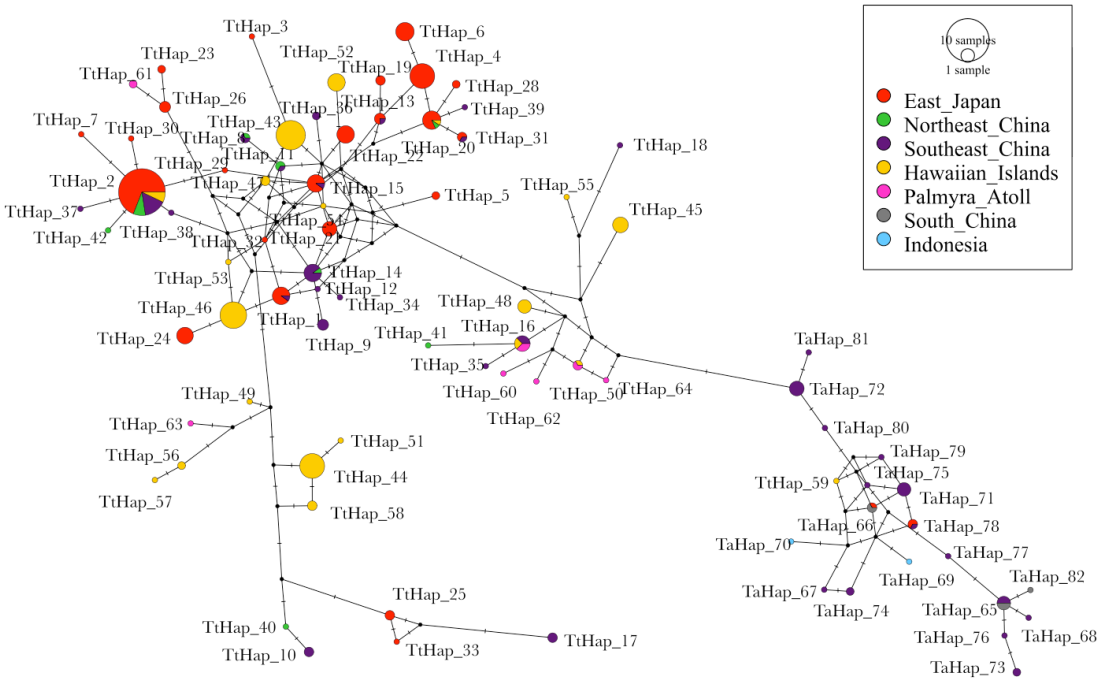


Figure 4.8. The Median-joining network tree for CBD and IPBD mtDNA control region haplotypes. Each circle represents a unique haplotype. The size of circle indicates the number of individuals having the haplotype and the colour shade indicates the proportion of each population within the haplotype. The number of hatch marks at the lines indicates the number of mutational steps separating the haplotypes. Solid circles indicate missing intermediate haplotypes.

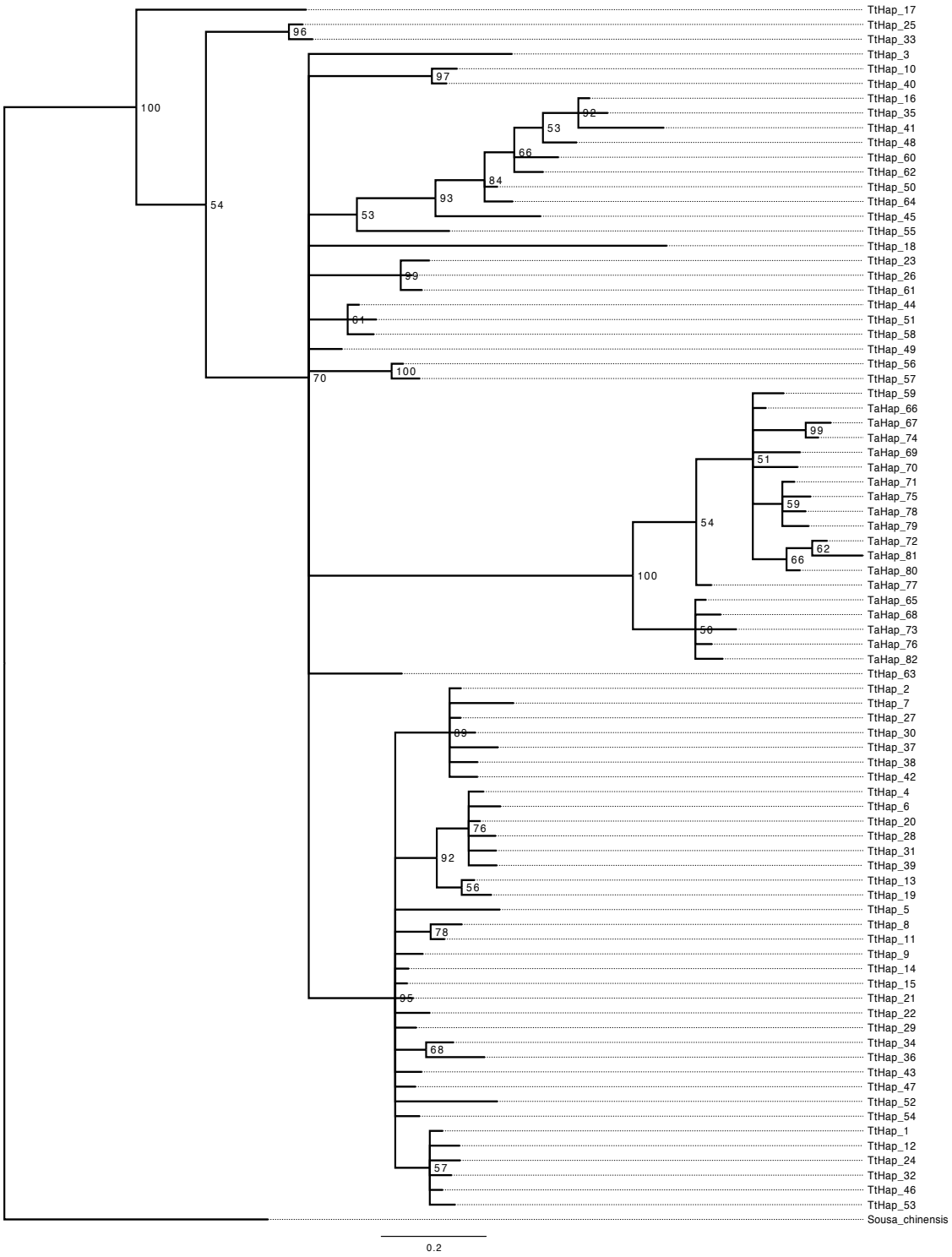


Figure 4.9. Phylogenetic relationship of the mtDNA haplotypes for the CBD and IPBD from the western and central North Pacific Ocean. The number at the branch indicates the bootstrap probability.

Population structure and expansion history for CBD inferred from mtDNA data

Most pairwise F_{ST} and Φ_{ST} comparisons were statistically significant (Table 4.10). Fisher's exact tests based on haplotype frequencies suggested the five putative populations were well differentiated, except for the comparison between Northeast and Southeast China (Table 4.11). The clear differentiation between the Hawaiian and Palmyra populations has been reported in the original paper (Martien et al. 2012); here the analysis further reveals that the Hawaiian and Palmyra populations were also differentiated from the western North Pacific populations. Within the western North Pacific region, the Northeast China population was the least differentiated, although the statistical insignificance could be largely due to deficient sample size, which was an issue for the Northeast China population sample.

A negative Tajima's D was estimated for all putative populations, although none of the values were significantly different from zero (Table 4.9). A negative Fu's Fs was estimated for the East Japan and Southeast China populations, but again none of the estimates was statistically significant. The only exception was when all samples were pooled together, the Fu's Fs estimate was negative and significantly different from zero (Table 4.9). The mismatch distributions for each putative population appeared to be multimodal (Fig. 4.10), even though fit to the expansion model could only be rejected for the Hawaiian and Northeast China populations with the demographic expansion model (Table 4.12).

Table 4.10. Pairwise F_{ST}/φ_{ST} comparisons among the five putative CBD populations in western-central North Pacific Ocean. The pairwise F_{ST} value is above the diagonal and the pairwise φ_{ST} value is below the diagonal.

			F_{ST}				
		n	East Japan	SE China	NE China	Hawaii	Palmyra
φ_{ST}	East Japan	160		0.04114**	0.02039	0.11421**	0.10727**
	SE China	49	0.07986**		0.01819	0.10365**	0.076**
	NE China	14	0.01974	0.00526		0.13426**	0.13486**
	Hawaii	119	0.15981**	0.05938**	0.09145**		0.10945**
	Palmyra	11	0.53322**	0.30374**	0.43446**	0.2427**	

*: P<0.05; **: P<0.01

Table 4.11. Pairwise non-differentiation exact P values estimated based on mtDNA haplotype frequencies (above the diagonal) or Tamura and Nei's distance model (below the diagonal).

	p				
	East Japan	SE China	NE China	Hawaii	Palmyra
East Japan		0.000±0.000 ***	0.000±0.000 ***	0.000±0.000 ***	0.000±0.000 ***
SE China	0.000±0.000 ***		0.242±0.015	0.000±0.000 ***	0.000±0.000 ***
NE China	0.001±0.000 **	0.253±0.021		0.000±0.000 ***	0.001±0.000 **
Hawaii	0.000±0.000 ***	0.000±0.000 ***	0.000±0.000 ***		0.000±0.000 ***
Palmyra	0.000±0.000 ***	0.000±0.000 ***	0.002±0.001 **	0.000±0.000 ***	

*: P<0.05, **: P<0.01, ***:p<0.001

Table 4.12. The parameters of the demographic and spatial expansion models for each putative population, estimated by the mismatch distribution analysis.

	Tau	95%CI	SSD	Mode SSD p value	Raggedness index (r)	p
Demographic expansion model						
Japan	6	(2.240—9.256)	0.02029	0.0925	0.04178	0.0861
Southeast China	4.4	(0.137—20.461)	0.02883	0.1543	0.04305	0.0696
Northeast China	0	(0—0.586)	0.80256	0	0.13211	0.9994
Hawaii	10.7	(5.609—14.5)	0.03509	0.0029	0.05379	0.0003
Palmyra	1.6	(0—16.305)	0.07867	0.1582	0.10876	0.269
Spatial expansion model						
Japan	4.64032	(2.185—7.97)	0.01657	0.4126	0.04178	0.5882
Southeast China	3.93602	(1.534—18.241)	0.02594	0.2514	0.04305	0.2757
Northeast China	4.61573	(1.003—9.904)	0.0718	0.2174	0.13211	0.4642
Hawaii	9.71783	(6.142—13.588)	0.02406	0.358	0.05379	0.516
Palmyra	10.046	(0.149—161.891)	0.07782	0.0617	0.10876	0.464

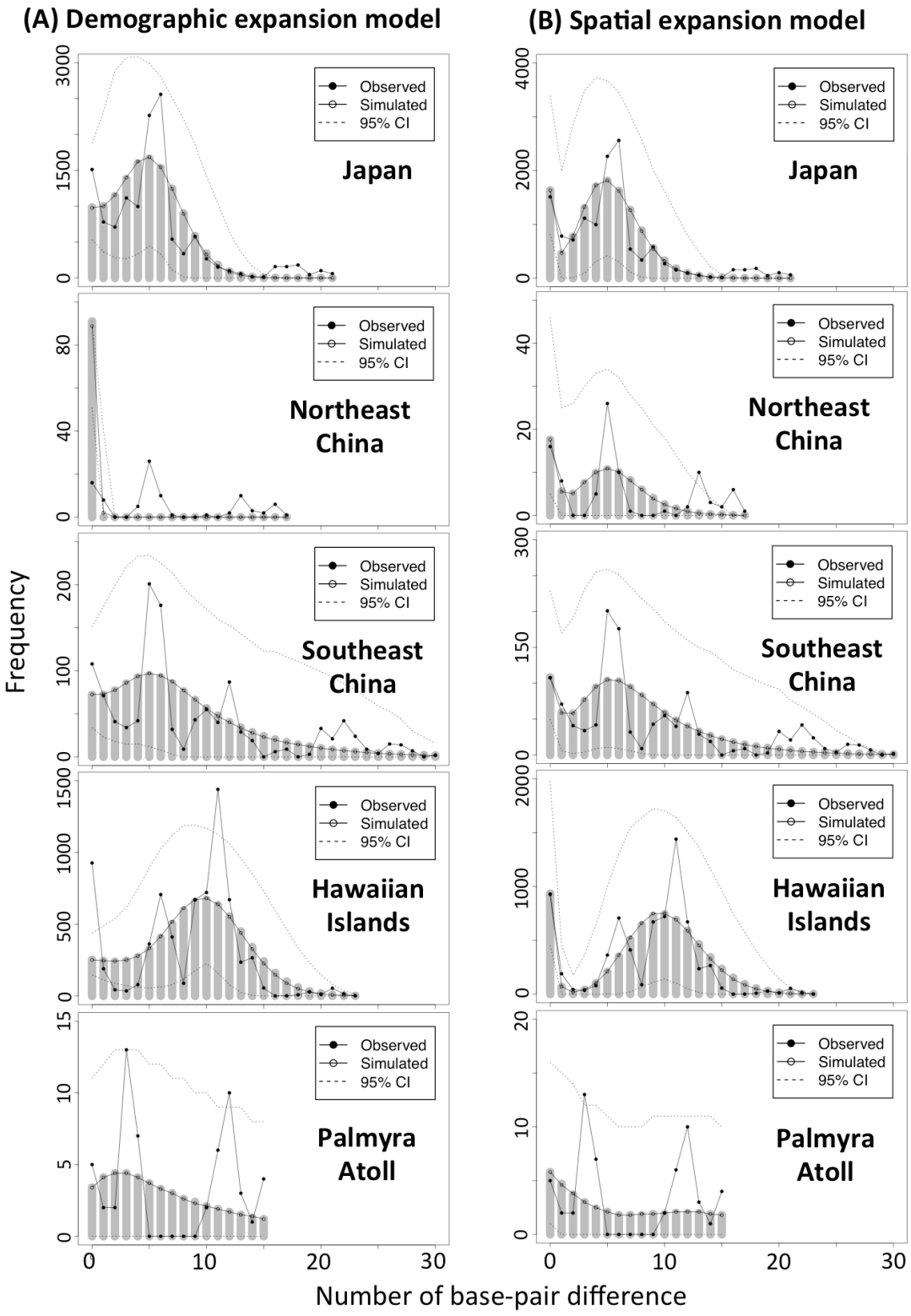


Figure 4.10. Observed and expected mismatch distributions for the CBD populations in the western and central North Pacific Ocean under the demographic (A) and spatial expansion models (B). The vertical bars (in grey) in the panels indicate the model frequency in each scenario.

Discussion

Together with the earlier publication which suggests significant population differentiation between the CBD found in the waters around the Hawaiian Islands and Palmyra Atoll (Martien et al. 2012), the data, for the first time, show genetic differentiation between shallow and deep regions of the coastal waters in the western North Pacific region, and significant differentiation between the western and central North Pacific populations. The data rejected the hypothesis that coastal CBD is absent in the western North Pacific Ocean due to the presence of their potential habitat competitor, the IPBD. The following paragraphs are devoted to discuss the compatibility between the genetic structure of CBD and the stock structure based on shipboard survey data, possible mechanisms that may result in such population structure, insights into the population dynamics of the CBD populations, potential interaction between CBD and IPBD, and conservation implications for the two species.

CBD populations in the western North Pacific Ocean

Miyashita (1993) studied the sighting records of several small cetaceans in the western North Pacific Ocean (approximate survey area: 25—50°N, 130°E—180°; excluded Sea of Japan) and concluded that CBD is mainly distributed in 30—42°N and west of 160°E, with a density gap at 142—145°E. The density gap is suggested as a boundary

separating the 'Japanese coastal' population (west of 142°E) and 'Japanese offshore' population (east of 145°E). The existence of a boundary is tentatively supported by a telemetry study that showed that the CBD population targeted by the Japanese coastal drive fishery, which is usually operated within 15—20 nautical mile from land (Kishiro and Kasuya 1993), is unlikely to utilise the waters further than 200 nautical miles from land (Tanaka 1987). If the Japanese coastal population truly exists, the East Japan samples should be from this population, since most of the samples were collected from dolphins caught in the coastal drive fishery.

The Geneland analysis further suggests the southern range of this 'Japanese coastal' population could be extended further south to the eastern coast of Taiwan (22—25°N, east of 121°E), and to avoid confusion it is then called the "East Coast Cluster". The eastern coasts of Taiwan and Japan (between 22—42°N) are together embedded in a unique oceanic biogeographic province, of which the main characteristic is sharing the speedy, warm, relatively high saline Kuroshio Current flowing northeastwardly from Luzon in the Philippines to the eastern coast of Japan year-round (Barkley 1970, Wyrtki 1975, Spalding et al. 2012) (Fig. 4.11). Although it is still uncertain what type of habitat this East Coast Cluster CBD population utilises and where that habitat is, it is very likely that the strong, constant Kuroshio Current plays a crucial role in defining this habitat (Tanaka 1987). Similar population structure where there is connectivity along the eastern coasts of Taiwan and Japan has been suggested for short-finned pilot whales

(*Globicephala macrorhynchus*) (Chen et al. 2014), Risso's dolphins (*Grampus griseus*) (Chapter 2) and Fraser's dolphins (Chapter 3). However, it is possible that there is further fine-scale population sub-division within the cluster, as seems to be the common pattern for CBD elsewhere in the world (e.g., Mirimin et al. 2011, Martien et al. 2012, Richards et al. 2013, Gaspari et al. 2015a). In this study, the sample size for eastern Taiwan is too small (n=4) to reveal such pattern, even if it does exist.

On the other hand, Kasuya et al. (1997) found a subtle difference in several life history traits (e.g., the body length at sexual maturity, the age of sexual maturity and the interval of breeding) between CBD caught in Taiji (eastern Japan) and Iki (southwestern Japan), suggesting CBD populations between the eastern and western coasts of Japan are differentiated (cited in Kasuya 2011). This hypothesis is tentatively supported by Hayano (2013), who studied 520bp mtDNA control region sequences in 42 CBD from the eastern and western coasts of Japan and found that seven of the 10 samples collected from the western coast were grouped in a unique phylogenetic cluster with a bootstrap support value of 71%. The FCA and Geneland results also support the differentiation of CBD populations between the western and eastern coasts of Japan (i.e., between the Sea of Japan and the Pacific coast of Japan), although the sample size from the population west of Japan is too small for robust inference. However, it seems likely that the CBD either side of Japan are differentiated populations, as such differentiation pattern has

been reported for other cetaceans, e.g., in minke whales (*Balaenoptera acutorostrata*) (Abe et al. 2000) and Dall's porpoises (*Phocoenoides dalli*) (Hayano et al. 2003).

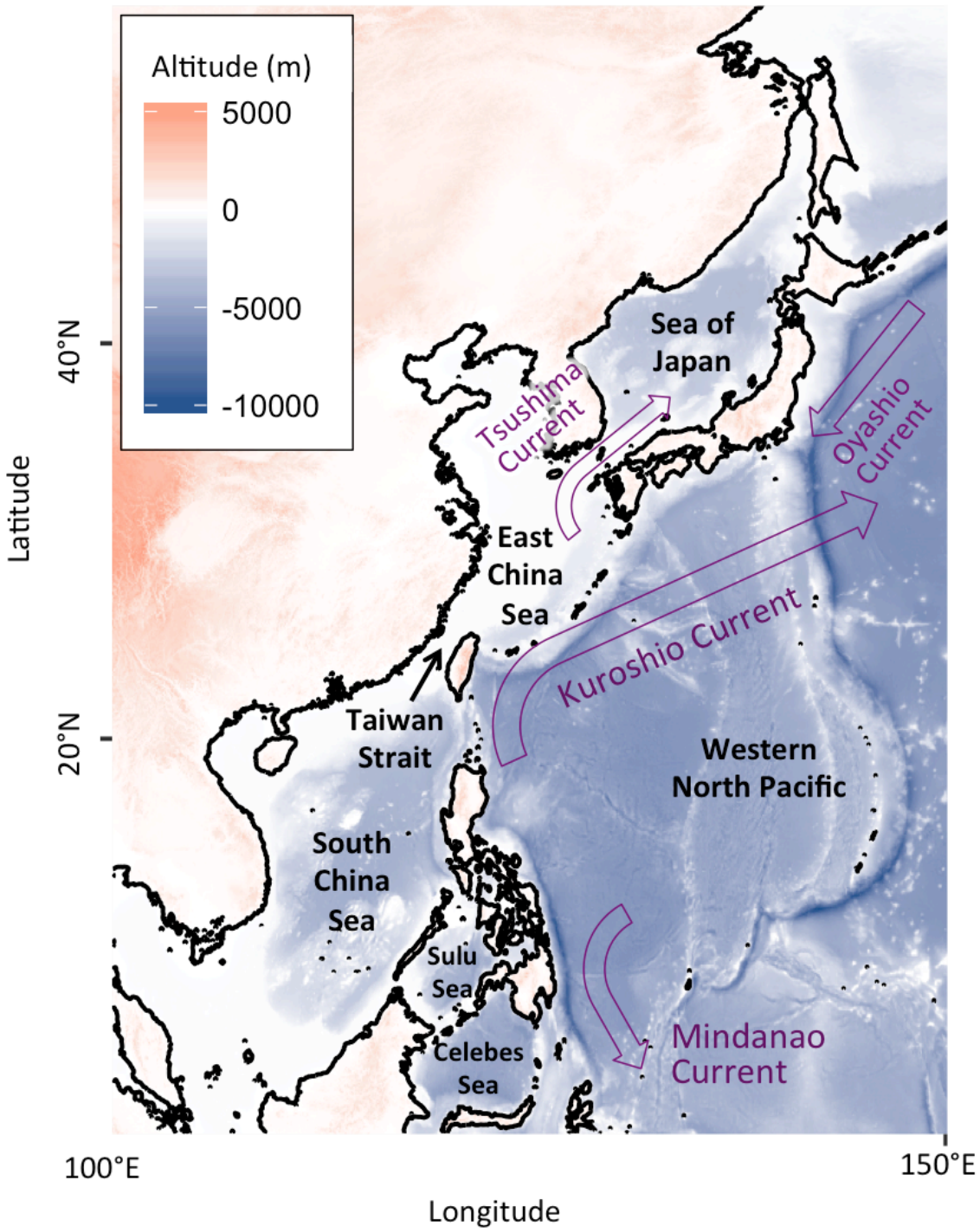


Figure 4.11. A map of eastern Asian waters showing the abyssal topography and the major surface ocean currents mentioned in this study.

The results further reveal that there may be another coastal CBD population in the vicinity of the Taiwan Strait (west Taiwan). Although its relationship with the CBD population from west Japan (in the Sea of Japan) is ambiguous, the differentiation pattern appears to be correlated with the distribution of shallow continental shelves (the western coast of Taiwan and Japan; the West Coast Cluster) and steep continental slopes (the eastern coasts of Taiwan and Japan; the East Coast Cluster) along the western coasts of the North Pacific Ocean (Fig. 4.11). The differentiation between 'inshore (shallow-water)' and 'offshore (deep-water)' populations is a typical scenario for CBD, and it has been attributed to habitat specialization, and perhaps is a result of independent colonization of the coastal populations (Hoelzel et al. 1998, Tezanos-Pinto et al. 2009, Moura et al. 2013, Louis et al. 2014b, Gaspari et al. 2015b, Lowther-Thieleking et al. 2015). However, for this West Coast Cluster, the samples were collected exclusively from the eastern side of the Taiwan Strait, but it is reasonable to expect this population is also distributed along the other side of the strait, and possibly further north in the East Sea or south to the South China Sea. This would be consistent with the geographic adjacency, the similarity of physical and biological environment, and the brisk activity of coastal currents in the region (Spalding et al. 2007, Cho et al. 2009), although further subdivision of this West Coast Cluster can be expected as discussed in the earlier paragraph. Earlier studies for bottlenose dolphins in the coastal region of the western North Pacific Ocean mainly focused on the ecologic, morphologic

and genetic differences between CBD and IPBD (Gao et al. 1995; Wang et al. 1999, 2000; Yang et al. 2005; Kurihara and Oda 2007), providing limited insight into the population differentiation within the CBD species. The study highlights the need for further careful investigations into CBD in this region, whether it is the distribution, habitat preferences, or behaviours of the dolphins to shed more light on the evolutionary mechanisms driving the CBD populations in Asian coastal waters to differentiate.

Conversely, there is insufficient evidence to determine the presence and the precise range of the 'Japanese offshore' (30—42°N, 145°E—180°) and the 'Southern offshore' (23—30°N, 127°E—180°) populations, the two offshore CBD populations proposed by Miyashita (1993). Since both of these 'offshore' populations are suggested as having an extensive range into the vicinity of the central North Pacific Ocean, one might suspect they are connected with, or even the same as, the populations in the central Pacific Ocean, given their geographic proximity. Martien et al. (2012) claim that the CBD inhabiting Hawaiian waters are isolated from other Pacific populations, and this seems to support this idea. However, the possibility cannot be excluded, that the CBD found around the Hawaiian Islands are in fact another coastal population, given that there have been a number of studies that suggest oceanic cetacean species established isolated coastal population(s) around the Hawaiian Islands (Andrews et al. 2010, Courbis et al. 2014, Martien et al. 2014). In addition, the FCA and Geneland results suggest a fairly distinct population of CBD inhabiting Philippine waters, though

the sample size is too small for strong inference, and one of the samples could possibly be a hybrid. Dolar et al. (2006) reported that the CBD population in central Philippine waters (Sulu Sea and the Tañon Strait) was found exclusively in shallow and intermediate waters inside of the shelf break. Although various lines of evidence indicate the possibility, more data are needed to resolve this question.

The dynamics of the CBD populations in the western North Pacific

The $N_e\mu$ estimated for the West Coast Cluster and the East Coast Cluster are only about 1/4—1/10 of the $N_e\mu$ estimated for the more pelagic central Pacific populations ($N_e\mu$=1.52—3.45; Martien et al. 2012). This agrees with an earlier report that suggests the N_e for coastal CBD populations tends to be smaller than the N_e for pelagic populations (Louis et al. 2014a). The calculation showed the N_e/N estimates for both the West Coast and East Coast Cluster are similar in magnitude, ranging between 0.042 and 0.118, and the range seems to agree with the estimate proposed by a meta-analysis of N_e/N for wildlife populations (N_e/N=0.1—0.11) (Frankham 1995). However, the actual N_e/N for both populations can be even smaller than what have been calculated, since the N used for this calculation did not include the CBD from Taiwanese waters and the Sea of Japan, while the N_e from genetic data did.

Low N_e/N implies a presence of biased reproductive success, biased sex ratio, a recent population bottleneck, or that the population is highly age-structured (Nunney 1993, Hedrick 2005, Charlesworth 2009). Biased reproductive success and biased sex ratio may be the causes of low N_e/N, as such bias appears to be common in cetacean species, including bottlenose dolphin (Krützen et al. 2004, Cerchio et al. 2005, Frasier et al. 2007, Green et al. 2011, Wiszniewski et al. 2012, Nichols et al. 2014). However, the low N_e/N can be a result of artifacts since 1) the program Migrate may end up suggesting an underestimated $N_e\mu$ if the population size is not consistent, namely when the size was increasing or fluctuating through times (Beerli 2009), and 2) the N estimate reported in earlier studies was estimated based on a rather basic transect line survey method, which is now known to be insufficient for estimating marine mammal abundance (Alpizar-Jara and Pollock 1996, Okamura et al. 2012). The negative Tajima's D and Fu's Fs estimates and the decent fitting to the unimodal population expansion models for the East Japan (the East Coast Cluster) and the Southeast China (the West Coast Cluster) populations can be regarded as a sign of population expansion in the past, and therefore suggesting the population size is inconsistent through time. Conversely, the Tajima's D and Fu's Fs estimates are statistically insignificant and the mismatch distribution appears to be multimodal in either the demographic or spatial model, that suggests the population expansion, if it ever happened, was mild and progressive, and therefore the influence of an inconsistent population size to the $N_e\mu$

may be limited. Nevertheless, further analyses using other coalescent computer programs assuming no constant population size, such as LARMAC (Kuhner 2006), IMa2 or IMa2p (Hey 2010, Sethuraman and Hey 2015), and BEAST (Drummond and Rambaut 2007), would be useful to confirm the accuracy of $N_e\mu$ estimates and the impact of population size change in the past (Beerli 2009).

Possible mechanisms that shape CBD population structure in the western North Pacific

The Migrate analysis suggests that long-term gene flow between the East and West Coast Clusters is limited to less than one migrant per generation. On the other hand, the GeneClass analysis identified three contemporary first-generation migrants in both West and East Coast Clusters, suggesting the presence of on-going gene flow between the two populations. The Kuroshio Current could play an important role in promoting gene flow, given that the current itself and its branch currents constantly drive the surface waters in and out the shallow coastal region (Cho et al. 2009, Matsuno et al. 2009, Jan et al. 2010), and it has been suggested that the movement of CBD can be driven by the flow of the Kuroshio Current (Tanaka 1987). If this is correct, the interchange of CBD between the two populations may be a contemporary phenomenon, because during the glacial period the influence of the Kuroshio Current on the coasts of the eastern Asian

Continent was weakened as the flows to the East China Sea and South China Sea were limited (Ijiri et al. 2005, Jiang et al. 2006). The Tsushima Warm Current, a branch of Kuroshio Current carrying warm water into the Sea of Japan through the Tsushima Strait, was even suspended during the Last Glacial Maximum (Itaki et al. 2004). Therefore the oceanography of the region may not have promoted connectivity during the glacial period as much as today, and the lack of warmer water introduced by the Kuroshio current from the south could have generated contrasting physical conditions between the shallower western coastal and the deep eastern continental slope habitats. This habitat distinction may have further reduced connectivity during that period. Low levels of gene flow have been reported between the coastal and pelagic populations in the eastern North Atlantic Ocean (Louis et al. 2014a), and among the regional populations around the Hawaiian Islands (Martien et al. 2012), and it is hypothesised as a result of assortative mating, due to the constrained preference of natal habitat, specialised diet, and possibly, cultural familiarity (Hoelzel et al. 1998; Möller et al. 2007; Cantor and Whitehead 2013; Louis et al. 2014a, b). In the case of this study, although the strong correspondence between population structure and contrasting oceanographic features (*i.e.*, shallow continental shelves *versus* sharp continental slopes) implies population-specific resource preference, it seems more likely to be a result of historic isolation (possibly during the glacial period) than a result of deliberate assortative mating.

Sympatric relationship between IPBD and CBD

The mtDNA data agree with previous studies (Wang et al. 1999, Kakuda et al. 2002, Natoli et al. 2004, Yang et al. 2005, Kita et al. 2013, Moura et al. 2013) showing clear phylogenetic differentiation between IPBD and CBD. The microsatellite data also exhibit a distinct difference between IPBD and CBD. On the other hand, the microsatellite data also provide some indication of limited gene flow between these two species, indicating three individuals that might have hybrid ancestry. However, the possibility cannot be eliminated, that the interbreeding was between CBD and other delphinid species that are closely related to IPBD but live sympatrically with the CBD, such as pantropical spotted dolphins (*Stenella attenuata*), striped dolphins (*S. coeruleoalba*), spinner dolphins (*S. longirostris*), common dolphins (*Delphinus* spp.), and Fraser's dolphins (LeDuc et al. 1999, Kingston et al. 2009, Möller et al. 2008, McGowen 2011, Amaral et al. 2012). This has the potential to produce misleading results when the signal for hybridisation is weak, as in the case of the study. In fact, among four potential hybridisations between the *Tursiops* congeneric species (three from this study and one from Martien et al. 2012), one of the putative hybrid animals was similar in appearance to Fraser's dolphin, and two (one from eastern Taiwan and the other from Hawaii) were sampled from a region where the occurrence of IPBD has never been documented (Yang et al. 1999, Chou 2007, Baird et al. 2013). Since there is evidence of polyphyly among the *Tursiops-Stenella-Delphinius* complex of species

(LeDuc et al. 1999, Kingston et al. 2009, McGowen 2011, Amaral et al. 2012), the evidence for hybridisation therefore needs to be interpreted carefully.

It has been proposed that there are at least six IPBD populations in Japanese waters (Amano 2007, Brownell and Funahashi 2013). Kakuda et al. (2002) studied the genetic structure of IPBD from Mikura Island (about 200km south of Tokyo) using mtDNA control region sequences and concluded that the dolphins were genetically similar to the IPBD in Taiwanese waters. Hayano (2013) used the same genetic marker and reported a clear population differentiation among Mikura Island, Amakusa, Amami and Ogasawa Islands. The residency of Amakusa, Mikura Island, and Kagoshima Bay populations has been proposed based on photo-identification analysis (Shirakihara *et al*. 2002, Kogi *et al*. 2004, Nanbu *et al*. 2006). Morisaka et al. (2005) found significant geographic variation in the whistles among dolphin populations around Amakusa, Mikura Island and Ogasawa Islands. In Taiwanese waters, the distribution of IPBD is seemly discontinuous: current field observations and records of fishery interactions showed that this species aggregates around the Penghu archipelago (in the Taiwan Strait, west of Taiwan), and the coastal waters off Nan-Wan, southeast of Taiwan (Wang et al. 1999, Wang 2000).

The genetic data for IPBD were obtained from three putative aggregation sites in Taiwanese and Japanese waters: Amakusa (western Japan), Mikura Island (eastern Japan) and western Taiwan; and the FCA result showed distinct clustering for those

samples. The data may be consistent with the population structure for IPBD in coastal Japanese and Taiwanese waters proposed by Hayano (2013), but to verify the hypothesis, further examination using more samples from the same and further sites is necessary.

Conservation implications

The results indicate that there are at least two populations of CBD distributed parapatrically in the coastal waters around Taiwan and Japan, corresponding to the distribution of shallow continental shelf or deep continental slope habitats. Although the analyses detected some recent immigration activities, the long-term estimates show limited gene flow between the two populations. This potentially agrees with earlier analyses that show habitat specialisation plays an important role in differentiating inshore and offshore populations (Hoelzel et al. 1998, Möller et al. 2007, Louis et al. 2014b). In addition, the two populations are perhaps confronted by different forms of anthropogenic threats. It is therefore justifiable to manage them as separate CBD populations.

One of the most acknowledged threats to the CBD in the coastal waters of the western North Pacific Ocean is the small-scale whaling fishery, which regularly operated in Japanese waters on a yearly basis (Perrin et al. 2005, Kasuya 2007, Kasuya

2011). The small-scale whaling appears to target the East Coast Cluster, since the catch of CBD today is limited to three prefectures along the Pacific coasts of Japan, namely Wakayama, Shizuoka and Okinawa (Kasuya 2011). The intensified whale watching activities along the east coast of Taiwan, if unregulated, could also create unnecessary harassment to this population (Hoyt 2001, Perrin et al. 2005, Parsons 2012). For the West Coast Cluster CBD, as well as for the IPBD, it is foreseeable that habitat loss/degradation, pollutant accumulation, acoustic disturbances and entanglement in fisheries would be the main threats, as such impacts have been identified for other coastal cetacean species, *i.e*., the Indo-Pacific humpback dolphin (*Sousa chinensis*) and finless porpoise (*Neophocaena phocaenoides*), in the same region (Perrin et al. 2005, Jefferson et al. 2009, Choi et al. 2013, Slooten et al. 2013). An earlier study suggests that regional genetic diversity for IPBD in Japanese waters is low (Hayano 2013) which means the IPBD populations can be more vulnerable, regarding some IPBD populations are known to be disturbed by whale watching activities (Matsuda et al. 2011). In addition, the interaction between IPBD and the West Coast Cluster CBD may also be a factor influencing the population trend of both IPBD and CBD, since the analysis shows that IPBD and the West Coast Cluster CBD likely co-exist in the coastal regions along the eastern Asian Continent and can be competing for the same (or similar) habitat resource. It is therefore proposed that further investigations on the CBD and IPBD in

this region are certainly needed, in order to strengthen the basis of scientific knowledge and consequently to provide more useful information for conservation management.

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Appendices

Appendix 4.1. List of the samples acquired for this study. Note not all sample in this list were used in the study. The samples analysed are indicated as 'Y' in 'MS' (microsatellite genotyping) and 'mtDNA' (mtDNA haplotype) columns. Abbreviations for the Contributors: Es-Bank, the Center for Enviornmental Studies at Ehime University (Japan); NTU, National Taiwan University; SWFSC, Southwest Fisheries Science Center (USA).

Appendix 4.2. Sample information for the common bottlenose dolphin (CBD) and Indo-Pacific bottlenose dolphin (IPBD) samples used in the mtDNA analyses in this study.

Appendix 4.3. Presence of null alleles, number of alleles, allelic richness, inbreeding coefficient (F_{IS}), observed heterozyogsity (H_O) and expected heterozygosity (H_E) for the 24 microsatellite loci in the bottlenose dolphins examined in this study. The loci marked by asterisk are discarded from further analyses.

Appendix 4.4. Polymorphic sites in the bottlenose dolphin mtDNA control region haplotypes. The dot indicates identical site to the top sequence and the dash indicates an insertion–deletion event. The number in the top row indicates the position of the variable site in the 388bp sequence.

Appendix 4.1.

Longitute	Latitiute	ID	Species	Location	Population	Sample Source	Contributor	Year	Sex	MS	mtDNA
135.950142	33.593888	EW01297	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1986	M	Y	TtHap_1
139.558056	33.844444	EW01354	*Tursiops aduncus*	Mikurajima, Tokyo	East Japan	Stranding	Es-Bank	2001	M	Y	TaHap_66
130.391667	32.612222	EW04792	*Tursiops aduncus*	Off Habojima Is., Kami-amakusa, Kumamoto	Sea of Japan	Stranding	Es-Bank	2004	M	Y	TaHap_78
130.114722	32.558889	EW04898	*Tursiops aduncus*	Off Tsuhji-shima Is., Amakusa, Kumamoto	Sea of Japan	Stranding	Es-Bank	2007	M	Y	TaHap_78
119.596992	23.571382	487	*Tursiops aduncus*	Penghu	Taiwan	Bycatch	NTU	2000	F	Y	TaHap_72
121.674164	25.202864	790	*Tursiops aduncus*	Taipei	Taiwan	Stranding	NTU	2005	M	Y	TaHap_71
120.902556	24.808667	1112	*Tursiops aduncus*	Hsinchu	Taiwan	Stranding	NTU	2004	M	Y	TaHap_72
120.858794	24.702706	TSDU04	*Tursiops aduncus*	Miaoli	Taiwan	Stranding	NTU	2011	M	Y	TaHap_74
127.788611	26.482778	EW01286	*Tursiops truncatus*	Onna, Okinawa	East Japan	Captive	Es-Bank	2003	F	Y	N/A
138.786389	38.06	EW01338	*Tursiops truncatus*	Niigata	East Japan	Captive	Es-Bank	1993	F	Y	N/A
138.903889	34.600833	EW01342	*Tursiops truncatus*	Shimoda, Shizuoka	East Japan	Captive	Es-Bank	1995	M	Y	N/A
138.903889	34.600833	EW01344	*Tursiops truncatus*	Shimoda, Shizuoka	East Japan	Captive	Es-Bank	1998	F	Y	N/A
139.472889	35.297064	EW01345	*Tursiops truncatus*	Eno-shima Island, Kanagawa	East Japan	Captive	Es-Bank	1999	F	Y	N/A
138.903889	34.600833	EW01347	*Tursiops truncatus*	Shimoda, Shizuoka	East Japan	Captive	Es-Bank	1999	F	Y	N/A
138.903889	34.600833	EW01348	*Tursiops truncatus*	Shimoda, Shizuoka	East Japan	Captive	Es-Bank	1999	F	Y	N/A
138.903889	34.600833	EW01349	*Tursiops truncatus*	Shimoda, Shizuoka	East Japan	Captive	Es-Bank	1999	F	Y	N/A
138.903889	34.600833	EW01350	*Tursiops truncatus*	Shimoda, Shizuoka	East Japan	Captive	Es-Bank	2000	F	Y	N/A
138.903889	34.600833	EW01351	*Tursiops truncatus*	Shimoda, Shizuoka	East Japan	Captive	Es-Bank	2000	F	Y	N/A
139.472889	35.297064	EW01356	*Tursiops truncatus*	Eno-shima Island, Kanagawa	East Japan	Captive	Es-Bank	2002	F	Y	N/A
138.903889	34.600833	EW04601	*Tursiops truncatus*	Shimoda, Shizuoka	East Japan	Captive	Es-Bank	2004	F	Y	N/A
138.903889	34.600833	EW04602	*Tursiops truncatus*	Shimoda, Shizuoka	East Japan	Captive	Es-Bank	2004	F	Y	N/A
138.903889	34.600833	EW04603	*Tursiops truncatus*	Shimoda, Shizuoka	East Japan	Captive	Es-Bank	2004	F	Y	N/A
138.730833	34.551667	EW04604	*Tursiops truncatus*	nakaki, minamizu, sizuoka	East Japan	Captive	Es-Bank	2004	M	Y	N/A
135.950142	33.593888	EW01288	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1986	M	Y	TtHap_2

Longitute	Latitiute	ID	Species	Location	Population	Sample Source	Contributor	Year	Sex	MS	mtDNA
135.950142	33.593888	EW01289	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1986	F	Y	N/A
135.950142	33.593888	EW01290	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1986	M	Y	TtHap_2
135.950142	33.593888	EW01291	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1986	M	Y	TtHap_3
135.950142	33.593888	EW01292	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1986	F	Y	N/A
135.950142	33.593888	EW01294	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1986	M	Y	TtHap_2
135.950142	33.593888	EW01295	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1986	F	Y	TtHap_5
135.950142	33.593888	EW01296	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1986	F	Y	TtHap_6
135.950142	33.593888	EW01299	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1986	M	Y	TtHap_7
135.950142	33.593888	EW01300	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1986	F	Y	TtHap_2
135.950142	33.593888	EW01302	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1986	M	Y	TtHap_4
135.950142	33.593888	EW01339	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1994	F	Y	N/A
135.950142	33.593888	EW01340	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1994	F	Y	N/A
137.502694	35.032933	EW04600	*Tursiops truncatus*	Aichi	East Japan	Stranding	Es-Bank	2004	M	Y	N/A
138.824583	34.613889	EW04825	*Tursiops truncatus*	Nakagi, Minami-izu, Sizuoka	East Japan	Stranding	Es-Bank	2005	F	Y	N/A
131.462222	31.806944	EW04842	*Tursiops truncatus*	Aoshima, Miyazaki	East Japan	Stranding	Es-Bank	2006	M	Y	N/A
135.950142	33.593888	EW05121	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1986	F	Y	TtHap_4
135.950142	33.593888	EW05122	*Tursiops truncatus*	Taiji, Wakayama	East Japan	Whaling	Es-Bank	1986	F	Y	TtHap_5
136.998069	36.795811	EW01352	*Tursiops truncatus*	Himi, Toyama	Sea of Japan	Stranding	Es-Bank	2001	F	Y	N/A
137.519167	37.700556	EW01353	*Tursiops truncatus*	Suzu, Ishikawa	Sea of Japan	Stranding	Es-Bank	2001	M	Y	N/A
139.337778	38.524167	EW01355	*Tursiops truncatus*	Tsuruoka, Yamagata	Sea of Japan	Stranding	Es-Bank	2001	F	Y	N/A
138.057558	37.022864	EW01357	*Tursiops truncatus*	Joetsu, Niigata	Sea of Japan	Stranding	Es-Bank	2002	F	Y	N/A
120.168281	23.485762	186	*Tursiops truncatus*	Chiayi	Taiwan	Seized	NTU	2001	M	Y	TtHap_2
120.168281	23.485762	187	*Tursiops truncatus*	Chiayi	Taiwan	Seized	NTU	2001	F	Y	TtHap_2
120.028194	23.105722	204	*Tursiops truncatus*	Tainan	Taiwan	Stranding	NTU	2003	M	Y	TtHap_2
120.20124	23.719825	270	*Tursiops truncatus*	Yunling	Taiwan	Seized	NTU	2002	M	Y	TtHap_2
120.20124	23.719825	271	*Tursiops truncatus*	Yunling	Taiwan	Seized	NTU	2002	F	Y	TtHap_8

Longitute	Latitiute	ID	Species	Location	Population	Sample Source	Contributor	Year	Sex	MS	mtDNA
120.20124	23.719825	274	*Tursiops truncatus*	Yunling	Taiwan	Seized	NTU	2002	F	Y	TtHap_9
120.20124	23.719825	280	*Tursiops truncatus*	Yunling	Taiwan	Seized	NTU	2002	F	Y	TtHap_16
121.909278	25.097167	282	*Tursiops truncatus*	Taipei	Taiwan	Stranding	NTU	2002	M	Y	TtHap_10
120.20124	23.719825	283	*Tursiops truncatus*	Yunling	Taiwan	Seized	NTU	2002	M	Y	TtHap_11
120.20124	23.719825	286	*Tursiops truncatus*	Yunling	Taiwan	Seized	NTU	2002	M	Y	TtHap_12
120.20124	23.719825	295	*Tursiops truncatus*	Yunling	Taiwan	Seized	NTU	2002	F	Y	TtHap_13
119.596992	23.571382	459	*Tursiops truncatus*	Penghu	Taiwan	Bycatch	NTU	2000	M	Y	TtHap_2
119.596992	23.571382	461	*Tursiops truncatus*	Penghu	Taiwan	Bycatch	NTU	2000	F	Y	TtHap_2
120.20124	23.719825	467	*Tursiops truncatus*	Yunling	Taiwan	Seized	NTU	2002	F	Y	TtHap_14
120.20124	23.719825	481	*Tursiops truncatus*	Yunling	Taiwan	Seized	NTU	2002	F	Y	TtHap_2
121.507673	23.494786	496	*Tursiops truncatus*	Hualien	Taiwan	Bycatch	NTU	2004	M	Y	TtHap_15
121.507673	23.494786	499	*Tursiops truncatus*	Hualien	Taiwan	Bycatch	NTU	2004	M	Y	TtHap_17
121.507673	23.494786	611	*Tursiops truncatus*	Hualien	Taiwan	Bycatch	NTU	2001	F	Y	TtHap_17
121.507673	23.494786	660	*Tursiops truncatus*	Hualien	Taiwan	Bycatch	NTU	2000	F	Y	TtHap_17
121.693361	25.204417	824	*Tursiops truncatus*	Taipei	Taiwan	Stranding	NTU	2004	F	Y	TtHap_2
121.361847	25.145481	840	*Tursiops truncatus*	Taipei	Taiwan	Stranding	NTU	2005	M	Y	TtHap_16
120.579583	24.393361	870	*Tursiops truncatus*	Taichung	Taiwan	Stranding	NTU	2004	M	Y	TtHap_14
120.772661	24.652847	1014	*Tursiops truncatus*	Miaoli	Taiwan	Stranding	NTU	2005	M	Y	TtHap_9
121.707272	25.165094	1070	*Tursiops truncatus*	Keelung	Taiwan	Stranding	NTU	2007	F	Y	TtHap_14
120.707917	24.573194	1107	*Tursiops truncatus*	Miaoli	Taiwan	Stranding	NTU	2004	M	Y	TtHap_8
122.005556	25.014217	TSDU01	*Tursiops truncatus*	Taipei	Taiwan	Stranding	NTU	2010	M	Y	TtHap_14
121.633833	25.240872	TSDU02	*Tursiops truncatus*	Taipei	Taiwan	Stranding	NTU	2010	M	Y	TtHap_10
120.08606	23.268242	TSDU09	*Tursiops truncatus*	Tainan	Taiwan	Stranding	NTU	2012	F	Y	N/A
123.5	9.5	2646	*Tursiops truncatus*	Philippines	Philippines	Bycatch	SWFSC	1993	F	Y	N/A
123.549135	9.568629	5560	*Tursiops truncatus*	Siaton	Philippines	Fishery?	SWFSC	1994	F	Y	TtHap_1
N/A	N/A	730	*Tursiops truncatus*	Taipei	Taiwan	Stranding	NTU	2002	U	N	TtHap_2
N/A	N/A	853	*Tursiops truncatus*	Miaoli	Taiwan	Stranding	NTU	2005	U	N	TtHap_18

Appendix 4.2.

Population	Location	Year	n (sampled/ used)	Sampling source	Presence of parent-offspring pairs	GenBank Assession No.	References
Common bottlenose dolphin (CBD), *Tursiops truncatus*							
East Japan	Taiji, Japan	1986	12/12	DF	Yes	TBD	This study
	Taiji, Japan	2005	165/148	DF	Yes	AB303154-74	Kita et al. 2013
Northeast China	Zhoushan, China	NA	8/8	FI	N/A	AF355585-86, AF459509, AF459513-14, AF459522	Yang et al. 2005
	Lianyungang, China	NA	5/5	FI	N/A	AF459510, AF459512, AF459515	Yang et al. 2005
	Qingdiao, China	NA	1/1	FI	N/A	AF355587	Yang et al. 2005
Southeast China	Hong Kong	1994	3/3	S	N/A	AF056220-21	Wang et al. 1999
	Philippines	1994	1/1	FI	No	TBD	This study
	Taiwan	1994-96, 2000-12	43/43	FI, S	N/A	AF056223-32, TBD	Wang et al. 1999, this study
	Dong Shan, China	NA	2/2	FI	N/A	AF459511, AF459523	Yang et al. 2005
Hawaii	Hawaii, HI	2000-06	22/22	BI	Yes (adjusted)	EF672700-23, EF672725	Martien et al. 2012
	Four-Islands, HI	2000-06	26/26	BI	Yes (adjusted)	EF672700-23, EF672726	Martien et al. 2012
	Oahu, HI	2000-06	30/30	BI	Yes (adjusted)	EF672700-23, EF672727	Martien et al. 2012
	Kauai, HI	2000-06	41/41	BI	Yes (adjusted)	EF672700-23, EF672728	Martien et al. 2012
Palmyra	Palmyra, HI	2000-06	11/11	BI	Yes (adjusted)	EF672700-23, EF672729	Martien et al. 2012
Total			370/353				
Indo-Pacific bottlenose dolphin (IPBD), *Tursiops aduncus*							
Japan	Mikura Island	2001	1/1	S	No	TBD	This study
	Amakusa	2004, 2007	2/2	S	No	TBD	This study
South China	Beihai, China	1995	6/6	FI	N/A	AF056233-34, AF459520	Wang et al. 1999, Yang et al. 2005
Southeast China	Taiwan	1994-2011	18/18	FI, S, C	N/A	AF056233-36, AF056239-43, TBD	Wang et al. 1999; this study
	Xiamen, China	TBD	5/5	FI	N/A	AF056233, AF056239-40	Wang et al. 1999, Yang et al. 2005
	Dong Shan, China	TBD	6/6	FI	N/A	TBD	Yang et al. 2005
Indonesia	Unknown location	NA	2/2	C	N/A	AF056237-38	Wang et al. 1999
Total			40/40				

Appendix 4.3.

Species	Common bottlenose dolphin								Common bottlenose dolphin							
Population	West Japan								East Japan							
n	4								32							
Locus	Null alleles	No. of alleles	Allelic richness	FIS	HO	HE	P	SD	Null alleles	No. of alleles	Allelic richness	FIS	HO	HE	P	SD
D14	no	5	1.857	0.455	0.5	0.857	0.09	0.03%	no	7	1.738	-0.017	0.75	0.738	0.58	0.05%
D22	no	5	1.857	-0.2	1	0.857	0.66	0.04%	no	10	1.862	0.205	0.688	0.860	0.12	0.06%
Dde59	no	2	1.429	-0.2	0.5	0.429	1	0.00%	no	6	1.771	0.233	0.594	0.771	0.22	0.05%
Dde65	no	4	1.786	-0.333	1	0.786	1	0.00%	no	6	1.801	-0.015	0.813	0.801	0.8	0.03%
Dde66	no	2	1.429	-0.2	0.5	0.429	1	0.00%	no	8	1.684	0.087	0.625	0.684	0.51	0.05%
Dde69	no	3	1.607	-0.286	0.75	0.607	1	0.00%	no	5	1.752	0.046	0.719	0.752	0.56	0.05%
Dde70	no	4	1.821	0.429	0.5	0.821	0.32	0.04%	no	16	1.921	-0.018	0.938	0.921	0.96	0.02%
Dde72*	no	4	1.75	0.368	0.5	0.75	0.31	0.05%	yes	15	1.899	0.448	0.5	0.899	0	0.00%
Dde84	no	5	1.857	0.455	0.5	0.857	0.09	0.03%	no	7	1.791	0.012	0.781	0.791	0.55	0.04%
EV14	no	5	1.893	0.182	0.75	0.893	0.47	0.05%	no	14	1.922	-0.052	0.969	0.922	0.41	0.04%
EV37*	no	5	1.857	0.455	0.5	0.857	0.08	0.03%	yes	16	1.909	0.212	0.719	0.909	0.02	0.01%
KWM12a*	no	4	1.786	0.053	0.75	0.786	1	0.00%	yes	9	1.782	0.344	0.516	0.782	0	0.00%
KWM1b	no	2	1.429	-0.2	0.5	0.429	1	0.00%	no	3	1.542	-0.097	0.594	0.542	0.61	0.05%
KWM2a	no	5	1.857	0.143	0.75	0.857	0.65	0.05%	no	7	1.758	0.011	0.75	0.758	0.77	0.04%
KWM2b	no	2	1.25	0	0.25	0.25	1	0.00%	no	5	1.46	0.187	0.375	0.460	0.17	0.03%
KWM9b	no	4	1.75	0	0.75	0.75	1	0.00%	no	5	1.714	0.082	0.656	0.714	0.36	0.05%
MK3	no	7	1.964	-0.043	1	0.964	1	0.00%	no	10	1.86	0.019	0.844	0.860	0.33	0.04%
MK5	no	5	1.857	-0.2	1	0.857	0.66	0.05%	no	12	1.843	-0.039	0.875	0.843	0.94	0.02%
Sco11	no	4	1.821	0.1	0.75	0.821	0.77	0.04%	no	8	1.82	-0.068	0.875	0.820	0.56	0.04%
Sco28	no	2	1.25	0	0.25	0.25	1	0.00%	no	4	1.282	0.338	0.188	0.282	0.02	0.01%
Sco55	no	1	1	NA	NA	NA	NA	NA	no	2	1.411	-0.222	0.5	0.411	0.38	0.05%
TexVet7	no	4	1.75	0	0.75	0.75	1	0.00%	no	5	1.614	-0.175	0.719	0.614	0.55	0.05%
AAT44	no	4	1.75	-0.412	1	0.75	1	0.00%	no	7	1.757	-0.033	0.781	0.757	0.91	0.03%
TexVet5*	no	5	1.933	-0.091	1	0.933	1	0.00%	no	8	1.853	0.002	0.852	0.853	0.39	0.04%
Mean		4	1.689	0.055	0.685	0.719				8.125	1.739	0.064	0.692	0.739		
SD		1.314	0.254		0.241	0.212				3.971	0.167		0.184	0.167		

Species	Common bottlenose dolphin								Indo-Pacific bottlenose dolphin							
Population	Taiwan								All samples							
n	28								7							
Locus	Null alleles	No. of alleles	Allelic richness	F_{IS}	H_O	H_E	P	SD	Null alleles	No. of alleles	Allelic richness	F_{IS}	H_O	H_E	P	SD
D14	no	7	1.788	0.049	0.750	0.788	0.325	0.04%	no	4	4	0.4	0.429	0.692	0.136	0.12%
D22	no	9	1.849	0.076	0.786	0.849	0.479	0.04%	yes	3	3	0.714	0.143	0.473	0.020	0.04%
Dde59	no	7	1.745	0.091	0.679	0.745	0.078	0.03%	no	4	4	0.25	0.571	0.747	0.226	0.14%
Dde65	no	7	1.832	0.057	0.786	0.832	0.612	0.05%	no	5	5	0.226	0.571	0.725	0.567	0.15%
Dde66	no	5	1.686	0.169	0.571	0.686	0.376	0.05%	no	3	3	-0.021	0.571	0.560	1.000	0.00%
Dde69	no	5	1.714	0.203	0.571	0.714	0.014	0.01%	no	4	4	-0.111	0.714	0.648	1.000	0.00%
Dde70	no	13	1.906	0.015	0.893	0.906	0.806	0.04%	no	4	4	0.094	0.571	0.626	1.000	0.00%
Dde72*	no	14	1.914	0.063	0.857	0.914	0.003	0.01%	no	5	5	0.314	0.571	0.813	0.472	0.17%
Dde84	no	6	1.768	0.023	0.750	0.768	0.328	0.04%	no	3	3	0.094	0.571	0.626	0.515	0.15%
EV14	no	12	1.856	0.083	0.786	0.856	0.394	0.05%	no	4	4	0.442	0.286	0.495	0.165	0.13%
EV37*	no	15	1.871	0.1	0.786	0.871	0.002	0.00%	yes	4	4	0.593	0.286	0.670	0.026	0.04%
KWM12a*	no	7	1.764	-0.029	0.786	0.764	0.043	0.03%	no	4	4	0.048	0.714	0.747	1.000	0.00%
KWM1b	no	3	1.6	-0.073	0.643	0.600	0.682	0.04%	no	2	2	0.143	0.429	0.495	1.000	0.00%
KWM2a	no	9	1.858	0.043	0.821	0.858	0.073	0.02%	no	5	5	0.324	0.571	0.824	0.415	0.14%
KWM2b	no	5	1.61	-0.114	0.679	0.610	0.363	0.05%	no	1	1	NA	NA	NA	NA	NA
KWM9b	no	7	1.715	-0.152	0.821	0.715	0.111	0.03%	no	3	3	0.143	0.571	0.659	0.675	0.15%
MK3	no	11	1.87	-0.068	0.929	0.870	0.869	0.04%	no	5	5	0.324	0.571	0.824	0.414	0.16%
MK5	no	10	1.877	-0.019	0.893	0.877	0.724	0.05%	no	4	4	0.262	0.571	0.758	0.063	0.08%
Sco11	no	6	1.692	0.229	0.536	0.692	0.034	0.02%	no	4	4	-0.355	1.000	0.758	0.513	0.16%
Sco28	no	4	1.355	-0.151	0.407	0.355	1	0.00%	no	2	2	0.143	0.429	0.495	1.000	0.00%
Sco55	no	2	1.103	-0.038	0.107	0.103	1	0.00%	no	2	2	-0.2	0.429	0.363	1.000	0.00%
TexVet7	no	7	1.681	-0.051	0.714	0.681	0.318	0.04%	no	3	3	0	0.714	0.714	0.362	0.17%
AAT44	no	7	1.794	-0.035	0.821	0.794	0.326	0.04%	no	2	2	-0.364	0.714	0.538	0.506	0.15%
TexVet5*	no	10	1.869	-0.069	0.929	0.869	0.721	0.05%	no	4	4	-0.034	0.714	0.692	0.850	0.10%
Mean		7.833	1.738	0.024	0.721	0.738				3.5	3.5	0.16	0.553	0.650		
SD		3.384	0.184		0.184	0.184				1.103	1.103		0.179	0.126		

Appendix 4.4

	Variable site																										
Haplotype ID	6	9	18	26	29	43	48	49	57	69	71	75	76	77	80	83	85	87	89	90	92	93	94	95	96	100	102
TtHap 1	G	A	C	C	C	G	T	T	A	G	A	A	T	T	A	T	C	T	C	A	A	T	G	C	G	G	T
TtHap 2	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 3	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	T	.	.	.
TtHap 4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.
TtHap 6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 7	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 10	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
TtHap 11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 16	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	A	.
TtHap 17	A	.	T	.	T	A	.	.	.	.	G	.	.	.	.	.	.	.	T	.	.	.	.	T	A	.	.
TtHap 18	.	.	T	.	.	A	.	.	.	.	.	G	C	C	.	C	.	G	.	.	.	C	A	T	.	.	.
TtHap 19	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 21	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 22	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 23	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	T	.	.	.
TtHap 24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 25	A	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	.	.	T	A	.	C
TtHap 26	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	T	.	.	.
TtHap 27	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 28	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 29	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 30	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 31	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 32	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 33	A	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	.	.	T	A	.	C
TtHap 34	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 35	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	A	.
TtHap 36	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

	Variable site																											
Haplotype ID	6	9	18	26	29	43	48	49	57	69	71	75	76	77	80	83	85	87	89	90	92	93	94	95	96	100	102
TtHap 37	.	.	.	G	T	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 38	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 39	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 40	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
TtHap 41	.	G	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	A	.
TtHap 42	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 43	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 44	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
TtHap 45	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	C	.	T	.	.	.
TtHap 46	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 47	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 48	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	A	.
TtHap 49	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
TtHap 50	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	A	.
TtHap 51	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
TtHap 52	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 53	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 54	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 55	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	T	.	.	.
TtHap 56	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.
TtHap 57	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.
TtHap 58	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
TtHap 59	A	.	T	.	T	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	T	A	A	.
TtHap 60	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	A	.
TtHap 61	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	T	.	.	.
TtHap 62	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	A	.
TtHap 63	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
TtHap 64	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	A	.

(Continues)

	Variable site																										
Haplotype ID	115	130	135	136	140	144	154	157	179	180	191	199	201	202	204	208	231	234	237	250	251	252	255	258	260	261	263
TtHap 1	-	T	T	A	A	T	T	G	A	T	T	A	T	A	C	A	T	G	C	T	C	T	C	A	C	A	T
TtHap 2	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
TtHap 3	-	.	.	.	.	.	.	.	.	C	C	.	.	.	T	.	.	.	.	.	.	C	.	.	A	.	.
TtHap 4	-	.	.	G	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 5	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
TtHap 6	-	.	.	G	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 7	-	G	.	.	G	G	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
TtHap 9	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 10	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.
TtHap 11	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 12	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 13	-	.	.	G	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 14	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 15	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 16	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C
TtHap 17	T	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	T	.	A	.	.	.	.	.	.
TtHap 18	-	.	C	.	.	.	.	.	G	.	C	.	.	.	.	.	C	.	T	.	A	.	.	G	.	.	.
TtHap 19	-	.	.	G	.	.	.	.	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 20	-	.	.	G	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 21	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 22	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 23	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	G	.
TtHap 24	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
TtHap 25	-	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	.	.	A	.	.	.	.	.	.
TtHap 26	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	G	.
TtHap 27	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
TtHap 28	-	.	.	G	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
TtHap 29	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 30	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
TtHap 31	-	.	.	G	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 32	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 33	-	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	.	.	A	.	.	.	.	.	.
TtHap 34	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

	Variable site																										
Haplotype ID	115	130	135	136	140	144	154	157	179	180	191	199	201	202	204	208	231	234	237	250	251	252	255	258	260	261	263
TtHap 35	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C
TtHap 36	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
TtHap 37	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
TtHap 38	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
TtHap 39	-	.	.	G	.	.	.	.	.	.	C	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
TtHap 40	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.
TtHap 41	-	.	.	.	.	.	.	.	T	.	C	.	.	.	.	T	.	.	.	C	.	.	.	.	.	.	C
TtHap 42	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
TtHap 43	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 44	-	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.
TtHap 45	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
TtHap 46	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
TtHap 47	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 48	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
TtHap 49	-	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	T	.	T	.	.	.	.
TtHap 50	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
TtHap 51	-	.	C	.	.	.	.	A	.	.	C	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.
TtHap 52	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	A
TtHap 53	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
TtHap 54	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TtHap 55	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	G	.	.	.
TtHap 56	-	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
TtHap 57	-	.	C	.	.	.	.	.	.	.	C	.	C	.	.	.	C	.	.	.	.	.	.	.	.	.	.
TtHap 58	-	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.
TtHap 59	-	.	.	.	.	.	C	.	.	.	C	T	.	.	T	.	C	.	.	.	G	.	.	-	T	.	.
TtHap 60	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
TtHap 61	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	G	.
TtHap 62	-	.	.	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.
TtHap 63	-	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.
TtHap 64	-	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.

(Continues)

	Variable site																										
Haplotype ID	264	265	266	268	269	270	279	280	281	288	289	291	294	296	300	311	315	339	343	356	359	376	377	378	379	380	381
TtHap 1	C	C	T	C	C	T	T	T	C	C	A	T	A	T	T	A	C	C	C	T	T	A	C	C	-	T	C
TtHap 2	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 3	T	.	.	T	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	C	.	G	T	.	-	.	T
TtHap 4	.	T	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 5	.	T	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	.	-	.	.
TtHap 6	.	T	.	.	T	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 7	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 9	T	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.
TtHap 10	T	T	C	T	T	.	.	C	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	-	C	.
TtHap 11	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 12	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.
TtHap 13	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 14	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.
TtHap 15	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 16	.	T	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	-	.	.
TtHap 17	T	T	C	T	G	.	C	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 18	T	T	.	T	T	.	C	C	.	.	G	C	.	.	.	.	.	.	.	C	.	.	.	T	-	C	.
TtHap 19	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 20	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 21	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.
TtHap 22	.	.	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 23	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	C	.	.	T	.	-	.	.
TtHap 24	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.
TtHap 25	T	.	.	T	.	.	C	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.
TtHap 26	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	C	.	.	T	.	-	.	.
TtHap 27	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.
TtHap 28	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 29	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 30	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.
TtHap 31	.	.	.	.	.	.	.	C	.	.	.	.	.	C	C	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 32	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 33	T	.	.	T	T	.	C	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.

	Variable site																										
Haplotype ID	264	265	266	268	269	270	279	280	281	288	289	291	294	296	300	311	315	339	343	356	359	376	377	378	379	380	381
TtHap 34	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.
TtHap 35	.	T	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	-	.	.
TtHap 36	.	.	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 37	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 38	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 39	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 40	T	T	C	T	.	.	.	C	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	-	C	.
TtHap 41	.	T	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	-	.	.
TtHap 42	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	.	-	.	.
TtHap 43	.	.	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T	.	-	.	.
TtHap 44	T	T	.	.	.	.	C	C	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 45	T	T	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	-	C	.
TtHap 46	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.
TtHap 47	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T	.	-	.	.
TtHap 48	.	T	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	-	.	.
TtHap 49	T	T	.	.	.	.	C	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T	.	-	.	.
TtHap 50	T	T	.	T	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	T	-	.	.
TtHap 51	T	T	.	.	.	.	C	C	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 52	T	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 53	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 54	.	.	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 55	T	.	.	T	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	-	.	.
TtHap 56	T	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	.	T	.	-	.	.
TtHap 57	T	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	.	T	.	-	.	.
TtHap 58	T	T	.	.	.	.	C	C	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	-	.	.
TtHap 59	T	T	C	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	-	.	.
TtHap 60	.	T	.	.	.	.	.	C	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	T	-	.	.
TtHap 61	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	C	.	.	.	.	-	.	.
TtHap 62	.	T	.	T	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	T	-	.	.
TtHap 63	T	T	.	T	.	.	C	.	.	.	G	.	G	.	.	.	.	.	.	C	C	.	T	.	-	.	.
TtHap 64	T	T	.	T	.	C	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	T	-	.	.

Chapter 5. Thesis Synthesis

The aims of this study were to increase the understanding of cetacean population genetic structure, diversity and dynamics in the North Pacific Ocean, to identify the potential biological and environmental factors that the findings may result from, and to highlight the needs of conservation management. In Chapters 2—4, I studied three species of dolphins of which population genetic data were regionally or globally deficient, and presented analyses for several species-specific issues in each chapter. For instance, in Chapter 2, the conflicting hypotheses proposed in earlier ecological studies regarding Risso's dolphin (*Grampus griseus*) population structure in the North Pacific were examined; in Chapter 3, the concordance of morphological and genetic differences between the Fraser's dolphins (*Lagenodelphis hosei*) found in Japanese and Philippine waters was confirmed; and in Chapter 4, the hypothesis that a coastal type of common bottlenose dolphin (*Tursiops truncatus*) is absent in the western North Pacific was rejected. Here I compare the findings with published cetacean genetic studies to seek further insights into the spatial and temporal factors that may influence population genetic structure, diversity and dynamics, and attempt to highlight the conservation implications accordingly. I also discuss the limitations of this study, provide some

possible alternatives to overcome these limitations, and indicate research objectives for the future.

Population structure

This study, for the first time, revealed the population structure for the Fraser's dolphins and common bottlenose dolphins across the western North Pacific Ocean, and for Risso's dolphins in the Northern Hemisphere. As different species have different ecological or biological characteristics, it is reasonable to expect the three species to show different patterns of population structure even though they inhabit the same waters. This would be consistent with the different patterns of population structure found among the common bottlenose dolphin, short-beaked common dolphin (*Delphinus delphis*) and Atlantic white-sided dolphin (*Lagenorhynchus acutus*) with sympatric distributions along both sides of North Atlantic Ocean (Quérouil et al. 2007; Mirimin et al. 2009; Moura et al. 2013a; Banguera-Hinestroza et al. 2014; Louis et al. 2014a). In the case, although only common bottlenose dolphin populations showed a "nearshore-offshore" differentiation pattern, sampling for the other species did not permit a test for this potential pattern.

In other respects, there seems to be some common features across the population structure of the three studied species in western North Pacific waters. For instance, the dolphins that encountered at the eastern coasts of Taiwan and Japan are always

identified as from the same population in all three species. This may suggest common environmental drivers generating population structure for each of these species in this region. I suspect that the Kuroshio Current, a dominant ocean current in the western North Pacific that shapes the region's ecosystem and promotes mixing, is playing an important role in this circumstance. Ocean circulations are known to play an important role in shaping the population structure of many marine species explicitly relying on external forces to disperse their larvae or reproductive adults. For example, Knutsen et al. (2007) suggest that ocean currents that drift the eggs and larvae of Greenland halibut (*Reinhardtius hippoglossoides*) and promote gene flows in the North Atlantic Ocean. Coleman et al. (2011) found that the strength of continental boundary currents correlates positively with the coastal genetic connectivity in kelp (*Ecklonia radiata*). Schunter et al. (2011) studied the correlationship between the genetic data of comber (*Serranus cabrilla*) and the oceanographic data in the Mediterranean Sea, and found that oceanographic front does play an important role in the determination of the observed genetic flow. Similar conclusion is reported in Godhe et al. (2013), who studied the population genetic structure of common marine diatom (*Skeletonema marinoi*) in Scandinavian waters. The lack of significant genetic differentiation between the bluefin tuna (*Thunnus thynnus thynnus*) schools sampled in Tyrrhenian Sea and the Balearic Sea is suggested to be due to the presence of a connecting ocean circulation (Carlsson et al. 2004). Dolphins may not rely on ocean circulation to disperse, but their dispersal

could be restricted within the distribution range of their preferred prey species, which in turn are influenced by oceanographic processes (Ballance et al. 2006). Such a cascading effect has been suggested to result in the population structure of dusky dolphins (*Lagenorhynchus obscurus*) and common dolphins (Harlin-Cognato et al. 2007; Möller et al. 2011; Amaral et al. 2012a), and appears to be a potential mechanism for Risso's dolphin population differentiation between the Californian coasts and the Eastern Tropical Pacific (Chapter 2).

A recent study reports the genetic structuring of juvenile Atlantic cods (*Gadus morhua*) in the eastern North Sea-Skagerrak-Kattegat region may reflect the drift of egg and larvae with regional ocean currents, but the genetic structure for the adults appears to be governed by the natal homing behaviour (André et al. 2016). Natal homing behaviour, in a broader sense, site fidelity, is regarded as a behaviour assisting locally adapted individuals to return to a suitable habitat. Such behaviour has been reported in a number of marine fish species, including herring (*Clupea harengus*) (Corten 2002), salmonids (Hendry et al. 2004), and Atlantic cod (André et al. 2016), as well as in many cetaceans (e.g., Bräger et al. 2002; Baird et al. 2008; Gonzalvo et al. 2014; Mahaffy et al. 2015; also see discussion in Chapter 2). In an experiment testing the level of site fidelity and homing ability of five intertidal rock pool fish species (White & Brown 2013), it is found that the three specialist species exhibit high fidelity and strong homing

ability, while the non-specialist species showed low fidelity and poor homing abilities, suggesting an evolutionary link between site fidelity and resource specialisation.

With limited ecological and behavioural inferences available for the dolphin species studied in this thesis, it seems that the population structure is a result of resource specialisation, which is affected by the oceanographic structure in the North Pacific Ocean. Further studies on the distribution of the prey species, as well as on the population structure of other dolphin species inhabiting the region, should provide further insight. It would also be useful to investigate the physiological, behavioural and cultural perspectives of prey specialisation in marine mammals. However, it is essential to address a more fundamental question, how resource specialisation would result in assortative mating and ultimately create population structure in cetaceans.

Genetic diversity and effective population size

Table 5.1 compares genetic diversity among the common bottlenose dolphin, Risso's dolphin and Fraser's populations with published studies of these and other dolphin species inhabiting the North Pacific Ocean. Genetic diversity in the studied populations, either evaluated by the bi-parental microsatellite or by the matrilineal mitochondrial DNA markers, is in most cases comparable to that seen in 'offshore' dolphin populations, such as the white-sided dolphins inhabiting the high-seas regions of the North Pacific Ocean (H_E=0.78, h=0.99; Hayano et al. 2004), the bottlenose

dolphins found in California offshore waters (H_E=0.83, *h*=0.97; Lowther-Thieleking et al. 2015), or those stranded along the Irish coasts believed to be from the offshore North Atlantic Ocean (H_E=0.8, *h*=0.94; Mirimin et al. 2011). It is also interesting to note that, at least in common bottlenose dolphins, the genetic diversity for the coastal populations along the Pacific coasts seems to be higher than those along the Atlantic coasts. This may suggest that coastal populations along the Pacific coasts have been stable over a longer period of time than those in the Atlantic, concurring with earlier notions suggesting the North Pacific as a reserve of marine biodiversity and one of the centres of origin for marine species (Briggs 2003) and that the bottlenose dolphins (*Tursiops* spp.) may have originated in Australasian waters (Moura et al. 2013b). If, as previously suggested, the North Atlantic coastal dolphin populations represent independent colonisations from their neighbouring pelagic populations (Natoli et al. 2006; Amaral et al. 2012b; Louis et al. 2014b; Gaspari et al. 2015), their low genetic diversity (and possibly that of the spinner and spotted dolphin populations around the Hawaiian Islands), could reflect diversity lost during founder events (see Hoelzel et al. 1998; Natoli et al. 2004; Sellas et al. 2005).

While some of the estimates of effective population size are consistent with these putative founder events, others, with the exception of the estimate for the Risso's dolphin population in the Eastern Tropical Pacific, are lower than expected (Table 5.2). In earlier chapters I have argued that some estimates may be explained by rapid post-

founder expansions, particularly for the Risso's dolphin population in the western North Pacific and the Fraser's dolphin populations encountered in Japanese and Taiwanese waters. This would not be fully reflected in the Migrate analyses used to estimate N_e, since this method assumes equilibrium between migration and drift (Beerli 2009). Even so, the $N_e\mu$ estimates for those populations with no significant signs of population expansion are, in general, less than 1. As discussed in Chapters 2 and 4, the low $N_e\mu$ for the Risso's dolphin population found in Californian waters and for the common bottlenose dolphin populations along the western coasts of North Pacific Ocean, may reflect isolated populations in coastal habitat (Louis et al. 2014a).

Table 5.1. Genetic diversity estimated using microsatellite (MS) or mitochondrial DNA (mtDNA) data for selected dolphin populations to compare with the results.

Region	Population or location	n		Indices of Genetic diversity				Reference
		MS	mtDNA	H_E	H_O	h	π	
Grampus griseus								
North Pacific	Taiwan	49	42	0.711	0.688	0.891	1.33%	Chapter 2
	East Japan	72	33	0.705	0.68	0.956	1.51%	
	Sea of Japan	12	17	0.698	0.697	0.868	1.42%	
	Central-Northeast Pacific	7	0	0.743	0.69			
	Eastern Tropical Pacific	22	21	0.739	0.722	0.88	1.71%	
	Oregon-California coastal	73	22	0.691	0.666	0.96	1.89%	
North Atlantic	The Azores	0	35			0.926	1.54%	Chapter2
	British waters	18	18	0.592	0.548	0.503	0.11%	Gaspari et al. 2007
Mediterranean Sea	Mediterranean Sea	33	24	0.638	0.467	0.938	1.31%	Gaspari et al. 2007
Lagenodelphis hosei								
North Pacific	Japan	37	35	0.637	0.596	0.973	1.20%	Chapter 3
	Taiwan	43	42	0.702	0.573	0.958	1.20%	
	Philippines	17	10	0.653	0.609	0.911	1.20%	

Tursiops truncatus								
North Pacific	Taiwan/SE China	28	49	0.738	0.721	0.908	2.19%	Chapter 4
	East Japan	32	160	0.739	0.692	0.87	1.37%	
	West Japan	4	0	0.719	0.685			
	NE China	0	14			0.824	1.64%	
	Palmyra	11	11	0.695	0.656	0.909	1.80%	Martien et al. 2012
	Hawai'i	21	22	0.736	0.692	0.87	2.20%	
	4-Islands	25	26	0.741	0.744	0.779	1.90%	
	O'ahu	30	30	0.746	0.695	0.83	1.80%	
	Kaua'i/Ni'ihau	40	41	0.744	0.75	0.892	2.20%	
	California-offshore	69	69	0.83	0.81	0.968	2.30%	Lowther-Thieleking et al. 2015
	Offshore Southern California Bight	0	51			0.97	2.30%	
	Offshore San Diego	0	18			0.967	1.90%	
	California-coastal	64	64	0.55	0.55	0.744	0.50%	
	Gulf of California coastal	0	52			0.943	1.60%	
	Gulf of California offshore	0	32			0.863	1.30%	
North Atlantic	Virginia and North	87	100	0.677	0.661	0.761	1.28%	Rosel et al. 2009
	Southern North Carolina	50	51	0.645	0.624	0.756	0.33%	
	Charleston, SC and surrounding area	100	110	0.652	0.633	0.498	0.16%	
	Georgia	40	40	0.682	0.675	0.573	0.19%	
	Jacksonville	77	78	0.69	0.672	0.558	0.18%	
	Florida panhandle, Gulf of Mexico	77	72	0.652	0.627	0.754	0.93%	
	South Galicia	22	25	0.604	0.658	0.367	0.50%	Fernández et al. 2011
	Sado estuary	0	4			0.667	0.60%	
	North Galicia	14	18	0.786	0.762	0.856	0.13%	
	Mainland Portugal	7	16	0.832	0.804	0.908	1.20%	
	Basque Country	4	2	0.807	0.733	1	1.20%	
	Canary Islands	3	6	0.85	0.717	1	1.80%	
	The Azores	0	10			0.978	1.50%	
	Northeast Atlantic Coastal South	119	115	0.60	0.58	0.5	0.1%	Louis et al. 2014a
	Northeast Atlantic Coastal North	77	76	0.54	0.49	0.67	0.6%	
	Pelagic	52	101	0.77	0.73	0.93	1.4%	

	Shannon	46	44	0.559	0.602	0.274	0.50%	Mirimin et al. 2011
	Connemara-Mayo	12	12	0.477	0.458	0.53	0.80%	
	Cork	0	2			0.5	1%	
	'Stranded'	23	22	0.798	0.779	0.943	1.40%	
	East Abaco	31	31	0.612	0.638	0.613	0.58%	Parsons et al. 2006
	White Sand Ridge	3	3	0.745	0.611	1	1.52%	
	South Abaco	22	22	0.609	0.563	0.7	0.54%	
South Atlantic	Floriano´polis	8	8	0.19	0.23	0.75	0.45%	Fruet et al. 2014
	Laguna	10	10	0.21	0.15	0	0%	
	north of Patos Lagoon	19	19	0.2	0.19	0.543	0.67%	
	Patos Lagoon estuary	63	63	0.26	0.26	0.481	0.72%	
	South of Patos Lagoon and Uruguay	12	12	0.2	0.23	0.648	0.67%	
	Bahı´a San Antonio	12	12	0.19	0.18	0	0%	
Mediterranean Sea	North Adriatic	39	29	0.77	0.71	0.82	1.07%	Gaspari et al. 2015
	Central-South Adriatic	24	16	0.78	0.77	0.87	0.95%	
	Ionian	6	6	0.76	0.68	0.93	1.26%	
	Aegean	6	5	0.76	0.63	1	1.28%	
	Tyrrhenian	14	14	0.75	0.67	0.67	0.73%	
	Pelagic	52	51	0.73	0.70	0.90	1.3%	Louis et al. 2014a
Stenella longirostris								
North Pacific	Kure Atoll	3	3	0.718	0.71	0.395	0.24%	Andrews et al. 2010
	Midway Atoll	4	4	0.713	0.716	0.405	0.18%	
	Pearl & Hermes Reef	3	3	0.707	0.71	0.2	0.14%	
	French Frigate Shoals	5	5	0.762	0.75	0.491	0.40%	
	Ni'ihau	9	9	0.801	0.733	0.656	0.64%	
	Kaua'i	5	5	0.718	0.734	0.429	0.55%	
	O'ahu	6	6	0.737	0.726	0.582	0.48%	
	Maui Nui	5	5	0.732	0.727	0.461	0.43%	
	Kona Coast	12	12	0.747	0.742	0.721	0.88%	
	Samoa	13	13	0.814	0.856	0.975	1.98%	
Stenella attenuata								
North Pacific	Hawai'i	37	38	0.835	N/A	0.376	0.40%	Courbis et al. 2014
	4-Islands	26	27	0.826	N/A	0.527	0.60%	
	Oahu	26	27	0.794	N/A	0.145	0.10%	
	Kauai/Niihau	8	8	0.841	N/A	0.75	0.80%	
Lagenorhynchus obliquidens								
North Pacific	Japanese coastal waters	35	35	0.66	0.64	0.894	1.02%	Hayano et al. 2004
	Offshore North Pacific	24	24	0.78	0.8	0.993	2.04%	

Table 5.2. The indices of effective population size (Neμ) for selected small cetacean populations estimated using the program Migrate. Asterisks indicate potentially problematic estimates.

Species	Region	Population	Neμ	Reference
Grampus griseus	North Pacific	Eastern North Pacific	0.44—0.48	Chapter 2
		Eastern Tropical Pacific	3.2—3.62	
		Western North Pacific	0.36—0.38*	
Lagenodelphis hosei	North Pacific	Japan	0.31—0.36*	Chapter 3
		Taiwan	0.52—0.57*	
		Philippines	0.93—1.07	
Tursiops truncatus	North Pacific	East Coasts of Taiwan and Japan	0.37—0.44	Chapter 4
		West Coast of Taiwan	0.29—0.35	
		Palmyra	3.11—3.86	Martien et al. 2012
		Hawai'i	2.04—2.52	
		4-Islands	1.39—1.66	
		O'ahu	3.08—3.51	
		Kaua'i/Ni'ihau	3.01—3.69	
	North Atlantic	Virginia and North	0.92	Rosel et al. 2009
		Southern North Carolina	0.76	
		Charleston, SC and surrounding area	0.55	
		Georgia	0.98	
		Jacksonville	0.94	
		Florida panhandle, Gulf of Mexico	0.44	
Delphinus delphis	Mediterranean Sea	Black Sea	2.27	Natoli et al. 2008
		Ionian Sea	4.54	
		Alboran Sea	inf	
		Portugal	inf	
	South Pacific	Northern NSW	1.33–1.54	Möller et al. 2011
		Central NSW	1.41–1.78	
		Southern NSW	5.14–6.74	
Inia geoffrensis	Amazon River	Mamirauá	0.22–0.27	Hollatz et al. 2011
		Tefé	0.27–0.35	
Delphinapterus leucas	Arctic	Beaufort Sea	0.50–1.96	O'Corry-Crowe et al. 2010
		Svalbard	0.33–1.69	
Hyperoodon ampullatus	North Atlantic	Gully	0.14–0.16	Dalebout et al. 2006
		Labrador	0.30–0.33	
		Iceland	0.25–0.29	

Population dynamics and conservation

A sign of population expansion is detected in the Risso's and Fraser's dolphin populations, and the Migrate analysis results suggest a prevalence of northbound long-term gene flow for Fraser's dolphins in the western North Pacific Ocean, which may reflect a tendency for southern populations to explore further suitable habitat in the north with warm climate. The intensification of Kuroshio Current during the latest deglaciation is likely to be an important factor promoting the range expansion in Risso's and Fraser's dolphins in the western North Pacific Ocean (see Discussion in Chapters 2 and 3). The rapid expansion detected for Risso's dolphins around the British Isles may be attributable to a similar pattern of dispersal into warming northern regions, while it could also be the result of post-bottleneck expansion. Expansion time estimates suggest that the study populations started to expand around the early Holocene (~11,000 years ago), consistent with the estimations suggested for several other cetacean species (e.g., Banguera-Hinestroza et al. 2014; Louis et al. 2014b; Moura et al. 2014).

In contrast, all populations inhabiting tropical waters, or the 'refugia' sheltering marine species from extreme conditions during the glacial periods (e.g., the Azores and the Mediterranean Sea; Maggs et al. 2008), exhibit a stable population trend: high genetic diversity and no obvious sign of population expansion. The estimates further indicate that the Fraser's dolphin population in Philippine waters and the Risso's dolphin population in Eastern Tropical Pacific have a considerable effective population

size, suggesting that climate change through time has had less impact on these dolphin populations.

The findings in this study appear to support an earlier prediction which suggests that Risso's dolphins, Fraser's dolphins and common bottlenose dolphins might respond to water temperature increase during climate change with range expansion (MacLeod 2009). However, the distribution of cetaceans is mainly defined by their prey abundance and distribution, rather than oceanographic conditions (such as water temperature; Ballance et al. 2006, Amaral et al. 2012a), and climate change might not affect the distribution of their prey species in the same way. For instance, the increase of seawater temperature over recent decades has been shown to reduce the abundance of marine phytoplankton (Behrenfeld et al. 2006; Boyce et al. 2010), and the decrease of primary productivity can trigger a crisis to marine fishery sustainability (Perry et al. 2005; Brander 2010). The influence of climate change upon marine ecosystems is complicated, and ocean systems are being driven towards extreme conditions that the dolphin species may not have encountered before (Walther et al. 2002, Hoegh-Guldberg & Bruno 2010). It is therefore probably too early to conclude whether or not the dolphin populations studied here are threatened by global climate change.

Limitations of this study and possible alternatives

The lack of a sufficient number of samples from the regions of interest is perhaps the greatest limitation for most cetacean genetic studies, including the present study. As systematic collections of biopsy samples from wild population are not always available, the consensus is to use cetacean tissue samples that are collected opportunistically through stranding or bycatch events, although they are usually associated with inevitable problems, such as scattered sample size and ranges, and the uncertainty of sample origins (Bilgmann et al. 2011; Peltier et al. 2012). As a result, I was unable to evaluate the level of isolation for the Risso's and bottlenose dolphins living in the Sea of Japan, nor the level of differentiation for the two species inhabiting Philippine waters. Neither was I able to test whether inshore-offshore population structure also exists in the western North Pacific cetacean populations, due to the lack of samples collected from high sea regions. One possible way to overcome the problem of small sample sizes is to examine the population genetics using next generation sequencing (NGS) techniques, where small sample size can be compensated by genotyping an enormous number of single nucleotide polymorphism (SNP) loci with a high sequencing depth (Willing et al. 2012). However, there are limits to the potential for compensation by this method, and ultimately a larger sample size should be pursued wherever possible.

The availability of biological and ecological information for the dolphin populations studied here would facilitate the interpretation of the genetic findings. For instance, skull morphometric variation in bottlenose dolphins in Chinese/Taiwanese and Japanese waters (Wang et al. 2000; Kurihara et al. 2006) and the diet analysis for Fraser's dolphins in Taiwanese waters (Wang 2003; Wang et al. 2012) provide the potential for corroborative inference, though in this case neither correlated with the genetic structure found in this study. The absence of fine-scale ecological analysis for the bottlenose dolphins in the Taiwan Strait leaves open questions about how the two potential resource competitors could coexist. Moreover, a recent model analysis suggests sociality can be an important component for mammal population structure (Parreira & Chikhi 2015), but the lack of social structure data for the populations studied here prevents further analysis. Since genetic data alone provide only limited inference, projects providing greater supporting biological and environmental data have great potential, such as the ongoing humpback whale studies in the North Pacific (the SPLASH project, http://www.splashcatalog.org/; Calambokidis et al. 2008).

The concern of using a hypothetical molecular substitution rate to calculate the time of population expansion has been discussed in Chapter 2. Using an average mutation rate to calculate the effective population size (N_e) from the $N_e\mu$ estimates may also be problematic since the mutation rates appear to vary among microsatellite loci (Chakraborty et al. 1997). Therefore, the inferences derived from estimates involving

these assumptions, such as the time of expansion, N_e, and N_e/N ratio should be interpreted with caution. Calibration using species-specific ancient DNA (aDNA) samples provides fairly consistent estimations across mammal species (Ho et al. 2011). However, these estimates reflect "accelerated" rates that are relevant to recent timeframes (e.g. within the Holocene) and can be species-specific.

What's next?

In the last decade, the rapid development of NGS techniques has permitted biologists to obtain high quality, genome-wide sequencing data from non-model organisms at an affordable price and to explore further population genetic questions that are difficult to assess with traditional Sanger sequencing or microsatellite genotyping. There have been a number of reviews published regarding the application of NGS to molecular ecology (e.g., Tautz et al. 2010; Ekblom & Galindo 2011; Glenn 2011; Shokralla et al. 2012). In the case, as resource specialisation appears to be an important factor driving population differentiation in the species, NGS techniques could be used to identify candidate genes that associate with this type of differentiation (e.g., Hohenlohe et al. 2010; Moura et al. 2015), and possibly confirm the physiological significance of identified loci by studying gene expression patterns among populations (e.g., Goetz et al. 2010). It is also possible to learn more about individual diet using NGS techniques (see Pompanon et al. 2012).

There is an interesting ecological perspective on the formation of population structure pending further investigation. With the insights into the relationship between Risso's dolphins and pilot whales, and common bottlenose dolphins and Indo-Pacific bottlenose dolphins as potential resource competitors (Tezanos-Pinto et al. 2009; Bearzi et al. 2011), it seems the effect of resource competition has never been regarded as a possible ecological factor in the way that prey distribution or intrinsic population affinity (philopatry) to drive population structure in cetaceans. Genetic or genomic approaches might not be useful in this respect, but long-term field observations and temporal/spatial model simulations might be useful.

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