The Potential Distribution and Maternal Lineage of Two Cetaceans Species (*Grampus griseus* and *Pseudorca crassidens*) in the Subfamily Globicephalinae from the Thai Andaman Sea and the Gulf of Thailand

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Abstract: Although the existence of two cetacean species in and around Thai Seas from within the subfamily Globicephalinae, Risso’s dolphin *Grampus griseus* G. Cuvier, 1812, and false killer whale *Pseudorca crassidens* Owen, 1846, has been known for decades, current knowledge on the abundance, genetic diversity, and conservation status of these marine mammals is limited as these are rare oceanic species for Thailand’s territorial waters. Frozen skin tissue samples taken from six cetaceans (four Risso’s dolphins: two false killer whales) stranded along Thai coastlines were investigated. We aimed to identify the maternal lineage and connection of our samples throughout their distribution range. Accordingly, we analyzed the dataset of 110 and 50 mtDNA control region sequences of Risso’s dolphins and false killer whales, respectively. This dataset was retrieved from the online database of the National Center for Biotechnology Information (NCBI) and included six mtDNA sequences obtained from Thai Seas. Two unique haplotypes of Risso’s dolphins were found in the Thai Andaman Sea, whereas one haplotype identified as being from the Gulf of Thailand is a common haplotype shared with other regions of the Pacific Ocean. Two haplotypes were found for false killer whales from the Thai Andaman Sea, and these were also in common with other regions of the Indo Pacific Ocean. While shared haplotypes with other regions may imply inheritance from the same female ancestor, we speculate that distinct populations with unique genetic structures also exist in Thai Seas. Beneficially, our results could be used to monitor alterations of haplotypes or to assess the maternal genetic diversity of designated species in the future to establish baseline information for Thai Seas and adjacent waters.

Keywords: D-loop; population genetic; Risso’s dolphins; false killer whales

1. Introduction

The Thai Seas, which consist of the Thai Andaman Sea and the Gulf of Thailand, are known to be the natural habitat of 27 cetacean species including whales, dolphins, and porpoises [1–5]. Risso’s dolphin (*Grampus griseus* G. Cuvier, 1812) and false killer whale (*Pseudorca crassidens* Owen, 1846), closely related species within the subfamily Globicephalinae based on mitogenomic phylogenetic analysis, are also known to occupy the
Thai Seas [1,5–8]. There is a similarity in the distribution range that extends from coastal areas to oceanic depths in tropical and temperate waters; however, the cooler-temperate waters found between latitudes of 30–45° are preferred by Risso’s dolphins [9–12]. Water depth clearly affects the occurrence of this species as they exhibited a clear preference for the outer continental shelf at depths within a range of 300–1500 m in eastern Taiwanese waters [10,13–15]. It is unusual for this species to be found in the Gulf of Thailand where the average depth of the water is limited to only 45 m [10,16]. In contrast, the false killer whale, which is recognized as a warm temperate oceanic species, is more often sighted throughout the Thai Seas and adjacent waters when compared with Risso’s dolphin [1,8,17]. However, the number of stranded animals of both species along Thai coasts has been minimal since the first record in 1996 [1,18,19].

Currently, both species are listed in Appendix II of the Convention on International Trade in Endangered Species (CITES). According to the assessment of the International Union for Conservation of Nature (IUCN) Red List, Risso’s dolphin is now considered a species of least concern, while the status of the false killer whale is near threatened [20,21]. In fact, the populations of both species in many regions have been affected by a range of human activities such as fishery bycatch, intentional killings, loud anthropogenic sounds, and disturbance by the whale-watching boats of tourists [22–29]. Accordingly, high-intensity sonar and chemical contamination have been recognized as significant threats to the Risso’s dolphin population in the western Ligurian Sea. This has resulted in a decrease in the population size of these cetaceans [30]. Recently, the subpopulation of the false killer whale inhabiting the waters around the Hawaiian Islands has significantly declined as a result of the commercial fishing industry [31,32]. A high proportion of fishery-related scarring on the dorsal fins of false killer whales inhabiting the coastal waters of the main Hawaiian Islands also confirm the ongoing negative impacts of the fisheries located around this area [33].

The assessment of the regional conservation status of each species and an understanding of the potential threats to their existence are extremely important for the conservation of these species. Certain regional animal protection acts have been established to support the protection of these species, such as the Wildlife Conservation Act in Taiwan for Risso’s dolphin [34] and the Marine Mammals Protection Act in the United States for the false killer whale [20]. For Thailand, most of the cetaceans inhabiting Thai Seas are now being protected by the establishment of the Wild Animal Reservation and Protection Act (WARPA) of 1992 [35]. In recent decades, around 22 species of cetaceans, including the false killer whale, have been listed as protected marine animals by WARPA, while only two species of baleen whales are listed as conserved animals [35]. However, these lists do not cover all cetacean species that exist in Thailand’s waters. This is because there is insufficient data pertaining to some species that have recently been found in these waters, while there have also been very few records of stranded animals along Thai coasts, such as the Risso’s dolphins and humpback whales (Megaptera novaeangliae) [18].

Biological data related to the abundance and dispersal of each species, as well as the population structure, genetic variations, and the identification of specific threats for cetacean populations, will be needed to effectively evaluate their present conservation status and improve relevant conservation management strategies going forward [36–38]. Several studies on the local populations of Risso’s dolphins and false killer whales have been conducted using nuclear DNA microsatellite and mitochondrial DNA (mtDNA) markers to gain in-depth biological information. In these studies, information on the significant genetic differentiations in the populations of Risso’s dolphins inhabiting UK waters and the Mediterranean Sea was established without sharing mtDNA control region haplotypes between the populations of these animals, while a lower degree of genetic diversity was recorded for the population of UK dolphins [39]. Later, Chen, et al. [40] reported on the genetic differentiations among three populations of this species inhabiting the North Pacific Ocean. Based on mtDNA control region sequence analysis, these populations were also found to be genetically different from the populations inhabiting UK waters and the Mediterranean Sea in a previous study conducted by Gaspari et al. (2007) [39].
phylogeographic structure of false killer whales inhabiting the North Pacific Ocean, and specifically the coastal waters of the main Hawaiian Islands, revealed a unique set of haplotypes [41]. A further investigation on the false killer whale population structure that inhabit the Hawaiian Archipelago was conducted by Martien, et al. [42]. Three unique haplotypes were identified throughout the coastal waters of the main Hawaiian Islands and the northwestern waters of the Hawaiian Islands that did not share haplotypes with other locations. This phylogeographic pattern of mtDNA also suggests that they have a common history of colonization.

Importantly, the populations of Risso’s dolphins and the false killer whales that inhabit Thai waters have never been studied. These marine mammals are rare oceanic species for the Thai Seas; although, some sightings and incidences of stranding have been documented [1,18,19]. Since 1994, pods of false killer whales have often been sighted around the Similan Islands and the Racha Islands in the Thai Andaman Sea, as well as around Kho Tao in the Gulf of Thailand [1,19]. In contrast, there have been no recorded sightings for free-ranging Risso’s dolphins in these areas. While this species is thought to be a rare species for the Thai Seas, there have been only a few reports of stranded individual dolphins along Thailand’s coasts, such as in the coastal areas of Nakhon Si Thammarat Province, Prachuap Khiri Khan Province in the Gulf of Thailand, and Phuket Province in the Thai Andaman Sea [18]. Currently, published data pertaining to the abundance, population, genetic diversity, and conservation status of Risso’s dolphins and false killer whales inhabiting Thailand’s waters is considered insufficient, while any connection between the populations of these species inhabiting the Thai Seas and the adjacent waters remains unclear. Thus, a lack of information on these species has led to difficulties in setting up effective conservation strategies and protective regulations. Therefore, in order to collect relevant biological data and understand how the cetaceans found stranded along Thai coasts may be connected to others around the world, we have investigated the mtDNA control region haplotypes of four Risso’s dolphin and two false killer whale sequences based on the limited stranded samples provided by the Phuket Marine Biological Center, Thailand. The mtDNA control regions obtained from six specimens were sequenced in order to determine their matrilineally genetic relationship to cetaceans inhabiting other oceanic waters by retrieving the available sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank. This was done in order to observe the haplotype distribution of both species on a global scale.

2. Materials and Methods
2.1. Samples and DNA Extraction

In this study, six skin tissue samples of stranded cetaceans were provided by the Phuket Marine Biological Center, Phuket 83000, Thailand. This center has collected and stored tissue samples of all stranded cetaceans along Thailand’s coasts since 1990 (Table 1). All samples were preserved in 95% ethanol and stored at −20 °C. Four tissue samples of Risso’s dolphins were collected from both the Thai Andaman Sea (ADM) and the Gulf of Thailand (GOT), whereas tissue samples of only two false killer whales were collected from ADM. The skin samples were extracted using a DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to the method previously described [43,44] at the Faculty of Veterinary Medicine, Chiang Mai University, Thailand. Extracted DNA samples were prepared for purity evaluation using 2% agarose gel electrophoresis and were then visualized under ultraviolet light. DNA quantity was determined using a spectrophotometer in order to measure the absorbance ratio at 260–280 nm. Diluted samples (10 ng/µL) were stored at −20 °C for subsequent analyses. This study was approved of by the Animal Use Committee of the Faculty of Veterinary Medicine, Chiang Mai University, Thailand, in 2020 (S14/2563).
Table 1. Cetacean samples used in this study obtained from Thailand’s territorial waters.

| Species                  | Accession Number | Date of Collection | Location        | Sea  |
|--------------------------|------------------|--------------------|-----------------|------|
| Risso’s dolphin (Grampus griseus) | MZ401232         | 17 May 2006       | Phuket          | ADM  |
|                          | MZ401234         | 12 December 2009  | Phuket          | ADM  |
|                          | MZ401235         | 28 January 2010   | Nakhon Si Thammarat | GOT |
|                          | MZ401236         | 20 February 2011  | Nakhon Si Thammarat | GOT |
| False killer whale (Pseudorca crassidens) | MZ401168         | 26 June 2008      | Phuket          | ADM  |
|                          | MZ401169         | 24 June 2013      | Phuket          | ADM  |

ADM = the Thai Andaman Sea, GOT = the Gulf of Thailand.

2.2. mtDNA Control Regions

The mtDNA control regions of six samples (Table 1) were amplified using PCR primers: forword, 5’-CAT ATT ACA ACG GTC TTG TAA ACC-3’; and reverse, 5’-GTC ATA AGT CCA TCG AGA TGT C-3’ [45]. PCR reactions were conducted in 25 µL reaction volumes consisting of 1X reaction buffer, 2 mM MgCl₂, 0.4 mg/mL bovine serum albumin, 0.25 mM dNTPs, 0.4 µM of both forward and reverse primers, 5 U/µL Platinum Taq DNA polymerase (Invitrogen), and 10 ng/µL of the DNA sample. The PCR conditions were performed as follows: 95°C for 5 min, 40 cycles of 95°C for 30 s, 50°C for 45 s, 72°C for 1 min, and 72°C for 10 min. The amplicon size of all samples at 600 bp were visualized on 2% agarose gel electrophoresis. All PCR products obtained from the amplification were then sequenced by ATGC Co., Ltd., Pathum Thani, Thailand. The mtDNA control region sequences obtained from this study were visually prepared and edited manually using the MEGA-X program version 10.2.2 [46].

The mtDNA control region sequences of Risso’s dolphins and false killer whales available in the GenBank database were obtained to generate two datasets according to each species. These datasets were established using the following keyword searches: “mitochondrion/control region mtDNA/d-loop” + “Grampus griseus/Pseudorca crassidens”. The mtDNA sequences of both full length and partial length were presented through keyword searches, although only the D-loop Feature in the “Graphics” section was exported as a FASTA record. A total of 110 sequences of Risso’s dolphins and 50 sequences of false killer whales were obtained through designated keyword searches. The mtDNA control sequences of six studied samples were included in these datasets for subsequent analysis. The final two datasets and the accession numbers of all sequences are presented in Tables S1 and S2.

2.3. mtDNA Sequence Analysis and Phylogeographic Relationships

In our sequence samples, each species was aligned to the worldwide sequences in each dataset as implemented by Clustal W in MEGA X version 10.2.2 [46,47]. The haplotypes of each dataset were determined using DnaSP program version 6.12.3 [48]. Geographic maps of haplotype distribution were generated using the map function implemented in R studio [49]. The Median Joining Networks (MJNs) of each species were constructed using PopART program version 1.7 [50].

2.4. Phylogenetic Tree Construction

Phylogenetic trees of mtDNA control region sequences for each species were constructed using Bayesian Analysis implemented in the MrBayes program version 3.2.7 [51]. To identify the best tree evolutionary models for Risso’s dolphins and false killer whales, jModelTest version 2.1.10 was used to achieve a result of the best fit model established as HKY+G and GTR+I+G, respectively [52,53]. The total run length of Markov Chain Monte Carlo (MCMC) sampling at 10,000,000 iterations for each tree was performed, while samples were drawn for every 5000 iterations. Convergence diagnostics with an average standard deviation of the split frequencies below 0.01 were used. The first 2,500,000 iterations were discarded in the burn-in step. Mitochondrial control region DNA sequences of other cetacean species were used to serve as an out group for each phylogenetic tree: white-beaked dolphin (Lagenorhynchus
albirostris AJ554061), rough-tooth dolphin (Steno bredanensis NC 042716), and killer whale (Orcinus orca NC 023889). The phylogenetic trees obtained were then visualized by iTOL version 6.1.1 [54].

3. Results

3.1. mtDNA Control Region

A total of six mtDNA control region sequences were successfully amplified from the Thai samples (4 Risso’s dolphins: 2 false killer whales). Maximum sequence lengths of 491 bp for Risso’s dolphins and 484 bp for false killer whales were obtained in this study and deposited in GenBank (MZ401232, MZ401234-36, and MZ401168-69). The datasets of each species obtained from GenBank, including our new sequences, were of different lengths; however, for the purposes of comparison with all available sequences in each species, the length of each dataset was reduced and aligned with the consensus fragments of 390 bp for Risso’s dolphins and 323 bp for false killer whales.

3.2. Haplotypes and Phylogeographic Relationships

Overall, with regard to the global scale of mtDNA control region haplotypes, we identified a total of 58 variable sites: 81 haplotypes for Risso’s dolphins at 22 variable sites and 23 haplotypes for false killer whales. The haplotype distribution of each species is shown in Figures 1 and 2. For our samples, three haplotypes of Risso’s dolphins (haplotypes 2–4, Figure 1) and two haplotypes of false killer whales (haplotypes 1 and 2, Figure 2) were detected.

Figure 1. Map showing the geographic distribution of Risso’s dolphin mtDNA control region haplotypes. Each circle represents the haplotypes of Risso’s dolphins in each location. The two sizes of circles indicate the number of haplotypes: smaller, 1–2 haplotypes; larger, >2 haplotypes. The color indicating the haplotype and the proportion of color in each circle are related to haplotype frequency. Abbreviations for location are as follows: AZ, Azores Island; MED, Mediterranean; FR, France; SC, Scotland; UK, United Kingdom; IO, Indian Ocean; ADM, Thai Andaman Sea; GOT, Gulf of Thailand; PH, Philippines; TW, Taiwan; SCN, Southeastern China Sea; SOJ, Sea of Japan; JP, Japan; EJP, Eastern Japan; ETP, Eastern Tropical Pacific Ocean; OCC, Oregon-California Coastal.
The MJNs of Risso’s dolphin indicated multiple alternative connections among haplotypes worldwide (Figure 3). Many haplotypes associated with a small degree of frequency were connected to each other through one to five mutation steps, whereas some clusters of haplotypes were present in a specific geographic region. For example, haplotypes 5 and 18 were restricted to the North Atlantic region, while haplotypes 6, 10, and 13 were restricted to the Mediterranean Sea. Shared haplotypes also occurred across many locations such as haplotypes 5 and 19 that were shared between Eastern Japan, the Sea of Japan, and Taiwan. However, haplotypes shared between oceanic regions were not observed. For our Risso’s dolphin samples, two unique Thai haplotypes, namely haplotypes 3 and 4 obtained from the Thai Andaman Sea, were reported, while haplotype 2 obtained from the Gulf of Thailand is shared with specimens obtained from Eastern Japan, the Sea of Japan, Taiwan, and the Oregon-California Coastal region (Figure 3). A simpler pattern of MJNs was observed for false killer whale mtDNA control region haplotypes (Figure 4). The haplotypes were also connected to each other with one to five mutation steps. The sharing of haplotypes between the Atlantic and Pacific Oceans was not detected. Instead, it appeared to occur between the Indian and Pacific Oceans, wherein haplotype 2 included our false killer whale sample obtained from the Thai Andaman Sea and others obtained from the Indian Ocean and the territorial waters of Japan. According to the analysis of another sample of the false killer whale in our study, cetaceans of the Thai Andaman Sea also shared haplotypes with several locations in the Pacific Ocean (haplotype 1), i.e., the Central North Pacific Ocean, the Eastern Tropical Pacific Ocean, the territorial waters of Japan, and the coastal waters of the main Hawaiian Islands. This high frequency haplotype is likely to be the ancestral haplotype for the Indo Pacific Ocean as well as the haplotype 13 for the main Hawaiian and Northwestern Hawaiian Islands (Figure 4).
Figure 3. Median joining network (MJN) of all Risso's dolphin mtDNA control region haplotypes. The MJN was generated from 390 base pairs of Risso's dolphin mtDNA control region sequences and indicated that 81 haplotypes were found in 16 locations. Each haplotype is colored by location and the small black circles represent inferred haplotypes (not sampled). The size of each circle is proportional to its haplotype frequency, while nucleotide substitutions are shown at the branches as small transverse bars. Purple arrows indicate the haplotypes from the Thai Andaman Sea and the green arrow indicates the haplotype from the Gulf of Thailand. Abbreviations for location are as follows: AZ, Azores Island; MED, Mediterranean; FR, France; SC, Scotland; UK, United Kingdom; IO, Indian Ocean; ADM, Thai Andaman Sea; GOT, Gulf of Thailand; PH, Philippines; TW, Taiwan; SCN, Southeastern China Sea; SOJ, Sea of Japan; JP, Japan; EJP, Eastern Japan; ETP, Eastern Tropical Pacific Ocean; OCC, Oregon-California Coastal.
3.3. Phylogenetic Reconstruction

Bayesian phylogenetic reconstructions revealed a monophyletic taxon of mtDNA lineages for Risso’s dolphins and false killer whales from worldwide samples with a posterior probability of 1.00 for both species (Figures 5 and 6). In terms of Risso’s dolphin phylogeny, many clades of haplotypes were observed. Our two samples obtained from the Gulf of Thailand (haplotype 2) were clustered in a monophyletic clade with other samples obtained from the waters of Eastern Japan and Taiwan in the Pacific Ocean (Figure 5). This clade is also paraphyly to the unique haplotypes of Risso’s dolphins obtained from the Thai Andaman Sea (haplotype 3 and haplotype 4). With regard to false killer whales, there was only one large clade that contained all haplotypes. This included our two samples obtained from the Thai Andaman Sea (haplotype 1 and haplotype 2, Figure 6).
Figure 5. Bayesian phylogenetic tree of Risso’s dolphin based on 390 bp alignment of mitochondrial DNA control region. Accordingly, 96 sequences of Risso’s dolphins were acquired from the NCBI database, while four sequences were obtained from this study. The white-beaked dolphin (Lagenorhynchus albirostris AJ554061), rough-tooth dolphin (Steno bredanensis NC 042716), and killer whale (Orcinus orca NC 023889) were utilized as out groups. The circular bands of color around the tree indicate haplotype: first layer and location, second, third, fourth, and fifth layers (inner to outer). Purple arrows indicate samples from the Thai Andaman Sea and green arrows indicate samples from the Gulf of Thailand. Abbreviations of location are as follows: AZ, Azores Island; MED, Mediterranean; FR, France; SC, Scotland; UK, United Kingdom; IO, Indian Ocean; ADM, Thai Andaman Sea; GOT, Gulf of Thailand; PH, Philippines; TW, Taiwan; SCN, Southeastern China Sea; SOJ, Sea of Japan; JP, Japan; EJP, Eastern Japan; ETP, Eastern Tropical Pacific Ocean; OCC, Oregon-California Coastal.
Figure 6. Bayesian phylogenetic tree of false killer whales based on 323 bp alignment of mitochondrial DNA control regions. Accordingly, 48 sequences of false killer whales were obtained from the NCBI database, while two sequences were obtained from this study. The white-beaked dolphin (*Lagenorhynchus albirostris* AJ554061), rough-tooth dolphin (*Steno bredanensis* NC 042716), and killer whale (*Orcinus orca* NC 023889) were utilized as out groups. The circular bands of color around the tree indicate haplotype: first layer and location, second and third layers (inner to outer). Purple arrows indicate samples from Thai Andaman Sea. Abbreviations of location are as follows: ADM, Thai Andaman Sea; CNP, Central North Pacific Ocean; ENP, Eastern North Pacific Ocean; EP, East Pacific Ocean; ETP, Eastern Tropical Pacific Ocean; IP, Indo Pacific Ocean; JP, Japan; MHI, Main Hawaiian Islands; NWHI, Northwestern Hawaiian Islands; SP, South Pacific Ocean; WP, West Pacific Ocean; IO, Indian Ocean; AO, Atlantic Ocean; WNA, West Northern Atlantic Ocean; Unk, Unknown.

4. Discussion
4.1. Unique Haplotypes of Risso’s Dolphins and Potential Distribution in Thai Seas

According to our assessment of the unique mtDNA control region haplotypes of Risso’s dolphins inhabiting the Thai Andaman Sea, we found the potential presence of a different genetic unit when compared to other studied areas. These haplotypes were not included in previous genetic studies or other established databases; however, they did appear to be present in different habitats that are known to be home to a wide range of prey species. The topography of the Andaman Sea, including continental shelves with the preferred depth for this species, also support the possibility of the occurrence for a distinct Risso’s dolphin population in this region where the water depth is around 200–2000 m and deeper than the Gulf of Thailand [55]. Within a short geographic distance, many factors, such as divergent habitats, foraging specializations, site fidelity, and social structure, can be involved in shaping the population structure of cetaceans and specifically odontocetes [56–58]. This would be particularly true for Risso’s dolphin populations in eight regions according to the outcomes of a number of previous studies. These regions included the territorial waters of the UK, the
Mediterranean Sea, the Azores Islands, Taiwanese waters, the Sea of Japan, eastern Japan, Eastern Tropical Pacific waters, and the Oregon-California coastal region, all of which have indicated significant differentiations in mtDNA control regions ($F_{st}$, $p$ value < 0.05) across these differing habitats [39,40].

Differences in prey specialization and the foraging habitats of distinct populations have also been suggested as potential mechanisms for the genetic differentiations that have occurred among other cetaceans, i.e., killer whales and bottlenose dolphins [56,59,60]. Similarly, it is generally known that Risso’s dolphins feed primarily on a wide variety of cephalopod species that inhabit both the water column and the ocean floor [26,61]; however, differences in the main prey species within each region have been observed. A study of the stomach contents of Risso’s dolphins obtained from the Northern Atlantic region and the Faroe Islands revealed different types of squid as the main prey species during different periods of the year i.e., Todarodes sagittatus in September and Eledona cirrhosa in April [26]; whereas in the Mediterranean Sea, two types of squid in the family Histiotethidae, i.e., Histiotethis bonnellii and Histiotethis reversa, were reported as being representative of the primary diet of this dolphin species [62]. Unfortunately, the prey species found in the stomach contents of our samples obtained from the Thai Seas have never been identified. Therefore, we suggest that, in the future, the stomach contents of dead stranded cetaceans should be investigated in further studies. This information could provide even more supportive evidence for the existence of a distinct dolphin populations in relation to prey specialization.

Although the sighting and capture records of Risso’s dolphins in worldwide oceans have been reported by collecting all available data from 1950 to 2012 [1,10], the records from the Thai Andaman Sea and the Gulf of Thailand have not been included in any of the previous studies. In the last decade, there has been a lack of evidence to prove the existence of this species in Thai Seas, particularly in the waters of the Gulf of Thailand where the maximum water depth is limited to only 80 m [16]. Consequently, the Gulf of Thailand is thought to be an unfavorable area for Risso’s dolphins, which are known to prefer deeper waters [10]. In addition to the shallow waters of the gulf and the absence of reported sightings of free-ranging cetaceans in this area, our results also support the contention that the Gulf of Thailand might not be the core habitat of the Risso’s dolphin as the haplotype we found (haplotype 2) from two stranded dolphins in this area was also found in other regions of the Pacific Ocean, i.e., the waters of Eastern Japan, the Sea of Japan, Taiwan, and the Oregon-California Coastal region. Similarly, it is unusual for this species to be observed in western Taiwanese waters, where the water is much shallower than it is in eastern Taiwanese waters. Only four sightings of Risso’s dolphin groups and a few incidences of stranding were recorded in this area [34,63], while there was a significant number of sightings, at 1141, recorded in a survey conducted in the deeper waters of the Hualien and Shiri ports of east-central Taiwanese waters over a 17-year period [15]. Despite this species’ preference for water-depth [6,10,64,65], it is likely that the Risso’s dolphins found stranded in the Gulf of Thailand were transient individuals that may have strayed far from the pods that inhabit the waters outside the gulf in the South China Sea, where the water is known to be deeper than 1000 m [66,67].

Sightings of Risso’s dolphins in and around the Andaman Sea and adjacent waters, i.e., the Bay of Bengal and the southeastern coasts of Indonesia and Australia, have been scarcely noted [10]; however, samples obtained from these areas are rare and hard to obtain. In our study, although unique haplotypes were observed from the Thai Andaman Sea, more information is needed to confirm the existence of distinct populations inhabiting these areas. In the last decade, the recent emergence of this species in the Thai Seas and the lack of other biological information could lead to hard decisions being made on the establishment of accurate conservation status; thus, this species has not yet been considered for acceptance on WARPA’s animal protected lists of Thailand. We suggest that additional genetic studies involving even more samples of this species in these areas, particularly from the Thai Andaman Sea, would be beneficial in revealing further information of evidentiary value.
4.2. Maternal Lineage of False Killer Whales Inhabiting the Thai Andaman Sea and Other Regions

The existence of shared haplotypes of false killer whales between the Thai Andaman Sea and other areas within the Pacific Ocean was observed in our study. Haplotype 1 in our study is an ancestral haplotype that is widely distributed across the Pacific Ocean, including MHI, as it appears to exhibit the greatest degree of frequency and is located in the middle of the MJNs. In the previous study, three unique haplotypes of this species were thought to be found only in MHI and NWHI as demographical isolated populations, while these haplotypes were not found elsewhere in the Pacific Ocean [42]. However, in that study, not only were three unique haplotypes recorded around the Hawaiian Archipelago, but another haplotype was also shared between Northern Australia and MHI. It may also be possible that the false killer whale population around the Thai Andaman Sea has shared female ancestors with other regions. Apparently, this haplotype has persisted and survived even in diverse habitats, though more samples should be investigated to confirm the common haplotype of this population within this area and perhaps the existence of even more haplotypes.

Although pods of free-ranging false killer whales have often been sighted in the Thai Seas [1,19], a connection of maternal lineage between the Thai Andaman Sea and the Gulf of Thailand has never been revealed. This is because no relationship could be established from samples obtained from the Gulf of Thailand and from stranded individual cetaceans along both coasts. Sightings and stranding incidences for this species were recorded around the Malay Peninsula, i.e., the Langkawi Archipelago, Tioman Island, and Sabha, Malaysia, all of which were close to our study area [68]. However, there is limited knowledge on the population of this species throughout these areas. In addition, their genetic information has rarely been investigated. Only two sequences of unknown location from the Indo Pacific Ocean have been deposited in the online database (NCBI), though this haplotype (haplotype 20) is closely related to our samples with differences in one to two nucleotide positions. With regard to the matrilineal social systems of this species [69], the remarkably low diversity of the mtDNA control region has been revealed across many regions, i.e., MHI (hd = 0.395 ± 0.043 to 0.554 ± 0.053), NWHI (hd = 0.105 ± 0.092), and ETP (hd = 0.676 ± 0.052) [42]. Thus, we speculate that very few haplotypes have occurred around the Thai Seas, while haplotype 20 in our study is also likely to be found in populations around these areas.

The number of deaths and stranded cetaceans along Thai coasts have been reported annually. Fishing equipment has been identified as posing the greatest potential threat to these mammals [70], though the specific threat for two species, namely Risso’s dolphins and the false killer whale, is poorly understood. We suggest that monitoring through active surveys, i.e., line transect surveys on abundance, sight effort records, and photo-identification studies, on free ranging cetaceans of both species in the Thai Seas should be initiated to evaluate the abundance, distribution pattern, and potential threats for each species. Studies such as these have previously been performed involving other species such as the Irrawaddy dolphin (Orcaella brevirostris), the Indo Pacific humpback dolphin (Sousa chinensis), and the Indo Pacific finless porpoise (Neophocaena phocaenoides) [2,3]. Additionally, further genetic studies involving potential molecular markers, such as nuclear DNA microsatellites, are urgently needed to assess their genetic diversity and population structure. This information will help to improve vital and relevant conservation measures for Thailand’s cetacean populations.

Evaluation of the conservation status and establishment of effective conservation strategies for both species have been a challenge for ecologists and conservation biologists, though the achievement of which may currently be impossible as little is known about these marine mammals in the Thai Seas. To support the conservation of these cetaceans in these areas, we are providing useful genetic information to enable researchers to better understand their distribution and the connection of maternal lineage in the Thai Seas and oceanic waters throughout the world. In the past, there has been limited published data on Risso’s dolphins in and around the Malay Peninsula. Consequently, findings on the unique haplotypes for our samples obtained from the Thai Andaman Seas indicate the presence of
new genetic evidence for the potential distribution and existence of distinct populations in this area. Moreover, the haplotypes of both species include shared haplotypes of the false killer whales, which could be used to monitor alterations in haplotypes or maternal genetic diversity in the future. Importantly, our findings can contribute to the establishment of baseline genetic information for the Thai Seas and adjacent waters.

5. Conclusions

Our study highlights include valuable information on the mtDNA control region haplotype for both Risso’s dolphins and false killer whales in comparisons made between the Thai Seas and oceans throughout the world. We have also provided new genetic information for both species derived from cetaceans that were stranded along Thai coasts. Despite the limited number of samples in our study, two unique haplotypes of Risso’s dolphins obtained from the Thai Andaman Sea were observed. While the unique haplotypes of this species may infer the existence of local dolphin populations around the Thai Andaman Sea and adjacent waters, the shared haplotype of both species, including that of the false killer whales that inhabit the Thai Seas and other locations, also indicates a connection and potential distribution of the maternal lineage throughout the distribution range of this species. However, in order to identify the existence of local dolphin populations, more information pertaining to population structure, abundance, distribution range, and an understanding of the prey species derived from the stomach contents of the cetaceans in these areas will be needed. Furthermore, more samples from these areas will need to be obtained and investigated in order to gain in-depth population information and to develop and establish effective conservation strategies for Thai cetaceans.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14040257/s1, Table S1: The accession number of Risso’s dolphins (Grampus griseus) used in this study; Table S2: The accession number of false killer whales (Pseudorca crassiden) used in this study. The references [7,39–42,71–83] are cited in the supplementary materials.

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