

University of Groningen

Molecular ecology and evolution of the porpoises

Ben Chehida, Yacine

DOI:
[10.33612/diss.181589330](https://doi.org/10.33612/diss.181589330)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2021

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):
Ben Chehida, Y. (2021). *Molecular ecology and evolution of the porpoises*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen. <https://doi.org/10.33612/diss.181589330>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Chapter 7

Synthesis

Yacine Ben Chehida

The objective of this thesis was to advance our knowledge about the evolutionary history of the porpoise family, in particular the factor that shaped their evolution at the macro- and microevolutionary time scale. At the microevolutionary scale, I have studied the processes shaping the population genetic structure and gene flow for some specific species, sub-species, and populations with the idea to contribute to their conservation. Among the key results of my PhD, the following are among the most significant:

- I provided the first unambiguous estimation of the porpoise phylogeny using complete mitogenomes (Chapter 2).
- Similar to the macro-evolutionary processes reported for other Delphinidae, porpoises radiated during the Late Miocene and Pliocene as a response to past environmental changes. The mechanisms driving species diversification in the northern hemisphere are mirrored in the southern hemisphere with the poorly-known species with a possible parallel evolution related to the colonization of coastal vs. oceanic habitats (Chapter 2).
- At the microevolutionary scale, all intra-specific subdivisions were shaped during the Quaternary glaciations. Our results suggest that the evolutionary history is richer and more complex than previously suspected for many taxa, as many species displayed unreported genetic subdivisions (Chapter 2, 3, 4, and 6).
- The level of genetic diversity within species is primarily determined by the number of intraspecific subdivisions. Once such subdivisions are accounted for, mitochondrial genetic diversity turned out to be a good proxy of the conservation status reported in many porpoise taxa (Chapter 2).
- Harbor porpoise from the North Atlantic (*P. p. phocoena*) formed a coherent genetic cluster during the LGM suggesting that populations on both sides of the Atlantic belong to the same subspecies. Habitat modeling suggests that during the expansion of the polar front, *P. p. phocoena* occupied southern waters in the North Atlantic and then recolonized the North Atlantic and the North Sea following the retreat of the last Pleistocene glaciers. However, contrary to many other cetacean species and previous beliefs, spatial patterns of genetic diversity did not exhibit spatial patterns expected under the hypothesis of a leading-edge effect associated with a rapid northward expansion following the post-glacial ice retreat. Instead, spatial trends in genetic diversity, including the pattern of isolation by distance (IBD), were more consistent with local variation in census population size which suggests that a migration-drift equilibrium has been established, erasing any signal of postglacial expansion (Chapter

3).

- The distinct lineage of porpoises occupying the Iberian waters has been losing mitochondrial genetic diversity at an alarming rate over the past 30 years. This drastic reduction might mirror a potential population collapse of this unique lineage which warrants further study (Chapter 4).
- Harbor porpoises from the Black and Azov Seas form a homogeneous genetic unit despite the previously reported morphological heterogeneity. Population genetic simulations suggest that this lack of genetic subdivision is not related to any time-lag effect between demographic and genetic subdivision (the so-called "*grey zone*" of population differentiation), but more likely reflects a genuine signal of panmixia. Morphological differences between the Black Sea and Azov Sea porpoises may reflect other biological processes (Chapter 5).
- Analyses of the population genetic variation of the highly threatened finless porpoise along the Yangtze River (China) revealed the presence of a cryptic subdivision with three genetically distinct populations. Quantification of the genetic diversity within these populations showed clear evidence of a population decline. Patterns of gene flow inferred in this study also suggested that these three populations are becoming increasingly fragmented, possibly related to increasing anthropogenic habitat disturbances. Finally, demographic inferences using coalescent modeling suggested that the Yangtze finless porpoises colonized the Yangtze River from a few founder individuals during the last ice age, followed by a population fragmentation during the Holocene. In accordance with previous results, I inferred from the genetic data a drastic population collapse within the last 50 years, consistent with the boom of Chinese Modern Economy Development. These echoed the numerous conservation concerns emphasized for this taxon (Chapter 6).

A visual summary of the key results for the harbor porpoises can be found in Figure 7.1. In this synthesis, I will discuss some of these findings in more detail to connect the different knowledge gathered among the chapters of this thesis. I will also provide some perspective. In the first part, I will discuss the process of speciation in the Phocoenidae along the so-called speciation continuum. In the second part, I will discuss the conservation implications of some of the results of this thesis. In the third and last part, I will discuss the reasons for using "classical" molecular markers (i.e. mitochondrial DNA and microsatellites) despite the popularization over the last years of genome-scale datasets, related to the dramatic

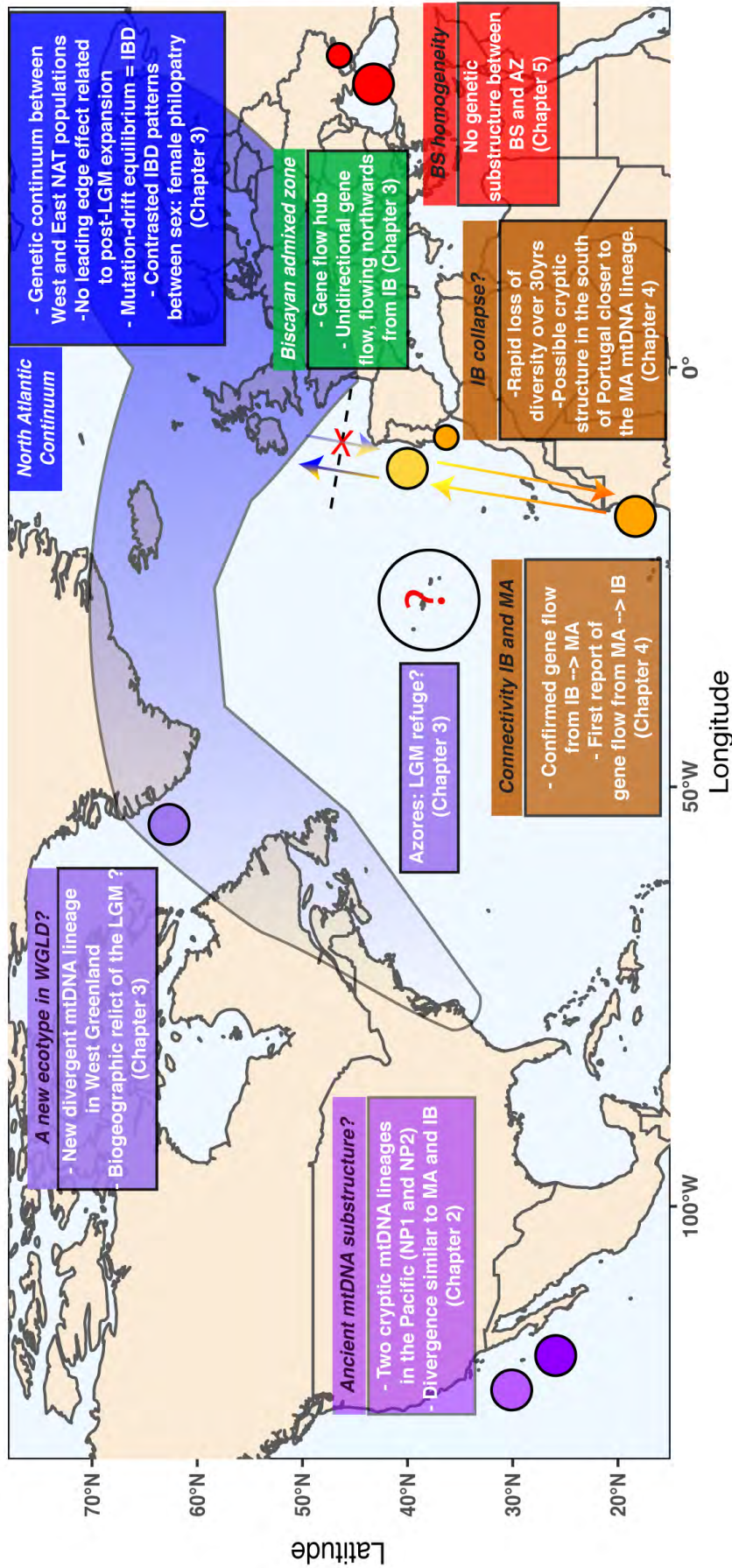


Figure 7.1: Map summarizing the main discoveries related to evolutionary history of the harbor porpoises. NP = North Pacific; NAT = North Atlantic; MA = Mauritania; IB = Iberia; BS = Black Sea; AZ = Azov Sea; IBD = isolation by distance; LGM = last glacial maximum. The circles correspond to different genetic groups identified in this thesis. The ellipse represents approximately the geographical range *P. phocoena*. The white-blue gradient color depicts the pattern of isolation by distance. The dashed line represents the position of the hybrid zone in the Bay of Biscay. The arrows depict gene flow direction. A cross on the top of an arrow means that gene flow does not occur in the direction of the arrow. The empty black circle surrounds the Azores. The question mark next to the empty circle means that the Azores might have been a glacial refuge for the harbor porpoise during the LGM.

reduction of the sequencing costs with the development of next-generation sequencing (NGS) technologies (Cammen, et al., 2016; Morin, et al., 2020b). I will then discuss the limitations of classic genetic markers and explain why the field has to move on to genomic scale studies.

1. Porpoise diversification along the speciation continuum

Like other odontocetes families (Steehan, et al., 2009; McGowen, 2011; McGowen, et al., 2019), the radiation of extant porpoise species occurred from the late Miocene to the early Pleistocene (between 5.5 and 2.5 Million years ago; Mya) in response to environmental changes and habitat diversification. Speciation in the porpoise family likely was similarly mainly mediated by historical variation in their environment and by the colonization of new ecological niches (Steehan, et al., 2009; Marx and Uhen, 2010; McGowen, 2011; McGowen, et al., 2019). Past climatic changes impacted the ocean temperature, currents, distribution of nutrient-rich waters, areas of high primary production, and thus the distributions of prey and predator species (Pastene, et al., 2007). The reallocation of these resources forced cetacean species to colonize new habitats, facilitating allopatric divergence and possibly triggering speciation (Pastene, et al., 2007). Due to their small size and demanding reproductive strategy (for a cetacean), porpoises rely on a regular food intake to meet their demanding life history traits (Lockyer, 2007; Hoekendijk, et al., 2017). They are thus even more sensitive to environmental changes. While their tremendous need for food is expected to favor intra- and interspecific competition, such competition could be mitigated by colonizing and adapting to new ecological niches. The antitropical distribution of the four temperate porpoise species (Dall's and harbor in the northern hemisphere vs. Burmeister's and Spectacled in the southern hemisphere) is an excellent illustration of such a scenario: it is likely the consequence of the past climate changes forcing populations to colonize new habitats. In the same manner, the parallel evolution observed between coastal and pelagic species in the Northern and Southern hemispheres (Chapter 2) reflects how specialization to different ecological niches is driving the divergence of the different porpoise taxa. I also observed that the mechanisms operating at the macroevolutionary timescales are also taking place at a microevolutionary timescale within species as attested by the multiple sub-species and ecotypes that diverged and adapted to different habitat conditions within the last Million years (Chapter 2).

The divergence in the porpoise family follows the concept of speciation continuum (Nosil, 2012b; Stankowski and Ravinet, 2021) where each pair of species, subspecies, ecotypes, or populations are at a different stage along this continuum of genetic divergence (Figure 7.2).

Pairs of lineages are expected to display different levels of reproductive isolation ranging from diverging entities that can fully interbreed to taxa that are completely reproductively isolated. Discussing the case of *Drosophila*, Wu (2001) suggested that the speciation process can be subdivided into four main stages (Figure 7.3). In stage 1, diverging entities show potentially differential adaptation but reproductive isolation is absent. In stage 2, reproductive isolation starts to occur but in case of secondary contact diverging entities may fuse back or not. In stage 3, gene flow between diverging entities can still occur but reproductive isolation is already strong enough to prevent them from fusing back. Stage 4 is the ending state of the divergence process, reproductive isolation is complete and gene flow is not possible anymore. In practice, the process of speciation being continuous, there are no sharp boundaries between these stages (Figure 7.2). Instead, they should be interpreted as practical delimitations informative on how far speciation proceeded. A particularly important area in this continuum, is the “grey zone” of speciation, an area that can encompass both the stage 2 and 3 (Figure 7.3), where different criterion to define species do not provide the same species delineation (De Queiroz, 2007). Here, inspired by these four stages, I will discuss the process of diversification in the porpoise family (Figure 7.3). However, in addition to these four stages, I will consider one additional stage corresponding to the “grey zone” of population differentiation (hereafter stage 0, Chapter 5, see also Bailleul et al. 2018).

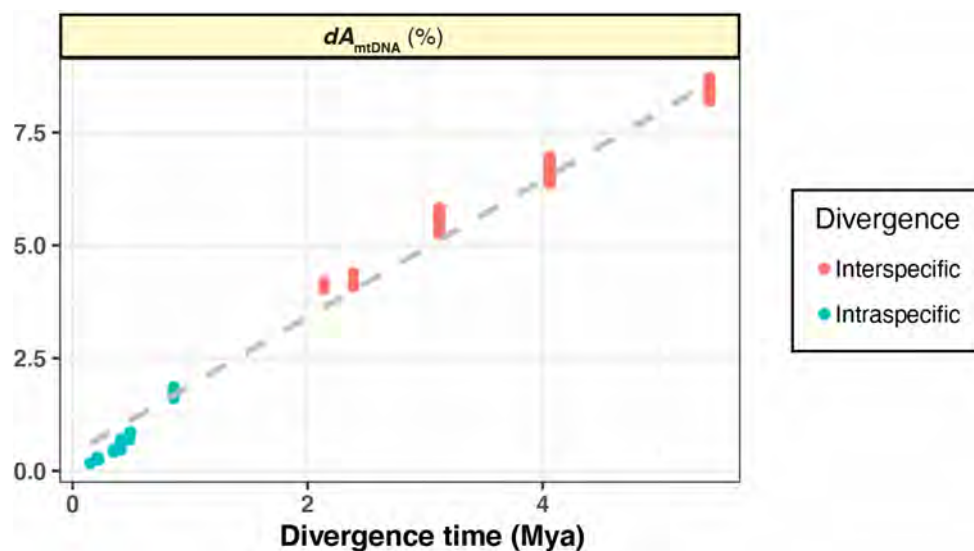


Figure 7.2: Continuum of genetic divergence in the porpoise family. The plots show the evolution of the mitochondrial net genetic divergence (Nei, 1987) between pairs of taxa as a function of the divergence time estimated in chapter 2 (Table S2.5). The grey dashed line shows the regression line suggesting that the level of divergence increases linearly with the divergence time.

- **Stage 0: The grey zone of population differentiation**

At the earliest stage of divergence across the speciation continuum, when two lineages just start to split, they become demographically independent from one another, but the two groups may still be genetically homogeneous (Bailleul, et al., 2018). This time lag between demographic and evolutionary process, called the “grey zone” of population differentiation, is due to the time required for genetic drift to significantly change alleles frequencies and built up genetic differentiation between separating populations (Chapter 5). Assuming neutrality and no gene flow, differentiation between diverging populations depend on the strength of genetic drift, which is itself inversely proportional to the effective population sizes (N_e 's). When N_e 's are large, the strength of drift on the evolution of allele frequencies is reduced. These allele frequencies may remain unaffected for some generations, due to this incomplete lineage sorting. Therefore, the identification of significant genetic differentiation may not always be possible using genetic markers. This creates a "grey zone" of population differentiation where most genetic markers will show limited statistical power to uncover population genetic differentiation. These markers will provide an apparent signal of panmixia despite the demographic independence of two diverging populations. In chapter 5, I discussed the likelihood that such a "grey zone" of population differentiation could explain the observed panmixia in Black and Azov Sea harbor porpoises. The simulations performed, modeling the impact of genetic drift and gene flow on two diverging units, showed that genetic panmixia was the most likely hypothesis. Indeed, given the low N_e observed in the porpoises, any “grey zone” of population differentiation would be very short (< 20 generations).

- **Stage 1: Emerging genetic differentiation but fusion in case of secondary contact**

Once the "grey zone" of population differentiation is passed, lineage sorting becomes efficient and differentiation builds up among diverging groups. Genetic markers can thus capture a significant amount of differentiation in the allele frequencies, as measured typically for example by using F_{ST} statistics. The diverging entities enter stage 1 (Figure 7.3) and are often referred to as "populations" since individuals from distinct populations are still fully interfertile with no or very weak reproductive isolation. Gene flow between populations is thus highly permissible in case of geographic contact or migration between them. Assuming a model of allopatry, when genetic divergence builds up, differences in allelic frequencies and lineage sorting gradually increases. Neigel et al. (1986) showed that during this process, the relationship between two diverging taxa will be described by a polyphyly, then by a paraphyly, and ultimately by a

reciprocal monophyly. In this stage, the phylogenetic relationships between diverging taxa will be principally depicted by polyphyly or paraphyly because divergence did not proceed long enough to allow monophyly to appear. The four genetic groups of Yangtze finless porpoises identified in chapter 6 have recently diverged during the last millennia and could represent taxa at this early stage of the speciation continuum. Their net mitochondrial divergence is quite low ($dA \leq 0.05\%$, based on the control region) and they are still sharing many mitochondrial haplotypes. However, mitochondrial and nuclear F_{ST} values were all highly significant suggesting that, although most of the observed mtDNA haplotypes were not fixed within each group, alleles and haplotypes frequencies were still significantly different.

- **Stage 2: Most genetic markers are differentiated, local adaptation may occur, issue uncertain in case of secondary contact.**

A step forward in the speciation continuum, at stage 2 (Figure 7.3), the relationships between taxa become often depicted by reciprocal monophyly and the level of genetic divergence increases substantially. Allelic frequencies are expected to be highly differentiated between groups and genetic clustering methods will distinguish these groups unequivocally using neutral markers. At this point, taxa are often referred to as subspecies or ecotypes and may display divergent adaptation, reflected by ecological, behavioral, and/or morphological differences. In the genic view of speciation (Wu, 2001) at this stage, a certain degree of reproductive isolation exists between taxa, but they can still fuse or diverge in case of secondary contact. It would also correspond to the beginning of the "grey zone" of speciation (De Queiroz, 2007).

In this thesis, I discussed that on one side, major glaciation events resulted in large habitat fragmentation promoting allopatric divergence at the origin of the divergence between taxa. On the other side, habitat releases during the post-glacial periods have also opened new ecological niches and created ecological opportunities that may have enhanced genetic divergence between subspecies (Steeman, et al., 2009; Slater, et al., 2010). The case of the finless porpoise depicts particularly well how the last ice age and habitat specialization drove the diversification of this species of Phocoenidae (Chapter 6). The finless porpoise colonized the Yangtze River from the Yellow Sea at the beginning of the Holocene once the Yangtze river was freed from the ice. Since, the Yangtze finless porpoise has rapidly diverged from the East Asian finless porpoise by adapting to the freshwater environment (Zhou, et al., 2018).

Previous works have shown that harbor porpoises present multiple similar cases of ecotypes/subspecies. For example, in the North Atlantic and adjacent Seas (including the Black

Sea) multiple subspecies diverged from each other. They display distinct ecology, morphology, and genetic make-up, suggesting they adapted to the local specificities of their respective environments (Rosel, et al., 1995; Fontaine, et al., 2007; Fontaine, et al., 2014; Fontaine, et al., 2017; Nielsen, et al., 2018). For instance, it is thought that the upwelling porpoises from Iberia and Mauritania have diverged from those in the Black Sea and those in the North Atlantic during the last ice age, adapting to the upwelling ecosystems (Fontaine, et al., 2014; Fontaine, 2016). In this thesis, I identified also a fourth mitochondrial lineage displaying a similar level of divergence as the three others (North Atlantic, Upwellings, and the Black Sea). This haplotype was found in one porpoise off Greenland and which may belong to a distinct ecotype adapted to more offshore habitats (see Chapter 3). In the other porpoise species, I revealed intraspecific mitochondrial subdivisions with the same level of divergence as observed between intraspecific divisions in finless or harbor porpoises (see chapter 2). For example, I observed highly divergent haplotypes within the spectacled porpoises (SP1 and SP2), Dall's porpoises (DP1 and DP2), and North Pacific harbor porpoises (NP1 and NP2). The level of net mitochondrial divergence for all the intraspecific groups found in chapters 2 and 3 (from 0.2% to 1.9%) is typical subspecies levels of mitochondrial net divergence (Rosel, et al., 2017).

While these divergent mitochondrial lineages are suggestive that cryptic subdivisions may exist, an alternative hypothesis is possible. Divergent mitochondrial lineage may reflect past subdivisions that may no longer exist. Divergent taxa may still persist and segregate within a population, even at low frequency due to incomplete lineage sorting. For example, Morin et al. (2021) recently analyzed the genetic structure of the North Pacific harbor porpoises using hundreds of SNPs along the genome and observed no genetic subdivision at these nuclear markers despite the two divergent mitochondrial lineages segregating within this group. The same could also be true for the divergent mitochondrial lineage identified in chapter 3 in one Greenlandic porpoise. However, in contrast with the North Pacific harbor porpoises, preliminary analyses of the possibly distinct offshore porpoise ecotype recently discovered using satellite telemetry in Greenland waters (Nielsen, et al., 2018) was found to be genetically distinct at the nuclear genome, based on thousands of SNPs obtained from RADseq for 68 porpoises (Nielsen, et al., 2019). This suggests that the mitochondrial lineage uncovered in chapter 3 is indeed pointing toward a new distinct ecotype that split from the other Atlantic porpoises during the LGM. This points toward the need for additional studies using genome scale markers to assess the evidence of possible subdivisions obtained from a single locus like the mitochondrial genome.

- **Stage 3: Most markers are genetically differentiated, fusion unlikely in case of secondary contact.**

Further along the speciation continuum, at stage 3 (Figure 7.3), reproductive isolation becomes stronger, leading to reduced hybrid fitness and hybrid inviability. At this stage of the speciation continuum, diverging taxa reached a point where fusion is not possible anymore, but rare hybrids can still occur, with rare events of backcrossing that could generate gene flow. At this point, the evolving biological entities come closer to what is commonly referred to as different species or "good species". This stage encompasses the "*grey zone*" of speciation, where barriers to gene flow between species remain partially permeable (Roux, et al., 2016). Thus, hybrids can still be observed, but the species are sufficiently divergent so that their fertility is strongly reduced. Even in the rare case where hybridization leads to a fertile progeny (usually females, but not male hybrids), there may be no significant contribution from these hybrids to the parental gene pools, and thus reduced or no gene flow between species.

In the porpoise family, a couple of species pairs might correspond to this stage, such as the Indo-Pacific and the Narrow-ridged finless porpoises. Although the study of Zhou et al. (2018) revealed no evidence of gene flow between these two species, their overlapping distribution in the Taiwan Strait and their low level of divergence ($dA = 0.86\%$; see also Rosel, et al., 2017) suggest that gene flow may still be possible between them. The case of the North Pacific and North Atlantic harbor porpoises is another interesting example. Currently considered as different subspecies, they represent a good candidate of a pair of taxa plausibly in the middle or beyond the "*grey zone*" of speciation given their level of divergence (higher than between the officially recognized distinct species of finless porpoises), their ecological/morphological differences, and geographical isolation. Since their distribution ranges do not overlap, there is currently no way to tell what could happen in the case of secondary contact. With the ongoing climate change and the re-opening of the Bering Strait, it will be interesting to continue tracking possible evidence of secondary contact between Pacific and Atlantic harbor porpoise.

The Dall's and harbor porpoises, which diverged within the last three Mya might also correspond to this "*grey zone*" of speciation. Evidence of recurring natural hybridization between female Dall's porpoise with male harbor porpoise in waters surrounding British Columbia in Canada indicates that pre and post-zygotic barriers are still incomplete between these two sister species (Willis, et al., 2004). The viability/fertility of these hybrids is still unknown, but stranded hybrids have been documented carrying a fetus, presumably resulting from the back-crossing between a female hybrid and a male harbor porpoise (Crossman, et al., 2014).

- **Stage 4: Highly differentiated species**

The last stage of the speciation continuum (Figure 7.3) implies that complete reproductive isolation has evolved. Species are unquestionably distinguished as two separate evolutionary entities. Hybridization, if it is still occurring, should be extremely rare, and hybrids are expected to be unviable at this point. If occasionally F1 or F2 hybrids are still fertile, they should be quickly eliminated by selection against the hybrids. Thus, they do not contribute to the parental gene pool. The full reproductive isolation characteristic of this last stage is expected between any recognized porpoise species. For example, porpoise species from the Northern and Southern hemispheres diverged more than four Mya and are completely isolated ecologically and geographically. This suggests these species are completely reproductively isolated, however, this cannot be formally assessed in cetacean species. Nevertheless, alternative approaches, such as the statistical framework developed by Roux et al. (2016), can be applied to estimate the amount of gene flow between pairs of population, subspecies, and species from genomic data. More generally, as for other cetacean groups (Foote, et al., 2016; Arnason, et al., 2018; Westbury, et al., 2020), genomic data will be instrumental to advance further our knowledge of the processes governing the divergence of the porpoise species and estimate their position along the speciation continuum.

Finally, it is worth mentioning that there is accumulating evidence supporting the idea that the achievement of complete reproductive isolation is a very slow process in cetacean (Arnason, et al., 2018; Westbury, et al., 2020). Westbury et al. (2020) showed there is a long-time lag between the split and the complete cessation of gene flow among many species and even families of toothed whales. The aforementioned study estimated the splitting time between the harbor and finless porpoise at 5.3 Mya based on a single whole genome per species, thus confirming the divergence time obtained in chapter 2 from complete mitogenome data. However, their results also indicated that gene flow and incomplete lineage sorting between these species may have persisted up to four million additional years. They attributed this long delay before complete isolation to the high mobility of the cetacean, the very conserved architectures of cetacean genomes resulting in a slow accumulation of genomic incompatibilities, and the absence of obvious geographical barriers in the marine realm (Westbury, et al., 2020).

Moreover, the frequent observation of hybrids between congeneric cetacean species corroborates the idea that barriers to interbreeding are not absolute among cetaceans, further challenging the process of speciation in this group and raising the question of how they can

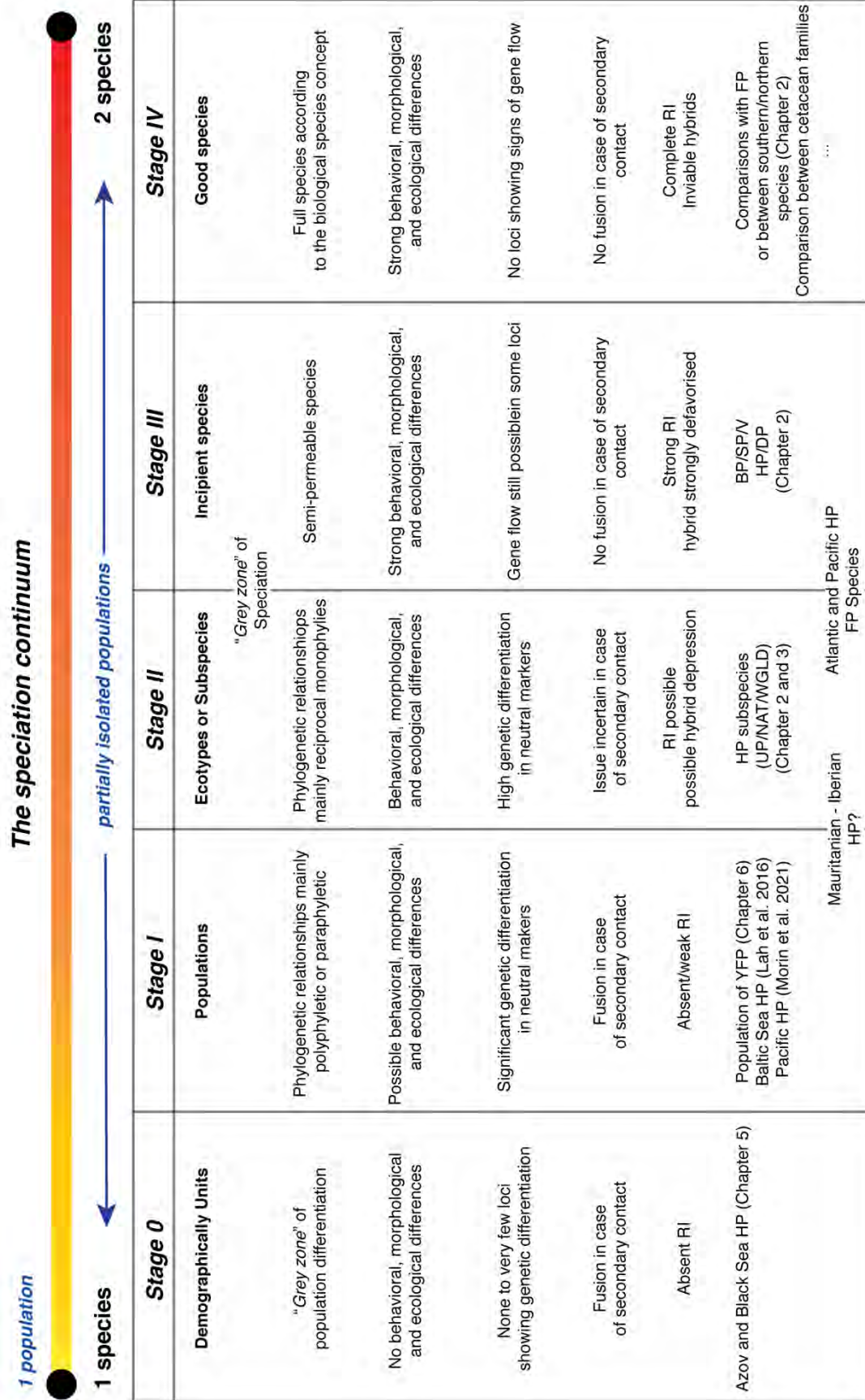


Figure 7.3. Description of the five stages of biological divergence along the speciation continuum in the porpoise family described in section 8.2. RI, reproductive isolation; HP, harbor porpoise; NAT; North Atlantic harbor porpoise; UP, upwelling harbor porpoises; WGLD, West Greenlandic harbor porpoise; FP, finless porpoise; YFP, Yangtze finless porpoise; DP, Dall's porpoise; BP, Burmeister's porpoise; SP, spectacled porpoise; V, vaquita.

keep their genetic integrity (Crossman, et al., 2016). It has been advanced that the stability of the karyotype of cetacean genomes, despite their long evolutionary history (Fordyce, 2018), is an indicator of a lack of strong post-mating barriers to hybridization (Crossman, et al., 2016). Moura et al. (2020) also showed that the explosive radiation of many Delphinidae species occurred in presence of extensive gene flow, potentially leading to a case of hybrid speciation (Amaral, et al., 2014). More generally, the rapid radiation of many species of toothed whales, including porpoises, might have been boosted by the presence of gene flow as it is now acknowledged for many other taxa (Marques, et al., 2019). Analogous evolutionary mechanisms might also explain the radiation of the infraorder of cetacean. Again, the application of genome-wide data can shed some light on the ins and outs of these evolutionary processes in cetaceans, including porpoises.

2. General conservation implications and perspectives

Many porpoise species, subspecies, and populations have recently undergone dramatic population size declines as a consequence of human activities (Carlén, et al., 2021). That is why some species like the finless porpoises, harbor porpoises from the Black Sea and Baltic Sea, or the vaquita are considered as highly endangered by the IUCN. In contrast, other taxa like the Dall's porpoise and the North Atlantic harbor porpoises, are listed as *Least Concern* by the IUCN. However, certain human activities such as fisheries are increasingly affecting these taxa and could put them at risk too in a near future (Reeves, 2003). The spectacled and Burmeister's porpoises have been recently classified as *Least Concern* and *Near threatened*, respectively, but knowledge about these taxa is very lacunar (especially about abundance) to provide a reliable conservation status or population trends (Félix, et al., 2018; Dellabianca, et al., 2018).

Genetic analyses based on traditional markers (principally mtDNA and microsatellites) have been (and are still) used abundantly to assist population assessments and conservation policies. They provided information on cetacean biology that could not have been gathered otherwise using direct observations. Such information includes surveying genetic variation, understand population structure and dispersal behaviors, estimate population trends, levels of inbreeding, gene flow, assess impacts of past climate changes on natural populations to forecast possible future evolution with the ongoing environmental changes, or identify evolutionary significant units and define management units (Morin and Dizon, 2018). For example, genetics was instrumental to define the management units used in Dall's (IWC, 2002) and harbor porpoises (Donovan and Bjørge, 1995). I will discuss below how the knowledge obtained

during this thesis can help inform or sharpen future porpoise management plans.

Yangtze finless porpoise

The chapter 6, dedicated to the conservation genetics of the Yangtze finless porpoise, provides probably one of the most concrete examples of the usefulness of genetic approaches to infer population trends and understand the impact of human activities on a small cetacean species. This study explores the past and contemporaneous demographic processes that shaped the genetic structure of the critically endangered populations of finless porpoises in the Yangtze River in China. Genetic clustering methods on microsatellites identified at least three genetically distinct populations. Each of these populations displayed an extremely low contemporary N_e which is consistent with the rapid decline they underwent during the recent decades. It was believed that the ongoing fragmentation of their habitat over the last decades resulted mainly from the Chinese economic boom along the Yangtze River. This assumption was supported by demo-genetic inferences using the approximate Bayesian computation analysis that highlighted a massive population contraction, up to a factor of 50, in each population occurring within the last 50 years. However, I also highlighted that environmental changes during the Holocene affected the connection among the different lakes of the Yangtze River. These past changes initiated the fragmentation of the finless habitat before the Chinese industrialization and made the Yangtze finless porpoises particularly vulnerable to the current anthropogenic activities. Further insight on the conservation genetics of these porpoises was provided by the genetic clustering methods which revealed that porpoises in between the three identified populations consist of an admixed group whose genetic makeup is a mixture of the genetic ancestry of the three other populations. Subsequently, the analysis of connectivity between populations showed that highly asymmetric gene flow occurs via the intermediate admixed population, but not enough to ensure effective gene flow between the three populations at the extremities. These results imply that populations are losing connectivity, and underlined the pivotal role of the admix population in connecting the other populations. From an applied perspective, these results help to realize the crucial roles of this admixed population acting as a hub to connect by gene flow the distinct populations along the Yangtze River. Conservation policies decided to prioritize the conservation efforts on this admix group in order to maximize population connectivity between adjacent lakes and mitigate the ongoing habitat fragmentation (Mogensen, 2019; Huang, et al., 2020). Also, based on the genetic structure highlighted in chapter 6, several individuals were translocated to maximize genetic diversity in different reserves dedicated to the protection of the finless porpoises (Ding, et al., 2019; Mei, et al.,

2021). These measures have borne fruits as the first signs of population recovery are becoming visible for the Yangtze finless porpoises since their implementation (Huang, et al., 2020).

North Atlantic harbor porpoise

In chapter 3, I explored the evolutionary history, population structure, and the processes driving dispersal behavior in the harbor porpoises with a principal focus on the *P. p. phocoena* sub-species. I showed that porpoises form this genetic continuum stretched from the northern part of the Bay of Biscay to the East coasts of the United States with no apparent barriers to gene flow. However, I showed that individual dispersal was geographically restricted and it was thus insufficient to genetically homogenize this continuum leading to a highly significant pattern of isolation by distance (IBD). Therefore, conceptually managing such a continuum can be challenging because defining discrete conservation units in a geographically continuous system is not straightforward. Classical concepts of population (Waples and Gaggiotti, 2006) are thus difficult to apply in such a continuum under IBD (Rousset, 1997). Under such a system, panmixia holds only at the scale of the "neighborhood". A significant level of genetic differentiation is expected between any part of the continuum that exceeds the neighborhood size and can thus be considered as potential distinct management units. Defining management units in such conditions is a delicate task. Additional complicating matters include a geographical variation of the IBD strength (Fontaine, et al., 2007) and a differential IBD between sex and age classes (Fontaine, et al., 2017). Therefore, in order to plan management and conservation efforts for *P. p. phocoena*, the joint IMR-NAMMCO workshop on the status of harbor porpoises (2019) used the results presented in chapter 3 and stressed the need to define efficient and practical management units across the North Atlantic. They based their assessment using multiple lines of evidence such as genetic, demographic, behavioral, ecological information, but also on the genetic and demographic connectivity between geographic areas. It was also stressed that such an endeavor must be supported by strong international collaborations to efficiently monitor porpoises over their complete distribution range.

The IMR-NAMMCO workshop (2019) identified some knowledge gaps that must be filled to keep improving the monitoring of harbor porpoises in the North Atlantic. It was suggested that genetic analyses will be essential to fill some of these gaps. First, due to the growing impact of commercial fisheries and bycatch on harbor porpoises, there is a crucial need to define and adjust management units that account for these issues. From a theoretical point of view, as local variation in IBD strength is a function of local individual density, it must be strongly influenced by the bycatch rates. To this date, the impact of bycatch in such a biological

continuous system under IBD has not been properly assessed. Such an investigation is fundamental to evaluate the status of the local demes across the North Atlantic, to tailored conservation strategies, and to identify the areas that are at high risk of local depletion, which in turn could disrupt gene flow and fragment populations. Second, the genetic structure around certain areas such as the Irish waters, Faroe Islands, or around Greenland remained poorly known and must be studied in greater detail. Third, the study presented in chapter 3 is based on 10 microsatellites and a quarter of the mitogenomes. However, genomics data are known to provide greater resolutions than traditional molecular markers at detecting subtle and unreported population structure. For example, Lah et al. (2016) showed that genetic structure in the Baltic Sea could be identified using 2,872 SNPs but not with 13 microsatellite loci. Therefore, I expect that future studies will use more systematically genome-wide markers obtained using affordable NGS protocols, such as amplicon sequencing for Genotyping-in-Thousands method (Campbell, et al., 2015), to provide increased power to study fine scale population structure in harbor porpoises (Meek and Larson, 2019; Morin, et al., 2021). Finally, the genetic structure and abundance of harbor porpoises are expected to change over time, particularly in the context of the ongoing climate change. The genetic structure described in chapter 3 was obtained for a sampling cohort ranging between 1990 and 2000. There is, therefore, a critical need to assess how the genetic structure has evolved over the last 30 years. Such genetic time series would allow to understand the temporal variations of the genetic structure and to adjust the management protocols accordingly.

Another interesting insight to the conservation of the North Atlantic harbor porpoises was provided by complementing the population genetic inferences with the Aquamap environmental suitable habitat modeling which provided a window on how habitat evolved in the past and how they would change in a near future, by 2050 under the most aggressive scenario of the Intergovernmental Panel on Climate Change (Schwalm, et al., 2020). Under this climate model, while not changing dramatically, the abiotic environmental suitability for harbor porpoises will be shifted slightly northward in a near future. This kind of approach can help anticipate how the species is going to change its spatial structure in the future and then devise conservation strategies accordingly. However, the predictive habitat model used in this thesis can be improved at least in two ways. First, it does not consider primary production and shift in prey distribution, which are major drivers of the range of cetaceans (see chapter 1). Their inclusion might improve the predictions. Second, the model does not consider the distinct ecological preferences of each ecotype and thus assumes that harbor porpoise is monotypic. Not taking into account local adaptation and ecological preferences can lead to inaccurate range

prediction as a set of variables can be relevant for one ecotype and can be inappropriate for another (Hällfors, et al., 2016). For example, *P. p. meridionalis* and the porpoise from Greenland seem to favor more offshore and deeper water than other harbor porpoises. The inclusion of these observations to predict the habitat of *P. p. phococena* can distort the inferred suitable habitats by suggesting that areas with deep waters are highly favorable for *P. p. phococena*, while they are not. In the future, these habitat models could be greatly refined by modeling suitable habitats for each ecotype separately. In addition, supplementing these models with genotype association analyses on genomics data can further improve considerably the accuracy of the predictions (Razgour, et al., 2019).

A distinct ecotype in West Greenland waters?

Using satellite tracking, Nielsen et al. (2018) showed that some harbor porpoises from West Greenland display an offshore behavior that contrasts strikingly with the coastal behavior documented for the neighboring populations of *P. p. phocoena*. The authors argued that by displaying a large prey diversity (Heide-Jørgensen, et al., 2011) and different migratory and diving behavior, harbor porpoises from West Greenland (WGLD) could belong to a unique oceanic ecotype. The genetic distinctiveness report in one WGLD individual carrying a very divergent mitochondrial haplotype (Chapter 3) as well as in another preliminary study based on thousands of SNPs data (IMR-NAMMCO, 2019; Nielsen, et al., 2019) lend additional support to their morphological and behavioral distinctiveness and suggest that these porpoises could indeed form a genetically distinct ecotype. The joint IMR-NAMMCO (2019) recommended considering harbor porpoise from west Greenland as a separate management unit but it was also underlined that more research was needed. Indeed, many questions remain open like how differentiated are porpoises from WGLD compared to the other populations from the North Atlantic? Are they all genetically different? Is there a genomic signature of local adaptation? How big is this genetically distinct population? Do they all display this offshore behavior? How ecologically different are they? Further studies are required to characterize its peculiar evolution and demographic trends, as well as refine its ecology and behavior to understand how distinct/unique these porpoises are. Like most porpoises, bycatch is a threat to Greenlandic harbor porpoises. Besides, these porpoises are traditionally hunted around Greenland (Teilmann and Dietz, 1998). Whether these direct catches are sustainable or not remains unknown. However, to this day, the hunt is not regulated in Greenland as there are no limits on the maximal number of catches. It could represent a threat, especially if this particular population

is small and isolated. Although demographic modeling seems to show that overall the number of porpoises is relatively stable in Greenland, more research efforts must be carried out to assess the impact of both bycatch and direct catches (IMR-NAMMCO, 2019).

The upwelling porpoises *P. p. meridionalis*

The Iberian and Mauritanian harbor porpoise constitute a distinct group specialized to the upwelling environment in the meridional waters of the North-East Atlantic. They are genetically, morphologically, and ecological distinctive from other harbor porpoises (chapter 2,3,4; see also Fontaine et al., 2014). Consequently, although these populations are currently considered as separate management units (IMR-NAMMCO, 2019), there is accumulating evidence indicating that they should be considered as a distinct subspecies (Smeenk, et al., 1992; Donovan and Bjørge, 1995; Fontaine, et al., 2014; Fontaine, 2016; Pierce, et al., 2020). Fontaine et al. (2014) suggested reclassifying the Iberian and the Mauritanian porpoises as a new subspecies because of the level of mitochondrial divergence as large as the divergence observed between subspecies currently recognized in the North Atlantic (*P. p. phocoena*) and in the Black Sea (*P. p. relicta*). Even if there is no formal taxonomic description yet, Fontaine et al. (2014) suggested the name *P. p. meridionalis*. Since then, several demands have been introduced to consider them as a separated subspecies, but officially this new classification has not been adopted yet (Pierce, et al., 2020).

A collapsing Iberian population of *P. p. meridionalis*?

The estimated census size of the Iberian population is among the lowest (~2,900 individuals) and so is the local density (Hammond, et al., 2013; Hammond, et al., 2017). This echoes to the low genetic diversity and N_e reported for this population (see chapter 2, 3, and 4). The analyses performed in chapter 4 indicated also a sharp decline in mitochondrial genetic diversity over the last two generations (Figure 4.2) that may raise concerns about the conservation status of the Iberian porpoise (Pierce, et al., 2020). This decline of genetic diversity combined with their dependence on very specific environmental conditions potentially expose them to genetic, demographic, and environmental stochastic forces, which could represent a great threat to these porpoises. However, the primary threat remains the high bycatch rates due to the importance of fishing off Portugal and Spain (Pierce, et al., 2020). In this regard, the International Whaling Commission reviewed in May 2020 the situation of the Iberian harbor porpoise and agreed that bycatch mortality was unsustainably high (Pierce, et al., 2020). Iberian fisheries have one of

the highest rates of bycatch in the world (up to 10%) and population dynamics models showed that a safe limit on removals would be about 25 animals per year, far from the current rates (Zerbini, et al., 2011; IMR-NAMMCO, 2019; Pierce, et al., 2020). This called for 1) concrete and immediate action to regulate fishery bycatch in the Iberian waters to ensure the survival of this unique population and 2) the reclassification of these porpoises (jointly with the Mauritanian ones) as a distinct subspecies to assess and define their IUCN status which is likely highly threatened.

The admixture zone in Bay of Biscay

Populations of *P. p. meridionalis* from Iberian waters and those from *P. p. phocoena* further North form a hybrid zone in the northern part of the Bay of Biscay. Such a tension zone is of particular interest for both evolutionary and conservation purposes. Coalescent modeling showed that harbor porpoises from the North Atlantic and the upwelling came back into contact during the last millennia (Fontaine, et al., 2010; Fontaine, et al., 2014). This contact zone with mixed genetic ancestry from both subspecies extends from the Bay of Biscay to the Celtic Sea and in the Channel and then vanishes further North (Fontaine, et al., 2017).

From an evolutionary perspective, hybrid zones are known to be "natural laboratories" to study evolutionary processes and in particular local adaptation, reproductive barriers, and speciation (Hewitt, 1988). The presence of such a hybrid zone in the Bay of Biscay indicates that *P. p. meridionalis* and *P. p. phocoena* are not fully reproductively isolated. However, its relatively narrow size suggests that selective processes may limit its extension and could suggest that speciation might have begun (Hewitt, 2001). The future fate of this hybrid zone is still unsure. In the long term, it can persist under certain conditions (Barton and Hewitt, 1985) or on the contrary disappear. One possible outcome is that the Iberian population will get incorporated to *P. p. phocoena* over time because dispersal is unidirectional flowing from Iberia northward into the hybrid zone (chapter 3 and 4). This unidirectional dispersal can lead gradually to the assimilation of the Iberian population by *P. p. phocoena*, thus resulting in the disappearance of the Iberian porpoises. The considerable difference in habitat and population size between the two populations makes this scenario particularly plausible. However, if local adaptation is strong enough, it could counter this effect and allow the two taxa to co-exist (Barton and Hewitt, 1985). The application of genomics data combined with cline analyses in the future can provide important insights on the properties of this hybrid zone (Gompert, et al., 2017). For example, it can help determine whether local adaptation is occurring or not. Moreover, it can help investigate whether hybrid individuals exhibit signs of fitness decline or

not. Answering these questions is crucial to understand which evolutionary scenario is maintaining this hybrid zone. Furthermore, it will be important to track how this hybrid zone has evolved over the last 30 years. It has been already noticed that the Iberian population has lost half of its mitochondrial genetic diversity over the last three decades. It will be interesting to see how the admixture zone in the Bay of Biscay has evolved.

From a conservation perspective, understanding the fine scale population structure along the hybrid zone will help better monitor the harbor porpoises along the North Atlantic. This hybrid zone plays an important role in the population dynamics of the harbor porpoises in the Atlantic because it serves as a hub for gene flow between *P. p. phocoena* and the Iberian population (Chapter 3). It should therefore be carefully monitored. Besides, hybrid zones are known to be particularly sensitive to climate change (Taylor, et al., 2015). A thorough study of this area will help to assess how the ongoing climate change is impacting the harbor porpoise. For example, the generalization of the time series presented in chapter 3 including microsatellites and more individuals continually sampled across the Bay of Biscay and the adjacent regions can enable to test whether this hybrid zone shifted towards the north over the last 30 years.

The African (or Mauritanian) population of P. p. meridionalis

The African population of harbor porpoises is highly isolated and constitutes the rear edge of the species distribution located in one of the most productive marine habitats, the Mauritanian upwelling. They are closely related, yet genetically distinct, from the Iberian porpoises (see chapters 2, 3 and 4). As such, they form a second population of *P. p. meridionalis*. Like the Iberian porpoises, the African porpoises are morphologically larger (~2m) than the typical size reported for two other subspecies (1.5m) in the North Atlantic (*P. p. phocoena*) and in the Black Sea (*P. p. relicta*), or even compared with the harbor porpoises from the North Pacific (*P. p. vomerina*; Read, 1999; Fontaine, 2016). These porpoises are primarily found off of the Mauritania coast, but their range stretches from Morocco to the Gambia (Boisseau, et al., 2010; IMR-NAMMCO, 2019). Like the Iberian population, it is thought that their larger body size is related to their specialization to the upwelling ecosystem (Smeenk, et al., 1992). Besides, very little is known about the relationship that exists between these two upwelling populations. How connected are they? The phylogenetic inference of chapter 4 revealed the first evidence of migration from the Mauritanian population into the present-day Iberian population, suggesting this latter is not as isolated as previously thought.

Also, the haplotype network revealed that the two migrating individuals displayed one mitochondrial haplotype (haplotype 10, Figure S4.2) that was different from other Mauritanian haplotypes by at least two mutations, supporting the idea that additional cryptic populations might exist between the Iberian and African populations. This result suggests that population structure among the upwelling porpoises might be more complex than previously thought and raise several additional questions. For example, are there additional populations than currently suggested, based on our sparse sampling? What is the connectivity among them? Are there individuals continuously distributed between Iberia and Africa? If so, is there a contact zone between both? Due to their large distribution along the North West African coasts, can we expect a pattern of IBD, similar to the one observed in *P. p. phocoena*? From a conservation point of view, answering these questions is fundamental. Currently, Mauritanian porpoises are considered as a separated management unit but there is still very limited data available about their range, population genetic structure, abundance, bycatch, and other threats (IMR-NAMMCO, 2019). As a result, the status of this population is highly uncertain. So far, the data available comes from stranded animals, some of which show evidence of being bycaught (Van Waerebeek and William, 2007). However, how sustainable are these incidental catches is currently unknown. The data deficiency for this population meant that it is currently impossible to estimate either abundance or the impact of bycatch (IMR-NAMMCO, 2019). The joint IMR-NAMMCO workshop (2019) stressed that genetic inference would be paramount to provide first estimates of population abundance. The genetic diversity at both mtDNA and microsatellites quantified in this thesis is low and similar to the one observed in other populations known to be under threat (like Iberia or the Black Sea; Chapters 2, 3, and 4). Also, the N_e value estimated of 16 (95% CI:6-31; Table S3.6) is the lowest estimated in any harbor porpoise population, which probably reflects their low private allelic richness (Table S3.5 and Figure S3.13) raise additional concerns. Therefore, like all other populations, it is crucial to rapidly assemble information to assess the status of the Mauritanian porpoise and design efficient actions to preserve it.

3. Limitations and perspectives

The use of “classic” genetic markers in the age of genomics

All the chapters in this thesis relied on two types of molecular markers to explore the patterns of genetic variation in the porpoises: mitogenomes (chapters 2, 3, 4, 5, and 6) and microsatellites (chapters 3, 5, and 6). These traditional molecular markers greatly improved our

knowledge about the phylogeny, phylogeography, and conservation genetics of the porpoises (Figure 7.1).

The advent of NGS and their increasing applications to non-model taxa in the last five years have opened up new avenues to 1) better address questions that have been studied with traditional molecular markers and 2) explore new ones inaccessible so far using traditional markers (Luikart, et al., 2018). NGS gives access to millions of loci spread across the genome and most of the conservation genetics and evolutionary biology addressed in this thesis could have been addressed with genomics markers. Indeed, genome-scale markers are known to often outperform classical markers because they provide many more markers and a more representative picture of the variation along the genome (Funk, et al., 2012; McMahon, et al., 2014; Loire and Galtier, 2017; Gagnaire, 2020). However, in this thesis, the application of classical genetic approaches was still necessary for three main reasons:

1. First, I used in this thesis classic genetic markers to remain consistent with previous studies and to use previously published data and combine them with the new data produced in this thesis. Indeed, chapters 3, 5, and 6 comprised both published and newly generated data sets. The comparisons with those past studies would not have been possible without using the same molecular markers. In chapter 3, for instance, it allowed the comparison of the 768 harbor porpoises from the East Atlantic, from Fontaine et al. (2014), with the 173 new harbor porpoises from the West Atlantic using a common set of genetic markers.
2. Second, many studies presented in this thesis rely on extensive sampling of hundreds of individuals, offering a precise representation of the spatial distribution of the genetic variation. Alternative genomics approaches such as the RADseq or whole genome sequencing remain too expensive to sequence hundreds of individuals (especially when I started this thesis).
3. Finally, NGS methods are highly sensitive to DNA quality, as many of the samples used in this thesis come from degraded tissues, it would have limited the number of samples I would have been able to use.

Emerging NGS approaches, such as genotyping-by-sequencing (GBS) approaches, such as the Genotyping-in-Thousands by sequencing (GTseq) approach (Campbell, et al., 2015), are promising alternatives that can now overcome the sample quality and the cost issues highlighted. GBS facilitates the collection of genome-scale data at similar costs to traditional markers (Meek and Larson, 2019). For example, a large number of individuals can be genotyped for a panel of a few hundred SNPs in a single Illumina HiSeq lane. GBS can be

applied even to poor quality samples (Morin, et al., 2021). It also permits the identification of short genomic regions containing multiple SNPs segregating as haplotypes (called microhaplotypes). These microhaplotypes are phased, providing much more information than the same number of bi-allelic SNPs. Thus, for a similar sequencing effort, they provide more statistical power per locus to address population genetics questions (Baetscher, et al., 2018). All these properties make GBS approaches particularly attractive for future conservation genetics projects (Baetscher, et al., 2018). Morin et al. (2021) demonstrated the applicability of GBS in the study of the genetic structure of the Pacific harbor porpoises. Using 271 highly degraded samples spread continuously across the Pacific American coasts, these authors extracted 292 loci as microhaplotypes. This study illustrated the unprecedented power of GBS to detect fine population subdivisions (Morin, et al., 2021).

Consequently, here I advocate that the fields of cetacean conservation genetic and phylogeography must now move to genomics because classical markers come with a certain number of limitations and genomics approaches can overcome many of them (Luikart, et al., 2018). For example, classical genetic data sets, composed of a dozen molecular markers, are just a very small sample from the whole genome (Gagnaire, 2020). Using such a low number of loci comes with inevitable high sampling errors due to the stochastic nature of some evolutionary processes. This may lead to low power or biased inferences that may not be representative of population or species evolutionary history (Loire and Galtier, 2017). The massive number of independent loci provided by genomics datasets enable to easily bypass these limitations. Besides the increasing resolution and precision, genomics can address previously intractable questions (Luikart, et al., 2018). For example, genomics can advance tremendously our understanding of the roles of selective processes in shaping the genetic variation across the genome, explore the genetic basis of adaptation, identify candidate genes involved in speciation or measure fitness and inbreeding depression (Nosil, 2012a; Luikart, et al., 2018; Robinson, et al., 2019; Gagnaire, 2020). In addition to answering these fundamental evolutionary questions, genomics permits to estimate more accurately genetics parameter of relevance for conservation, such as delineating more confidently the species tree, identifying fine-scale population subdivision, or estimating N_e and its temporal variation, genetic differentiation, gene flow, admixture, inbreeding, relatedness, inbreeding depression, demographic history, phylogenetic relationships, etc. This holds the promise of assisting more reliably the conservation of cetacean (McMahon, et al., 2014; Teixeira and Huber, 2021).

Today, the field of cetacean genomics is still in its infancy but it is growing rapidly (Cammen, et al., 2016). A dedicated international workshop during the World Marine Mammal

Conference in December 2019 (WMMC 2019) held in Barcelona showed the growing interest of cetacean biologists in the field of genomics. The increasing number of genomics studies covering a various spectrum of topics attests well to this rising interest (Yim, et al., 2014; Foote, et al., 2016; McGowen, et al., 2019; Morin, et al., 2020a; Morin, et al., 2020b; Moura, et al., 2020; Morin, et al., 2021). Accordingly, the number of draft reference genomes available for cetaceans has rapidly increased over the last years (Morin, et al., 2020a). For porpoises, a couple of genomics studies have already been published (Lah, et al., 2016; Zhou, et al., 2018; Autenrieth, et al., 2018; Morin, et al., 2020b; Morin, et al., 2021) and three reference genomes are available (Autenrieth, et al., 2018; Zhou, et al., 2018; Morin, et al., 2020b). In addition, preliminary results on the harbor porpoise evolutionary history (Fontaine and Ben Chehida, 2019) and the phylogenomics of the complete porpoise family (Fontaine, et al., 2019) based on low coverage whole genome resequencing data were presented during the WMMC 2019. Consequently, due to the great opportunities offered by genomics over traditional markers, similar studies should (and will certainly) become commonplace in the coming years.

From conservation genetics to conservation genomics

Genomics is expected to provide new insights informative to hone conservation plans (Funk, et al., 2012; McMahon, et al., 2014; Meek and Larson, 2019; Morin, et al., 2020a). As previously mentioned genomic resources provide the tools to explore thoroughly a broad spectrum of questions (Luikart, et al., 2018). In addition, these kinds of information can tremendously help conservation programs by offering an improved estimation of numerous parameters of relevance for conservation. However, its greatest potential lies in the study of functional genetic variation and the ability of genomics to uncover the basis of adaptation (Funk, et al., 2012; McMahon, et al., 2014; Loire and Galtier, 2017; Razgour, et al., 2019; Teixeira and Huber, 2021).

So far, the best illustration of the usefulness of genomics to inform porpoise conservation is the example of the vaquita (Morin, et al., 2020b). In order to prevent the extinction of the vaquita, a high quality chromosomal-level reference genome (the most complete cetacean genome to date) was generated. Based on this reference genome, Morin et al. (2020b) analyzed the genomic diversity and historical demography of the vaquita. The very low nuclear genomic diversity they reported echoed to the low mitochondrial level of genetic diversity of the vaquita I highlighted in chapter 2. However, the application of the pairwise sequentially Markovian coalescent (PSMC) indicated that the vaquita population remained low and stable for the last ~300,000 years, a demographic trend not captured using the skyline plot

approach. Mitochondrial genetic diversity provided resolution only since its time to the most recent common ancestor dating back to 3,000 years before the present (Figure 2.5). This is a perfect illustration of the additional power provided by the analysis of whole genome variation that includes several thousand loci to inform demographic inferences. In addition, the same study highlighted that the level of heterozygosity was evenly distributed across the whole genome. The coalescent simulations performed indicated that this pattern likely resulted from a long-term small population size rather than a recent severe bottleneck or inbreeding. They concluded that the long persistence at a low size allowed the vaquita to purge highly deleterious mutations from the population (Robinson, et al., 2019). These results are vital for the conservation of the vaquita because they suggest that this species on the brink of extinction is not a dead-end species doomed to extinction due to its very low genetic diversity (Morin, et al., 2020b; Whibley, 2021). Instead, it strongly indicates that if bycatch (i.e. the main threat to vaquita) could be totally interrupted, the inbreeding depression should not be considered as a concern for the recovery of the vaquita (Morin, et al., 2020b).

The generalization of the simulation framework used by Morin et al. (2020b) could be extended to study all species of porpoises. A comparison between endangered and non-endangered taxa could be particularly insightful to shed some light on the genome-wide dynamics associated with inbreeding depression in porpoise taxa. Combine with the inference of demographic change, it could help to figure out whether taxa at a small census size, N_e , or range suffer from severe deleterious mutations or if these mutations are purged from the genome as a result of the long-term persistence like the vaquita (Robinson, et al., 2019; Morin, et al., 2020b).

Finally, genomics data can shed light, by using genome scans, on how some populations are locally adapting to the specificity of their environment (Funk, et al., 2012; Gagnaire, et al., 2015; Oleksiak, 2018; Gagnaire, 2020). That could be, for example, the case for the Iberian, Mauritanian, or Greenland populations. If local adaptation occurs in these populations, that would confirm that they represent separate evolutionary significant units and would warrant the elaboration of specific long-term conservation measures. Also, I highlighted a total genetic homogeneity between Azov and Black Sea harbor porpoises (Chapter 5) despite reported morphological differences (Gol'din, 2007; Gol'din and Vishnyakova, 2015). Genome scale analyses can be applied to investigate potential signatures of local adaptation in the genome of either of these porpoises and could represent the basis to define two distinctive conservation units (Funk, et al., 2012).

Other approaches and concluding remarks

The different chapters of this thesis provided an overview of the usefulness of genetic tools to identify conservation issues and assist conservation practice. However, although genetic analyses have been identified as potentially high value to identify conservation units in cetaceans (Martien, et al., 2019), it is just one facet, not a panacea for all conservation problems. Genetic approaches must be complemented with approaches from other fields. Other types of data such as field observations, morphometric, satellite tracking, trace elements, stable isotopes, or stomach content can provide a very rich source of information that is complementary to genetic analyses. These would highly benefit conservation practices. For example, the application of high-resolution digital acoustic tags was a revolution to study the foraging behavior of harbor porpoises (Wisniewska, et al., 2016; Hoekendijk, et al., 2017). The usage of such diverse types of data and methods holds the potential to reveal fundamental aspects of the elusive biology of porpoises but can also provide unprecedented insights that can considerably help the conservation of porpoises. For instance, currently, the NOAA defines management units based on a total of 11 sources of evidence, genetic being just one of them (Martien, et al., 2019). The joint NAMMCO-IMR workshop (2019) gathered scientists studying genetic, acoustic, ecotoxicology, physiology, population biology, life history, behavior, modeling, and feeding ecology. The fundamental need to develop multidisciplinary research integrating these different forms of data was strongly stressed. By providing a more comprehensive picture of the ecology, stock structure, population dynamics, and evolution of the populations using such a multifaceted approach would guide better future assessment and conservation of the harbor porpoise and other porpoise species.

References

Amaral, A.R., *et al.* Hybrid speciation in a marine mammal: the clymene dolphin (*Stenella clymene*). *PLoS ONE* 2014;9(1):e83645.

Arnason, U., *et al.* Whole-genome sequencing of the blue whale and other rorquals finds signatures for introgressive gene flow. *Science Advances* 2018;4(4):eaap9873.

Autenrieth, M., *et al.* High-quality whole-genome sequence of an abundant Holarctic odontocete, the harbour porpoise (*Phocoena phocoena*). *Mol Ecol Resour* 2018;18(6):1469-1481.

Baetscher, D.S., *et al.* Microhaplotypes provide increased power from short-read DNA sequences for relationship inference. *Molecular Ecology Resources* 2018;18(2):296-305.

- Bailleul, D., *et al.* Large-scale genetic panmixia in the blue shark (*Prionace glauca*): a single worldwide population, or a genetic lag-time effect of the “grey zone” of differentiation? *Evol Appl* 2018;1-42.
- Barton, N.H. and Hewitt, G.M. Analysis of Hybrid Zones. *Annual Review of Ecology and Systematics* 1985;16(1):113-148.
- Boisseau, O., *et al.* Encounter rates of cetaceans in the Mediterranean Sea and contiguous Atlantic area. *Marine Biological Association of the United Kingdom. Journal of the Marine Biological Association of the United Kingdom* 2010;90(8):1589.
- Cammen, K.M., *et al.* Genomic Methods Take the Plunge: Recent Advances in High-Throughput Sequencing of Marine Mammals. *J. Hered.* 2016;107(6):481-495.
- Campbell, N.R., Harmon, S.A. and Narum, S.R. Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. *Molecular Ecology Resources* 2015;15(4):855-867.
- Carlén, I., Nunny, L. and Simmonds, M.P. Out of Sight, Out of Mind: How Conservation Is Failing European Porpoises. *Frontiers in Marine Science* 2021;8:13.
- Crossman, C.A., Barrett-Lennard, L.G. and Taylor, E.B. Population structure and intergeneric hybridization in harbour porpoises *Phocoena phocoena* in British Columbia, Canada. *Endangered Species Research* 2014;26(1):1-12.
- Crossman, C.A., Taylor, E.B. and Barrett-Lennard, L.G. Hybridization in the Cetacea: widespread occurrence and associated morphological, behavioral, and ecological factors. *Ecol Evol* 2016;6(5):1293-1303.
- De Queiroz, K. Species Concepts and Species Delimitation. *Syst. Biol.* 2007;56(6):879-886.
- Dellabianca, N., Pitman, R.L. and Braulik, G. *Phocoena dioptrica*. The IUCN Red List of Threatened Species. 2018.
- Ding, W., Mei, Z. and Kexiong, W. A reserve network of translocation brings hope and offers opportunities for the recovery of the critically endangered Yangtze finless porpoise. In, *World Marine Mammal Conference 2019 (WMMC 2019)*. Barcelona, Spain; 2019.
- Donovan, G.P. and Bjørge, A. Harbour porpoises in the North Atlantic: edited extract from the report of the IWC Scientific Committee. Dublin: Rep. Int. Whal. Commn; 1995.
- Félix, F., *et al.* *Phocoena spinipinnis*. IUCN Red List of Threatened Species. 2018.
- Fontaine, M.C. Harbour Porpoises, *Phocoena phocoena*, in the Mediterranean Sea and Adjacent Regions: Biogeographic Relicts of the Last Glacial Period. *Adv. Mar. Biol.* 2016;75:333-358.
- Fontaine, M.C., *et al.* Rise of oceanographic barriers in continuous populations of a cetacean: the genetic structure of harbour porpoises in Old World waters. *BMC Biol.* 2007;5(1):30.

- Fontaine, M.C. and Ben Chehida, Y. Influence of the reference genome and first insights into the evolutionary history of the harbor porpoises. In, *World Marine Mammal Conference (WMMC 2019). Genomics of Marine Mammals Workshop III*. Barcelona, Spain.; 2019.
- Fontaine, M.C., *et al.* Evolutionary history of the porpoise family (Phocoenidae): a perspective from mitogenomes and whole genomes. In, *World Marine Mammal Conference (WMMC 2019)*. Barcelona, Spain; 2019.
- Fontaine, M.C., *et al.* Postglacial climate changes and rise of three ecotypes of harbour porpoises, *Phocoena phocoena*, in western Palearctic waters. *Mol. Ecol.* 2014;23(13):3306-3321.
- Fontaine, M.C., *et al.* Mixing of porpoise ecotypes in southwestern UK waters revealed by genetic profiling. *R Soc Open Sci* 2017;4(3):160992.
- Fontaine, M.C., *et al.* Genetic and historic evidence for climate-driven population fragmentation in a top cetacean predator: the harbour porpoises in European water. *Proc. Biol. Sci.* 2010;277(1695):2829-2837.
- Foote, A.D., *et al.* Genome-culture coevolution promotes rapid divergence of killer whale ecotypes. *Nat Commun* 2016;7:11693.
- Fordyce, R.E. Cetacean Evolution. In, *Encyclopedia of Marine Mammals*. Academic Press; 2018. p. 180-185.
- Funk, W.C., *et al.* Harnessing genomics for delineating conservation units. *Trends in Ecology & Evolution* 2012;27(9):489-496.
- Gagnaire, P.-A. Comparative genomics approach to evolutionary process connectivity. *Evol Appl* 2020;13(6):1320-1334.
- Gagnaire, P.-A., *et al.* Using neutral, selected, and hitchhiker loci to assess connectivity of marine populations in the genomic era. *Evol Appl* 2015;8(8):769-786.
- Gol'din, P.E. Growth, proportions and variation of the skull of harbour porpoises (*Phocoena phocoena*) from the Sea of Azov. *J. Mar. Biol. Ass.* 2007;87(01):271-222.
- Gol'din, P.E. and Vishnyakova, K.A. Differences in skull size of harbour porpoises, *phocoena phocoena* (cetacea), in the Sea of Azov and the Black Sea: evidence for different morphotypes and populations. *Vestnik Zoologii* 2015;49(2).
- Gompert, Z., Mandeville, E.G. and Buerkle, C.A. Analysis of Population Genomic Data from Hybrid Zones. *Annual Review of Ecology, Evolution, and Systematics* 2017;48(1):207-229.
- Hällfors, M.H., *et al.* Addressing potential local adaptation in species distribution models: implications for conservation under climate change. *Ecol Appl* 2016;26(4):1154-1169.
- Hammond, P., *et al.* Estimates of cetacean abundance in European Atlantic waters in summer 2016 from the SCANS-III aerial and shipboard surveys. In.: Wageningen Marine Research; 2017.

- Hammond, P.S., *et al.* Cetacean abundance and distribution in European Atlantic shelf waters to inform conservation and management. *Biological Conservation* 2013;164:107-122.
- Heide-Jørgensen, M.P., *et al.* Harbour porpoises respond to climate change. *Ecol Evol* 2011;1(4):579-585.
- Hewitt, G.M. Hybrid zones-natural laboratories for evolutionary studies. *Trends in Ecology & Evolution* 1988;3(7):158-167.
- Hewitt, G.M. Speciation, hybrid zones and phylogeography - or seeing genes in space and time. *Mol. Ecol.* 2001;10(3):537-549.
- Hoekendijk, J.P.A., *et al.* Resilience of harbor porpoises to anthropogenic disturbance: Must they really feed continuously? *Marine Mammal Science* 2017;34(1):258-264.
- Huang, J., *et al.* Population survey showing hope for population recovery of the critically endangered Yangtze finless porpoise. *Biological Conservation* 2020;241:108315.
- IMR-NAMMCO. North Atlantic Marine Mammal Commission and the Norwegian Institute of Marine Research. In, *Report of Joint IMR/NAMMCO International Workshop on the Status of Harbour Porpoises in the North Atlantic*. Tromsø, Norway; 2019.
- IWC. Report of the Standing Sub-Committee on Small Cetaceans. In, *Journal of Cetacean Research and Management* 4. 2002. p. 325-338.
- Lah, L., *et al.* Spatially Explicit Analysis of Genome-Wide SNPs Detects Subtle Population Structure in a Mobile Marine Mammal, the Harbor Porpoise. *PLoS ONE* 2016;11(10):e0162792.
- Lockyer, C. All creatures great and smaller: a study in cetacean life history energetics. *Journal of the Marine Biological Association of the United Kingdom* 2007;87(4):1035-1045.
- Loire, E. and Galtier, N. Lacking conservation genomics in the giant Galápagos tortoise. *bioRxiv* 2017:101980.
- Luikart, G., *et al.* Population Genomics: Advancing Understanding of Nature. In, *Population Genomics*. 2018. p. 3-79.
- Marques, D.A., Meier, J.I. and Seehausen, O. A Combinatorial View on Speciation and Adaptive Radiation. *Trends in Ecology & Evolution* 2019;34(6):531-544.
- Martien, K.K., *et al.* The DIP delineation handbook: a guide to using multiple lines of evidence to delineate demographically independent populations of marine mammals. 2019.
- Marx, F.G. and Uhen, M.D. Climate, Critters, and Cetaceans: Cenozoic Drivers of the Evolution of Modern Whales. *Science* 2010;327(5968):993-996.
- McGowen, M.R. Toward the resolution of an explosive radiation—A multilocus phylogeny of oceanic dolphins (Delphinidae). *Mol. Phylogenet. Evol.* 2011;60(3):345-357.
- McGowen, M.R., *et al.* Phylogenomic Resolution of the Cetacean Tree of Life Using Target Sequence Capture. *Syst. Biol.* 2019;31(1.1FC):2553.

- McMahon, B.J., Teeling, E.C. and Höglund, J. How and why should we implement genomics into conservation? *Evol Appl* 2014;7(9):999-1007.
- Meek, M.H. and Larson, W.A. The future is now: Amplicon sequencing and sequence capture usher in the conservation genomics era. *Molecular Ecology Resources* 2019;19(4):795-803.
- Mei, Z., *et al.* Thresholds of population persistence for the Yangtze finless porpoise: implications for conservation managements. *Integrative Zoology* 2021.
- Mogensen, L. Identifying key threats and conservation requirements for the Critically Endangered Yangtze finless porpoise. *Doctoral thesis, UCL (University College London)*. 2019.
- Morin, P.A., *et al.* Building genomic infrastructure: Sequencing platinum-standard reference-quality genomes of all cetacean species. *Marine Mammal Science* 2020a;20:1361.
- Morin, P.A., *et al.* Reference genome and demographic history of the most endangered marine mammal, the vaquita. *Molecular Ecology Resources* 2020b:1-13.
- Morin, P.A. and Dizon, A.E. Genetics, Management. In, *Encyclopedia of Marine Mammals*. Elsevier; 2018. p. 410-416.
- Morin, P.A., *et al.* Population structure in a continuously distributed coastal marine species, the harbor porpoise, based on microhaplotypes derived from poor quality samples. *Mol. Ecol.* 2021.
- Moura, A.E., *et al.* Phylogenomics of the genus *Tursiops* and closely related Delphininae reveals extensive reticulation among lineages and provides inference about eco-evolutionary drivers. *Mol. Phylogenet. Evol.* 2020;146:106756.
- Nei, M. *Molecular Evolutionary Genetics*. New York: Columbia University Press; 1987.
- Neigel, J.E. and Avise, J.C. Phylogenetic relationships of mitochondrial dna under various demographic models of speciation. *Evolutionary process and theory* 1986:515-534.
- Nielsen, N.H., *et al.* The Greenlandic harbour porpoise – a separate ecotype? In, *World Marine Mammal Conference (WMMC 2019)*. Barcelona, Spain; 2019.
- Nielsen, N.H., *et al.* Oceanic movements, site fidelity and deep diving in harbour porpoises from Greenland show limited similarities to animals from the North Sea. *Marine Ecology Progress Series* 2018;597:259-272.
- Nosil, P. The genomics of ecological speciation. In, *Ecological Speciation*. Oxford University Press; 2012a. p. 169-191.
- Nosil, P. The speciation continuum. In, *Ecological Speciation*. Oxford University Press; 2012b. p. 192-213.
- Oleksiak, M.F. Adaptation without boundaries: population genomics in marine systems. In, *Population Genomics*. Springer; 2018. p. 587-612.

- Pastene, L.A., *et al.* Radiation and speciation of pelagic organisms during periods of global warming: the case of the common minke whale, *Balaenoptera acutorostrata*. *Mol. Ecol.* 2007;16(7):1481-1495.
- Pierce, G.J., *et al.* Is Iberian harbour porpoise (*Phocoena phocoena*) threatened by interactions with fisheries? 2020.
- Razgour, O., *et al.* Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections. *Proceedings of the National Academy of Sciences* 2019;116(21):10418.
- Read, A.J. Harbour porpoise *Phocoena phocoena* (Linnaeus, 1758). In, *Handbook of marine mammals*. London: Academic Press San Diego; 1999. p. 323-350.
- Reeves, R.R. *Dolphins, Whales and Porpoises*. IUCN; 2003.
- Robinson, J.A., *et al.* Genomic signatures of extensive inbreeding in Isle Royale wolves, a population on the threshold of extinction. *Science Advances* 2019;5(5):eaau0757.
- Rosel, P.E., Dizon, A.E. and Haygood, M.G. Variability of the mitochondrial control region in populations of the harbour porpoise, *Phocoena*, on interoceanic and regional scales. *Canadian Journal of Fisheries and Aquatic Sciences* 1995;52(6):1210-1219.
- Rosel, P.E., *et al.* Examining metrics and magnitudes of molecular genetic differentiation used to delimit cetacean subspecies based on mitochondrial DNA control region sequences. *Marine Mammal Science* 2017;33(Special Issue):76-100.
- Rousset, F. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* 1997;145(4):1219-1228.
- Roux, C., *et al.* Shedding Light on the Grey Zone of Speciation along a Continuum of Genomic Divergence. *PLoS Biol.* 2016;14(12):e2000234.
- Schwalm, C.R., Glendon, S. and Duffy, P.B. RCP8.5 tracks cumulative CO₂ emissions. *Proceedings of the National Academy of Sciences* 2020;117(33):19656-19657.
- Slater, G.J., *et al.* Diversity versus disparity and the radiation of modern cetaceans. *Proc. Biol. Sci.* 2010;277(1697):3097-3104.
- Smeenk, C., Leopold, M.F. and Addink, M.J. Note on the harbour porpoise *Phocoena phocoena* in Mauritania, West Africa. In.; 1992.
- Stankowski, S. and Ravinet, M. Defining the speciation continuum. *Evolution* 2021.
- Steeman, M.E., *et al.* Radiation of Extant Cetaceans Driven by Restructuring of the Oceans. *Syst. Biol.* 2009;58(6):573-585.
- Taylor, S.A., Larson, E.L. and Harrison, R.G. Hybrid zones: windows on climate change. *Trends Ecol Evol* 2015;30(7):398-406.
- Teilmann, J. and Dietz, R. Status of the harbour porpoise in Greenland. *Polar Biology* 1998;19(3):211-220.

- Teixeira, J.C. and Huber, C.D. The inflated significance of neutral genetic diversity in conservation genetics. *Proceedings of the National Academy of Sciences* 2021;118(10):e2015096118.
- Van Waerebeek, K. and William, P. Conservation status of the Northwest African population of the harbor porpoise. In.: *Convention on the Conservation of Migratory Species of Wild Animals*; 2007.
- Waples, R.S. and Gaggiotti, O. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Mol. Ecol.* 2006;15(6):1419-1439.
- Westbury, M.V., *et al.* Speciation in the face of gene flow within the toothed whale superfamily Delphinoidea. 2020;18:1469.
- Whibley, A. Genome insights give cause for optimism in the ongoing battle to save the vaquita. *Molecular Ecology Resources* 2021.
- Willis, P.M., *et al.* Natural hybridization between Dall's porpoises (*Phocoenoides dalli*) and harbour porpoises (*Phocoena phocoena*). *Canadian Journal of Zoology* 2004;82(5):828-834.
- Wisniewska, D.M., *et al.* Ultra-High Foraging Rates of Harbor Porpoises Make Them Vulnerable to Anthropogenic Disturbance. *Curr. Biol.* 2016;26(11):1441-1446.
- Wu, C.-I. The genic view of the process of speciation. *Journal of Evolutionary Biology* 2001;14(6):851-865.
- Yim, H.-S., *et al.* Minke whale genome and aquatic adaptation in cetaceans. *Nat. Genet.* 2014;46(1):88-92.
- Zerbini, A.N., *et al.* A Bayesian assessment of the conservation status of humpback whales (*Megaptera novaeangliae*) in the western South Atlantic Ocean. *J. Cetacean Res. Manage.* 2011(3):131-144.
- Zhou, X., *et al.* Population genomics of finless porpoises reveal an incipient cetacean species adapted to freshwater. *Nat Commun* 2018;9(1):1276.

