Genomic variability and population structure in shelled pteropods

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Summary

Rising atmospheric CO₂ concentrations cause ocean acidification, a decrease in pH that threatens the existence of many marine calcifying organisms. Shelled pteropods are marine planktonic snails that are regarded as bioindicators of ocean acidification because their thin aragonitic shells are susceptible to dissolution. Despite their small body size, shelled pteropods play an important role in the ocean’s carbonate budget and in marine food webs worldwide. Experiments have been conducted on shelled pteropods to assess their short-term responses to ocean acidification, but little is known about their evolutionary potential to adapt to long-term environmental changes. It is not possible to directly observe the evolutionary process due to difficulties in maintaining pteropods in lab cultures and their relatively long generation times. However, we can gain insight into factors affecting their adaptive potential by analysing levels of standing genetic variation within populations, gene flow between populations, and demographic fluctuations during their evolutionary history from genomic data.

While pteropods live in an open ocean environment and are hypothesised to have high effective population sizes and dispersal potentials, it is unknown if pteropod species are genetically homogeneous across their broad spatial ranges, or composed of several distinct populations. Given their roles as bioindicators, it is necessary to accurately assess their species boundaries because different species have different evolutionary trajectories and may have different sensitivities. In this thesis, I aimed to assess the spatial distribution of genetic variation within the shelled pteropod genus Limacina, to gain insight into the drivers of population structure in the open ocean and to obtain a better understanding of their evolutionary history and adaptive potential. Limacina bulimoides was chosen as a focal species because of its broad subtropical distribution and high abundance across the globe. Hence, levels of genetic variability in this species could be assessed at a population level across various spatial scales.

In Chapter 2, we assessed the population structure of L. bulimoides across a latitudinal transect in the Atlantic Ocean using partial DNA sequences of two barcoding genes, namely the mitochondrial cytochrome oxidase I (COI) and nuclear ribosomal 28S genes. Genetic differentiation of L. bulimoides across the sampling sites was compared to their shell shape variation and placed within the context of their abundance along an equivalent transect sampled two years later. We uncovered two dispersal barriers, one across the equatorial upwelling region between 15°N and 4°S, supported only by differentiation at the nuclear 28S locus, and the other dispersal barrier in the southern subtropical gyre, at 15-18°S, which was supported by both barcoding genes and shell shape variation. The locations of these dispersal barriers were congruent with regions of low abundance, supporting the hypothesis that areas of suboptimal habitat may function as barriers to dispersal in holoplanktonic organisms.
In **Chapter 3**, we developed a target capture approach to investigate genome-wide variation in the pteropod *L. bulimoides*, which was also tested on related pteropod species *L. trochiformis*, *L. lesueurii*, *L. helicina* and *Heliconoides inflatus*. A 2.9 gigabase draft genome of *L. bulimoides* was generated, and used in conjunction with a draft transcriptome to develop a set of genome-wide target capture probes, comprising 2812 single copy nuclear genes, including conserved protein coding regions, the 28S rDNA sequence, ten mitochondrial genes, 35 candidate biomineralisation genes and 41 non-coding regions. These probes were successful in obtaining detailed genomic information from the target species *L. bulimoides* with 97% of the targets being recovered.

In **Chapter 4**, we applied the target capture probes developed in **Chapter 3** to analyse spatial patterns of divergence of *L. bulimoides* across the global ocean. Genomic variation was studied with 107,214 single nucleotide polymorphisms (SNPs) from across 161 individuals, while shell shape variation was analysed using geometric morphometric analyses of shell images. We identified three distinct lineages, which we called the Atlantic, Indo-Pacific and Pacific lineage, based on their geography. We found no evidence of recent gene flow between the three lineages, not even between the Indo-Pacific and Pacific lineages that occur sympatrically in the North Pacific. The timing of divergence between the lineages was estimated to be during the mid-Pleistocene transition around 1 million years ago, while the fluctuations in population size within lineages coincided with known glacial-interglacial transitions. Shell shape was subtly different but overlapping between the lineages, and could not be used to distinguish them. However, we identified tissue pigmentation within the North Pacific individuals of the Pacific lineage as a potential distinguishing trait from the sympatric Indo-Pacific lineage. Hence, the circumglobal *L. bulimoides* is actually composed of three reproductively isolated lineages with more restricted distribution patterns that partially overlap.

In **Chapter 5**, we focused on the genome-wide diversity of 142 *L. bulimoides* individuals of the Atlantic lineage, with 97,425 SNPs obtained using target capture probes. The three populations that were tentatively identified in **Chapter 2** were confirmed by the genome-wide analysis, namely the North, Equatorial and South Atlantic populations, with no evidence of recent gene flow between them. The dispersal barriers between the three populations were narrowed down to 14-15°N and 15-18°S. The presence of narrow dispersal barriers and absence of genetic mixing suggests that (bio-)physical barriers, natural selection, or a combination of both could be keeping populations apart, although more analyses are required to identify the processes maintaining this population structure. The mitochondrial and nuclear signals were incongruent for some individuals, which suggest (ancient) mitochondrial introgression between populations.

In **Chapter 6**, we demonstrated a new method in which alcohol-based handgel, which was widely distributed during the COVID-19 crisis, was used to position shelled pteropods under the microscope for standardised photographs and mor-
Summary

Phometric analysis. The new method was more efficient than previous positioning methods used. There is potential for broader application of this method for the taxonomic identification, and the morphological and ontogenetic study of other small molluscs and planktonic organisms.

In Chapter 7, I summed up the findings from Chapters 2-6 and point to future research directions. My thesis has shown that genome-wide markers provide additional insights into the population structure and evolutionary history of L. bulimoides compared to barcoding genes. I found that L. bulimoides is not genetically homogeneous across its range, but is composed of at least three reproductively isolated lineages across the Atlantic, Indian and Pacific Oceans. Detailed analysis of the Atlantic lineage revealed further population structure, with three distinct populations separated by narrow dispersal barriers. Looking forward, the methods used to access genomic information in L. bulimoides can be applied to other shelled pteropods, including species in (sub)polar regions, which are already experiencing the effects of a rapidly acidifying ocean.
Samenvatting

De toenemende hoeveelheid CO₂ die door menselijke activiteiten in de atmosfeer wordt gebracht leidt tot verzuring van de oceaan. Dit is een proces waarbij het zeewater geleidelijk zuurder wordt, waardoor het voor kalkvormende organismen steeds moeilijker wordt om hun schaaltjes of schelpen te bouwen. Pteropoden, ook wel zeevlinders genoemd, zijn een groep planktonslakken die extreem gevoelig lijken te zijn voor oceaanverzuring vanwege hun dunne huisjes gemaakt van aragoniet (een zeer oplosbare vorm van calciumcarbonaat). Ook al zijn zeevlinders slechts enkele millimeters tot een centimeter groot, ze spelen een belangrijke rol in de mariene voedselketen en het carbonaat budget van de oceaan. Korte termijn experimenten hebben aangetoond dat zeevlinders te lijden hebben onder oceaanverzuring. Echter, we weten niet of zeevlinders zich kunnen aanpassen op de lange termijn. Het is niet mogelijk om het evolutionaire proces direct te observeren want het is zeer moeilijk om zeevlinders in het lab te kweken en ze hebben een relatief lange generatietijd (ongeveer een jaar). Maar we kunnen wel inzicht krijgen in factoren die hun aanpassing op de lange termijn bepalen, zoals de hoeveelheid genetische variatie binnen en tussen populaties, de mate van uitwisseling tussen populaties, en fluctuaties in populatiegrootte gedurende hun evolutionaire geschiedenis. Dit kan worden afgeleid uit het DNA.

Pteropoden leven in de open oceaan en algemeen wordt aangenomen dat ze grote effectieve populaties hebben en zich zeer goed kunnen verspreiden. Echter, we weten niet of soorten genetisch homogeen zijn over het hele gebied waarin ze voorkomen, of dat ze uit verschillende populaties of (onder)soorten bestaan. Omdat zeevlinders worden gezien als bio-indicatoren of graadmeters van oceaanverzuring is het belangrijk om de diversiteit aan soorten goed te kennen. Verschillende soorten hebben namelijk een verschillende evolutionaire geschiedenis en kunnen daarom verschillend reageren op veranderingen in hun leefomgeving. In dit proefschrift heb ik gekeken naar de verspreiding van genetische variatie in het genus Limacina om meer inzicht te krijgen in de processen die een rol spelen in het ontstaan van verschillende populaties en soorten in de open oceaan, alsnog hun potentieel om zich te kunnen aanpassen aan een toekomstige oceaan. Mijn onderzoek heeft zich met name gericht op Limacina bulimoides omdat deze soort in grote aantallen voorkomt en een wereldwijde verspreiding heeft. Hierdoor kon ik de genetische variatie goed bestuderen aan de hand van voldoen- de individuen uit verschillende oceaanbakkers.

In Hoofdstuk 2 hebben we de populatiestructuur van L. bulimoides bestudeerd langs een noord-zuid transect in de Atlantische Oceaan aan de hand van twee barcoding genen, namelijk het mitochondriale cytochrom oxidase I (COI) gen en het nucleaire ribosomale 285 gen. We hebben genetische differentiatie tussen populaties vergeleken met morfologische variatie in schelpvorm, en ook gekeken naar de abundantie langs een vergelijkbaar transect dat twee jaar later is bemonsterd.
Samenvatting

We ontdekten twee dispersie barrières: één barrière was gelegen in het gebied van de equatoriale oceaanstromen tussen 15°N en 4°S en werd alleen ondersteund door het nucleaire 28S gen. De tweede barrière was in de Zuid-Atlantische gyre tussen 15°S en 18°S, en werd ondersteund door beide genen en door verschillen in schelpvorm. De locaties van deze barrières overlapten met een afname in populatie dichtheden. Dit ondersteunt de hypothese dat suboptimale gebieden mogelijk leiden tot dispersie barrières in het plankton van de open oceaan.

In Hoofdstuk 3 hebben we een ‘target capture’ methode ontwikkeld om variatie verspreid over het hele genoom van de pteropode L. bullimoides te onderzoeken. Daarnaast hebben we deze methode getest op de verwante soorten L. trochiformis, L. lesueurii, L. helicina en Heliconoides inflatus. Op basis van een eerste gefragmenteerd genoom van 2.9 gigabase en een transcriptoom van L. bullimoides zijn de capture probes ontwikkeld. Deze probes omvatten 2.812 ‘single copy’ nucleaire genen, de 28S rDNA gen, tien mitochondriale genen, 35 kandidaat biominalisatie genen en 41 niet-coderende stukken. De probes waren succesvol om variatie over het hele genoom van L. bullimoides te onderzoeken (97% van de beoogde DNA sequenties zijn teruggevonden).

In Hoofdstuk 4 zijn de ‘target capture probes’, die we hebben ontwikkeld in Hoofdstuk 3, gebruikt om de ruimtelijke verspreiding van genoom variatie in L. bullimoides over de wereldwijde oceaan te onderzoeken. Genoom variatie is bestudeerd aan de hand van 107.214 ‘single nucleotidie polymorphisms’ (SNPs) van 161 individuen, terwijl hun schelpvorm werd onderzocht met een geometrische morfometrische analyse op basis van gestandardiseerde foto’s. We ontdekten drie evolutsionele lijnen van verwantschap, die we de Atlantische, Indo-Pacifische en Pacifische lijn hebben genoemd op basis van hun verspreidingen. We vonden geen enkel bewijs van genetische uitwisseling tussen deze drie lijnen, zelfs niet tussen de Indo-Pacifische en Pacifische takken welke samen voorkomen in de Noord Pacifische Oceaan. Op basis van genetische differentiatie schatten we dat de lijnen zijn gesplitst tijdens het midden-Pleistoceen, ongeveer 1 miljoen jaar geleden. Fluctuaties in populatiegrootte van de lijnen lijken samen te vallen met transities tussen ijstijden en interglaciaal. De schelpvorm verschilde subtiel tussen de verschillende lijnen maar vertoonde ook overlap, en kan daarom niet gebruikt worden als een morfologisch kenmerk. Echter, we hebben pigmentvlekken op de ‘vleugels’ van pteropoden van de Pacifische lijn ontdekt, die een mogelijk kenmerk zijn om de Pacifische lijn van de sympatrische Indo-Pacifische lijn te kunnen onderscheiden. Dus hoewel L. bullimoides een wereldwijde verspreiding heeft, bestaat deze ‘soort’ in feite uit drie reproductief geïsoleerde lijnen (wellicht zelfs drie afzonderlijke soorten) met meer beperkte en deels overlappende verspreidingspatronen.

In Hoofdstuk 5 hebben we de genoom-wijde variatie van 142 L. bullimoides individuen uit de Atlantische Oceaan onderzocht, op basis van 97.425 SNPs verkregen middels de ‘target capture probes’ uit Hoofdstuk 3. De resultaten van deze genoom-wijde studie bevestigen dat de Atlantische lijn bestaat uit drie verschillende popu-
Samenvatting


In HOOFDSTUK 6 beschrijven we een nieuwe methode, gebruikmakend van de alcohol-handgel die ruim voor handen was tijdens de COVID-19 crisis, om zeevinders te positioneren onder de microscoop en gestandaardiseerde foto’s te maken voor morfometrische analyses. De nieuwe methode bleek veel efficiënter dan de eerder gebruikte methodes. Daarnaast zou deze methode kunnen worden toegepast voor morfologische identificatie en ontogenetische studies van andere kleine mollusken en plankton.

In HOOFDSTUK 7 heb ik alle bevindingen van HOOFDSTUKKEN 2-6 samengevat en richtingen voor toekomstig onderzoek aangegeven. Mijn proefschrift heeft laten zien dat genoom-wijde merkers meer inzicht geven in de populatiestructuur en evolutionaire geschiedenis van L. bulimoides dan de barcoding genen die gewoonlijk gebruikt worden. Ik heb gevonden dat L. bulimoides niet genetisch homogeen is over het hele verspreidingsgebied, maar bestaat uit tenminste drie genetisch geïsoleerde evolutionaire lijnen in de Atlantische, Indische en Pacificse Oceaan. Binnen de Atlantische lijn vonden we nog verdere ruimtelijke structuur met drie populaties gescheiden middels twee nauwe dispersie barrières. Kijkend naar de toekomst, zie ik dat de nieuwe methoden die ik heb gebruikt om te kijken naar variatie binnen het genoom van L. bulimoides ook kunnen worden toegelaten op andere zeevinders, wat met name interessant zal kunnen zijn voor de (sub)polaire pteropode soorten die op dit moment al te lijden hebben onder de steeds zuurder wordende oceaan.
Author contributions

CHAPTER 2
LQC, TMPB, EG and KTCAP contributed to the study design. EG and KTCAP collected samples for the study. LQC and TMPB collected molecular and morphometric data. All authors analysed the data. LQC wrote the manuscript, with input from all authors. All authors approved of the final manuscript.

CHAPTER 3
LQC, TMPB, MC, MK, IS, GH and KTCAP contributed to the study design. KTCAP provided samples used in this study. FM and PRS analysed sequence data and contributed to the capture design. LQC, TMPB, MK and IS contributed to the molecular work. LQC, TMPB, MC, GH and KTCAP contributed to bioinformatic analyses and manuscript writing. All authors provided feedback and approved of the final manuscript.

CHAPTER 4
LQC, GH and KTCAP designed the study. EG and KTCAP contributed samples used in this study. LQC collected the molecular data while GS collected the morphometric data. LQC, MC, GH and KTCAP contributed to the bioinformatic analyses while LQC and GS analysed the morphometric data. LQC, MC, GH, EG and KTCAP contributed to the manuscript writing. All authors provided feedback and approved of the manuscript.

CHAPTER 5
LQC, GH and KTCAP designed the study. LQC, EG and KTCAP collected samples for the study. LQC collected the molecular data, while GS collected the morphometric data. LQC, GS, GH, and KTCAP contributed to data analyses and LQC, GH, JH and KTCAP contributed to manuscript writing. All authors provided feedback and approved of the manuscript.

CHAPTER 6
LQC and KTCAP designed the study. GS processed the specimens for photography, micro-CT scanning and DNA barcoding. LQC wrote the manuscript and all authors provided feedback and approved of the final manuscript.
About the author

Le Qin was born on 2nd September 1993 in Singapore, where she spent most weekends of her childhood exploring the beaches and various nature reserves. This is where her love for nature and science all began. She got her first taste of research in junior college, when she participated in the Science Research Programme, investigating the distribution and diets of intertidal limpets at the Tropical Marine Science Institute (TMSI) at the National University of Singapore (NUS). After graduating from junior college, Le Qin continued at TMSI, this time to study the formation of hairs on some marine mussel species, together with her twin sister. She was awarded a Loke Cheng-Kim scholarship to pursue a Bachelor’s degree in Zoology at Fitzwilliam College, University of Cambridge, England from 2012 to 2015. There were many opportunities for fieldwork and experiments, including studying the secretions of dock beetle larvae and habitat choice in estuarine shrimp, and from these Le Qin decided that working with live animals was probably not so suitable for her. A volunteering stint at the Mollusc collections of the Natural History Museum in London further solidified her view that dead animals were much more cooperative. Seeking a return to her marine roots, she wrote her final year thesis on the relative importance of various habitats to commercially important demersal fish, and the case for including these habitats in marine protected areas.

Le Qin then continued with a Master’s degree in Biosystematics based at the Natural History Museum and Imperial College London from 2015 to 2016, where she completed three research projects in a diverse range of fields, including fly metagenomics, tissue visualisation techniques for tapeworms, and a project on the biogeography of riverine snails, supervised by Ellinor Michel and Jon Todd. Following the completion of her Master’s degree, Le Qin returned to Singapore and kept busy with sorting out deep sea benthic organisms at the Keppel Corporate Lab (NUS), while looking out for exciting PhD opportunities. As fate would have it, Ellinor and Jon forwarded her an email about the opening for this current PhD position at Naturalis Biodiversity Center, and she seized her chance. In October 2017, she started her PhD project in Leiden on the population structure of shelled pteropods, under the supervision of Katja Peijnenburg and Galice Hoarau. There were many adventures along the way, such as living in the northernmost country she’d ever been to (which was not as cold or terrifying as she imagined) and embarking on a 25-day research cruise across the Atlantic Ocean to collect plankton for her genomic analyses. With her work, she gained greater insight into the distribution of genetic variation in pelagic snails, and facilitated the application of genome-wide techniques in non-model planktonic organisms. Le Qin will join the lab of Roger Butlin in April this year to continue working on snails, this time on the role of chromosomal inversions in the adaptation and speciation of intertidal periwinkles.
About the author

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