Contributions to phenotypic plasticity and DNA methylation in molluscs with special emphasis on the highly invasive New Zealand mud snail *Potamopyrgus antipodarum* (Gray, 1843)

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Ich widme diese Dissertation meinen Eltern und meinem Bruder,

die für mich immer eine Quelle der Liebe,

der Kraft und der Inspiration sind und bleiben werden -

ganz gleich, wie weit weg ich lebe.

Ihr seid mein Zuhause und ich liebe euch!

♦

I dedicate this dissertation to my parents and my brother,

who are and always will be my source of love, strength and inspiration –

no matter how far away I live.

You are my home and I love you!

Contents

I. SUMMARY

English

Increasing environmental changes primarily due to anthropogenic impacts, are affecting organisms all over the planet. In general, scientists distinguish between three different ways in which organisms can respond to environmental changes in their habitat: extinction, dispersal and adaptation. An example of organisms which are highly adaptable and can easily cope with new and changing environments are invasive species which are able to colonize new habitats with only few individuals. To successfully survive in their new environment, invasive species adapt fast to novel abiotic and biotic parameters, such as different temperature regimes. Phenotypic plasticity which enables organisms to quickly modify their phenotype to new environmental conditions, explains the success in adaptation of invasive species.

While underlying mechanisms of phenotypic plasticity are not fully understood, one possible "motor" of phenotypic plasticity is epigenetics. Especially DNA methylation could explain the fast changes of the organism's phenotype due to plasticity when experiencing changing environments, as invasive species do. DNA methylation could even contribute to the adaptation of invasive species via phenotypic plasticity, especially with clonally reproducing species. Methods such as common garden experiments with clonally reproducing species are a useful tool to differentiate between phenotypic plasticity and genetic adaptation because the confusing effects of genetic variation are lowered in clonally reproducing species.

Our overall goal was to evaluate the genetic adaptive potential of New Zealand mud snail (*Potamopyrgus antipodarum*) populations from Europe since they went through an extreme bottleneck after colonizing Europe only 180-360 generations ago. Seemingly, two different clonal lineages colonized Europe because two 16 s rRNA and cytochrome b haplotypes were found across different European countries, haplotypes t and z. The NZMS is a highly successful invasive species that is nowadays nearly globally distributed. The shells of the NZMS show a habitat-dependent high variability and are a fitness-relevant trait. The high variability in shell morphology is due to both genetic variation and phenotypic plasticity. To disentangle genetic from environmental effects on the shell morphology NZMS, we conducted a common garden experiment. We kept asexually reproducing females from eleven European populations in climate cabinets with three different temperatures to produce offspring. We compared shell size and shape across three generations using the geometric morphometrics approach. Furthermore, we estimated reaction norms, maternal effects, broad-sense heritability, the coefficient of genetic variation (CV_A) and evolvability

(I_A) in shell size and shape across different temperature conditions. Additionally, we investigated the reproductive rate of the parental generation.

Results showed that the shell morphology of the parental generation differed across populations. In contrast, the shell morphology of offspring generations became more similar. The reaction norms of the F1 generation were rather variable across the three temperatures. However, we were able to observe a haplotype-dependent pattern across the reaction norms suggesting a restricted genetic differentiation among NZMS in Europe. We detected high heritability values in size indicating a high genetic influence. Heritability values for shape were lower than in size. Generally, heritability varied slightly depending on temperature. Size seemed to have a higher evolvability than shape. However, the values of all our calculations were very low which indicates that the European NZMS populations are genetically diminished. The reproductive rate of the parental generation was rather haplotype than temperature dependent. In summary, we were able to display that the NZMS is capable to plastically adapt its shell morphology to different temperatures showing significant differences between the two haplotypes. Nevertheless, the low evolvability values indicate that little genetic variation has formed since the arrival of the NZMS in Europe and therefore, European NZMS seem to have a reduced ability to react to selection.

These results implied that phenotypic plasticity is important for the adaptation to different environmental conditions in the NZMS and maybe other molluscan species. Since classical experimental approaches can only describe the resulting phenotypes, we also intended to shed more light on the mechanistic side of environmentally induced phenotypic modifications using DNA methylation analysis. Although molluscs represent one of the most diverse taxa within the metazoan and are found in many different habitats, our knowledge of the DNA methylation in molluscs is scarce. Therefore, we aimed at deepening and summarizing our understanding about DNA methylation in molluscs. Publicly available molluscan genomic and transcriptomic data of all eight mollusc classes was downloaded to search for DNA methyltransferases (DNMTs 1-3) responsible for DNA methylation. Additionally, we estimated the normalized CpG dinucleotide content (CpG o/e) indicating the presence/absence and the frequency of DNA methylation in the genome. The CpG o/e ratio refers to the level of DNA methylation in the genome. Based on the sensitivity of methylated cytosines to mutate into thymine residues, species having a high germline methylation in genomic regions over evolutionary time, also have a lower CpG content, which is called CpG depletion. In contrary, species with a limited germline methylation in genomic regions over evolutionary time, show a higher CpG content and lack CpG depletion. The presence or absence of CpG depletion can be calculated with the CpG o/e ratio. Ultimately, the goal of our analyses was to gain insight into the evolution of methylation in molluscs.

We detected DNMTs in all eight mollusc classes and in most of the species. It is therefore plausible that the last common ancestor of molluscs has already had the enzymatic machinery which is needed for DNA methylation. However, various species did not possess the complete DNMT toolkit indicating evolutionary modification in DNA methylation. In general, we found a wide distribution of the bimodal CpG o/e pattern in six mollusc classes, resulting from CpG depletion. The genes in these groups seem to be divided into genes with a high degree of methylation and genes with a lower degree of methylation. This implies that DNA methylation seems to be rather common in molluscs. Species of Solenogastres and Monoplacophora were not or only sparsely methylated. It seems that those mollusc groups have undergone a reduction in DNA methylation. We hope that our investigations will demonstrate the lacking knowledge in epigenetics of molluscs and encourage scientist to execute and continue genetic studies on molluscs.

Deutsch

Die zunehmenden Umweltveränderungen, die in erster Linie auf anthropogene Einflüsse zurückzuführen sind, wirken sich auf Organismen auf dem gesamten Planeten aus. Im Allgemeinen unterscheiden Wissenschaftler zwischen drei verschiedenen Arten, wie Organismen auf Umweltveränderungen in ihrem Lebensraum reagieren können: Aussterben, Abwanderung und Anpassung. Ein Beispiel für Organismen, die sehr anpassungsfähig sind und sich leicht an neue und sich verändernde Umgebungen anpassen können, sind invasive Arten, die in der Lage sind, neue Lebensräume mit nur wenigen Individuen zu besiedeln. Um in ihrer neuen Umgebung erfolgreich zu überleben, passen sich invasive Arten schnell an neue abiotische und biotische Parameter an, z. B. an unterschiedliche Temperaturregimes. Phänotypische Plastizität ermöglicht es Organismen, ihren Phänotyp schnell an neue Umweltbedingungen anzupassen und erklärt den Anpassungserfolg invasiver Arten.

Die Mechanismen, die der phänotypischen Plastizität zugrunde liegen, sind zwar noch nicht vollständig geklärt, ein möglicher "Motor" der phänotypischen Plastizität ist jedoch die Epigenetik. Insbesondere die DNA-Methylierung könnte die raschen Veränderungen des Phänotyps eines Organismus bei Veränderungen der Umwelt aufgrund von Plastizität erklären, wie es bei invasiven Arten der Fall ist. Die DNA-Methylierung könnte sogar zur Anpassung invasiver Arten durch phänotypische Plastizität beitragen, vor allem bei klonal reproduzierenden Arten. Methoden wie Common Garden Experimente mit klonal reproduzierenden Arten sind ein nützliches Instrument zur Unterscheidung zwischen phänotypischer Plastizität und genetischer Anpassung, da die unübersichtlichen Effekte der genetischen Variation bei klonal reproduzierenden Arten geringer sind.

Unser übergeordnetes Ziel war es, das genetische Anpassungspotenzial von europäischen Populationen der Neuseeländischen Zwergdeckelschnecke (NZMS) (*Potamopyrgus antipodarum*) zu untersuchen, da diese nach ihrer Besiedlung Europas vor nur 180-360 Generationen einen extremen genetischen Flaschenhals (engl. „genetic bottleneck“) durchliefen. Offenbar wurde Europa von zwei verschiedene klonale Linien der NZMS kolonisiert, da zwei 16 s rRNA- und Cytochrom b-Haplotypen in verschiedenen europäischen Ländern gefunden wurden, die Haplotypen t und z. Die NZMS ist eine äußerst erfolgreiche invasive Art, die heute fast auf der ganzen Welt verbreitet ist. Die Schalen der NZMS weisen eine Lebensraum-abhängige, hohe Variabilität auf. Zudem sind sie ein fitnessrelevantes Merkmal. Die hohe Variabilität der Schalenmorphologie ist sowohl auf genetische Variation als auch auf phänotypische Plastizität zurückzuführen. Um die genetischen und die Umwelteinflüsse auf die Schalenmorphologie der

NZMS zu trennen, führten wir ein Common Garden Experiment durch. Wir hielten sich asexuell fortpflanzende Weibchen aus elf europäischen Populationen in Klimaschränken mit drei verschiedenen Temperaturen, um Nachkommen zu produzieren. Mit Hilfe der geometrischen Morphometrie verglichen wir Größe und Form der Schalen über drei Generationen hinweg. Darüber hinaus schätzten wir Reaktionsnormen, maternale Effekte, die „broad-sense" Heritabilität, den Koeffizienten der genetischen Variation (CVA) und die Evolvierbarkeit (IA) der Schalengröße und -form unter verschiedenen Temperaturbedingungen. Außerdem untersuchten wir die Reproduktionsrate der Elterngeneration.

Die Ergebnisse zeigten, dass sich die Schalenmorphologie der Elterngeneration zwischen den Populationen unterschied. Im Gegensatz dazu ähnelten sich die Schalenmorphologie der Töchtergenerationen immer mehr. Die Reaktionsnormen der F1-Generation zeigten sich bei den drei Temperaturen recht unterschiedlich. Wir konnten jedoch ein vom Haplotyp abhängiges Muster bei den Reaktionsnormen feststellen, was auf eine begrenzte genetische Differenzierung unter den europäischen NZMS deutet. Wir berechneten hohe Heritabilitätswerte für die Schalengröße, was auf einen hohen genetischen Einfluss hinweist. Die Heritabilitätswerte für die Schalenform waren niedriger als für die der Schalengröße. Im Allgemeinen variierte die Heritabilität in Abhängigkeit von der Temperatur leicht. Die Schalengröße schien eine höhere Evolvabilität zu haben als die Schalenform. Die Werte aller unserer Berechnungen waren jedoch sehr niedrig, was darauf hindeutet, dass die europäischen NZMS-Populationen eine geringe genetische Variabilität besitzen. Die Reproduktionsrate der Elterngeneration wurde eher vom Haplotyp als von der Temperatur beeinflusst. Zusammenfassend konnten wir zeigen, dass die NZMS in der Lage ist, ihre Schalenmorphologie plastisch an verschiedene Temperaturen anzupassen, wobei es signifikante Unterschiede zwischen den beiden Haplotypen gab. Dennoch deuten die niedrigen Werte für die Evolvierbarkeit darauf hin, dass sich seit der Ankunft der NZMS in Europa nur eine geringe genetische Variabilität herausgebildet hat, so dass es scheint, dass die europäischen NZMS nur begrenzt auf Selektion reagieren kann.

Unsere Ergebnisse deuten außerdem darauf hin, dass die phänotypische Plastizität für die Anpassung an unterschiedliche Umweltbedingungen bei den NZMS und möglicherweise auch bei anderen Molluskenarten wichtig ist. Da klassische experimentelle Ansätze nur die resultierenden Phänotypen beschreiben können, haben wir eine Analyse der DNA-Methylierung durchgeführt, um auch die mechanistische Seite der umweltbedingten phänotypischen Veränderungen näher zu beleuchten. Obwohl Mollusken eines der vielfältigsten Taxa innerhalb der Metazoen darstellen und in vielen verschiedenen Lebensräumen vorkommen, ist unser Wissensstand über ihre DNA-Methylierung nur gering. Daher haben wir uns zum Ziel gesetzt, unsere Kenntnis über die DNA-

Methylierung bei Mollusken zu vertiefen und zusammenzufassen. Öffentlich zugängliche genomische und transkriptomische Daten aller acht Molluskenklassen wurden heruntergeladen, um nach DNA-Methyltransferasen (DNMTs 1-3) zu suchen, die für die DNA-Methylierung verantwortlich sind. Zusätzlich schätzten wir den normalisierten CpG-Dinukleotidgehalt (CpG o/e), der das Vorhandensein/die Abwesenheit und die Häufigkeit der DNA-Methylierung im Genom anzeigt. Das CpG o/e-Verhältnis bezieht sich auf den Grad der DNA-Methylierung im Genom. Da methylierte Cytosine häufiger zu Thyminrückständen mutieren, haben Arten, die im Laufe der Evolution eine hohe Keimbahnmethylierung in Genomregionen aufweisen, auch einen geringeren CpG-Gehalt, was als CpG-Verarmung (engl. „CpG depletion“) bezeichnet wird. Im Gegensatz haben Arten, die eine geringe Keimbahnmethylierung in genomischen Regionen im Laufe der Evolution hatten, einen höheren CpG-Gehalt und keine CpG-Verarmung auf. Das Vorhandensein oder Nichtvorhandensein von CpG-Verarmung lässt sich anhand des CpG o/e-Verhältnisses berechnen. Letztendlich war das Ziel unserer Analysen, einen Einblick in die Evolution der Methylierung bei Mollusken zu gewinnen.

Wir haben DNMTs in allen acht Molluskenklassen und in den meisten Arten nachgewiesen. Es ist daher plausibel, dass der letzte gemeinsame Vorfahre der Mollusken bereits über die enzymatische Maschinerie verfügte, die für die DNA-Methylierung erforderlich ist. Einige Arten besaßen jedoch nicht den kompletten DNMT-Baukasten, was auf eine evolutionäre Veränderung der DNA-Methylierung hindeutet. Im Allgemeinen fanden wir eine weite Verteilung der CpG-Verarmung in sechs Molluskenklassen. Die Gene in diesen Gruppen scheinen in Gene mit einem hohen Methylierungsgrad und Gene mit einem niedrigeren Methylierungsgrad unterteilt zu sein. Dies lässt darauf schließen, dass DNA-Methylierung bei Mollusken recht häufig vorkommt. Arten der Solenogastren und Monoplacophoren waren nicht oder nur spärlich methyliert. Es scheint, dass diese Molluskengruppen einen Rückgang der DNA-Methylierung erfahren haben. Wir hoffen, dass unsere Untersuchungen das fehlende Wissen über die Epigenetik von Mollusken aufzeigen und Wissenschaftler dazu ermutigen, genetische Untersuchungen an Mollusken durch- und fortzuführen.

II. INTRODUCTION

General introduction

Climate change and its consequences

Over the last decades, climate change has had a major and ever-increasing impact on the organisms living on this planet (Malhi et al., 2020; Magnan et al., 2021; Soravia et al., 2021; van Der Heide et al., 2021). While CO_2 levels in the atmosphere had been varying widely throughout the history of the Earth, the enormous consumption of fossil fuels and the exploitation of land and sea have increased atmospheric CO_2 to levels never observed in the past 400.000 years (Etheridge et al., 1996; Macfarling Meure et al., 2006; Lüthi et al., 2008; Inglis et al., 2015). It is now generally agreed that the rapidly advancing climate change and its impacts are man-made (Hansen et al., 2007; Pachauri & Reisinger, 2008). Climate change, the result of rising CO_2 levels, acts over a long time scale and at first sight, seems to appear a less prominent threat to the biodiversity on Earth compared to other imminent factors, such as habitat destruction or pollution. Climate change interacts and amplifies other threats and factors resulting in biodiversity loss (Thuiller, 2007). Accordingly, organisms all over the planet suffer from consequences arising from global warming like increasingly warmer oceans, rising sea levels and a growing number of stronger and more destructive weather events (Masson-Delmotte et al., 2021).

Organisms´ different responses to climate change

Different organisms respond differently to changing environments (Harmon & Barton, 2013). One possibility to respond to climate change is dispersal. Some species are able to shift to other habitats that are better suited to their living conditions (Bernstein et al. (IPCC) 2007; Chen et al., 2011; Bellard et al., 2012). As different as species are, they apparently also possess different dispersal capacities (Korner et al., 2005). The chances of a successful dispersal are higher in opportunistic and mobile species which are able to disperse and adapt faster to changing environmental conditions. In contrast to more sensitive species which may not have the ability to disperse and go extinct (Davis, 1986; McKinney & Lockwood, 1999; Malcolm et al., 2002). Sensitive species may even be displaced from their habitat due to dispersing opportunistic species. Dispersing species may also suffer from outbreeding depression which means that when the dispersing group reproduces with another genetically distant group, their fitness may be reduced (Frankham et al.,

2011). Also, dispersing organisms lead to changing species distributions, abundance and newly emerging between-species interactions and therefore to changing species communities (Parmesan & Yohe, 2003; Lawler et al., 2009). This way, climate change can even impact species that are not directly threatened by rising temperatures.

Another option to respond to climate change is adaptation. Some species might be able to adapt morphologically or behaviourally to changing environments (Kinnison & Hendry, 2001; Hairston et al., 2005). Morphological, physiological adaptation or adaptation concerning life-history traits can occur via genetic adaptation or phenotypic plasticity (Caswell, 1983; Bradshaw & Holzapfel, 2006; Williams et al., 2008; Whitman & Agrawal, 2009). Although Gienapp et al. (2008) considered the distinction between genetic adaptation and phenotypic plasticity to be important, the exact relationship of genetic adaptation and phenotypic plasticity remains controversial among scientists (Arnold et al., 2019).

Species which cannot adapt to changing conditions or relocate to another more suitable habitat are likely to go extinct. Until now, it is difficult to predict and still unclear how many species will go extinct due to climate change in the upcoming years (Thomas et al., 2004; Lewis, 2006; Urban, 2015). The IUCN Red List of Threatened Species lists around 10,967 species that are currently in danger of extinction due to climate change (IUCN, 2022).

Phenotypic plasticity and genetic adaptation

The outcome of natural selection of genotypes or beneficial alleles is called genetic adaptation. In simplified terms, individuals of a population that possess a beneficial allele which helps them to cope better with, for example, rising temperatures, have a fitness advantage over individuals who do not possess this allele. A high genetic variation, which is the existence of genotypically diverse individuals in population, increases the possibility that some individuals possess a beneficial allele for future climate conditions (Rieger et al., 1968). In populations, essential mechanisms that create genetic variance are genetic drift, recombination, genetic migration and mutation (Star & Spencer, 2013; Carja et al., 2014).

In contrast to many mechanisms which alter the genetic variance, such as mutations or recombination, phenotypic plasticity is a faster way of adaptation and easier to reverse (Star & Spencer, 2013; Carja et al., 2014; Burggren, 2016). Phenotypic plasticity – and also adaptive phenotypic plasticity – is the capability of an organism to develop various, comparably fit phenotypes in various environments (DeWitt et al., 1998). Although not all phenotypic plasticity is

adaptive, a species with a high phenotypic plasticity may be able to adapt to numerous habitats despite having only one genotype (Sultan, 1995; Pigliucci, 2001). This may lead to an increased potential of survival among diverse habitats (Price et al., 2003). Phenotypic plasticity has been identified to play the main role in the organisms´ phenotypic reaction to climate change and other man-made disturbances (Gienapp et al., 2008; Matesanz et al., 2010; Merilä & Hendry, 2014; Alberti et al., 2017). It is nowadays understood as genetically regulated, heritable and a possibly important mechanism contributing to the evolution of species (Bradshaw, 2006; Lande, 2009).

Although phenotypic plasticity brings many advantages, such as a fast and flexible adaptation to changing environments, it also has some costs and limits. The maintenance of the sensory and the regulatory mechanisms of phenotypic plasticity needs energy and other resources, and hence, might reduce the fitness of the organism (DeWitt et al., 1998; Auld et al., 2010). In natural populations, there seems to be ample genetic variation in a wide range of plastic responses. Different levels of phenotypic plasticity show different degrees of reversibility. Biochemical responses, for example, tend to be reversable within a short time frame (days to weeks), whereas developmental responses normally take longer to be reversed or are irreversible (one to various generations). Also, a trait may be plastic in response to one environmental factor, such as temperature, but not in response to another factor, for example, pH (Pigliucci et al., 2006). Despite the fact that varying forms of phenotypic plasticity may add to adaptive evolution in populations being confronted with new environmental conditions, phenotypic plasticity can also slow down adaptation because of the reduction of the selection pressure (Ghalambor et al., 2007; Grenier et al., 2016). This way, phenotypic plasticity may benefit the conservation of genetic variation within a population (Grenier et al., 2016). Still, plasticity is generally said to have a limited genetic variation which might hinder evolution (Via & Lande, 1985; Van Tienderen, 1991; Pigliucci et al., 2006). The evolvability of phenotypic plasticity may mainly be subject to the particular trait and the outcome of the environmental variation. However, some theoretical models point to a more difficult evolution of plasticity (Van Tienderen, 1991; DeWitt et al., 1998; Sultan & Spencer, 2002; Ernande & Dieckmann, 2004).

Controlled quantitative genetic experiments are required to measure the costs of phenotypic plasticity (Van Tienderen, 1991; Tauber & Tauber, 1992). Common garden experiments, for example, show this structure and enable to distinguish between genetic adaptation and phenotypic plasticity (Moloney et al., 2009; de Villemereuil et al., 2016). Additionally, they are increasingly used to investigate the degree to which genetic and environmental factors influence the achievement of invasive species to successfully colonize new habitats (Moloney et al., 2009). In common garden experiments, closely-related individuals are raised under different environmental

conditions to be able to differentiate genetic and environmental effects. Moreover, it is possible to evaluate genetic affiliation, environmental parameters and genotype-environment interactions causing phenotypic plasticity (DeWitt et al., 1998).

To diminish the costs of generating and maintaining a phenotypically plastic trait, selection may act on the novel phenotype in a new environment and establish it in the population. No further environmental stimuli would be needed after this point and the phenotype would become genetically fixed. The capacity of the population would increase because of the reduction of the costs of phenotypic plasticity. Those mechanisms are called genetic assimilation and genetic accommodation. Epigenetic modifications, such as DNA methylation are supposed to be mechanisms of genetic assimilation (Richards et al., 2017; Nijhout et al., 2021). Genetic accommodation includes genetic assimilation and describes that a new phenotype which developed either by a genetic or an environmental change, stays in a population if it is advantageous and if its expression rate is raised by selection or by the environment (Nijhout et al., 2021). Both mechanisms, genetic assimilation and genetic accommodation, may also explain the high adaptive abilities of invasive species which invade new habitats and adapt quickly which leads to extremely high numbers of individuals within a short time (Pigliucci et al., 2006).

Invasive species

Invasive species are able to colonize non-native, new habitats and reproduce effectively there. They adapt easily to the new environmental conditions and are detrimental to their new environment. A lot of invasive species show signs of rapid adaptation which makes them especially suited to investigate adaptation (Facon et al., 2006; Prentis et al., 2008). The human-induced climate change facilitates the invasion of non-native species to new habitats because they are almost always more plastic in their reaction to a higher obtainability of resources than native species (Davidson et al., 2011). There, native flora and fauna often suffers from the disastrous impact of invasive species which often win the battle for resources and faster reproduction (Huxel, 1999; Mack et al., 2000; Simberloff, 2000; Pimentel et al., 2001). Invasive species even seem to be able to disperse quicker than native species in some places (Averett et al., 2016). The reason for the success of invasive species is in many cases due to a high capacity for phenotypic plasticity (Davidson et al., 2011). Some investigations explain the success of invasive species as a consequence of natural selection, but such cases are rare (Tsutsui et al., 2000; Lee, 2002). Often, an increased reproductive performance allows invasive species to rapidly colonize new habitats (Parker et al., 2013). Moreover, in several invasive organisms the females reproduce asexually. This way, every

individuum is able to produce offspring and it additionally bypasses the time and resource consuming process of finding a suitable partner (Sakai et al., 2001; Frankham, 2005; Mergeay et al., 2006; Xie et al., 2010; Gibson et al., 2017). Therefore, invasive species represent one of the most threatening factor for the health and equilibrium of global ecosystems (Gherardi, 2007; Nentwig, 2008).

Epigenetic mechanisms including DNA methylation as a "motor" for phenotypic plasticity

The high variation in some traits may facilitate the fast adaptation to new habitats in invasive species (Chown et al., 2007; Davidson et al., 2011). Trait variation in invasive species may be explained by epigenetic or other non-genetic processes, particularly in clonal invasive species (Mounger et al., 2021). Different epigenetic mechanisms are known, such as histone modification, nucleosome remodelling, non-coding RNA-sequences and DNA methylation (Ho & Burggren, 2010; Becker & Workman, 2013). DNA methylation is an epigenetic process which plays a big role in gene expression and gene silencing (Razin & Riggs, 1980; Bird, 1984; Cedar, 1988; Bird, 2002; Jones & Baylin, 2002), as well as in the activation or repression of transposable elements (TE) (Niederhuth & Schmitz, 2017; Underwood et al., 2017). DNA methylation can be connected with environmental stress, such as extreme temperatures (Verhoeven et al., 2010; Marsh & Pasqualone, 2014; Naydenov et al., 2015). The activation or inactivation of TEs, regulated by DNA methylation, among other things, can explain the capability of rapid adaptation despite low genetic variance in invasive species (Schrader et al., 2014; Stapley et al., 2015).

While some questions concerning the connection of methylation and phenotypic plasticity have not been answered yet, the rapid activation and inactivation of genes may be a "motor" of phenotypic plasticity (Roberts & Gavery, 2012; Duncan et al., 2022). The majority of research in DNA methylation was carried out on model organisms, plants and some invasive species (Zilberman, 2008; Zhong, 2016; Hawes et al., 2018). Wu & Morris (2001) defined epigenetics as "The study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence.". It represents the investigations of heritable alterations in gene expression. Those changes – in contrast to mutations – do not alter the DNA sequence (Wolffe & Guschin, 2000).

Epigenetic mechanisms can change gene expression and therefore, the species phenotype when being activated by environmental changes (Duncan et al., 2014). In contrast to genetic

mechanisms, such as mutations, epigenetic changes may create an advantage when being confronted with changing environments, because the process of phenotypic adaptation would be faster, more dynamic and reversable (Burggren, 2016). Consequently, epigenetic and genetic mechanisms play an important role in the biological response to environmental changes and local adaptation (Keller et al., 2016). In natural populations, individuals which will reproduce and be preferred by natural selection are those whose methylomes result in the best adaptation to the current environment (Kalisz & Purugganan, 2004; Skinner, 2015).

The most studied and best investigated epigenetic mechanism so far is DNA methylation, and more precisely, the methylation of cytosine. DNA methylation is a biological process in which a DNA methyl transferase (DNMT) attaches a methyl group to the DNA (Lyko, 2018). The bases to which a methyl group can be attached are adenosine and cytosine. In animals, only the DNA methylation of adenosine and cytosine can be found. The majority of investigations deal with the methylation of cytosine transforming into a 5-methylcytosine. Although DNA methylation can be found in many eucaryotes, the patterns, quantity and genomic site of methylation differs between vertebrates and invertebrates. In mammals, the CpG dinucleotides are strongly methylated in the whole genome except for CpG islands. Since CpG islands generally overlay with promoter regions, they are often not methylated (Schübeler, 2015). In vertebrates, the methylated cytosine hinders the basal transcription machinery ubiquitous transcription factors from appending to the DNA and from transcribing the following region/gene and therefore leads to gene silencing (Figure 1a) (Bird & Wolffe, 1999). Contrarily, invertebrates show less and almost insignificant methylation rates at CpG dinucleotides. Most invertebrates show the so called "mosaic methylation" pattern with sites showing no or few methylation alternating with heavily-methylated sites (Bird et al., 1979; Tweedie et al., 1997; Suzuki & Bird, 2008; Schübeler, 2015). However, methylation in invertebrates occurs at sites which are commonly oriented to a group of gene bodies (transcribed sequence of a gene consisting of exons and introns) (Suzuki & Bird, 2008; Feng et al., 2010; Zemach et al., 2010; Provataris et al., 2018). In contrast to the methylation in exons, the methylation in intragenic regions in invertebrates is linked to a transcription activation (Figure 1b) (Feng et al., 2010; Zemach et al., 2010; Jones, 2012). Still, the causal effects still have to be proven (Schübeler, 2015).

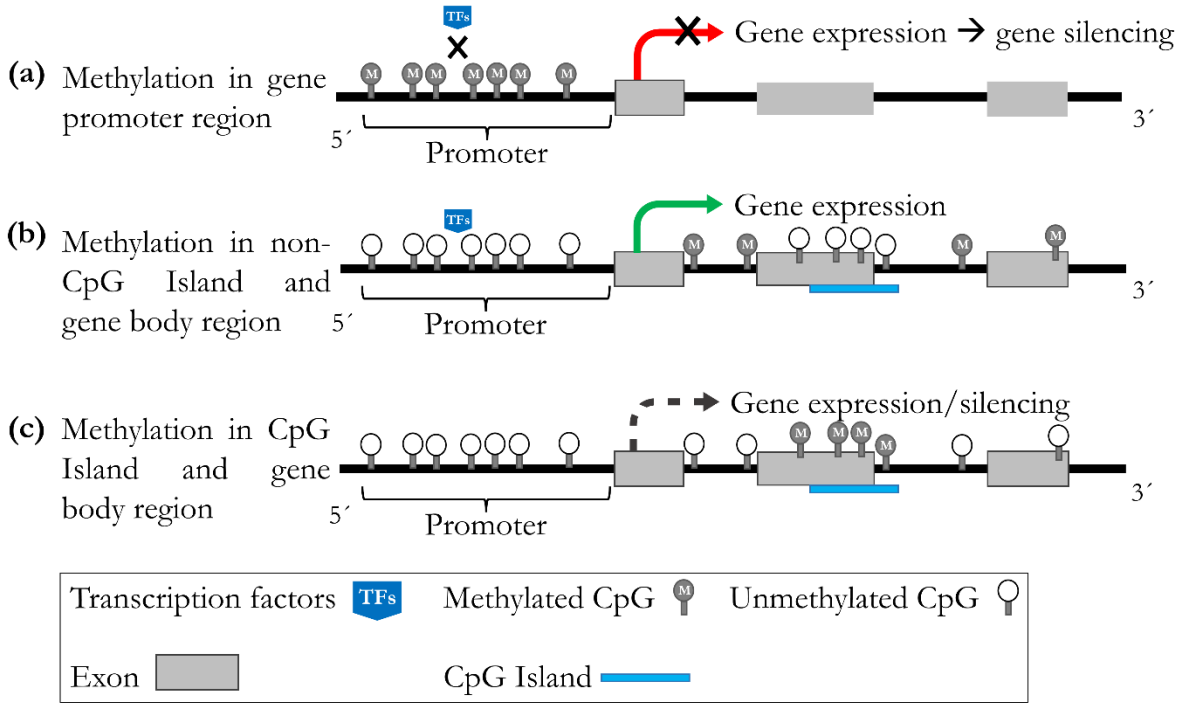

Figure 1. Simplified illustration of the correlations between DNA methylation and gene expression. (a) DNA methylation in the promotor region of the gene keeps transcriptional factors (TFs) from binding to the promotor region and hence the gene expression is suppressed. (b) DNA methylation in non-CpG Island and gene body regions leads to the activation of the gene transcription. (c) DNA methylation in CpG Island and gene body regions leads to the activation or repression of gene expression. Modified after Yang et al. (2016).

In the metazoa, the methyl group is attached to the DNA by two different types of DNA methyltransferases, DNTM1 and DNMT3 (Figure 2) (Goll & Bestor, 2005). DNMT 2 also known as tRNA aspartic acid methyltransferase, is not involved in the methylation of DNA, but in the methylation of a small RNA (Goll et al., 2006). DNMT3 is responsible for de novo methylation, DNMT1 for the conservation of already existing methylation (Jurkowska et al., 2011). Still, both DNMT1 and DNMT3 can contribute in de novo and maintenance methylation (Riggs & Xiong, 2004; Jones & Liang, 2009; Jeltsch & Jurkowska, 2014, 2016). Some invertebrates – mainly insects – possess only the DNMT1 and some paralogs which is therefore used for de novo and maintenance methylation (Bewick et al., 2017; Provataris et al., 2018).

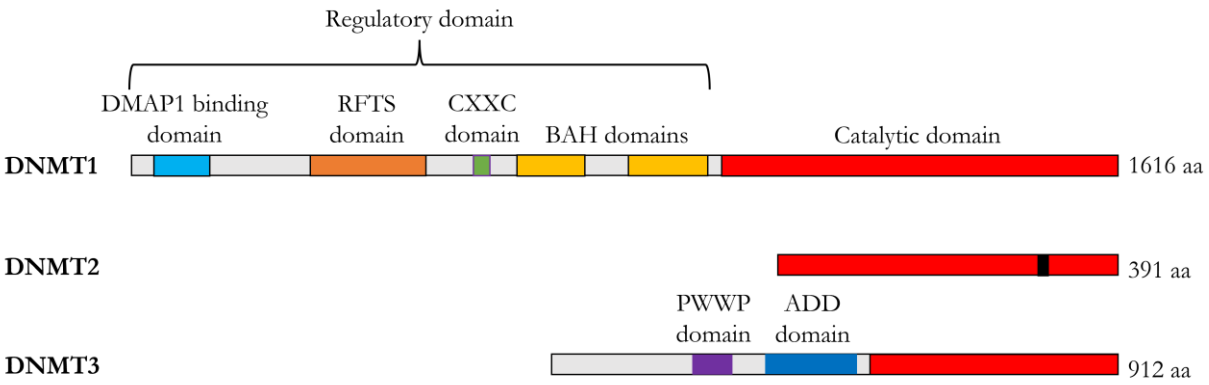

Figure 2. Members of DNA methyltransferase enzyme family. DNMT3a was taken as a model from DNMT3. Different colours display the conserved domains of all three DNMTs. The catalytic domain (red) can be seen in all the DNMTs. The small black region (CTF motif) in the catalytic region of DNMT2 differentiates DNMT2 from the other DNMTs. The amino acid numbers are representative for the respective DNMT in humans. Modified after Li & Zhang (2014) and Lyko (2018).

Apart from regulating the gene expression, DNA methylation is also used as a "epigenetic defence mechanism" by the genome to silence transposable elements (Slotkin & Martienssen, 2007). It also plays an important role among others in stress responses and carcinogenesis (Franco et al., 2008; Dowen et al., 2012), nutrition (Zhang, 2015), development (Geiman & Muegge, 2010; Smith & Meissner, 2013), diseases (Lu et al., 2013; Jin & Liu, 2018; Salameh et al., 2020), inactivation of chromosomes (Singer-Sam & Riggs, 1993), and adaptation including phenotypic plasticity (Flores et al., 2013; Schrader et al., 2014; Dubin et al., 2015; Stapley et al., 2015). Even limited DNA methylation can be seen as a motor for phenotypic plasticity by facilitating the adaptation of phenotypes to changing environments (Roberts & Gavery, 2012). There is still ambiguity among scientists about whether epigenetic processes, such as methylation rate and pattern are heritable from one generation to the other. Many investigations however, indicate that various epigenetic processes are heritable between generations (e.g. Jablonka & Lamb, 2002; Anway et al., 2005; Stouder & Paoloni-Giacobino, 2010; Greer et al., 2011; Ashe et al., 2012). DNA methylation seems to be a stable epigenetic mark because it survives various cell divisions and can be maintained during cell differentiation as a kind of epigenetic memory (Kim & Costello, 2017).

The New Zealand mud snail, *Potamopyrgus antipodarum* (Gray, 1843)

Origin and dispersal of the New Zealand mud snail

The New Zealand mud snail (NZMS) (Figure 3), *P. antipodarum*, belongs to the subclass Caenogastropoda and the family Tateidae. Figure 4 displays the phylogeny of the gastropods and shows the position of the NZMS within the class Gastropoda. The NZMS is originally from New Zealand where it can be found all over the country both in brackish water and freshwater in a variety of different habitats (Figure 5) (Winterbourn, 1970). The colour of the oblong shell ranges from light to dark brown and to grey. The NZMS is characterized by its high variation in shell morphology in its native range, New Zealand, but also in invaded countries (Figure 6) (Warwick, 1952; Winterbourn, 1970; Richards, 2002; Haase, 2003; Butkus et al., 2012). In New Zealand, the shell length varies between populations and the snail can grow up to 12 mm with the biggest individuals being four times the size of the smallest ones. In non-native areas, the NZMS can grow a shell length of maximum 6-7 mm (Winterbourn, 1970; Alonso & Castro-Diez, 2008). Some individuals possess spines as a shell armature to protect themselves from predation. Snails with spined shells have a higher possibility of dislodgement. The longer the spines of the shell, the more seston is captured and the higher the risk of dislodgement (Holomuzki & Biggs, 2005). Furthermore, the snail is able to close its long shell with a thin and corneous operculum (Gray, 1843; Duft, Schulte-Oehlmann, Tillmann, et al., 2003; Duft, Schulte-Oehlmann, Weltje, et al., 2003; Haase, 2003; Kistner & Dybdahl, 2014; Verhaegen, McElroy, et al., 2018; Verhaegen, Neiman, et al., 2018). *P. antipodarum* feeds on detritus, macrophytes and periphyton (Dorgelo & Leonards, 2001; Jensen et al., 2001; Alonso & Camargo, 2003; Duft, Schulte-Oehlmann, Tillmann, et al., 2003; Duft, Schulte-Oehlmann, Weltje, et al., 2003; Alonso, 2005).

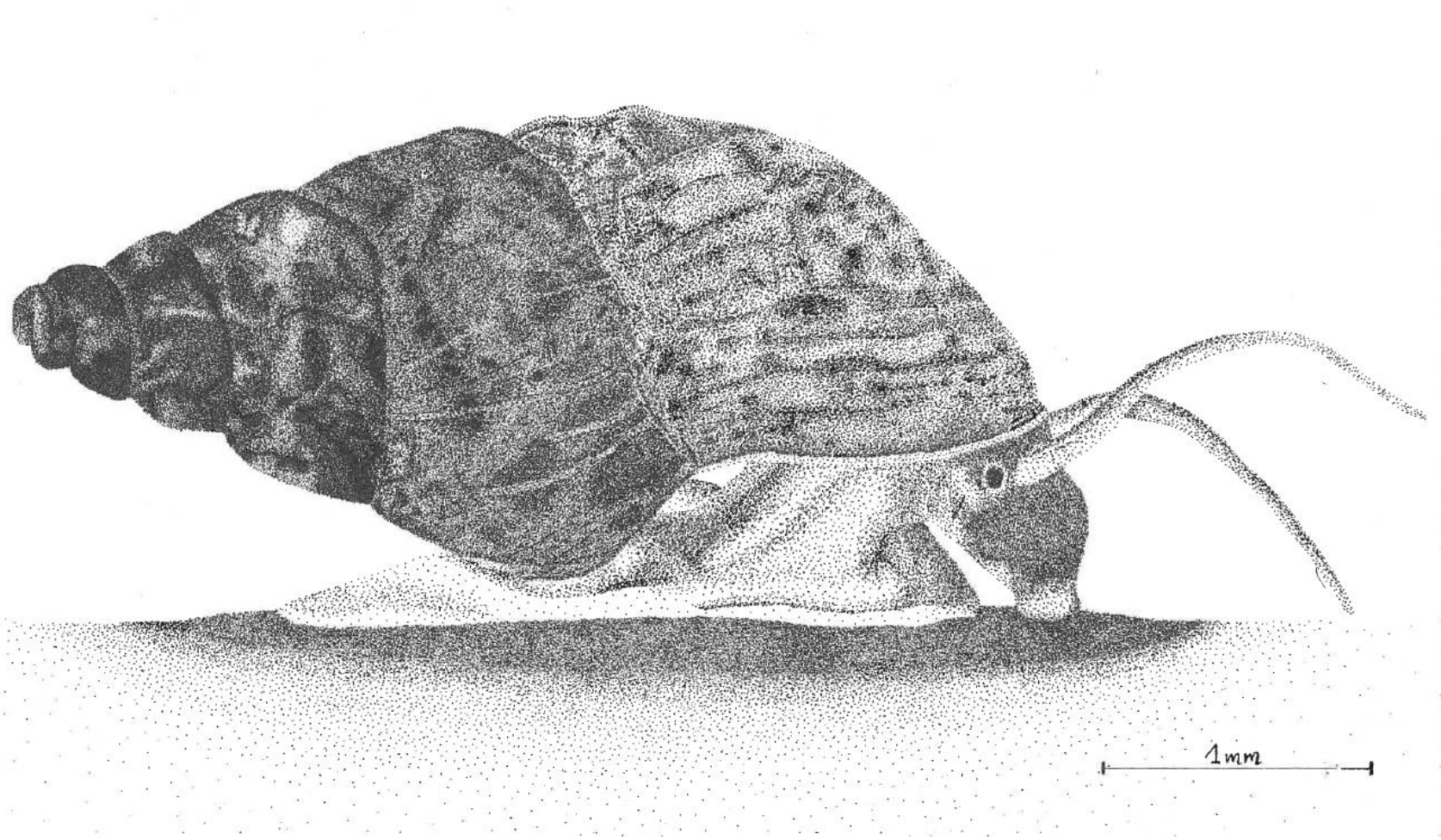

Figure 3. Drawing of the NZMS, *P. antipodarum*, in Indian ink. The size of the snail was estimated according to prior measurements of European NZMS. The drawing was made and modified after the original photo "Photo of the day (35): *Potamopyrgus antipodarum*" by Michael Maňas (2014) which can be found in the blog about gastropods of WordPress.com (https://gastropods.files.wordpress.com/2014/10/potamopyrgus-antipodarum.png).

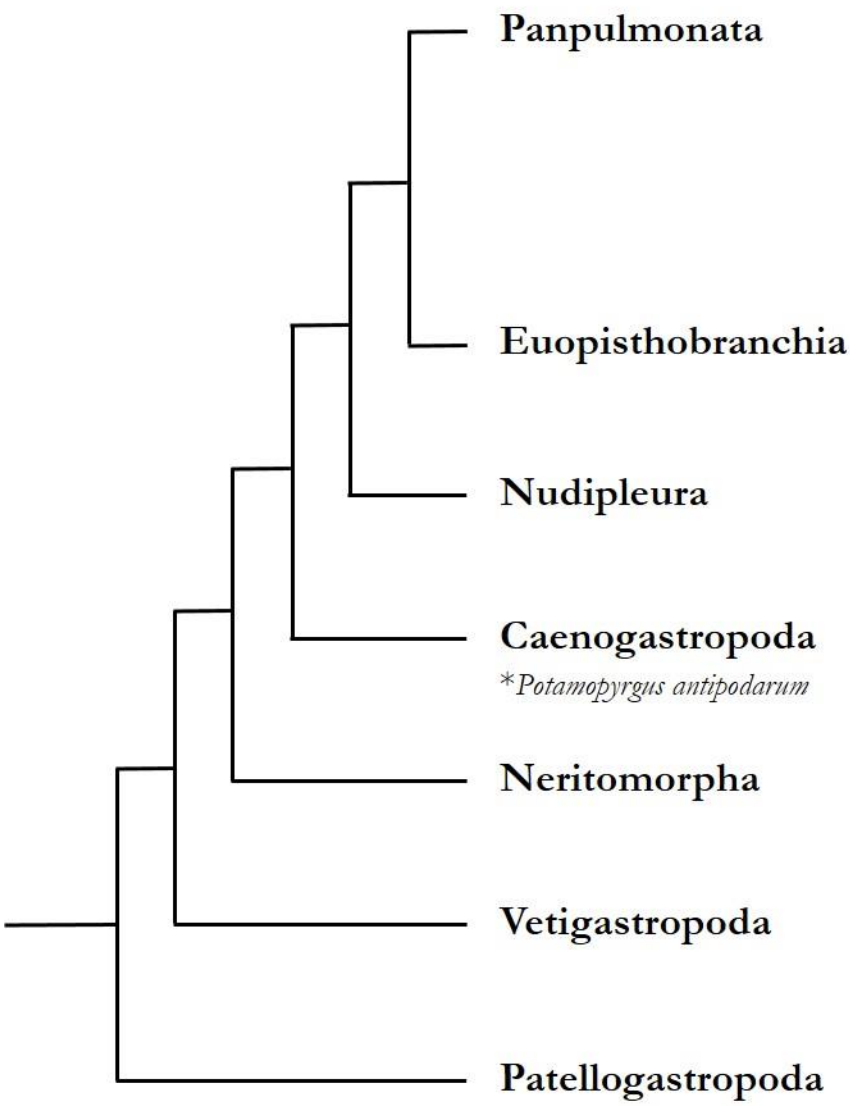

Figure 4. Gastropod phylogeny based on Kocot et al. (2011). The position of *P. antipodarum* within the gastropods is marked with an asterisk (*).

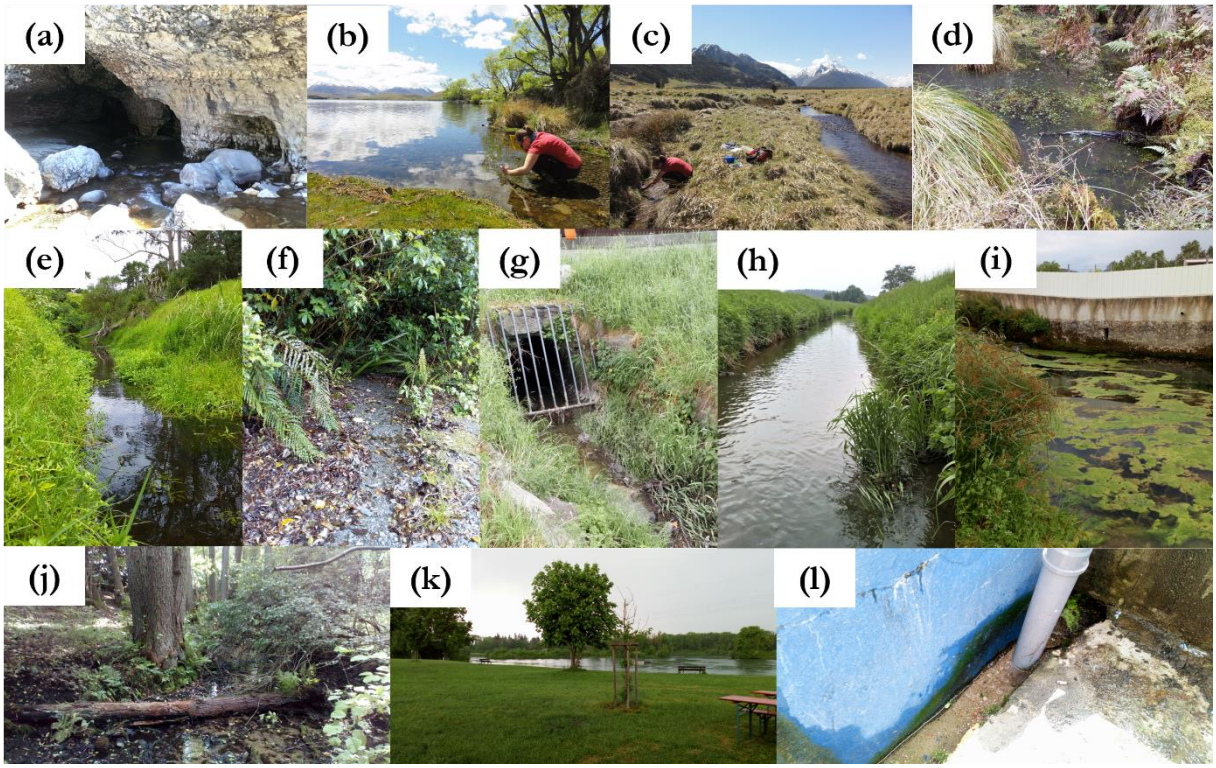

Figure 5. Different habitats of the NZMS in New Zealand (a, b, c, d, e, f) and Europe (g, h, i, j, k, l). The habitats vary in the type of water body, water depth, water temperature, water flow, salinity and water purity. (a) Cave Stream, Scenic Research, Canterbury, New Zealand. (b) Lake Alexandrina, Canterbury, New Zealand. (c) Lagoon Stream, next to Mount Cook Road, Canterbury, New Zealand. (d) Tarn/pool near Lake Haoroko, Southland, New Zealand. (e) Tributary to Mitimiti Stream, Northland, New Zealand. (f) Franz Joseph Glacier, West Coast, New Zealand. (g) Tributary of Memminger Ach, Memmingen, Southern Germany. (h) Kammel, Kammlach, Southern Germany. (i) Rio Alcaide, Porto de Mós, Leiria, Portugal. (j) Stream close to Klein Stresow, Rügen, Northern Germany. (k) Bathing lake Filzingen, Filzingen, Southern Germany. (l) Small stream in Póvoas village, Leiria, Portugal.

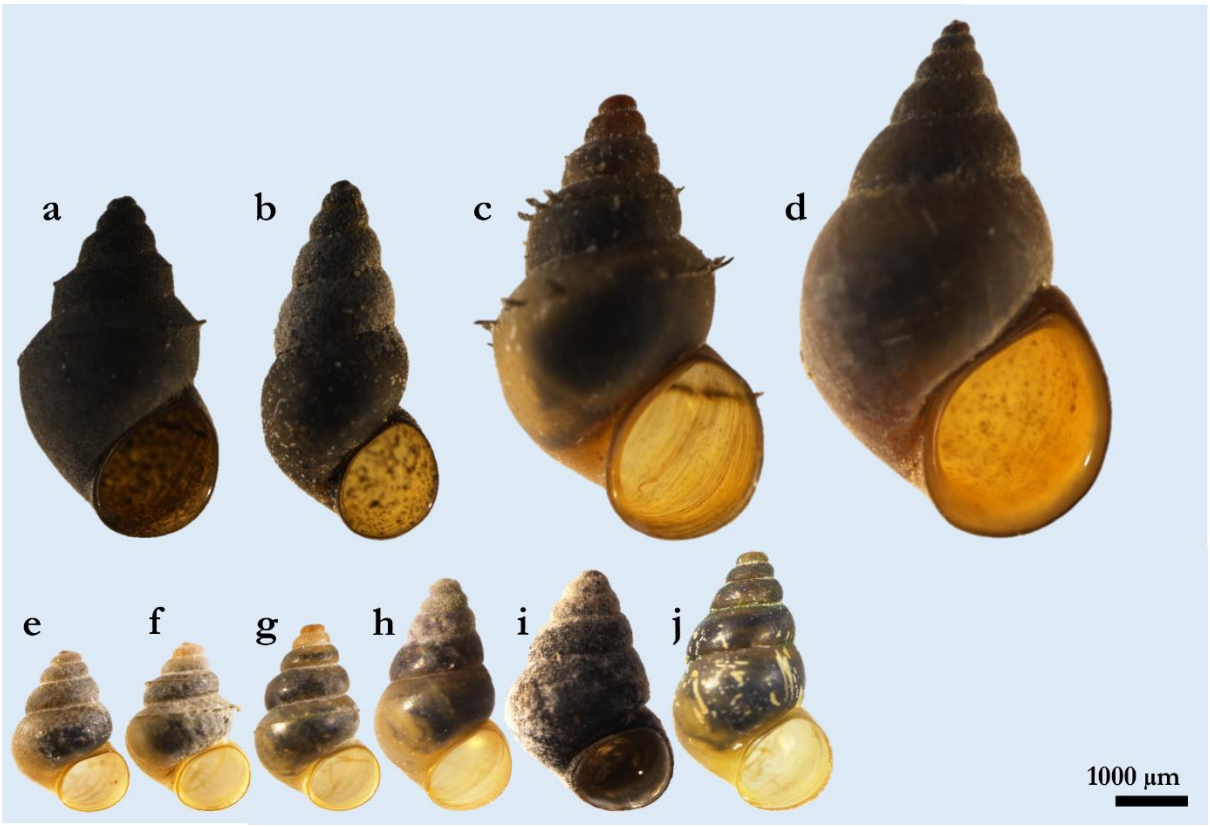

Figure 6. Shells from the NZMS from different habitats across New Zealand (a – d) and Europe (e – j). Shells of NZMS from New Zealand can grow four times as large as the shells of European NZMS.

The NZMS reproduces sexually and asexually in its native habitat and is ovoviviparous which means that the mother snail produces eggs that hatch within its body. The mother snail caries its offspring in a brood pouch until the embryos hatch and crawl out of the pouch (Jokela, Lively, Dybdahl, et al., 1997). Once adulthood is reached, the shell does not grow or change anymore (Verhaegen, McElroy, et al., 2018). The sexually reproducing females and males in New Zealand have a diploid chromosome set. Asexually reproducing females are tri- or tetraploid and they grow their offspring by apomictic parthenogenesis meaning that all offspring are clones to their mother (Lively, 1987; Phillips & Lambert, 1989; Wallace, 1992; Dybdahl & Lively, 1995; Neiman et al., 2011; Liu et al., 2012; Paczesniak et al., 2013). Asexually reproducing lineages of the NZMS repeatedly emerged from diploid individuals (Neiman et al., 2011, 2012; Paczesniak et al., 2013; Soper et al., 2013). In regions where the snail has been newly introduced, the females only reproduce asexually (Ponder, 1988; Hauser et al., 1992; Hughes, 1996; Jacobsen et al., 1996; Duft et al., 2007). Asexual female NZMS can reproduce all year around up to 70 embryos per brood although the number of offspring may fluctuate periodically (Schreiber et al., 1998; Duft et al., 2007; McKenzie et al., 2013; Verhaegen et al., 2021). The fecundity among populations varies due

to different environmental factors, such as water temperature, salinity and current (Verhaegen et al., 2021).

For New Zealand, Neiman et al., (2011) showed that triploid males are occasionally and repeatedly produced by triploid females. Although the triploid males produce sperm, it remains unclear if their sperms are able to fertilize female ova. The spermatozoa of asexual males have significantly more phenotypic variation and the probability is higher that they show extensive structural abnormalities (Jalinsky et al., 2020). Therefore, triploid males are considered asexual until now, but it remains to be investigated if they are really infertile (Soper et al., 2013). Some male individuals have also been found in Europe, but there was no proof of sexual reproduction (Wallace, 1979, 1985). European NZMS seem to be strictly parthenogenetic (Jacobsen & Forbes, 1997).

Since the NZMS reproduces both sexually and asexually in New Zealand, the genetic and the morphological variation is understandably higher than in Europe (Städler et al., 2005; Verhaegen, 2018). Over time, as more and more clonal lineages develop, the difference of morphological variation between sexually and asexually reproducing snails diminishes (Neiman et al., 2005; Paczesniak et al., 2013; Verhaegen, Neiman, et al., 2018). Across New Zealand, Neiman & Lively (2004) found 45 different genotypes from 20 different populations from 638 individuals using microsatellite genotyping. The result was confirmed by Neiman et al. (2011) sequencing a portion of the mitochondrial cytochrome-b gene 513 individuals. Städler et al. (2005) identified 17 different haplotypes among 65 snails from 15 different populations in New Zealand using 16S rRNA sequencing. In contrast, European female NZMS reproduce only asexually, we observe a low genetic variance. In the UK, 3 genotypes were found among 66 individuals from eleven populations using minisatellites (Hauser et al., 1992). Investigating 10 Danish populations using RAPDs, Jacobsen et al. (1996) identified two clonal lineages among 117 individuals. In various European countries, two mitochondrial haplotypes were detected (Verhaegen, McElroy, et al., 2018; Butkus et al., 2020) along which ten SNP (single nucleotide polymorphism) genotypes across 425 snails from 21 regions were detected (Verhaegen, McElroy, et al., 2018). When species colonize a new habitat, it may result in a low genetic diversity which is called the founder effect (Shirk et al., 2014). Especially invasive species which colonize new habitats with only few individuals, have a high probability to suffer from low genetic diversity. However, despite invading a new habitat with few individuals and low genetic variation, many invasive species did not experience the founder effect (Groves & Burdon, 1986; Novak, 2005; Wares, 2005; Dlugosch & Parker, 2008). Despite the low genetic variation in the European NZMS, there is also a reduced but still existent,

morphological variation observed. A possible explanation could be phenotypic plasticity (Verhaegen, McElroy, et al., 2018).

The New Zealand mud snail as an invasive species and model organism

The NZMS is a perfect example of an invasive species. As mentioned above, invasive species are well-suited model species to study adaptability and dispersal potential. Originating from New Zealand, the NZMS found its way to Europe in the mid-19th century, presumably in the ballast water of ships (Ponder, 1988; Smith, 1889). It was first documented in the river Thames in England and invaded the mainland of Europe around 1900 (Smith, 1889; Hubendick, 1950). From there it spread rapidly almost all over the European continent colonizing a variety of different habitats (Figure 5) (Bondesen & Kaiser, 1949; Hubendick, 1950; Lucas, 1959; Real, 1973; Wallace, 1985). Within the continents, the NZMS presumably not only spread through the ballast water of ships, but also through predators since it is known to survive the gut passage of fish and birds (Bruce et al., 2009; Naser & Son, 2009; Butkus & Rakauskas, 2020). Städler et al. (2005) confirmed that the European NZMS invaded from New Zealand. Over time, it was furthermore introduced to many other continents and countries all over the world, such as Australia, North America, Japan, Russia and Chile (Ponder, 1988; Bowler, 1991; Shimada & Urabe, 2003; Lucy & Graczyk, 2008; Alonso & Castro-Díez, 2012; Collado, 2014) and recently discovered in the North of Africa (Taybi et al., 2021). Subsequently, the NZMS is considered one of the most alarming alien species in Europe (Nentwig et al., 2018).

Being able to colonize a new habitat with only a single individual is clearly a high advantage (Barrett & Richardson, 1986). Apart from this, scientists found several further reasons for the NZMS showing such a high capacity to adapt to new and changing environments. The NZMS seems to have almost no controlling and regulating forces, such as predation and parasites, that would inhibit its rapid population growth (Alonso & Castro-Diez, 2008). Moreover, it prevails very well against competitors in the invaded habitats in the early stages of invasion. The high level of reproduction helps the asexual snail to flood newly invaded regions with offspring and the high tolerance level to physio-chemical factors helps to persist against various environmental conditions in the new habitat. The consequence of the high adaptive potential and resilience of the NZMS leads to a competition for resources with native species which the NZMS usually wins (Zaranko et al., 1997). In the USA and in Chile, endemic macroinvertebrate communities – and also other snail species, for example the closely related *Pyrgulopsis robusta* – face negative consequences by the

NZMS (Richards, 2004; Kerans et al., 2005; Riley et al., 2008; Collado, Aguayo, et al., 2019; Collado, Vidal, et al., 2019).

Another trait that helps the NZMS to be so adaptive, is its highly variable shell morphology (Haase, 2003; Kistner & Dybdahl, 2013, 2014; Verhaegen, McElroy, et al., 2018; Verhaegen, Neiman, et al., 2018). Even though genetic factors mainly determine shell morphology, both size and shape are in parts also influenced by environmental factors. Also brood size as proxy for fecundity shows a correlation with shell size; with shape only under extreme conditions (Verhaegen, Neiman, et al., 2018). The relationship between morphology, habitat and fecundity seems to be very complex and further investigation is needed. Despite the clonal kinship in asexually reproducing individuals, both shell size and fecundity vary among populations (Verhaegen et al., 2021). Water temperature, latitude and components concerning food disposability influence the size and shape of the shell (Verhaegen, McElroy, et al., 2018). It may also be possible that the shell morphology is connected to the reproductive rate of the snail. Shell size and number of brooded embryos are positively related because the bigger the shell of the snail, the more space the brood pouch can take up in its body. Consequently, the bigger the brood pouch, the more embryos fit in it and the more offspring can be produced (McKenzie et al., 2013; Verhaegen, McElroy, et al., 2018). A bigger and more stable shell also gives protection against its destruction caused by predators or after dislodgement but at the same time may also increase the risk of dislodgement with higher flow rate (Holomuzki & Biggs, 2006; Verhaegen et al., 2019).

Apart from investigations about the invasiveness of the NZMS (e.g. Alonso & Castro-Diez, 2008; Murria et al., 2008; Krodkiewska et al., 2021), the NZMS is also used as a model organism in investigations about the maintenance of sex (e.g. Lively, 1987; Neiman & Lively, 2005) and in ecotoxicology (e.g. Morley, 2008; Stange et al., 2012; Völker et al., 2014). *P. antipodarum* represents a highly adaptive and resistant brackish and freshwater organism whose further study could certainly shed light on open questions regarding invasive species.

Introduction to individual studies

Differentiation of genetic adaptation and phenotypic plasticity in the morphologically variable shell of the NZMS

Common garden experiments are promising approaches to study adaptation and to disentangle genetic from environmental effects to comprehend and foresee adaptive responses to changing environments (Moloney et al., 2009; de Villemereuil et al., 2016). Common garden experiments have been widely applied with investigations in plants (Linhart & Grant, 1996). Lately, various common garden experiments have also increasingly been carried out with animals, such as mammals (e.g. Suzuki et al., 2019; Bittner et al., 2021), fish (e.g. Hamel et al., 2020; Ramesh et al., 2021) and invertebrates (e.g. Bodensteiner et al., 2019; Iwabuchi & Gosselin, 2020). Breeding and rearing the study organism is the main challenge of the common garden technique (de Villemereuil et al., 2016). It is also often not easy to relate the outcome of common garden experiments to natural populations (Verhaegen, McElroy, et al., 2018). Despite these challenges, with the help of common garden experiments, scientists are able to investigate genetic basis of phenotypes from different populations while excluding environmental impacts. Common garden experiments aim at investigating local adaptation and the adaptive potential in different traits, for example, life history or morphological traits (e.g. Jensen et al., 2008; Kawakami et al., 2011; Pascoal et al., 2012). At the same the impact of different environmental parameters, such as salinity or temperature, on these traits can be analysed with aquatic organisms (Narum et al., 2013; DeFaveri & Merilä, 2014).

Due to the production of clonal offspring, the low genetic variation in invasive NZMS populations and the high adaptive potential, invasive NZMS represent a very appropriate model organism for studying and differentiating between genetic adaptation and phenotypic plasticity (Mergeay et al., 2006; Xie et al., 2010). The high variability of shell size and shape of the NZMS is connected to genetic adaptation, as well as to phenotypic plasticity (Kistner & Dybdahl, 2013) and could be an important factor for the high adaptability of the NZMS. It has already been shown in laboratory and field studies that different biotic and abiotic parameters have an influence on the shell morphology of the NZMS, such as predation, flow rate, parasitism and water depth (Jokela & Lively, 1995; Jokela, Lively, Dybdahl, et al., 1997; Haase, 2003; Levri et al., 2005; Holomuzki & Biggs, 2006). Dybdahl & Kane (2005) suggest to study the importance of adaptation by keeping invasive species from an environmental gradient in a common garden setting.

We set up a common garden experiment to disentangle genetic from environmental effects on the variability of the shell morphology of the NZMS. We investigated the influence of three

different temperatures on shell size and shape of NZMS from different European populations to test the following hypotheses: (1) shells of snails from diverse populations would become more similar in the same environments; (2) across the three temperatures, the reaction norm slopes would be similar; (3) the variability of the phenotypic traits could be mainly declared by environmental impacts, and therefore, we expect a low broad-sense heritability and (4) snails that were reared under lower temperatures would establish larger and more globular shells and have lower reproductive rates which can be explained with the temperature-size rule (Männer et al., 2022). The temperature-size rule says that organisms put more energy into their growth period. The organisms become mature at a larger body size and therefore reproduce later (Atkinson, 1994, 1995; Atkinson et al., 2003; Angilletta et al., 2004). Rearing a second daughter generation (F2), we were able to compare the shell morphology across three generations. Additionally, the effect of the three different temperatures on heritability and the reproductive rate of the parental generation were analysed.

DNA methylation patterns in molluscs

Although it has been shown that DNA methylation has already been present in the ancient eucaryotes´ genomes, the level and the target regions of DNA methylation vary across nowadays eucaryotes (Zemach et al., 2010). DNA methylation has already been investigated in animals, fungi and plants (Bird, 2002), for example in Bird & Wolffe (1999), Bestor (2000), Finnegan et al. (2000), Hsieh (2000), Martienssen & Colot (2001). The majority of investigations including phenotypic plasticity and DNA methylation were carried out on plants, model organisms and invasive species (Zilberman, 2008; Zhong, 2016; Hawes et al., 2018). Thereby, the impact of changing environmental conditions on the DNA methylation patterns of different organisms has already been shown (e.g. Putnam et al., 2016; Ibañez et al., 2021), as well as the impacts of a changing methylation rate on the pattern of phenotypic plasticity (Bossdorf et al., 2010). In the Antarctic worm, *Spiophanes tcherniai*, small temperature alterations increased the number of methylated cytosine and therefore the DNA methylation in general (Marsh & Pasqualone, 2014).

CpGs sites are DNA regions where a guanine nucleotide goes after a cytosine nucleotide. Especially vertebrates and mammals show a high amount of methylated CpGs in the whole genome. There are some regions that build an exception to this rule and lack methylation: so-called CpG islands. CpG islands frequently overlap with promotor regions and therefore are thought to miss methylation (Jeltsch, 2010; Schübeler, 2015). The quantity of methylation, the methylation patterns and the sites where methylation takes place in the genome vary between vertebrates and

invertebrates (Provataris et al., 2018). Unlike in vertebrates, the methylation in invertebrates is rather low and insignificant at CpG sites. They represent the "mosaic methylation" pattern, composed of regions alternately high in methylation or low in methylation (Bird et al., 1979; Tweedie et al., 1997). The main sites in the genome of invertebrates where DNA methylation is happening, are CpGs in exons and introns of gene bodies (Feng et al., 2010; Zemach et al., 2010). The expression "gene body" includes exons and introns and relates to the transcribed sequence of a gene. Insects for example, show a rather variable gene body methylation (Suzuki & Bird, 2008; Glastad et al., 2011). In some insect groups, the DNA methylation has probably even been lost or massively reduced (Provataris et al., 2018).

Although representing one of the most diverse and the second-largest phylum in invertebrates and being economically and medically a highly important invertebrate group (Ponder et al., 2002; Haszprunar & Wanninger, 2012; Rosenberg, 2014), the general knowledge and the investigation of the DNA methylation in molluscs is rather scarce. Until now, nothing is known about the distribution of DNMTs across all mollusc classes. Before the publication of the paper of Männer et al. (2021), DNA methylation had just been investigated in some molluscan species. Analyses of DNA methylation in molluscs have been done in seven bivalves, one cephalopod and eight gastropods. Zhang et al. (2020) gave an overview of the DNA methylation in molluscs concerning growth and development. Fallet et al. (2020) summarized the available insights on DNA methylation and histone modification in molluscs in a review. They found that both types of epigenetic mechanisms play a big role in the molluscan development and that both are influenced by environmental conditions. The quantity of DNA methylation is also quite diverse in molluscs. Furthermore, they declared the importance of broadening our current knowledge in the DNA methylation in molluscs in the future as it may explain the role of DNA methylation in the adaptation and evolution in molluscs.

To investigate the presence and degree of DNA methylation in molluscs, we analysed the presence/absence of DNMTs and the CpG observed/expected (o/e) ratio using genomic and transcriptomic data of all mollusc classes (Männer et al., 2021), following the procedure of Provataris et al. (2018). The CpG o/e ratio is a value which estimates the level of methylation occurring in the genome of individual species and is based on the susceptibility of methylated cytosines to mutate into thymine residues (Coulondre et al., 1978). Species that possess a high germline methylation in genomic regions over evolutionary time, have an increased possibility of showing a steady decrease of CpG dinucleotides (CpG depletion). In those genomes, CpGs are underrepresented and are referred to as a bimodal CpG o/e distribution. In contrast, species having a limited germline methylation in genomic regions over evolutionary time, show a higher CpG

content and therefore lack CpG depletion, also called an unimodal CpG o/e distribution (Coulondre et al., 1978; Bird, 1980; Sved & Bird, 1990; Wiebauer et al., 1993; Fryxell & Moon, 2005; Provataris et al., 2018). The CpG o/e ratio has been validated and successfully applied. The connection to the methylation status and the gene expression has been shown in various organisms (Suzuki et al., 2007; Elango & Yi, 2008; Park et al., 2011; Sarda et al., 2012; Dixon et al., 2014), molluscs among them (Gavery & Roberts, 2010; Fneich et al., 2013; Gavery & Roberts, 2013; Wang et al., 2014; Adema et al., 2017; Venkataraman et al., 2020). Diverse methylation patterns have already been shown in other invertebrate groups, such as insects (Bewick et al., 2017; Provataris et al., 2018). Therefore, we similarly expect a varying methylation pattern in the highly diverse group of the molluscs. This investigation aims to shed light on the methylation status of molluscs and broaden our knowledge to open and initiate more scientific epigenetic analysis in molluscs.

III. MATERIAL AND METHODS

Differentiation of genetic adaptation and phenotypic plasticity in the morphologically variable shell of the NZMS

Sample collection

We collected the snails for our common garden experiment along a northeast-southwest gradient along Europe. We collected adult snails from Northern Germany and Southern Germany (three populations each) and Portugal (four populations) (Figure 7). The Spanish population was collected and sent by Dr. Marian Ramos and Fernando García Guerrero from the National Museum of Natural Sciences in Madrid, Spain. For our analysis, we grouped the Spanish and the Portuguese populations under the term "Iberian Peninsula". Collection dates, localities, coordinates and habitat types are summarised in Table 1. The majority of the snails was collected by filtering the ground substrate of the water body through a small fishing net. The ground substrate was then poured into a white tray to sort the snails among the ground substrate that was taken with the net. Additionally, water temperature and salinity of the water bodies were measured in each habitat - except for the Spanish population (Table 1). The water temperature of the habitats ranged between 12.5°C and 24.0°C. All snails were collected from freshwater habitats. In small plastic jars filled with water from the collection site, we transported the snails to the laboratory in Greifswald where the snails were stored in small 700 ml aquariums at 20°C until the start of the experiment.

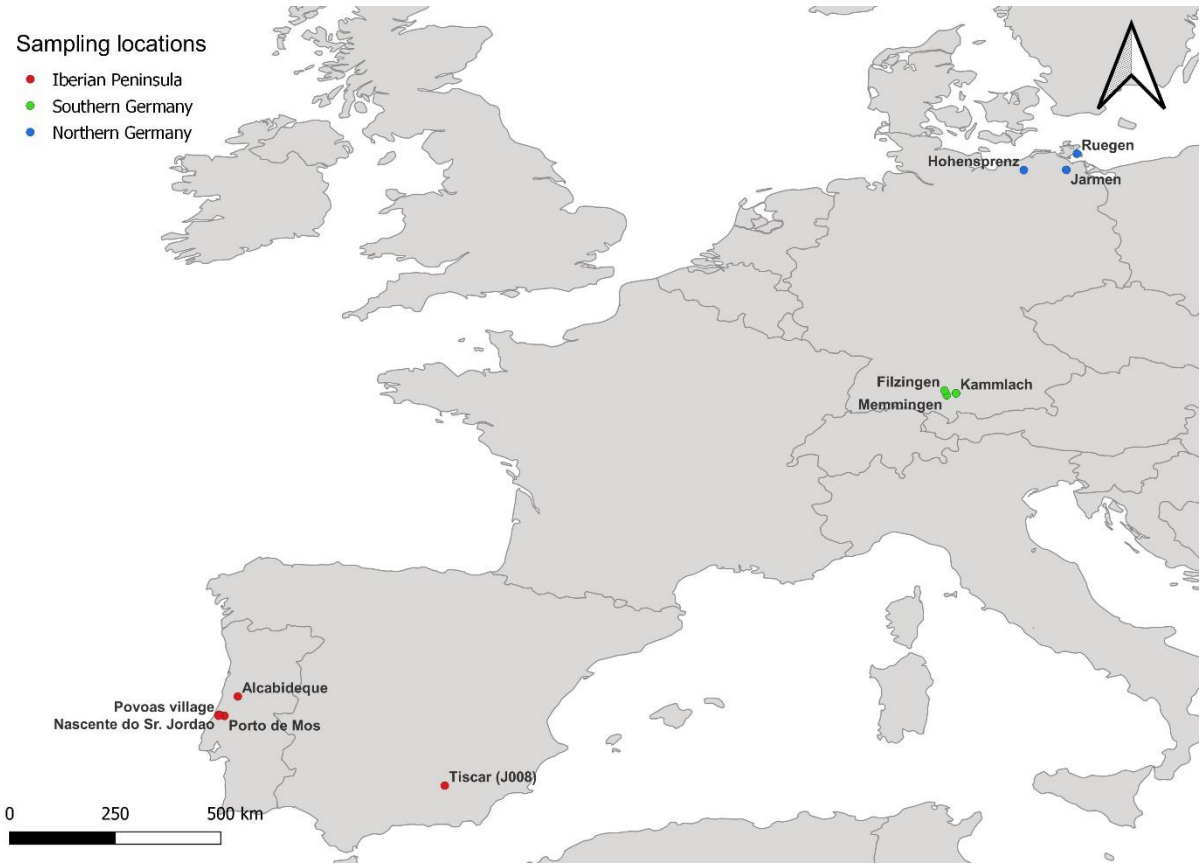

Figure 7. Sampling sites along the northeast-southwest gradient within Europe.

Table 1. Sampling site details of the 11 populations used in the common garden experiment.

Population	Region	Collection Date	Coordinates	Type of water body	Temperature at collection [°C]	Salinity at collection [ppm]
Hohensprenz	Northern Germany	02.07.2018	53°55´25.428" N 12°11´57.354" E	Stream	23.2	284
Jarmen		29.06.2018	53°55´44.952" N 13°19´0.540" E	Lake	21.5	309
Rügen		29.06.2018	54°21´19.421" N 13°35´52.402" E	Stream	15.7	309
Filzingen	Southern Germany	15.05.2018	48°8´7.697" N 10°6´55.371" E	Lake	17.8	203
Kammlach		17.05.2018	48°3´57.483" N 10°25´3.568" E	Stream	14.2	244
Memmingen		14.05.2018	48°1´3.165" N 10°10´19.766" E	Small river	12.5	270
J008 (Tiscar, Spain)	Iberian Peninsula	14.07.2018	37°46´05.0" N 3°01´26.8" W	Trough	-	-
Alcabideque		20.06.2018	40°6´23.311" N 8°27´54.130" W	Lake	17.0	404
Nascente do Sr Jordão		21.06.2018	39°37´41.958" N 8°57´29.599" W	Stream	20.1	211
Porto de Mós		21.06.2018	39°35´46.049" N 8°49´2.906" W	Small river	24.0	257

Póvoas Village		22.06.2018	39°36´7.236" N 8°58´12.964" W	Spring	17.8	146

Common Garden Experiment

With the common garden experiment, we aimed to analyse the reaction of the shell morphology on different temperatures (Moloney et al., 2009; de Villemereuil et al., 2016). For the experiment, we put one mother snail each in a small glass jar with 3 ml of sand covering the ground and filled with 250 ml of artificial freshwater (salt content: 0.5 ‰) (Figure 7). Additionally, we added a stone for the snail to take shelter and some pieces of shell from the marine bivalve *Arenomya arenaria* for chalk uptake to the aquarium. To sterilise both stones and shells before adding them to the aquarium, they were boiled beforehand in water. For better handling, we placed each ten glass jars in opaque trays (Figure 8). The opaque trays also prevented light from entering from the below. The trays were rotated within the climate cabinet after each snail maintenance, such as the glass jars within the trays to avoid any influence of the position in the climate cabinet on the experiment´s outcome (Figure 9). Each six mother snails per population were placed in the three climate cabinets with different temperatures (15°C, 19°C and 23°C) to produce offspring. We chose the three temperatures according to the habitat temperatures that the snails normally experience during their reproductive peak (Verhaegen et al., 2019). We also had to take care not choosing the temperatures too high or too low, because we wanted the snails to produce offspring in the course of our experiment and the NZMS reduces its reproduction with extreme temperatures (Dybdahl & Kane, 2005; Gust et al., 2011).

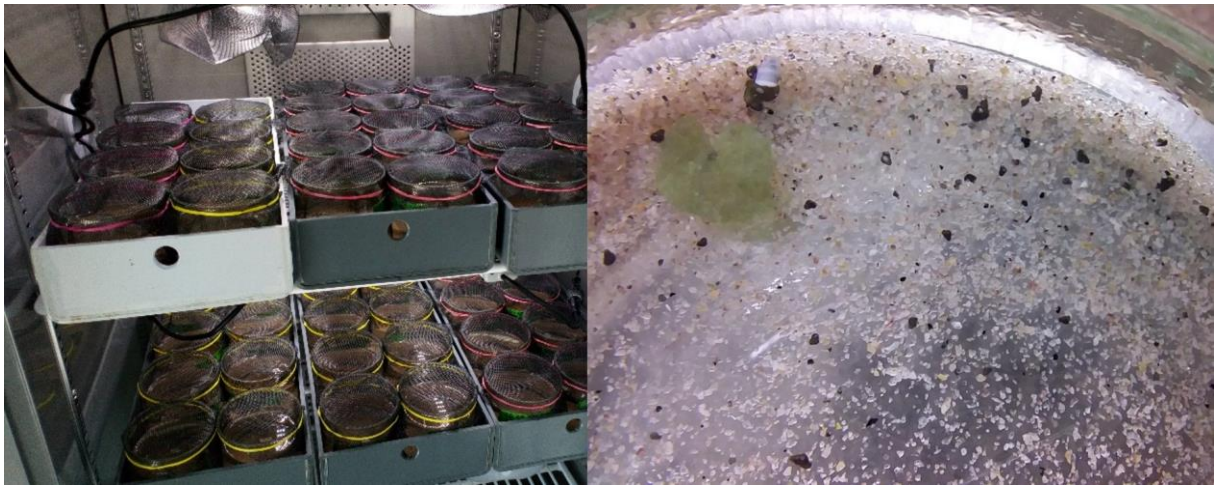

Figure 8. Set up of the common garden experiment in one of the three climate cabinets (left). Every mother snail was placed in a small glass aquarium to produce offspring/clones (right). The mother is marked with a white dot of nail polish.

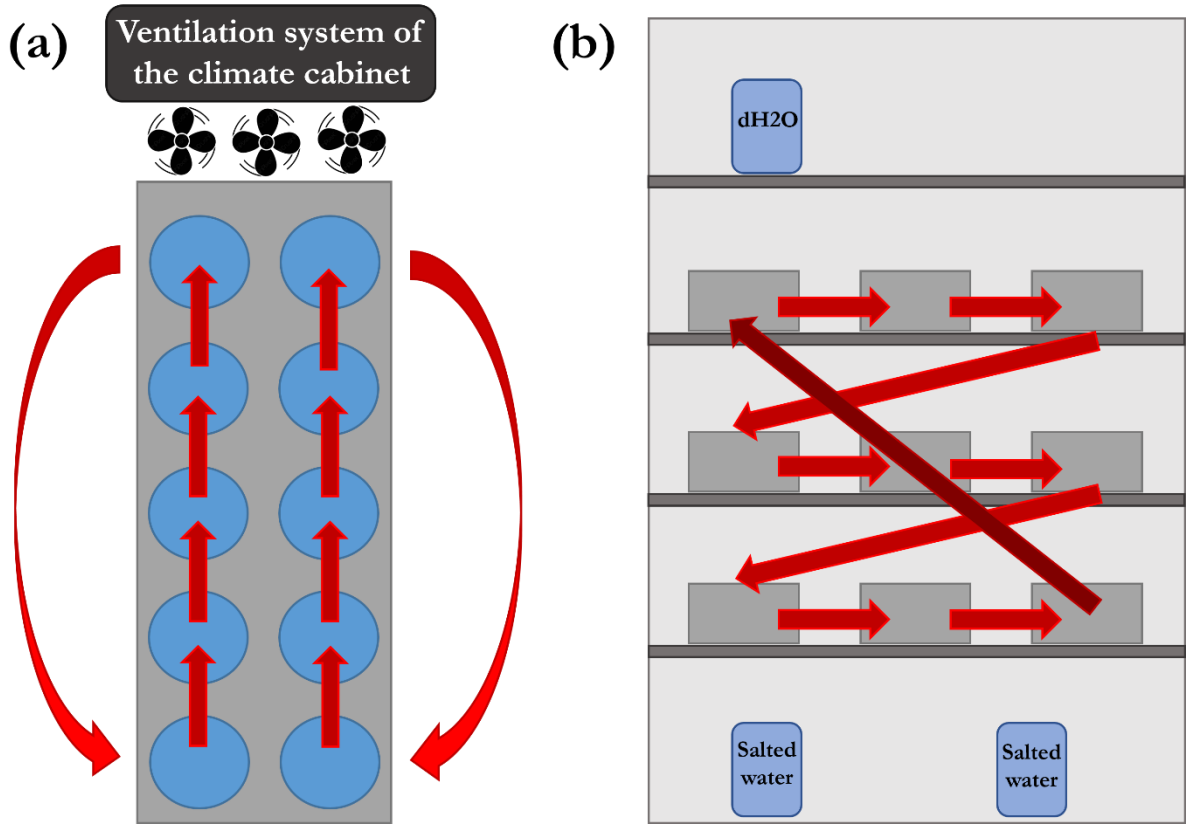

Figure 9. Weekly rotation of the (a) glass jars within each tray and (b) the trays within each climate cabinet after the snail maintenance.

We fed the snails and changed the water first once a week and changed it to once every ten days due to logistical circumstances. The snails were fed with Spirulina flakes (JBL Spirulina Premium). We created an artificial day/night rhythm in the climate cabinets (16 hours day and 8 hours night). The snail maintenance was conducted only during the day hours to avoid any disturbances during the night hours, such as light pollution when opening the climate cabinets.

Among other factors which influenced the shell morphology of the NZMS, such as flowrate, sunlight and nitrate concentration in the water, also temperature had an effect on the shell shape. Furthermore, once the NZMS reaches adulthood, the shell does not grow any further. (Verhaegen, McElroy, et al., 2018). We analysed the shell morphology of the daughter snails based on the developmental temperature of the respective climate cabinet they were born and raised in. Daughter snails grew up and remained in the glass jar of their mother snail. To differentiate between mother and daughter snails, once the daughter snails reached adulthood, we marked the mother snails with a dot of nail polish on their shells. When the mother snail reached ~ten offspring, we fixed the mother snail in ethanol (96%, MEK). When a daughter snail reached

adulthood, it was fixed in ethanol. Some daughter snails (F1) were placed in a separate glass jar to produce a second daughter generation (F2). Unfortunately, the Northern and Southern German populations of the F1 generation did not produce a sufficient number of offspring within the given time. We decided to end the experiment for the F1 snails rearing a F2 generation in January 2021 for all F1 mother snails except for the ones that were able to produce a sufficient number of offspring. The offspring were kept in the experiment until they reached adulthood. As only two Portuguese populations reached a suitable number of offspring across all three temperatures, we continued analyses that include the F2 generation, only with those two Portuguese populations. We fixed the last snail, and therefore finished our common garden experiment, on the 6th of June, two years after the start of the experiment.

The hatchlings of the NZMS hatch consecutively, rather than simultaneously over several weeks. This is why a split-brood design is inherently difficult to implement and impossible at our scale. Still, we do not see a problem with our approach (Hurlbert, 1984) because the invasive European populations that reproduce asexually are genetically very homogeneous (Verhaegen, McElroy, et al., 2018; Butkus et al., 2020; Weetman et al., 2002). The number of populations in our experiment is higher than the number of initial mother snails because studies already proposed that the variation between populations would be greater than the variation within them (Dybdahl & Kane, 2005; Kistner & Dybdahl, 2014).

Geometric morphometrics

The photos of the snail shells were taken with a Nikon SMZ25 stereomicroscope provided with a Nikon DS-Ri2 camera (Nikon, Tokio, Japan). A petri dish filled with silicone-inlay was used to hold each snail with its aperture facing upwards and with the coiling axis oriented horizontally (Figure 10). We measured one of the two size parameters, length, parallel to the coiling axis with the NIS-Elements Ar 4.51 imaging software (Niko, Tokio, Japan). The second size parameter, centroid size, was calculated using the Integrated Morphometrics Package CoordGen 8.

We used the Geometric morphometrics approach to determine the shell shape of the snails. An advantage of this approach is the calculation of shape without the influence of size. During the process the effects of size, rotation and position are filtered out by the Procrustes superimposition (Mitteroecker & Gunz, 2009; Tatsuta et al., 2018). After converting the photos into TPS files, we put 16 landmarks on each shell in such a way that their shape was well described using the programs tpsUtil64 version 1.78 and tpsDig version 2.31 (https://life.bio.sunysb.edu/morph/, downloaded

1st of March 2021). We identified 43 snails with damaged shells and shells overgrown with algae. We excluded those snails from further analysis because we were not able to place the landmarks in an unequivocal way. The measurements of the shells were always limited to the same person (L. Männer) to prevent a measurement bias due to variable handling of different labourers (Schilthuizen & Haase, 2010). Still, we quantified the repeatability of the shell measurement and the following procedure (see below) with 20 shells that were photographed twice over a two-week period. Both, the repeatability of size and shape measurement were not significantly different and therefore, showing a clear repeatability of the process. We tested the repeatability of size with a paired two-samples Wilcoxon test, $V(19) = 147$, $p = 0.12$ (size) and the repeatability of the shape measurements with the Integrated Morphometrics Package, Two Group 8: Goodall´s $F = 0.70$, $p = 0.91$ (Sheets, 2014).

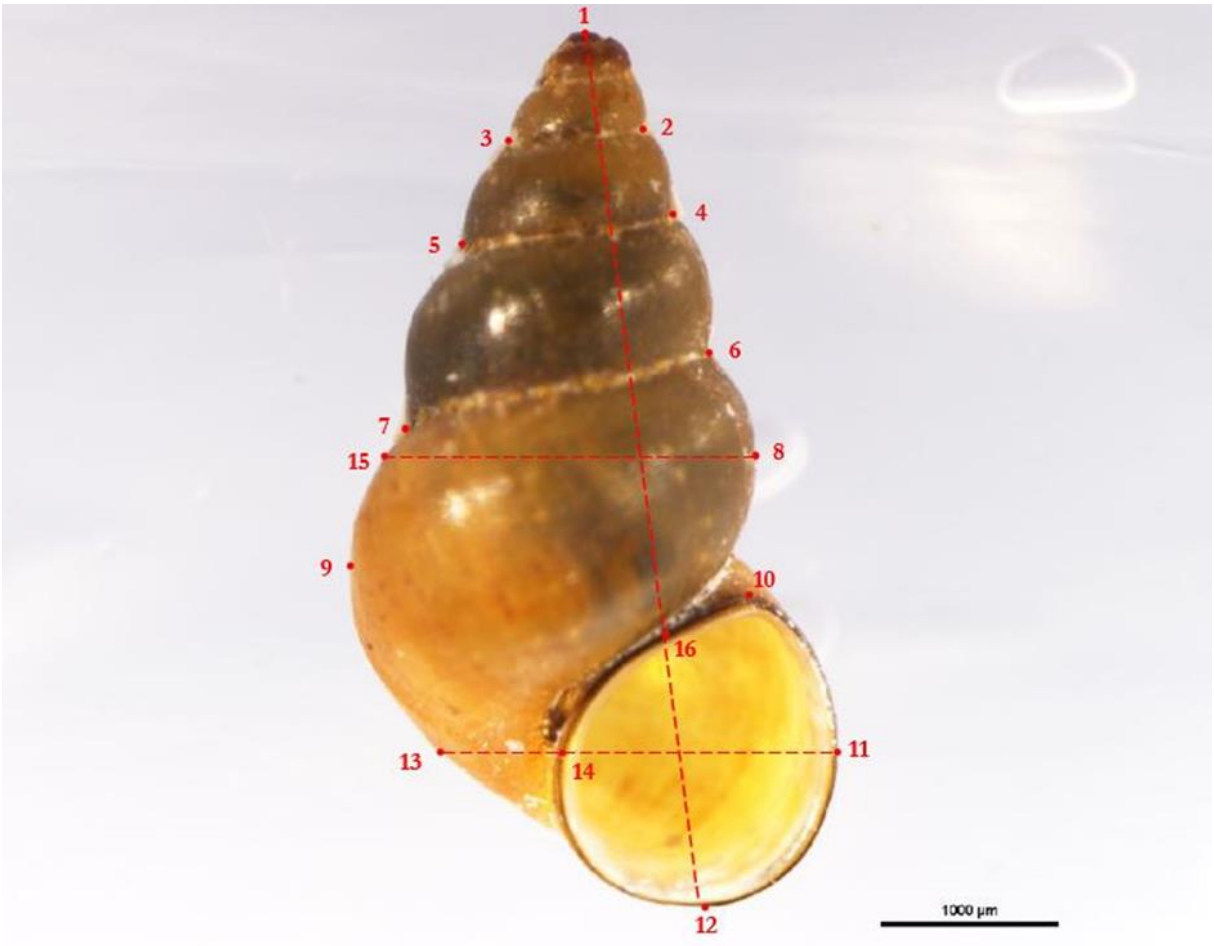

Figure 10. Location of the 16 landmarks. Dashed auxiliary lines represent help lines that display how landmarks 13-16 were created.

As mentioned above, we used the Integrated Morphometrics Package CoordGen 8 to execute the Procrustes superimposition, determine the centroid sizes and the measure of size of the geometric morphometrics framework. Additionally, we performed a principal component analysis (PCA) to control a posteriori for outliers after setting the landmarks (Figure 10). Based on the scatter plots of the first two principal components (PC; named relative warps in the context of geometric morphometrics), if we detected an outlier, we controlled the respective snail on the particular photo for possible damage or algae growth on the shell. Only snails that presented a damaged shell on the photo were excluded from further analysis. Additionally, we checked our data for allometry. Allometry describes the correlation of the change in the size of body parts with the whole body size (Huxley & Teissier, 1936; Gayon, 2000) and as Levinton (1988, page 305) defines it: "the relationship between changes in shape and overall size". Therefore, a non-linear connection of size and shape results from different rates of growth of distinct body parts (Huxley & Teissier, 1936; Nakagawa et al., 2017; Outomuro & Johansson, 2017). PC values were standardized accordingly with Regress 8 to compare the shape without the influence of the environment (shape corrected for allometry) with the uncorrected shape with the environmental influence (Sheets, 2014). All subsequent PCAs were conducted with both data sets, uncorrected and corrected, using PCAGen8. However, we did not detect large distinctions between the results of the non-standardised and the standardised PC values and therefore, described only the results with the non-standardised PC values. We executed our calculations on the first three PCs which revealed a total variance between 53.8% and 66.8% depending on the data set. Therefore, we used PCs 1-3 in our analyses to investigate shell shape (Table 2).

Using PCAGen8, we visualised the change and the meaning of the PC values in deformation grids. Deformation grids display the two extremes of the respective PC value within a dataset. This means that the two individuals that are located on the outer extremes of the PC variation are represented with a dot and an arrowhead.

Table 2. Variance of different non-standardised datasets described by the first 3 principal components (PCs) and of the sum of all three PCs. P = parental generation, F1 = first daughter generation, F2 = second daughter generation.

	PC 1	**PC 2**	**PC 3**	**Sum**
P + F1 + F2	0.298	0.247	0.123	0.668
P + F1	0.225	0.197	0.139	0.561
P	0.241	0.169	0.128	0.538
F1	0.237	0.177	0.136	0.55
F2	0.319	0.203	0.109	0.631

Life history

We analysed the reproductive rate to investigate the influence of environmental factors on the reproduction of the NZMS. The reproductive rate was defined as the days until the snails of the parental and the F1 generation produced ideally ~ten offspring. We included only mother snails that produced 1) seven or more offspring in the course of the experiment or 2) three to six offspring if they outlived not less than 18 months in the experiment. Mother snails that reached the above-mentioned parameters were fixed in ethanol. Mother snails that did not manage to produce the desired number of offspring or died before the end of the experiment without reaching the ten offspring, were excluded from the analysis. Using generalised linear models (GLMs), we tested the effect of temperature of the climate cabinets, water temperature difference between the habitat at collection time and climate cabinet, haplotype, population and size and shape parameter on the reproductive rate. The water temperature of the Spanish population at the time of collection was not measured and therefore we excluded the snails of the Spanish population from the analysis.

16S rRNA sequencing

We defined the mitochondrial lineages by sequencing a 16S rRNA fragment of ~500bp. Except for the populations from Northern Germany, we sequenced each three already photographed snails per population. We used the information about the Northern German populations from (Verhaegen, McElroy, et al., 2018). The low subsample of three individuals is sufficient because the two haplotypes t and z that occur in Europe are rarely found overlapping (Verhaegen, McElroy, et al., 2018; Butkus et al., 2020). DNA extraction was conducted by using the E.Z.N.A® Mollusc DNA Kit (Omega Bio-Tek Inc.). After crushing the complete snail, we extracted the DNA according to the instructions of the manufacturer. We executed the Polymerase chain reactions (PCR) in an overall volume of 10 µL which was composed of 1 µL of DNA solution (~20ng), 0.60 µL of 1% BSA, 5 µL of HS MyTaqTM RedMix (Bioline) and 0.20 µL of each primer (taken from a 10 pmol stock solution) 16Sar-L and 16Sbr-H (Palumbi, 1991).

The PCR temperature profile was a touch-down protocol. The conditions used in the PCR are visualised in Table 3. The PCR products were visualised on a 1% agarose gel and purified with an exonuclease I and shrimp alkaline phosphatase mix.

Table 3. The PCR steps with temperature and time. The PCR temperature profile was a touch-down-protocol.

PCR step	Temperature [°C]	Time [min:sec]	Cycles
Initial Denaturation	95	1:00	
Denaturation	95	0:20	
Annealing	60-51	0:20	10
Extension	72	0:30	
Denaturation	95	0:20	
Annealing	51	0:20	25
Extension	72	0:30	
Final Extension	72	5:00	

Statistical Analysis

We analysed all our data with R (R Core Team, 2021). To create plots, we used the package ggplot (Hadley, 2016). The statistical tests and resulting plots were run and created with the R packages lme4, lmtest, lmerTest and tidyverse (Zeileis & Hothorn, 2002; Bates et al., 2015; Kuznetsova et al., 2017; Wickham et al., 2019).

We compared shell sizes and shapes of the parental and the F1 snails between and within generations, temperatures, populations and haplotypes using non-parametric Kruskal-Wallis-tests. Non-parametric Kruskal-Wallis-tests were also used for the comparison of the shell morphology between and within the three generations (parental, F1 and F2), temperatures and the two Portuguese populations, Alcabideque and Póvoas village. Also, the reproductive rate was calculated using the non-parametric Kruskal-Wallis-test but only in the parental generation because the F1 generation produced too less offspring. After executing a Kruskal-Wallis-test, we always were running a Dunn's test which is doing pairwise comparisons between independent groups. The test was adapted for multiple testing by performing the "BH" technique by Benjamini & Hochberg (1995) to control for the false discovery rate. The Dunn´s tests were performed because the number of our mother snails was unbalanced and our data displayed heteroskedasticity.

During our analysis, we noted that the mother snails were not randomly allocated across the three temperatures. The mother snails that were assigned to the 19°C climate cabinet were significantly smaller in size than the mother snails from the 15° climate cabinet [Kruskal-Wallis chi-squared = 8.7404, df = 2, p 0 0.01265 (length); Kruskal-Wallis chi-squared = 7.1217, df = 2, p = 0.02842 (centroid size)]. Therefore, we decided to take the smallest six mother snails and their offspring in the F1 and the F2 generations out of our data set and further size analysis. The six mother snails and their offspring remained in the data set for shape analysis because we did not detect any significant difference in the shell shape of the mother snails across the three temperatures. After removing the six mother snails and their offspring from the data set, we did not observe any significant difference in the size parameter of the mother snails across temperature anymore.

We used the package "performance: Assessment of Regression Models Performance" to check for multicollinearity among the various explanatory variables (region, population, temperature and haplotype), for the two response variables (size and shape) and model types (generalised linear models and mixed effect models) (Lüdecke et al., 2021). We detected a high collinearity for the explanatory variables, region and population with all calculations (VIF > 10). When we compared the models calculating with region, respectively with population as fixed

factors using likelihood ratio tests (LRTs), the models including population displayed a significantly better outcome than the models that included region. Therefore, we ran only models with the variable population as fixed factor and eliminated the variable region from further modelling calculations. Additionally, we compared the collinearity of the two size parameters because the plots of length and centroid size displayed a high similarity. We decided to include both size parameters in our analysis because with varying shape along the observed geometrics, we describe size with both, a representative single distance measurement (length) and with a number based on a set of single size measures (centroid size).

We used generalized linear models (GLMs) to investigate the influence of the life history traits, size and shape of the parents´ generation. We decided to work with GLMs because they are robust against not normally distributed errors of the response variable. The fixed factors in the GLMs for analysing size and shape were haplotype, temperature and population. We added the fixed factors temperature difference, size and shape to the analysis of the life history traits. The temperature difference is calculated by subtracting the temperature in the respective climate cabinet from the water temperature in the habitat at the time of collection. We were able to include random factors with linear mixed effect models. This way we wanted to address the influence of the morphological traits of the F1 and the F2 generation and the reaction norm slopes of the F1 generation. To test the presence of a maternal effect in our data, we enclosed the mother snail ID as random factor. The comparison of the models was carried out using the Akaike information criterion (AIC). If the AIC values of different models varied by more than 2, we preferred the sparse model with a lower number in the degree of freedom. We changed the continuous factor temperature into a categorical factor to be able to better explain two and three-way connections and to consider possible linear curves (Mazé-Guilmo et al., 2016). For the selection of the best models, we worked with the effects package and its function "predictorEffects" to display the impact of the fixed variables (Fox, 2003). We ran the function "r.squaredGLMM" from the MuMIn package, version 1.43.17 to calculate the proportion of the total variance in both the random and the fixed factors (= conditional R^2) and of the fixed effects alone (= marginal R^2) (Nakagawa & Schielzeth, 2013; Johnson, 2014; Barton, 2021). To quantify the variance of the random factor in our models, the mother snail ID, we subtracted the marginal R^2 from the conditional R^2. The variance of the random factor indicates the possible effect of the mother snails and the circumstances in the respective jars. Using the mixed ANOVA with an interaction of temperature and population, we assembled reaction slopes to investigate the interaction of treatment and genotype. In the following step, we determined slopes using the function "lstrends" of the R

package "emmeans". Afterwards, a pairwise comparison of all slopes was executed, using the functions "pairs" to quantify significant distinctions and directional slope tendencies (Lenth, 2016).

Heritability

Heritability indicates how much of the variation in a trait can be described by genetic factors (Visscher et al., 2008). We calculated the broad-sense heritability (H^2) in the F1 and the F2 generation. The calculation of the broad-sense heritability includes the impact of dominance and epistasis (Visscher et al., 2008). The narrow-sense heritability (h^2) calculates the genetic variation explained with additive genetic values (V_A). As the NZMS reproduces asexually by rearing clones, we are not able to calculate the additive genetic variance. We determined the broad-sense heritability and therefore were not able to specify the additive genetic variance, but the "clonal repeatability" (Fischer et al., 2021). The R script to calculate heritability was provided by Fischer et al. (2021). We furthermore calculated the coefficient of genetic variation (CV_A) and the evolvability (I_A) for the size parameters. The CV_A represents a suitable constant to contrast studies investigating genetic variance and a parameter impartial of other reasons of variation because in comparison to heritability, the CV_A calculates the genetic variation standardised by the mean of the trait (Garcia-Gonzalez et al., 2012). The I_A, so the evolvability of a trait measures the predicted proportional answer to a unit strength of selection (Hansen et al., 2003; Hereford et al., 2004; Hansen et al., 2011). Unfortunately, we were not able to determine the CV_A and the I_A for the shape parameters PC 1-3. The PCs consist of small values close to or below zero. Calculating the CV_A and the I_A with the small PC values generated large CV_A and I_A values which were impossible to compare with the values of the size parameters.

We used linear mixed models to compute both the broad-sense heritability H^2 of the size and the shape parameter and the CV_A and I_A for only the size parameter for the F1 generation at the three different temperatures. To perform linear mixed models, we used the R package for linear models, lmer Test by applying its functions "lmer" and "VarCorr" (Kuznetsova et al., 2017). To compute broad-sense heritability, the coefficient of genetic variation CV_A and the I_A evolvability, we used the following formulas in accordance with Visscher et al. (2008; heritability) and Garcia-Gonzalez et al. (2012; CV_A and I_A; as eased by Houle (1992)):

$$H^2 = \frac{V_A}{V_A + V_{res}}$$

$$CV_A = \frac{\sqrt{V_A}}{\text{mean (trait)}}$$

$$I_A = \left(\frac{CV_A}{100}\right)^2$$

V_A = overall genetic variance;

V_{res} = variance within the clones.

Methylation patterns in molluscs

Data acquisition

We obtained genomic (predicted coding sequences (CDS) and proteins of structural annotations) and transcriptomic data which was publicly available at the year end of 2019 from different resources which can be seen in Supplemental Table S1 (Männer et al., 2021). During our investigation, we later included data that was accessible in 2020 to avoid losing essential taxa (Supplemental Table S1). We conducted our analysis mainly based on assembled and annotated (in the event of genomic data) sequences. As still some mollusc classes were not represented completely, we conducted de novo assemblies of RNAseq data and structural annotation of genome assemblies to present a taxonomically comprehensive dataset (Supplemental Table S6). In total, we obtained and assembled publicly accessible RNAseq data of 14 mollusc species (Supplemental Table S3). Using Trinity 2.11.0, we assembled the transcriptomes and additionally used BUSCO 4.1.4 with the dataset providedmetazoa_odb10 to come closer to the integrity of the transcriptome assemblies (Grabherr et al., 2011; Simão et al., 2015). Using GeMoMa 1.6.4, we furthermore executed structural annotations of publicly accessible genome assemblies from eight mollusc species (Keilwagen et al., 2016, 2018) (Supplemental Tables S5-S7). We assessed the quality of the definitive annotations by testing the annotation consistency with a custom script and BUSCO 4.1.4 (Supplemental Tables S2 and S8). We overall examined 31 genome annotations and 124 transcriptomes of 140 species in total representing data from all existing mollusc classes. The presence of DNMTs was analysed in 126 species, the CpG o/e ratio was calculated in 136 species. Supplemental Tables S1 and S2 contain the taxonomic information of the entire analysed species and information on data type and quality.

On Figure 11, the cladogram displays the current understanding of the phylogeny of molluscs to exhibit the phylogenetic distribution of the investigated taxa. The cladogram is based on the latest phylogenetic investigations of molluscs by Kocot et al. (2020). Only taxa that we analysed in this study, are part of the cladogram.

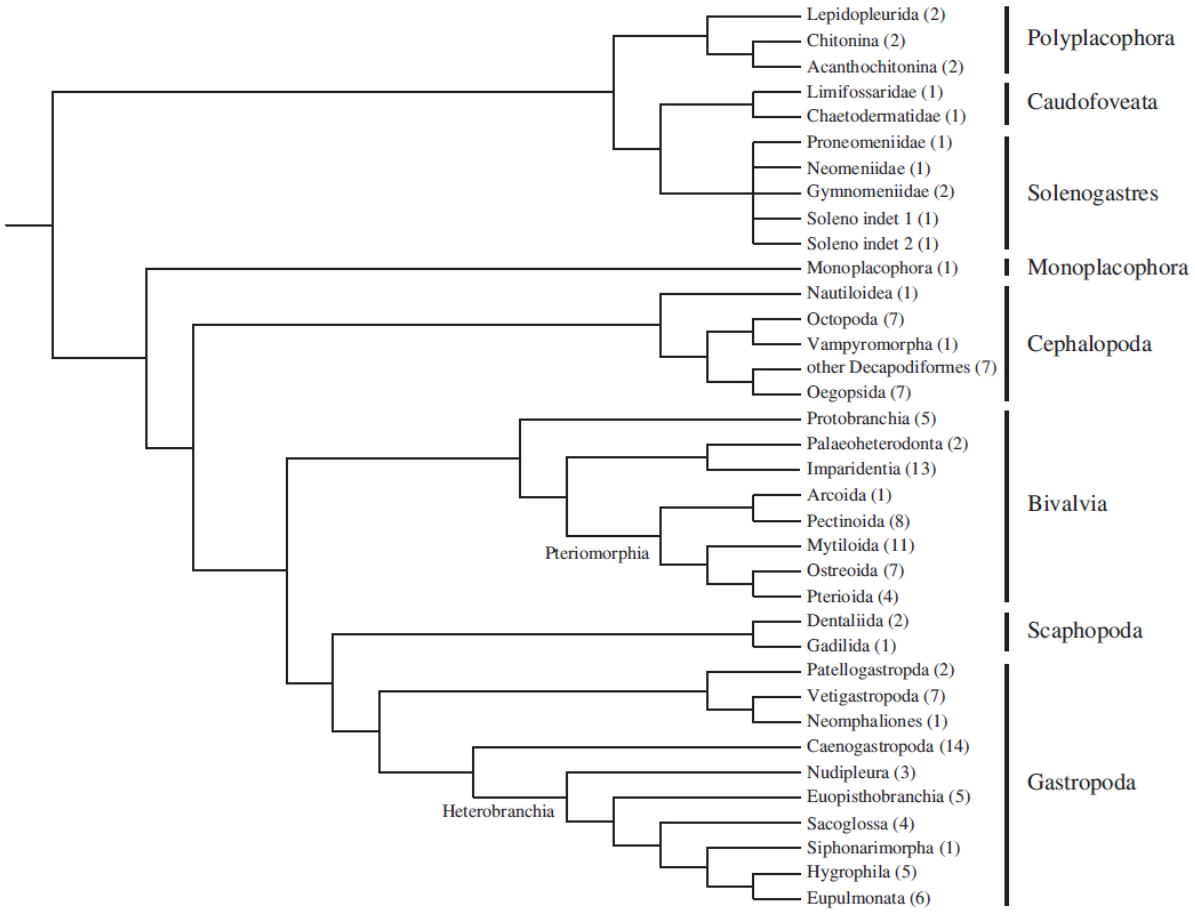

Figure 11. Cladogram demonstrating the phylogenetic distribution of the examined genomic data. Species investigated per taxonomic group are displayed by the numbers in brackets.

Identification of DNA methyltransferases

In this study, we focussed on determining genes coding for DNMT1 and DNMT3. We also searched for DNMT2 but we will not report in detail on the results of DNMT2. As DNMT2, also called tRNA aspartic acid methyltransferase 1, is not involved on the DNA level (Goll & Bestor, 2005; Goll et al., 2006; Jeltsch & Jurkowska, 2014; Lyko, 2018), we only show the results of the DNMT2 search. As explained by Provataris et al. (2018), we created Profile Hidden Markov Models (pHMMs). They were established from amino acid sequences of DNMT1 and DNMT3 from metazoa that we received from OrthoDB v. 8 (Kriventseva et al., 2015; Zdobnov et al., 2017), as searching for molluscan DNMTs did not create much output to build an appropriate search pattern. Using MAFFT LINS-i v. 7 (Katoh & Standley, 2013), we build alignments of each cluster of orthologous sequences and then, using hmmbuild (package of HMMER 3.1b2; www.hmmer.org), produced pHMMs from each alignment. Using fastatranslate of Exonerate 2.4.0 (Slater & Birney, 2005), we created the six reading frames from each transcriptomic sequence. The

predicted protein data, either genome or translated transcriptome, was looked up while using the produced pHMMs of DNMT1 and DNMT3. Using hmmscan (part of HMMER), we accordingly compared every candidate sequence of DNMT1 and DNMT3 that we sustained previously, with the Pfam-A database version 32.0. to distinguish their protein domains. We only continued to work with sequences which encompass a DNA methylase domain (Pfam accession number: PF00145) and/or DNMT1/DNMT3 specific domains, such as DNMT1-RFD (Pfam accession number: PF12047) or ADD_DNMT3 (Pfam accession number: PF17980). As DNMT1 and DNMT3 contain similar candidate sequences, we checked the remaining candidate sequences against the official gene data of *C. gigas* (Zhang et al., 2012). Candidate sequences whose best hit was not a DNMT of *C. gigas*, were eliminated from further analysis. In the end, we used blastp 2.6.0+ (Camacho et al., 2009) to compare the persisting candidate sequences against the non-redundant NCBI database. Only sequences which had their best match pertaining to metazoan species, were maintained.

Additionally, we wanted to check for a connection between the quality of the data and the identification of DNMTs. We therefore build generalized linear models with binary response variable (presence/absence) and logit link function in PAST 4.01 (Hammer et al., 2001). Quality was represented with independent variables such as BUSCO C (complete), 100 – BUSCO M (missing) = BUSCO present, number of contigs and total sequence length (Supplemental Table S2 and S4).

Calculation of normalised CpG dinucleotide content

CG dinucleotides show a lower occurrence in genes that were methylated in the past. In contrast, genes that did not show any presence of foretime methylation, possess a relatively higher amount of CG dinucleotides as outlined above (Bird, 1980). This leads to the conclusion that if a species owns both genes with methylation and genes without methylation, it will also show parts of the genome that are affected by CpG depletion and parts that are not. Compared to species that show a low or no amount of methylation. Those species only possess genes that are unchanged by CpG depletion. We estimated the CpG depletion of genes by quantifying the normalised CpG dinucleotide content (CpG o/e) using the following formula:

$$CpG\,\frac{o}{e} = \frac{P_{CpG}}{P_C * P_G}$$

P_{CpG} represents the amount of 5'-CpG-3 dinucleotides, P_C the amount of C nucleotides and P_G represent the amount of G nucleotides. To test if other parameters than cytosine DNA methylation show any influence, such as the GC content, we computed the normalised GpC dinucleotide content (Fryxell & Moon, 2005). We did not include sequences into the analysis of the normalised dinucleotide content that possessed less than 200 nucleotides or more than 5% of equivocal nucleotides.

Inferring the presence of DNA methylation based on CpG observed/expected distributions

We calculated the CpG o/e values of protein-coding sequences of molluscan species to infer the existence of DNA methylation. We checked the method of the CpG o/e distributions with the Gaussian mixture modelling software package Mclust version 5.4.6 with R 4.0.3 (Park et al., 2011; Scrucca et al., 2016; Song et al., 2017). Additionally, we fitted two Gaussian distributions to the CpG o/e and the GpC o/e distributions of the particular species. Provataris et al. (2018) built the basis of our argumentation on the presence of germline DNA methylation in the species´ protein-coding sequences:

1. A CpG o/e distribution is defined as bimodal if the total distinction of the means of the two Gaussian distributions adds up to at least 0.25 and if either of the means is less than 0.7. Furthermore, the ratio of data to the smallest of the fitted components should exceed 0.1. As the DNA methylation does not have an influence on the GpC dinucleotides, the guidelines of bimodality should not relate to the GpC o/e distribution. "Bimodal depleted" CpG o/e distributions that follow those guidelines can be seen in Figure 12a.

2. If the guidelines of bimodality are not completely met, the term "bimodality" is not used. Analyses in insects showed that some species displayed cytosine methylation confirmed by experimentation but did not meet the criteria for bimodal distribution. A large proportion of the data pertains to the smaller of the two fitted distributions (Glastad et al., 2011; Sarda et al., 2012; Cunningham et al., 2015). Based on the data of Zemach et al. (2010), we reduced the threshold value for the ratio of the smaller of the fitted normal distributions to ≥0.36. As with 1., those criteria should not apply for the respective GpC o/e distribution. Species that meet the above-mentioned guidelines show an "unimodal" CpG o/e distribution and are therefore "indicative of DNA methylation". An example of a mollusc species with unimodal CpG o/e distribution can be seen in Figure 12b.

3. If none of the guidelines under 1. and 2. was holding true, the confirmation for the presence of DNA methylation was too incomplete and therefore, inadequate. Mollusc species that did not meet the criteria, showed an "unimodal" CpG o/e distribution and were not "indicative of DNA methylation. Figure 12c displays the "unimodal" CpG o/e distribution of *Proneomenia custodiens*.

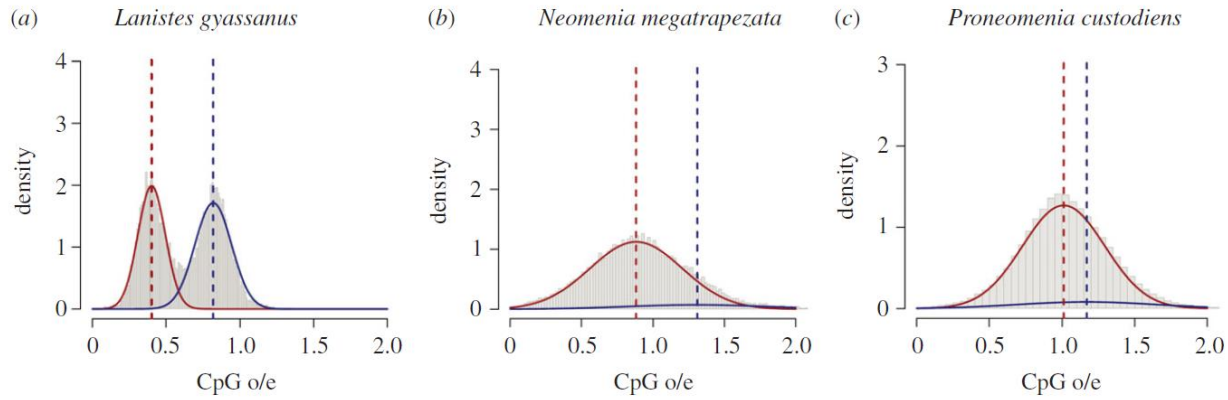

Figure 12. Three molluscan species as examples of three different CpG o/e distribution patterns in protein-coding sequences. The two Gaussian distributions fitted to the data are displayed by the red and the blue curve. The mean values of the fitted distributions are depicted with the equivalent red and blue dashed lines. (a) The characteristic bimodal CpG o/e distribution is represented by *Lanistes nyassanus*. One can clearly see the two different parts: one with low CpG o/e values (genes mainly shaped by CpG depletion) and one with high CpG o/e values (genes less influenced by CpG depletion). (b) *Neomenia megatrapezata* shows an "unimodal, indicative of DNA methylation" CpG o/e distribution. An explicit bimodality cannot be seen. Still, a compelling number of data containing components with low CpG o/e values form the typically big tail. (c) "Unimodal, not indicative of methylation" CpG o/e distribution is represented by *P. custodiens*. The means of the two fitted distributions look nearly alike and the ratio of the data which is part of the smaller portion is low.

IV. RESULTS

Differentiation of genetic adaptation and phenotypic plasticity in the morphologically variable shell of the NZMS

16S rRNA sequencing

Although 17 16s rRNA haplotypes were detected in New Zealand, Städler et al. (2005) only detected two haplotypes in Europe. Snail from our Portuguese populations possessed haplotype z. The Spanish and the German populations, however, owned haplotype t.

Morphological comparison of parental and F1 generations

In contrast to the F1 generation, the snails of the parental generation grew up under natural conditions. Since the shell of the NZMS does not change anymore once becoming adults, the temperature and the conditions of the common garden experiment did not have any effect on the shell morphology of the parental generation. However, the environmental of the habitat in which the snails of the parental generation grew up, might have influenced shell shape and size. The length of the snails of the parental generation did not vary across haplotypes, but across populations (Figure 13). The generalized mixed model that showed the lowest AIC value was the model which had temperature and population as fixed effects. In the parental snails, temperature and population (Table 4) explained the significant difference in shell length across the three climate cabinets. We could observe a similar outcome with the second parameter of size, centroid size (Figure 14; Table 5).

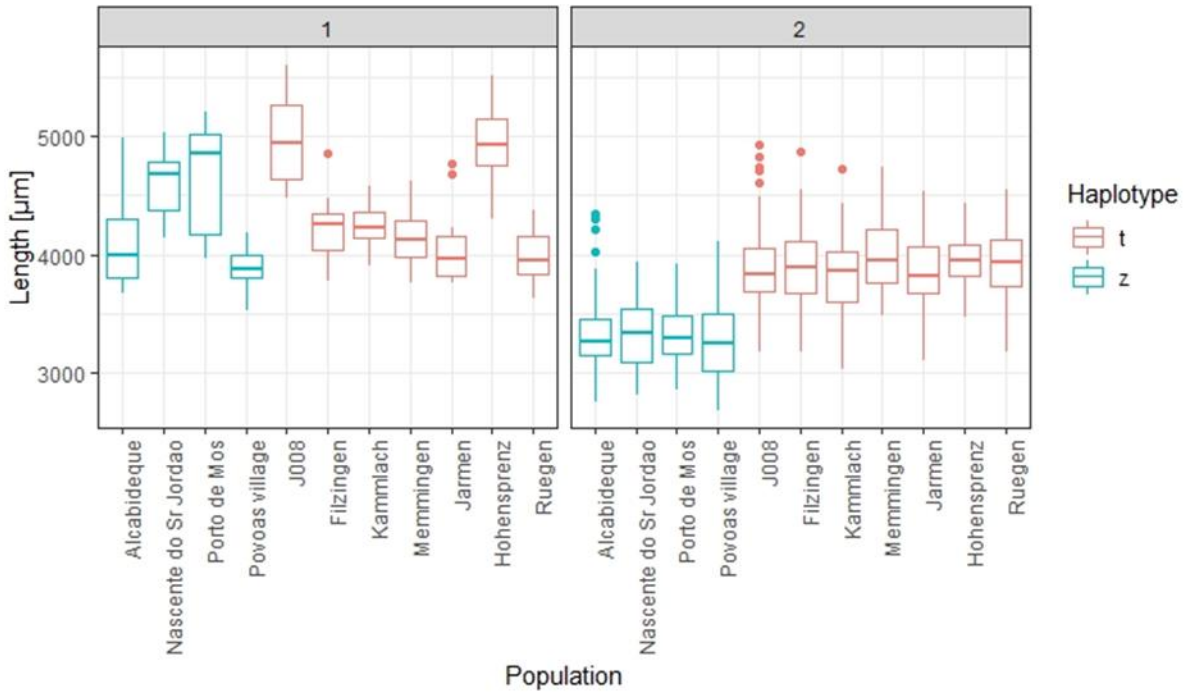

Figure 13. Length across populations of the parental (1) and the first daughter (2) generations.

Table 4. Generalized linear model of length including the fixed factors temperature and population in the parental generation. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	**Standard error**	**t-value**	**p-value**
Intercept	4193.47	79.12	53.001	< 2e-16 ***
Temperature 19°C	-174.21	60.79	-2.866	0.00477 **
Temperature 23°C	-130.46	57.41	-2.272	0.02451 *
Filzingen	127.10	106.34	1.195	0.23395
Hohensprenz	819.70	108.82	7.533	4.66e-12 ***
J008	889.59	104.47	8.515	1.79e-14 ***
Jarmen	-50.44	106.52	-0.474	0.63652
Kammlach	143.48	113.76	1.261	0.20921
Memmingen	58.39	104.47	0.559	0.57706
Nascente do Sr. Jordão	486.66	106.52	4.569	1.03e-05 ***
Porto de Mós	535.55	108.51	4.936	2.14e-06 ***
Póvoas village	-238.53	113.74	-2.097	0.03770 *
Rügen	-102.19	110.68	-0.923	0.35736

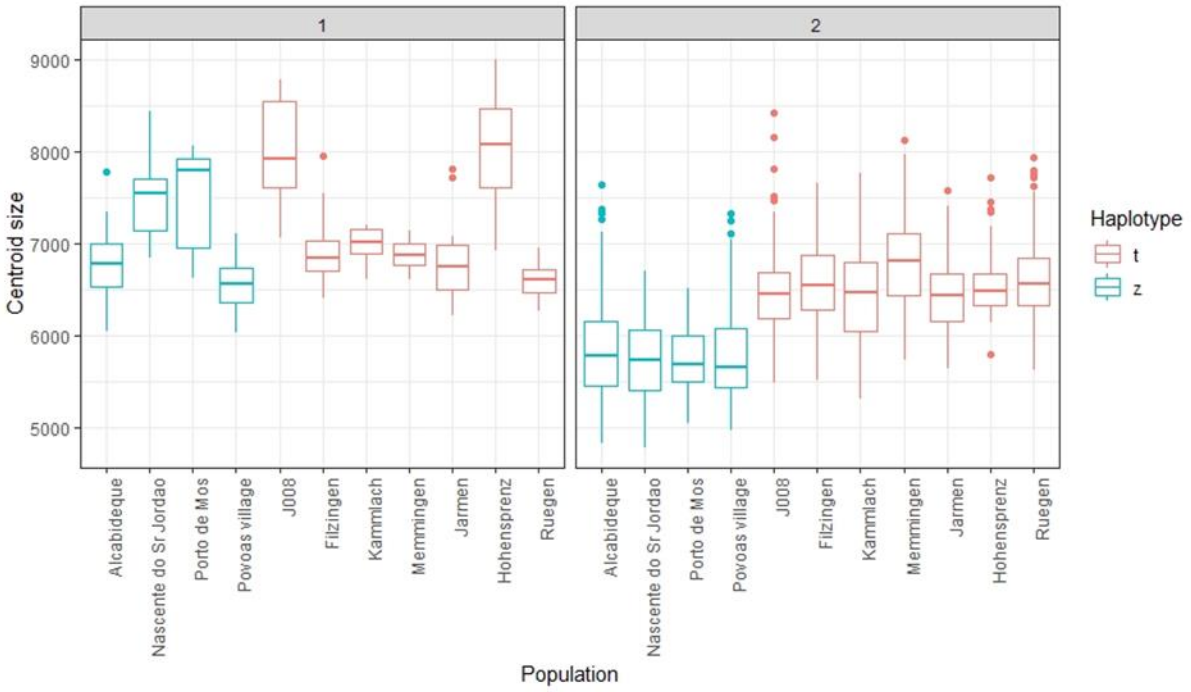

Figure 14. Centroid size across populations of the parental (1) and the first daughter (2) generations.

Table 5. Generalized linear model of centroid size including the fixed factors temperature and population in the parental generation. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	**Standard error**	**t-value**	**p-value**
Intercept	6935.206	106.019	65.415	< 2e-16 ***
19°C	-191.902	81.462	-2.356	0.01981 *
23°C	-230.054	76.925	-2.991	0.00327 **
Filzingen	130.950	142.496	0.919	0.35962
Hohensprenz	1207.240	145.812	8.279	6.96e-14 ***
J008	1199.862	139.984	8.571	1.29e-14 ***
Jarmen	-2.007	142.734	-0.014	0.98880
Kammlach	179.281	152.434	1.176	0.24145
Memmingen	92.245	139.985	0.659	0.51095
Nascente do Sr. Jordão	673.497	142.734	4.719	5.48e-06 ***
Porto de Mós	647.051	145.398	4.450	1.68e-05 ***
Póvoas village	-269.398	152.409	-1.768	0.07920 .
Rügen	-193.266	148.301	-1.303	0.19454

With regard to shape, populations and haplotypes showed a significant difference across PC1 in the parental generation [Kruskal-Wallis chi-squared = 71.73, df = 10, $p < 0.01$ (population); Kruskal-Wallis chi-squared = 51.19, df = 1, $p < 0.01$ (haplotype)] (Figure 15). Only populations, but not haplotypes differed significantly along PC 2 (Kruskal-Wallis chi-squared = 43.92, df = 10, $p < 0.01$) (Figure 16). As with PC 1, populations and haplotypes altered significantly along PC 3 [Kruskal-Wallis chi-squared = 67.946, df = 10, $p < 0.01$ (population); Kruskal-Wallis chi-squared = 49.846, df = 1, $p < 0.01$ (haplotype)] (Figure 17). The GLMs with the best AIC value were the models with population as fixed effect (Tables 6-8). The same AIC value was shown by the GLMs with the fixed factors population and haplotype. As the factors population and haplotype apparently intersected and the GLMs with only population as fixed factor had less degrees of freedom, we decided to display the GLMs with population as fixed factor.

The sample size n of the first offspring generation was 1158 for the shape analyses and 1111 for the size analyses. As in the parental generation, both length and centroid size showed a significant difference [Kruskal Wallis chi-squared = 227.84, df = 1, $p < 0.01$ (length); Kruskal Wallis chi-squared = 217.41, df = 1, $p < 0.01$ (centroid size)]. Furthermore, all the three PCs for shape of the F1 generation were significantly different from the parental generation [Kruskal Wallis chi-squared = 59.47, df = 1, $p < 0.01$ (PC 1); Kruskal Wallis chi-squared = 188.49, df = 1, $p < 0.01$ (PC 2); Kruskal Wallis chi-squared = 38.25, df = 1, $p < 0.01$ (PC 3)]. We displayed the changes of shape along the first three PCs across parental and first offspring generation F1 with deformation grids in Figure 18. Compared to the parental generation, the F1 generation became more alike in both size (Figures 13 and 14) and shape (Figures 15-17). However, this outcome was more noticeable in size than in shape.

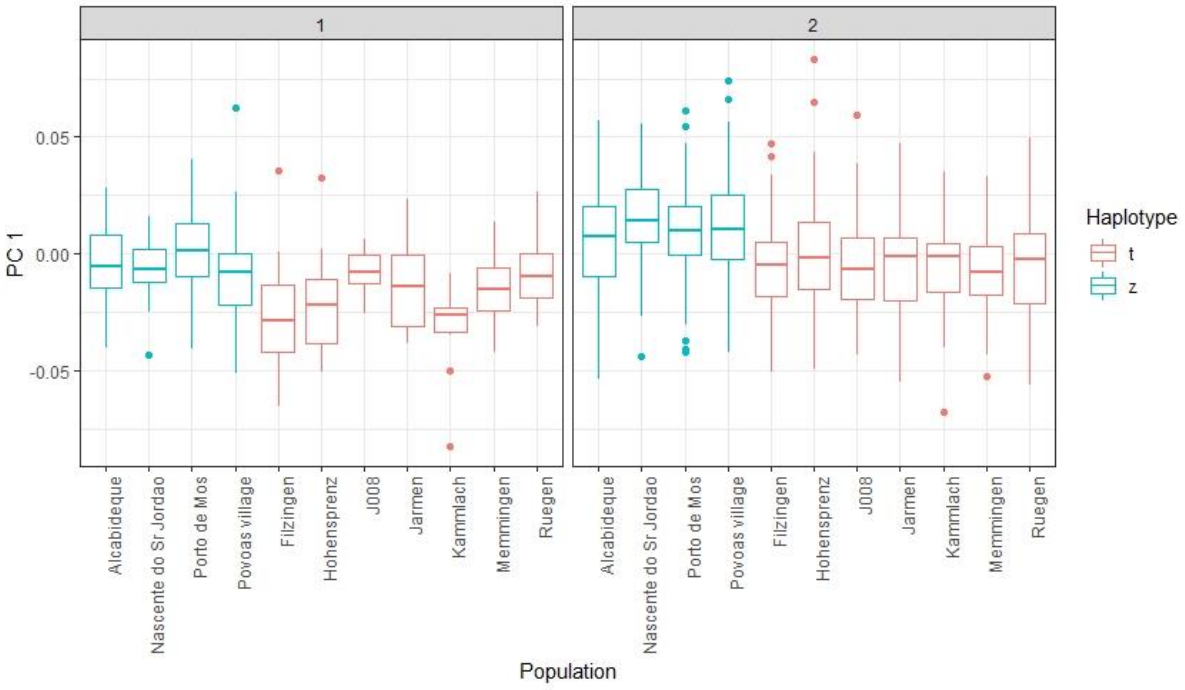

Figure 15. PC 1 for shape across populations of the parental (1) and the first daughter (2) generations.

Table 6. Generalized linear model of PC 1 for shape including the fixed factor population in the parental generation. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	**Standard error**	**t-value**	**p-value**
Intercept	0.0140459	0.0043153	3.255	0.001393 **
Filzingen	-0.0373179	0.0062906	-5.932	1.88e-08 ***
Hohensprenz	-0.0223248	0.0065241	-3.422	0.000795 ***
J008	-0.0160821	0.0062906	-2.557	0.011532 *
Jarmen	-0.0128257	0.0064006	-2.004	0.046831 *
Kammlach	-0.0375478	0.0068231	-5.503	1.51e-07 ***
Memmingen	-0.0212181	0.0062906	-3.373	0.000939 ***
Nascente do Sr. Jordão	0.0004233	0.0064006	0.066	0.947355
Porto de Mós	0.0138798	0.0064006	2.169	0.031644 *
Póvoas village	-0.0060304	0.0062906	-0.959	0.339231
Rügen	-0.0233737	0.0066637	-3.508	0.000592 ***

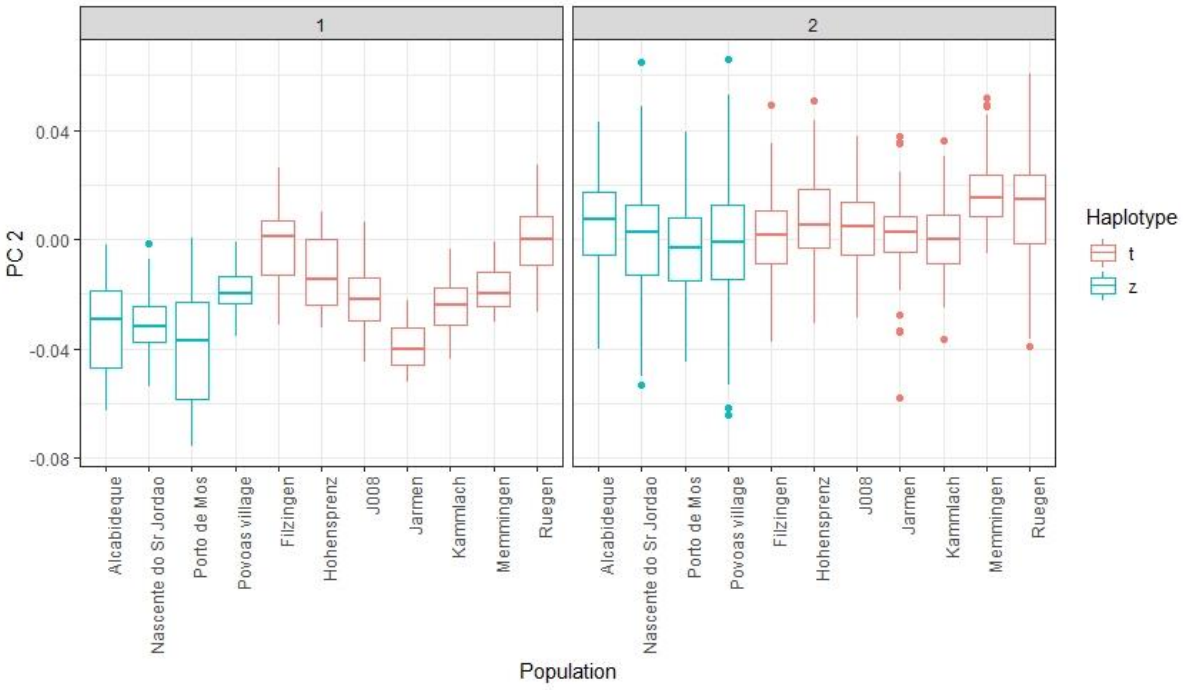

Figure 16. PC 2 for shape across populations of the parental (1) and the first daughter (2) generations.

Table 7. Generalized linear model of PC 2 for shape including the fixed factor population in the parental generation. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	**Standard error**	**t-value**	**p-value**
Intercept	-0.0058858	0.0040231	-1.463	0.145492
Filzingen	0.0202313	0.0058646	3.450	0.000723 ***
Hohensprenz	0.0139366	0.0060823	2.291	0.023292 *
J008	0.0034443	0.0058646	0.587	0.557858
Jarmen	-0.0117383	0.0059672	-1.967	0.050952 .
Kammlach	-0.0057496	0.0063610	-0.904	0.367461
Memmingen	0.0102367	0.0058646	1.746	0.082878 .
Nascente do Sr. Jordão	-0.0003347	0.0059672	-0.056	0.955348
Porto de Mós	-0.0010598	0.0059672	-0.178	0.859264
Póvoas village	0.0116155	0.0058646	1.981	0.049405 *
Rügen	0.0245915	0.0062125	3.958	0.000115 ***

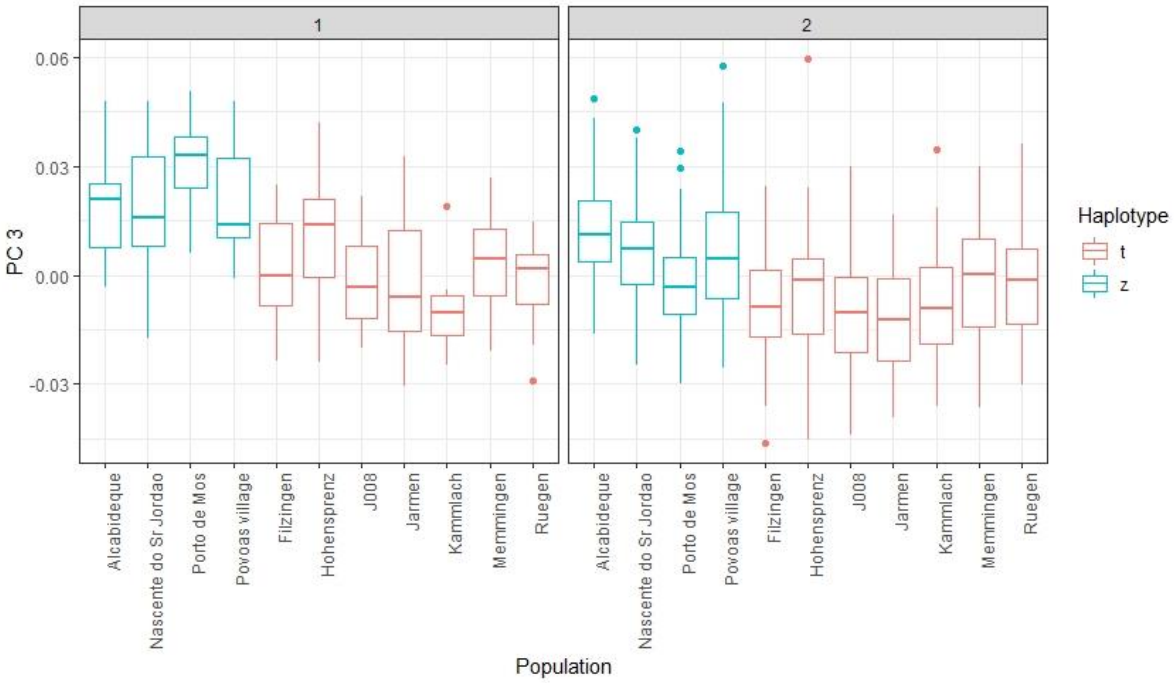

Figure 17. PC 3 for shape across populations of the parental (1) and the first daughter (2) generations.

Table 8. Generalized linear model of PC 3 for shape including the fixed factor population in the parental generation. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	**Standard error**	**t-value**	**p-value**
Intercept	0.008601	0.003218	2.672	0.008335 **
Filzingen	-0.015930	0.004692	-3.395	0.779960
Hohensprenz	-0.001362	0.004866	-0.280	0.779960
J008	-0.020800	0.004692	-4.433	1.75e-05 ***
Jarmen	-0.015057	0.004774	-3.154	0.001934 **
Kammlach	-0.013851	0.005089	-2.722	0.007237 **
Memmingen	-0.015991	0.004692	-3.408	0.000833 ***
Nascente do Sr. Jordão	0.002309	0.004774	0.484	0.629292
Porto de Mós	0.007071	0.004774	1.481	0.140583
Póvoas village	0.002207	0.004692	0.470	0.638669
Rügen	-0.026137	0.004970	-5.259	4.74e-07 ***

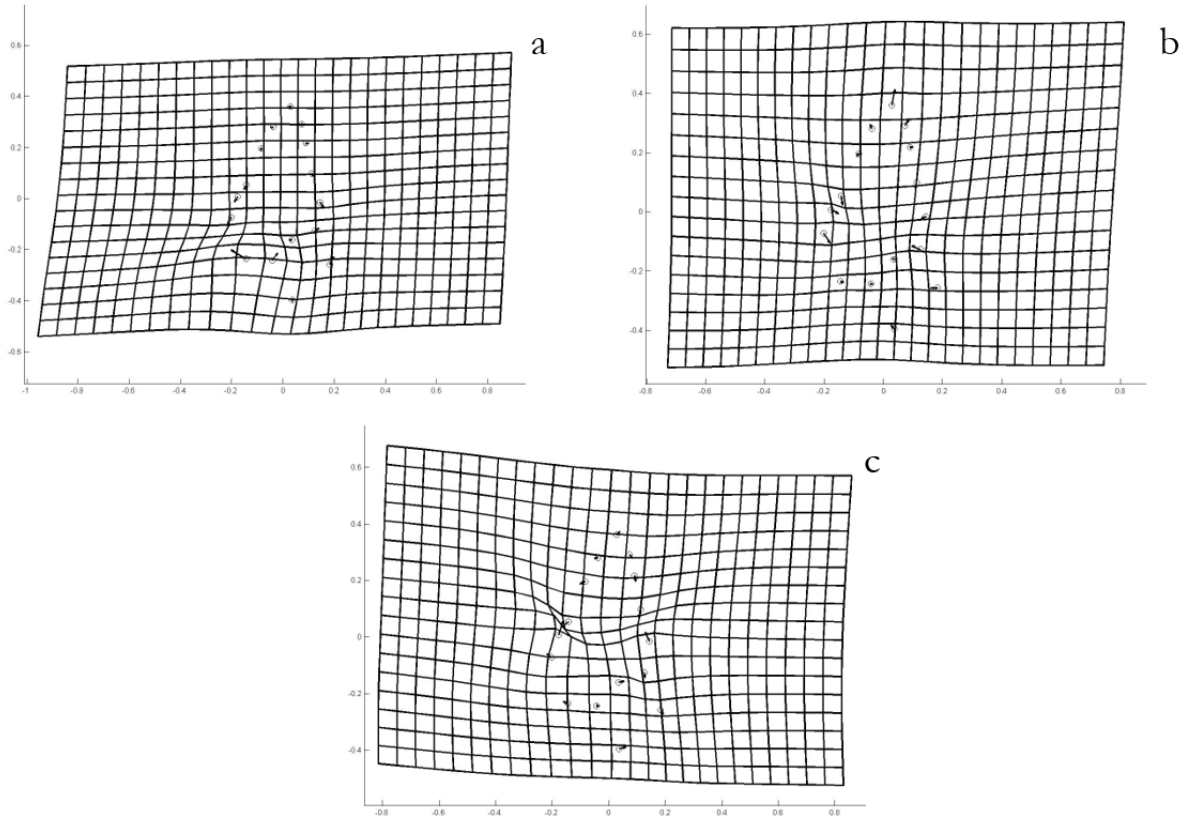

Figure 18. Deformation grids for PC 1 (a), PC 2 (b) and PC 3 (c) of the parental and the F1 generation. Grids show deformation from specimens with lowest (circle) to those with highest values (arrowhead). The values of PC 1 and PC 2 are generally higher in the F1 generation and thus, the shell shape of the F1 generation snails is represented by the arrowheads, the shape of the parental generation by the circles. The values of PC 3 are predominantly higher in the parental generation. Therefore, the shells of the parental generation have the shape as displayed by the arrowheads, the shells of the F1 generation as displayed by the circles.

Morphological traits in the offspring generation F1

The fixed factors of the most suitable linear mixed models of length for the F1 generation were temperature, haplotype and the interaction of temperature and haplotype (Table 9). The random factor, the ID of the mother snail, was included in all the models. For length, a significant impact could be observed with the interaction of temperature and haplotype. The outcome of centroid size was similar to the ones of length (Figures 19 and 20). The only difference is that only temperature already showed a significant impact on centroid size (Table 10). We calculated the marginal R^2 and the conditional R^2 of the linear mixed models for both size parameters and the proportions of variance by the random factor, the mother snail (Table 14).

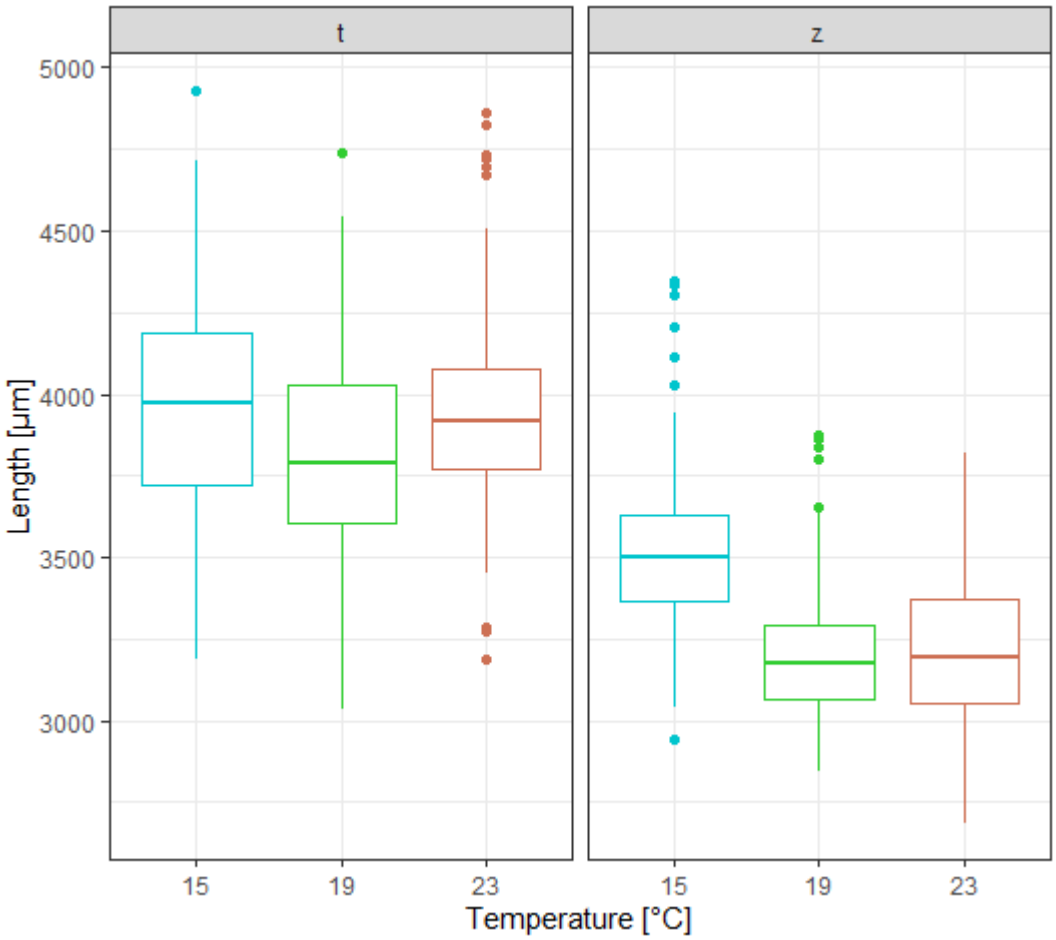

Figure 19. Length across temperature and haplotype of the F1 generation.

Table 9. Linear mixed model of shell length in the F1 generation including the fixed factors temperature, haplotype and the interaction of temperature and haplotype, besides the random factor the mother snail´s ID. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	**Standard error**	**df**	**t-value**	**p-value**
Intercept	3905.90	132.16	155.30	29.55	< 2e-16 ***
Temperature	0.34	6.68	152.99	0.05	0.96
Haplotype z	127.26	199.36	152.36	0.64	0.52
Temperature x Haplotype z	-38.18	10.24	151.06	-3.73	0.00027 ***

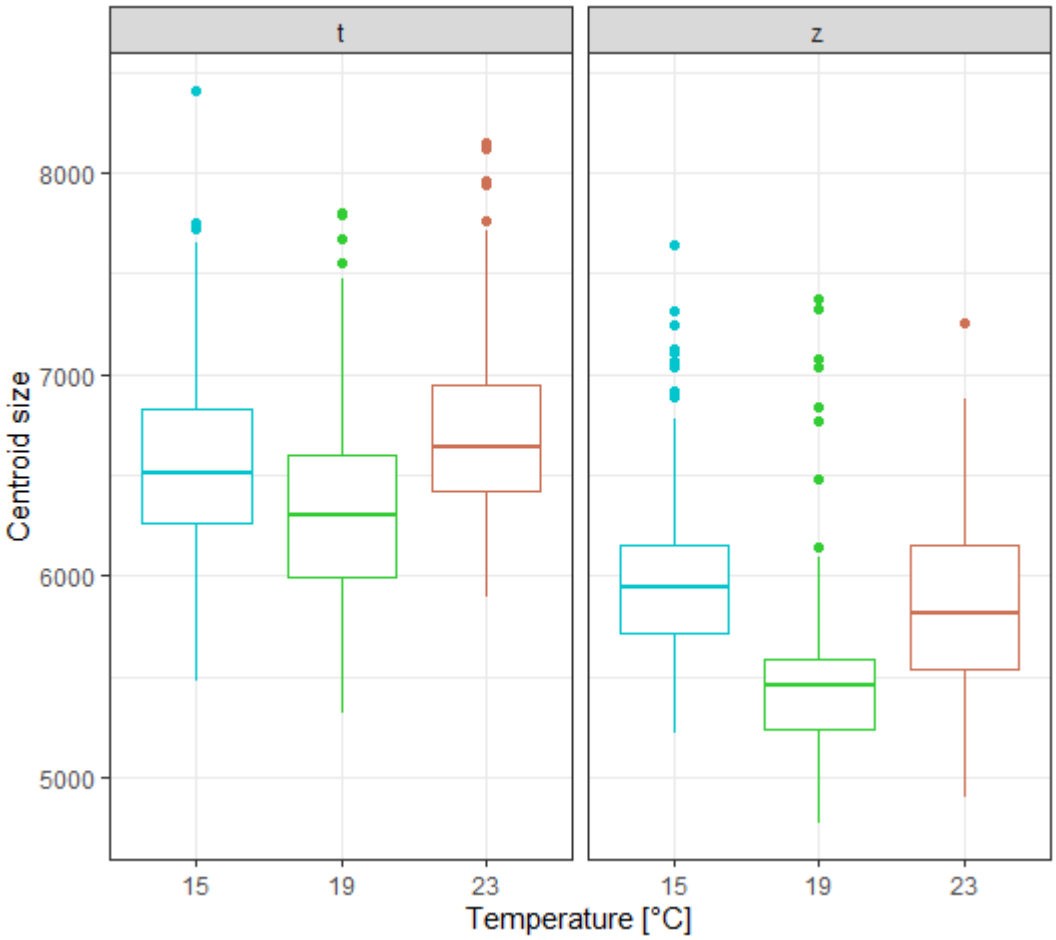

Figure 20. Centroid size across temperature and haplotype of the F1 generation.

Table 10. Linear mixed model of centroid size in the F1 generation including the fixed factors temperature and haplotype and the random factor of the mother snail´s ID. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	**Standard error**	**df**	**t-value**	**p-value**
Intercept	6101.19	209.75	160.61	29.089	< 2e-16 ***
Temperature	22.73	10.60	157.73	2.145	0.0335 *
Haplotype z	-18.55	316.00	156.59	-0.059	0.9533
Temperature x Haplotype z	-39.09	16.22	154.93	-2.410	0.0171 *

The best fitting linear mixed model of PC 1 for shape in the F1 generation was the one with haplotype as fixed factor which was showing a significant effect (Figure 21, Table 11). The best linear mixed model for PC 2 was the model with the fixed factors population and the interaction of temperature and haplotype. We observed significant effects on PC 2 with the fixed factors temperature, haplotype, the interaction of temperature and haplotype and every population, excluding Hohensprenz and Póvoas village (Figure 22, Table 12). For PC 3, the most appropriate model ran with the fixed factors temperature and haplotype which indicated a significant impact (Figure 23, Table 13). The marginal R^2 and the conditional R^2 as well as the proportion of variance by the random factor, the mother snail´s ID, of the shape parameter PC 1-3 can be seen in Table 14.

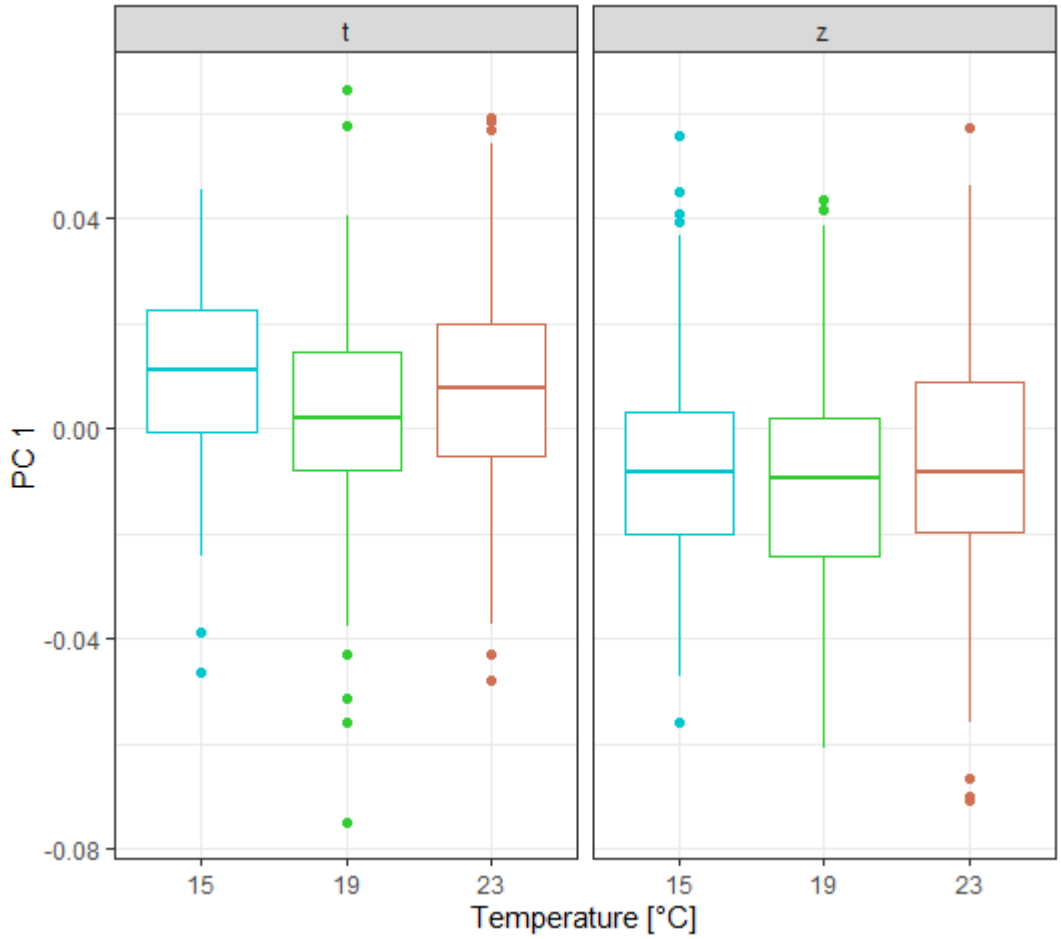

Figure 21. PC 1 for shape across temperature and haplotype of the F1 generation.

Table 11. Linear mixed model of PC 1 for shape in the F1 generation including the fixed factor haplotype and the random factor of the mother snail´s ID. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	Standard error	df	t-value	p-value
Intercept	0.0064	0.0013	162.93	5.12	8.62e-07 ***
Haplotype z	-0.0150	0.0019	155.26	-7.90	4.77e-13 ***

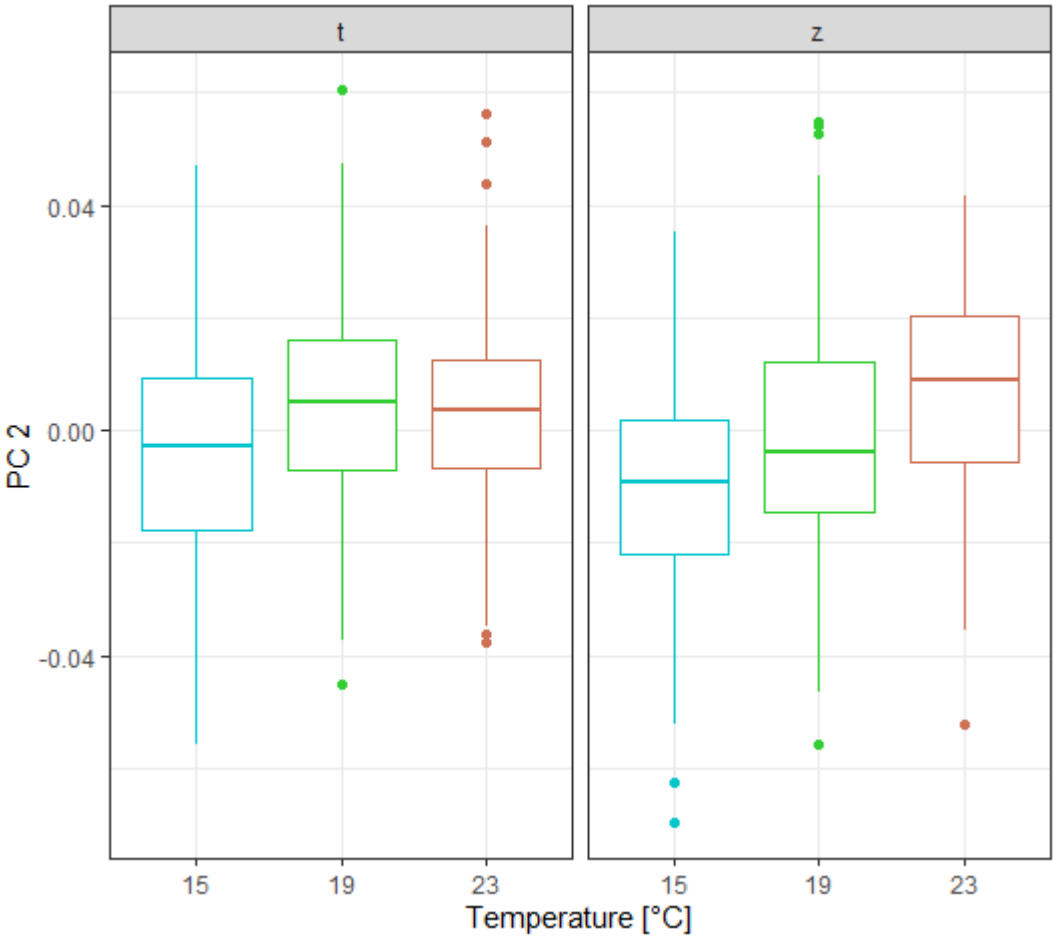

Figure 22. PC 2 for shape across temperature and haplotype of the F1 generation.

Table 12. Linear mixed model of PC 2 for shape in the F1 generation including the fixed factors temperature, haplotype, population and the interaction of temperature and haplotype as well as the random factor of the mother snail´s ID. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	**Standard error**	**df**	**t-value**	**p-value**
Intercept	-2.636e-02	8.314e-03	1.539e+02	-3.17	0.001836 **
Temperature	1.022e-03	3.838e-04	1.521e+02	2.66	0.008577 **
Haplotype z	-3.048e-02	1.232e-02	1.501e+02	-2.48	0.014452 *
Filzingen	1.106e-02	4.592e-03	1.497e+02	2.41	0.017281 *
Hohensprenz	2.821e-03	4.663e-03	1.503e+02	0.61	0.546153
J008	1.100e-02	4.447e-03	1.442e+02	2.48	0.014497 *
Jarmen	1.518e-02	5.031e-03	1.591e+02	3.02	0.002971 **
Kammlach	1.170e-02	4.649e-03	1.476e+02	2.52	0.012906 *
Memmingen	-3.590e-03	4.962e-03	1.516e+02	-0.72	0.470479
Nascente do Sr. Jordão	9.441e-03	4.182e-03	1.479e+02	2.26	0.025428 *
Porto de Mós	1.365e-02	3.915e-03	1.415e+02	3.49	0.000654 ***
Póvoas village	7.617e-03	3.922e-03	1.424e+02	1.94	0.054074 .
Temperature x Haplotype z	1.447e-03	5.881e-04	1.493e+02	2.46	0.015024 *

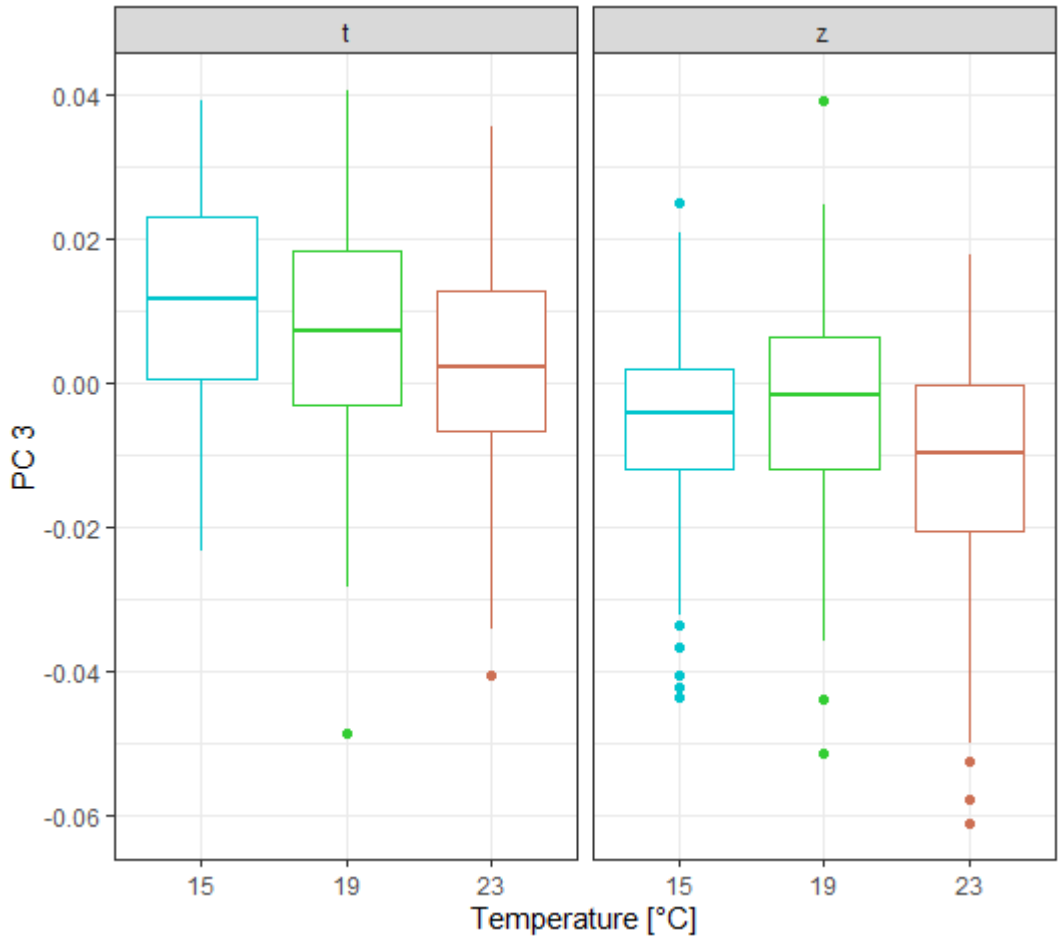

Figure 23. PC 3 for shape across temperature and haplotype of the F1 generation.

Table 13. Linear mixed model of PC 3 for shape of the F1 generation including the fixed factors temperature and haplotype and the random factor of the mother snail´s ID. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	**Standard error**	**df**	**t-value**	**p-value**
Intercept	2.736e-02	4.420e-03	1.586e+02	6.191	4.92e-09 ***
Temperature	-1.060e-03	2.206e-04	1.544e+02	-4.804	3.66e-06 ***
Haplotype z	-1.423e-02	1.440e-03	1.522e+02	-9.878	< 2e-16 ***

Table 14. Marginal R^2, conditional R^2 and the proportions of variance by the random factor (mother snail´s ID) of length, centroid size and PC 1-3 of the best fitted linear mixed models of the F1 generation.

	Marginal R^2	**Conditional R^2**	**Proportions of variance by the random factor [%]**
Length	0.55	0.76	21
Centroid size	0.42	0.65	23
PC 1	0.12	0.34	22
PC 2	0.18	0.49	31
PC 3	0.21	0.42	21

Morphological traits in the offspring generation F2

We could only examine Alcabideque and Póvoas village, two Portuguese populations with haplotype z, across all three generations, since the F1 generation did not produce a sufficient number of offspring in all populations within the given time. With the shape analysis of the Portuguese snails from all generations, we examined a sample size of n = 318 and for the size analysis a sample size of n = 300. The two parameters for size, length and centroid size differed significantly in comparison to the parental and the F1 generation [Kruskal-Wallis chi-squared = 68.81, df = 2, $p < 0.01$ (length); Kruskal-Wallis chi-squared = 18.16, df = 2, $p < 0.01$ (centroid size)]. In length, we did not observe a significant difference between the F1 and the F2 generation from the population Póvoas village (Figure 24). We only detected a significant difference in centroid size for Alcabideque between the parental and the F1 generations and between the F1 and the F2 generations (Figure 26). Taking the two Portuguese populations together, the snails of the parental generation were bigger in length than the two daughter generations. We observed the snails of the coldest temperature to be longer than snails from the 19°C and the 23° climate cabinet (Figure 25). The centroid size of snails from the F1 generation was smaller than the centroid size of the parental and the F2 generation – except for the snail from the 23°C climate cabinet which seemed to become smaller across generations. Despite this, we did not detect any temperature-dependent pattern across generations in centroid size (Figure 27). The parental generation was less diverse across the three temperatures than the F1 and the F2 generation. For length and for centroid size, the model with the lowest AIC, was the one with the interaction of temperature and population as fixed factor and the ID of the mother snail as random factor (Tables 15 and 16). The marginal R^2 and the conditional R^2 as well as the proportion of

variance by the random factor, the mother snail´s ID, of the size and for the shape parameter can be seen in Table 17.

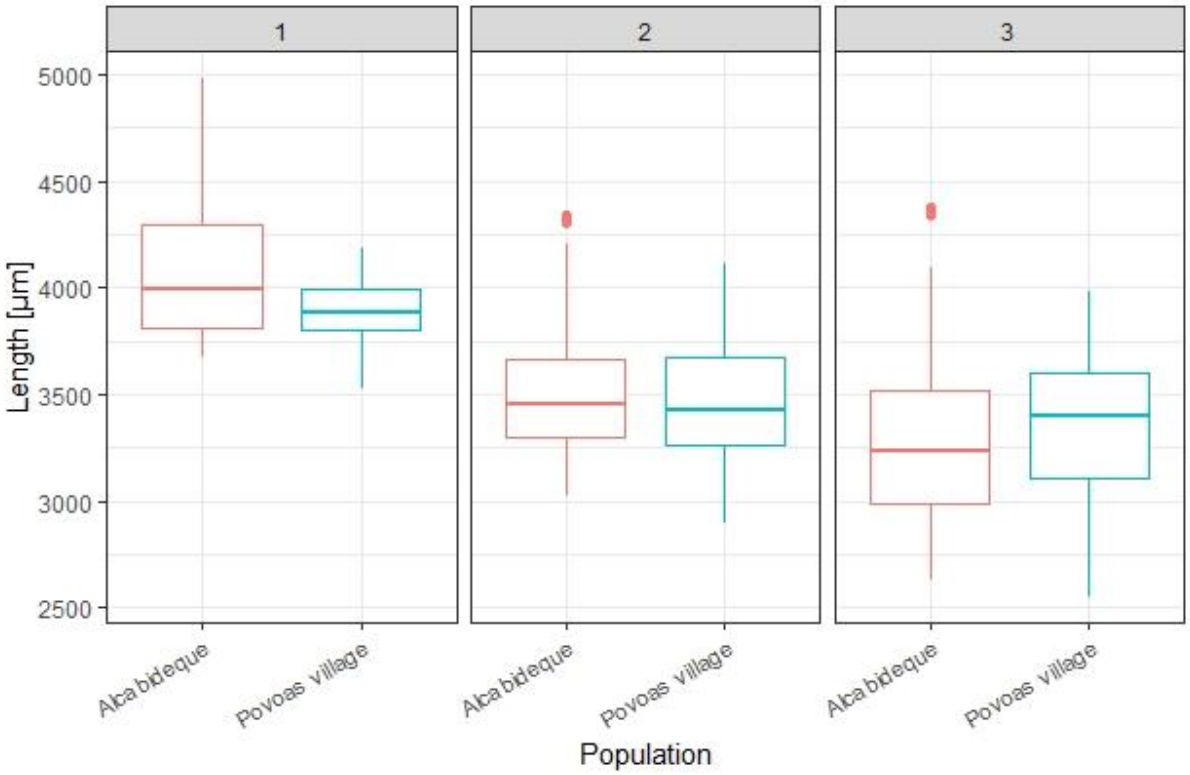

Figure 24. Length across the parental (1), the first daughter (2) and the second daughter (3) generations in the populations Alcabideque and Póvoas village.

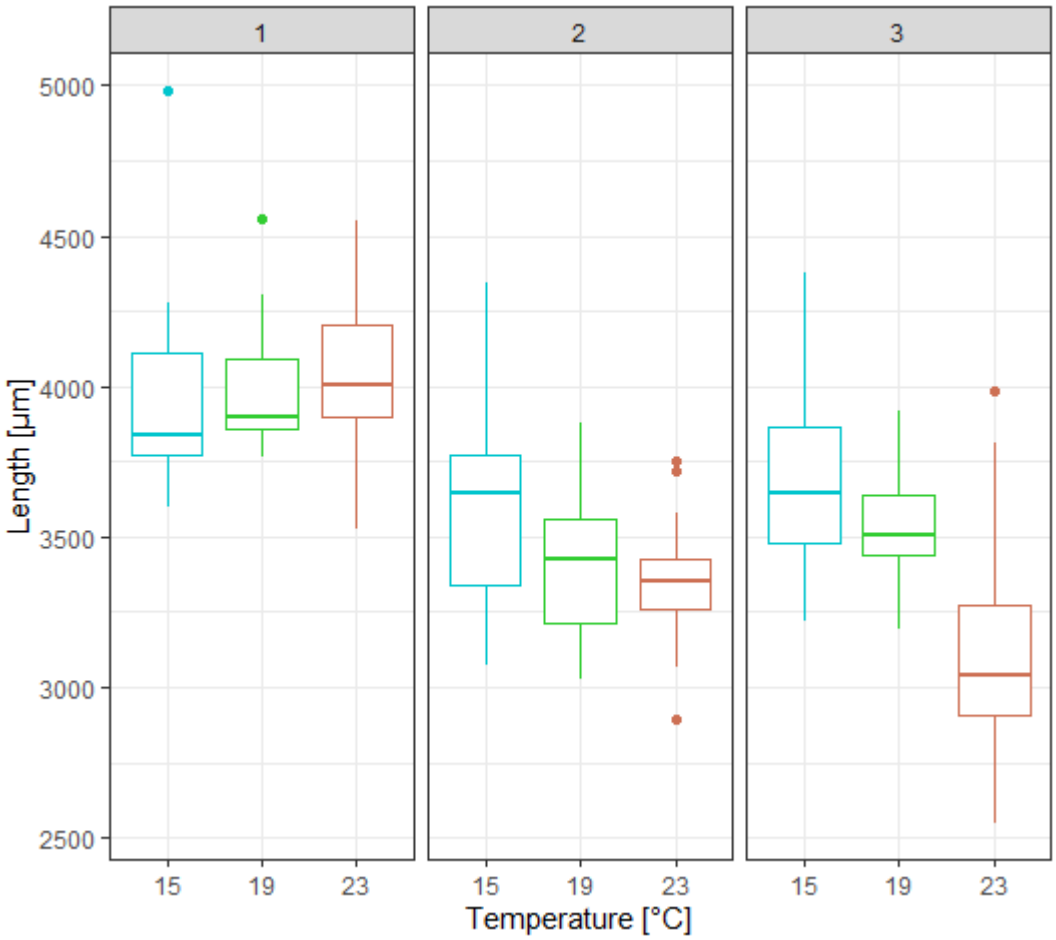

Figure 25. Shell length of the parental (1), the first daughter (2) and the second daughter (3) generations of the two Portuguese populations, Alcabideque and Póvoas village, across the three different temperatures.

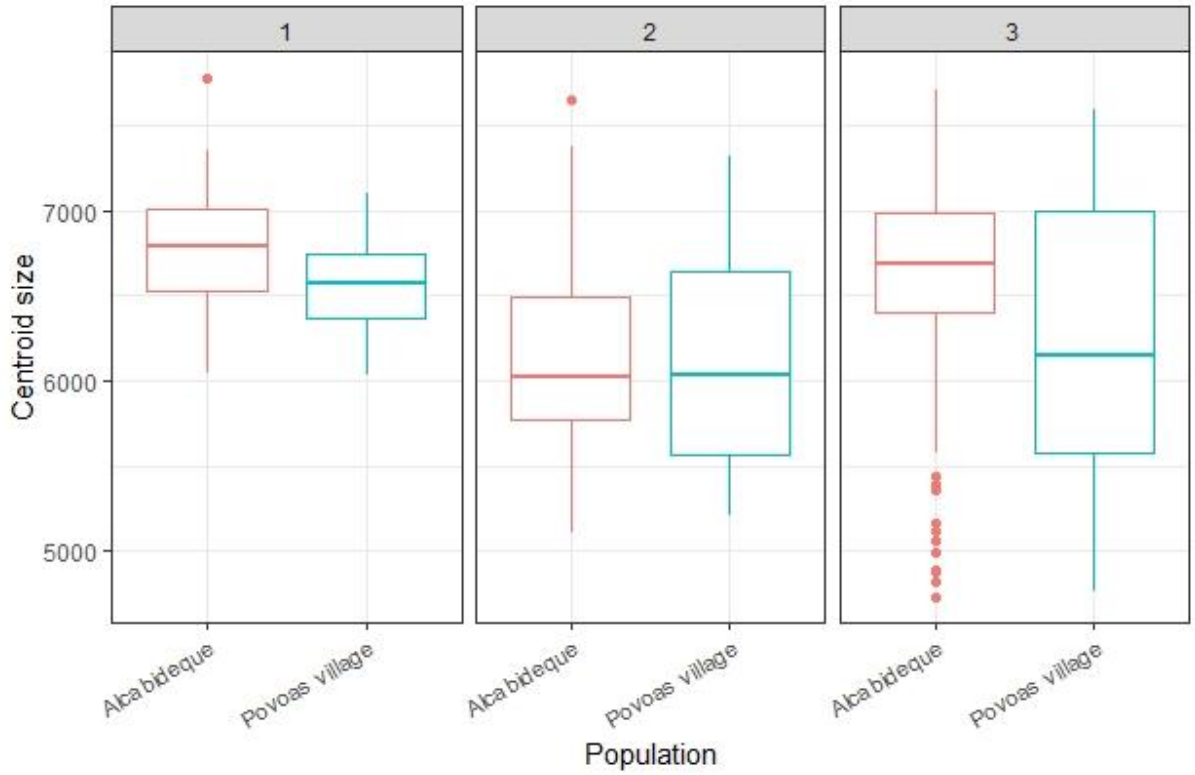

Figure 26. Centroid size across the parental (1), the first daughter (2) and the second daughter (3) generations in the populations Alcabideque and Póvoas village.

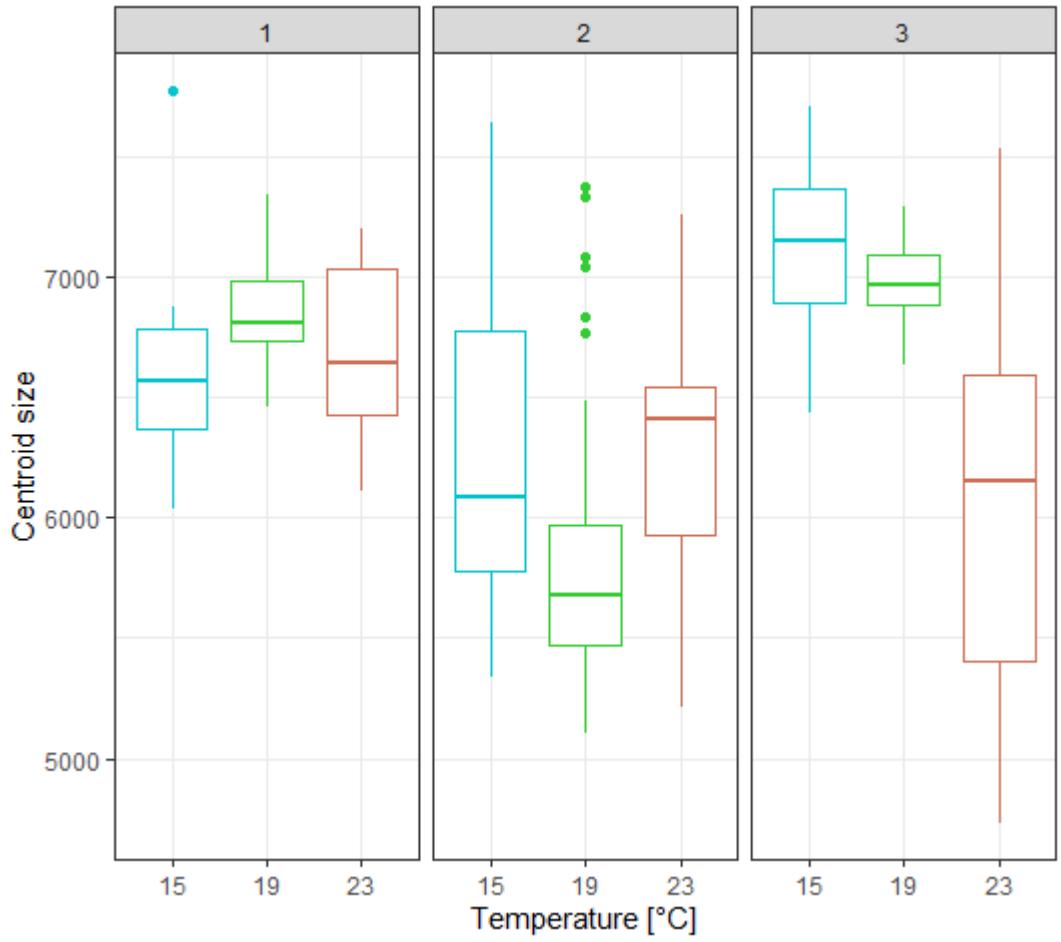

Figure 27. Centroid size of the parental (1), the first daughter (2) and the second daughter (3) generations of the two Portuguese populations, Alcabideque and Póvoas village, across the three different temperatures.

Table 15. Linear mixed model of length including the fixed factor, the interaction of temperature and population, and the random factor of the mother snail´s ID in the F2 generation. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	**Standard error**	**df**	**t-value**	**p-value**
Intercept	3998.42	102.54	34.04	38.995	< 2e-16 ***
Temperature 19°C	-478.26	130.75	35.34	-3.658	0.0008 ***
Temperature 23°C	-921.66	117.50	31.53	-7.844	6.64e-09 ***
Population Póvoas village	-432.96	122.38	34.10	-3.538	0.0012 **
Temperature 19°C x Population Póvoas village	457.38	237.73	40.28	1.924	0.0614 .
Temperature 23°C x Population Póvoas village	520.45	149.55	31.64	3.480	0.0015 **

Table 16. Linear mixed model of centroid size including the fixed factor, the interaction of temperature and population, and the random factor of the mother snail´s ID in the F2 generation. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	**Standard error**	**df**	**t-value**	**p-value**
Intercept	7344.55	187.48	39.22	39.174	< 2e-16 ***
Temperature 19°C	-387.93	238.82	40.48	-1.624	0.112
Temperature 23°C	-985.87	215.30	36.65	-4.579	5.23e-05 ***
Population Póvoas village	-315.69	223.76	39.24	-1.411	0.166
Temperature 19°C x Population Póvoas village	348.24	432.76	45.08	0.805	0.425
Temperature 23°C x Population Póvoas village	-502.16	274.02	36.71	-1.833	0.075 .

Table 17. Marginal R^2, conditional R^2 and the proportions of variance by the random factor (mother snail´s ID) of length, centroid size and PC 1-3 of the best fitted linear mixed models of the F2 generation.

	Marginal R^2	**Conditional R^2**	**Proportions of variance by the random factor [%]**
Length	0.47	0.65	18
Centroid size	0.32	0.63	31
PC 1	0.17	0.33	16
PC 2	0.40	0.54	14
PC 3	0.13	0.25	12

We observed a significant difference in PC 1-3 across the three generations [Kruskal-Wallis chi-squared = 65.32, df = 2, $p < 0.01$ (PC 1); Kruskal-Wallis chi-squared = 36.87, df = 2, $p < 0.01$ (PC 2); Kruskal-Wallis chi-squared = 37.49, df = 2, $p < 0.01$ (PC 3)] – with some exceptions. There was no significant difference in PC 1 between the F1 and the F2 generations of Alcabideque. Also, the parental and the F1 generation from Póvoas village did not vary significantly in PC 1 (Figure 28). The 23°C snails of the parental and the F1 generation showed higher PC1 values than snails from colder temperatures. In the F2 generation however, the 23°C snails had lower PC1 values than the snails from the other two colder temperatures (Figure 29). The model with the lowest AIC for PC 1 was the one with temperature and population as fixed factors (Table 18). For PC 2, no significant difference was detected between the parental and the F2 generation from Alcabideque and between the F1 and the F2 generations from Póvoas village (Figure 30). The snails from the 23°C climate cabinet showed higher PC 2 values in all generations (Figure 31). The best model for PC 2 contained temperature as fixed factor. (Table 19). In the populations of Alcabideque and Póvoas village, we did not monitor any significant difference in PC 3 between the F1 and the F2 generations (Figure 32). The PC 3 values became less heterogenous across temperatures in the F2 generation (Figure 33). For PC 3, the model having the interaction of temperature and population as fixed factors was the one with the lowest AIC (Table 20).

We illustrated the shape changes along the PC 1-3 of the parental, the F1 and the F2 generations using deformation grids (Figure 34). The parental and the F1 generations generally had higher PC 1 values than snails of the F2 generation resulting in smaller apertures and shells which are narrower at the bottom. Across temperature, also the snails from the 23°C climate cabinet of the parental and the F1 generations and snails from the 15°C and the 19°C climate cabinet of the F2 generation had comparably higher PC 1 values. We observed higher PC 2 values in the parental

generation than in the two daughter generations. The snails from the 23°C climate cabinet had higher PC 2 values in all three generations. Higher PC 2 values resulted in a shortened upper part and a broader lower part of the shell. Furthermore, snails with high PC 2 values show a bigger aperture. Concerning PC 3, the two daughter generations had higher values than the parental generation. Also, snails from the 23°C climate cabinet from the parental generation and snails from the 19°C climate cabinet from the F1 generation had higher PC 3 values than the snails from the same generation from different temperatures. Almost no difference was detected in the PC 3 values of the F2 generation across the three different temperatures. Higher PC 3 values showed in a narrower and shorter lower part of the shell.

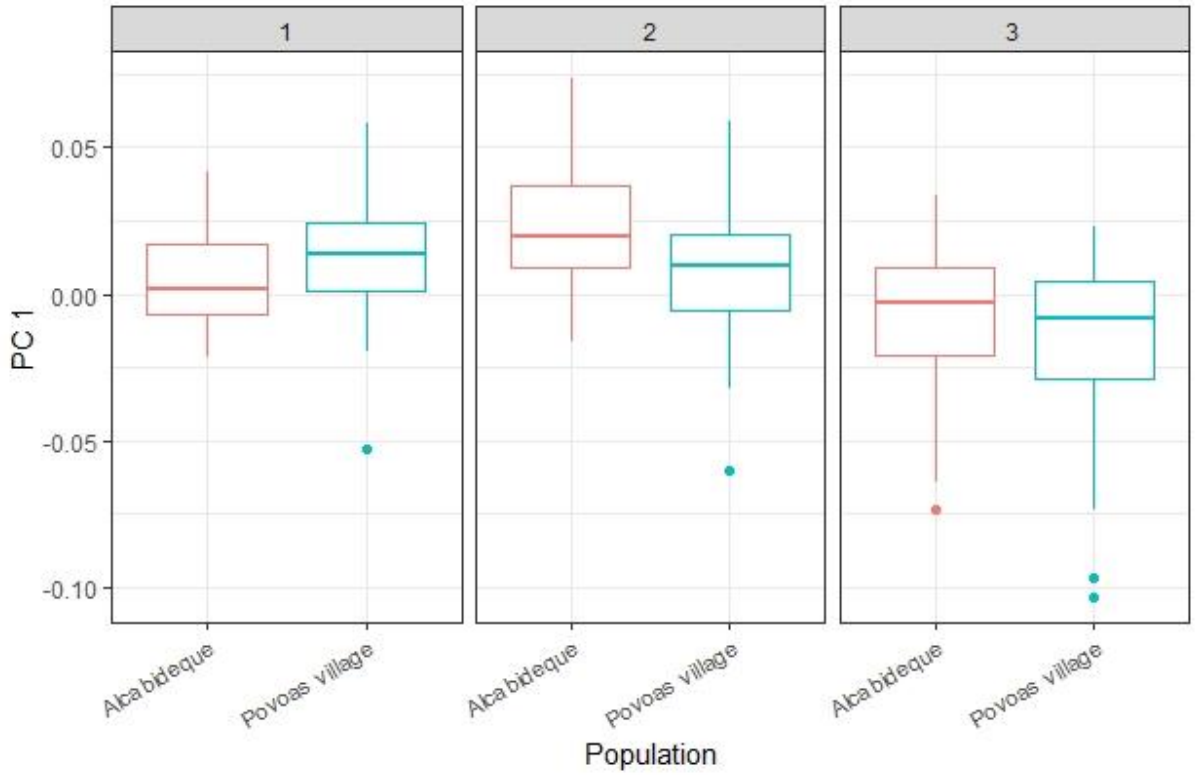

Figure 28. PC 1 for shape across the parental (1), the first daughter (2) and the second daughter (3) generations in the populations Alcabideque and Póvoas village.

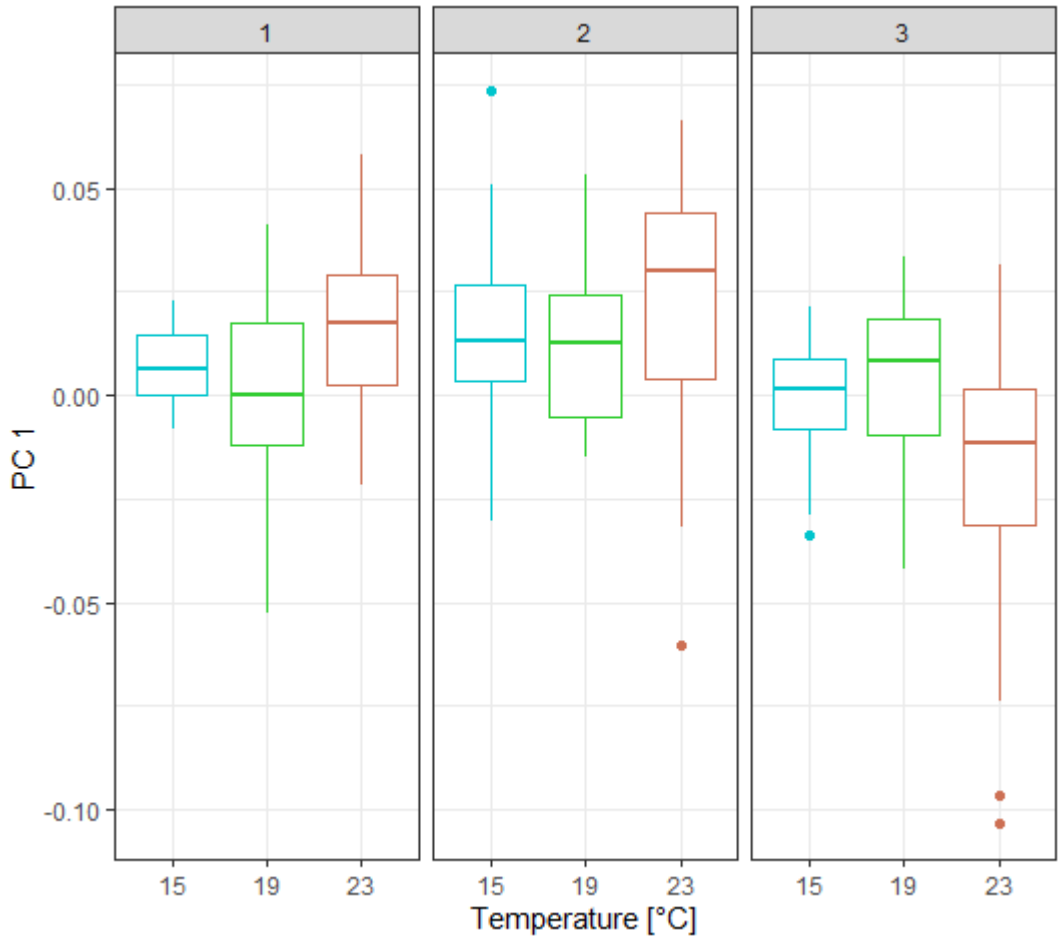

Figure 29. PC 1 for shape of the parental (1), the first daughter (2) and the second daughter (3) generations of the two Portuguese populations, Alcabideque and Póvoas village, across the three different temperatures.

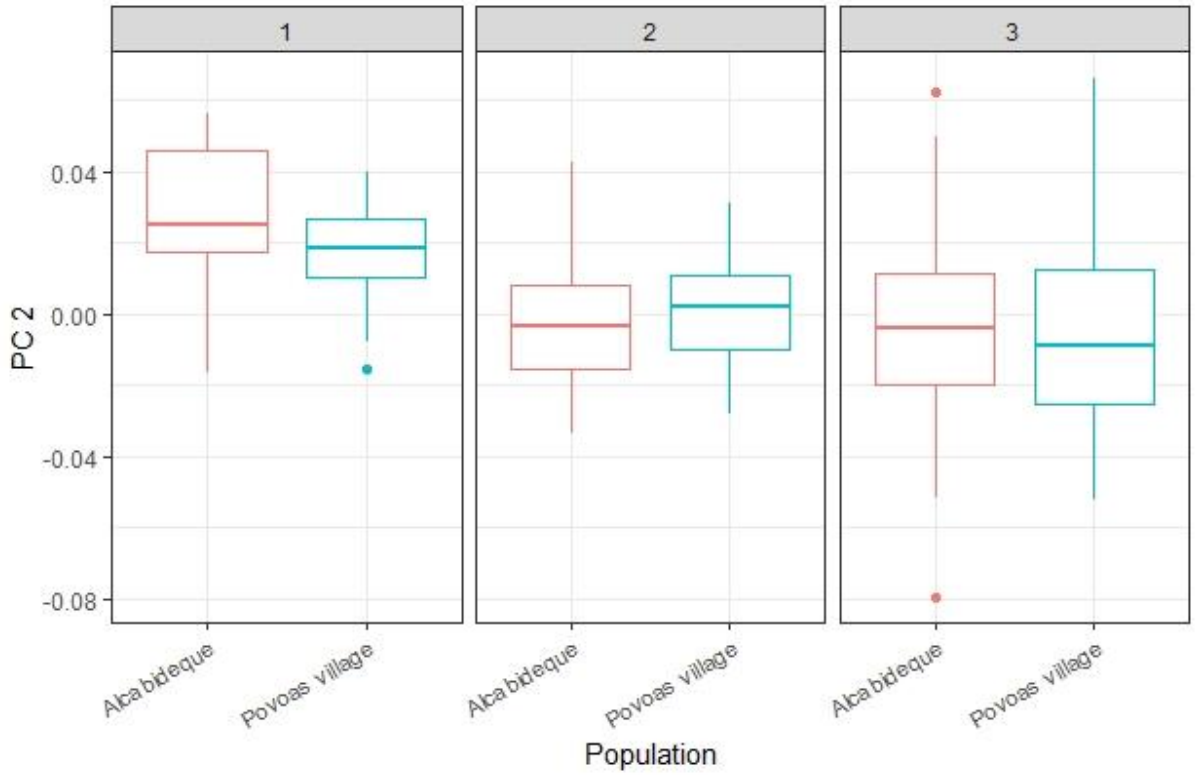

Figure 30. PC 2 for shape across the parental (1), the first daughter (2) and the second daughter (3) generations in the populations Alcabideque and Póvoas village.

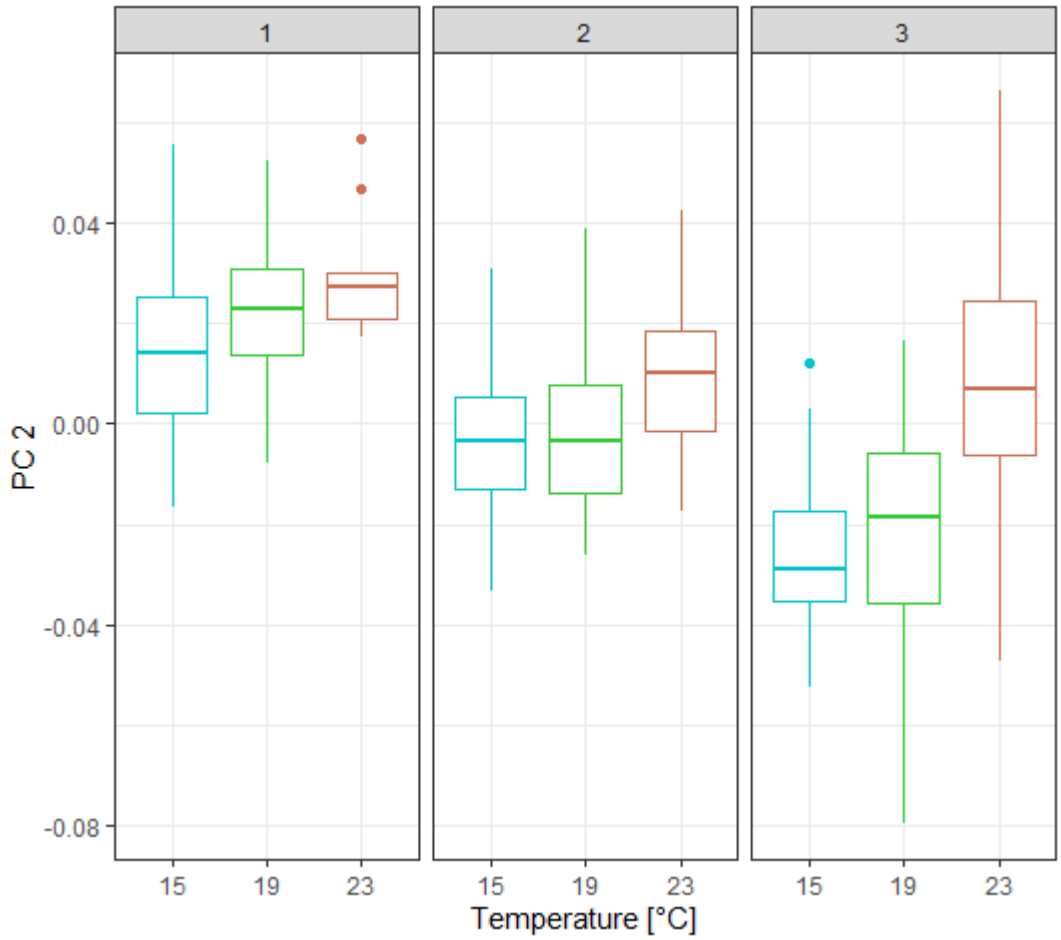

Figure 31. PC 2 for shape of the parental (1), the first daughter (2) and the second daughter (3) generations of the two Portuguese populations, Alcabideque and Póvoas village, across the three different temperatures.

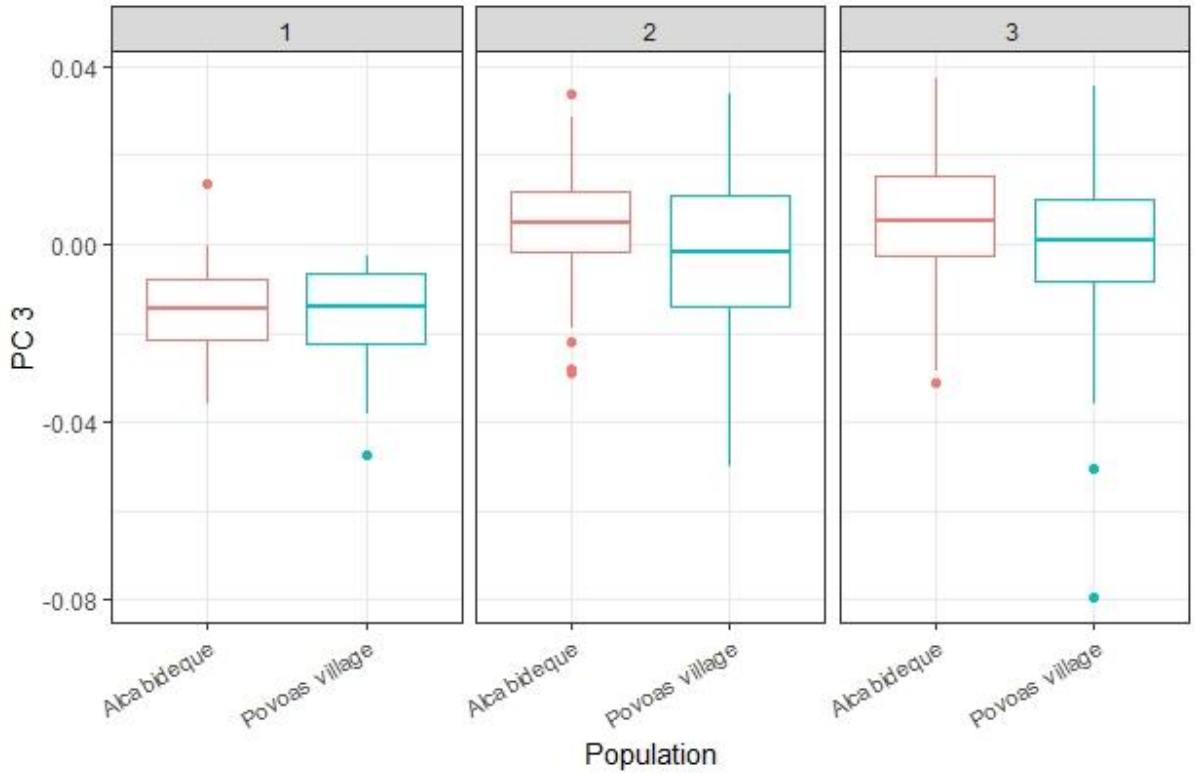

Figure 32. PC 3 for shape across the parental (1), the first daughter (2) and the second daughter (3) generations in the populations Alcabideque and Póvoas village.

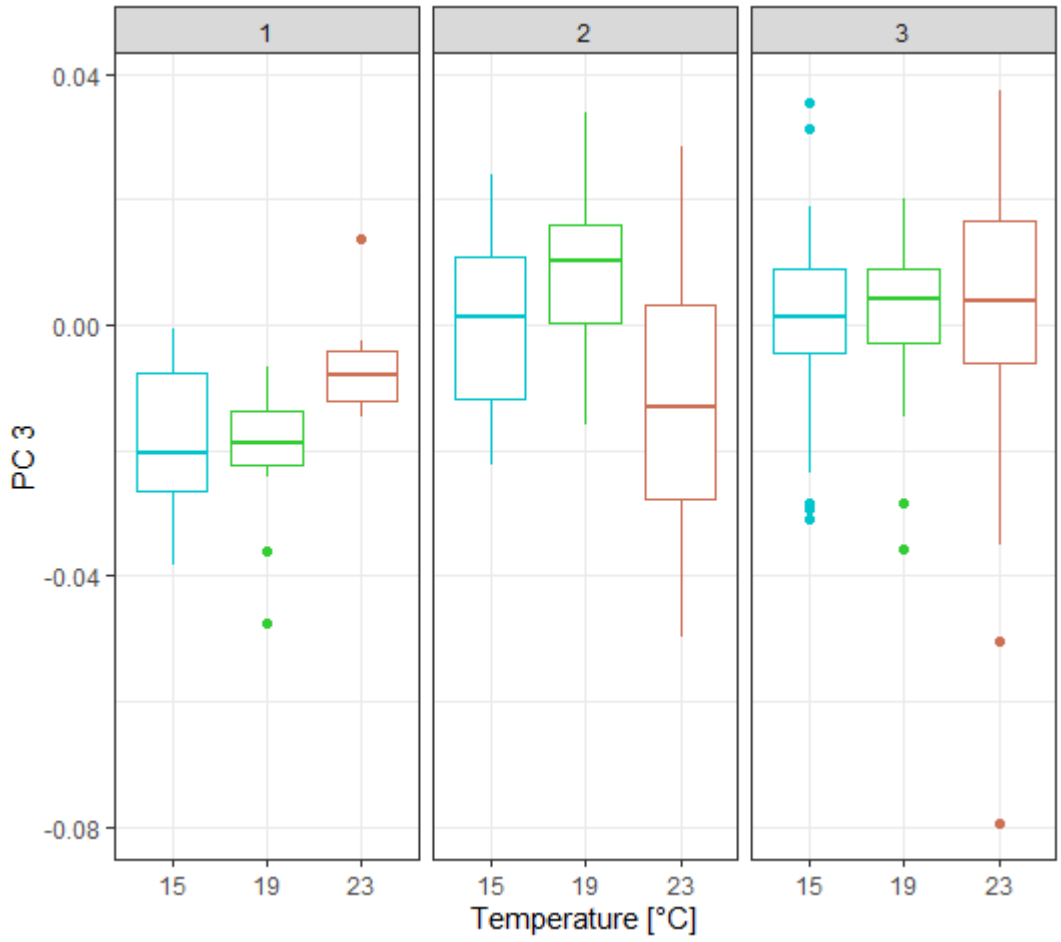

Figure 33. PC 3 for shape of the parental (1), the first daughter (2) and the second daughter (3) generations of the two Portuguese populations, Alcabideque and Póvoas village, across the three different temperatures.

Table 18. Linear mixed model of PC 1 for shape including the fixed factors of temperature and population and the random factor of the mother snail´s ID in the F2 generation. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	**Standard error**	**df**	**t-value**	**p-value**
Intercept	0.006646	0.005431	37.63	1.224	0.22865
Temperature 19°C	-0.002970	0.007105	43.81	-0.418	0.67798
Temperature 23°C	-0.019493	0.005452	36.25	-3.575	0.00101 **
Population Póvoas village	-0.010570	0.004793	34.48	-2.205	0.03420 *

Table 19. Linear mixed model of PC 2 for shape including the fixed factor temperature and the random factor of the mother snail´s ID in the F2 generation. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	**Standard error**	**df**	**t-value**	**p-value**
Intercept	-0.026731	0.004243	36.90	-6.300	2.49e-07 ***
Temperature 19°C	0.005941	0.006755	40.38	0.879	0.384
Temperature 23°C	0.036178	0.005291	32.81	6.838	8.64e-08 ***

Table 20. Linear mixed model of PC 3 for shape including the fixed factor, the interaction of temperature and population, and the random factor of the mother snail´s ID in the F2 generation. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	**Standard error**	**df**	**t-value**	**p-value**
Intercept	-0.004126	0.005113	41.72	-0.807	0.42419
Temperature 19°C	0.004699	0.006578	44.72	0.714	0.47879
Temperature 23°C	0.012300	0.005761	37.13	2.135	0.03940 *
Population Póvoas village	0.008110	0.006109	42.30	1.327	0.19149
Temperature 19°C x Population Póvoas village	-0.006041	0.009958	50.77	-0.607	0.54683
Temperature 23°C x Population Póvoas village	-0.022871	0.007345	37.75	-3.114	0.00351 **

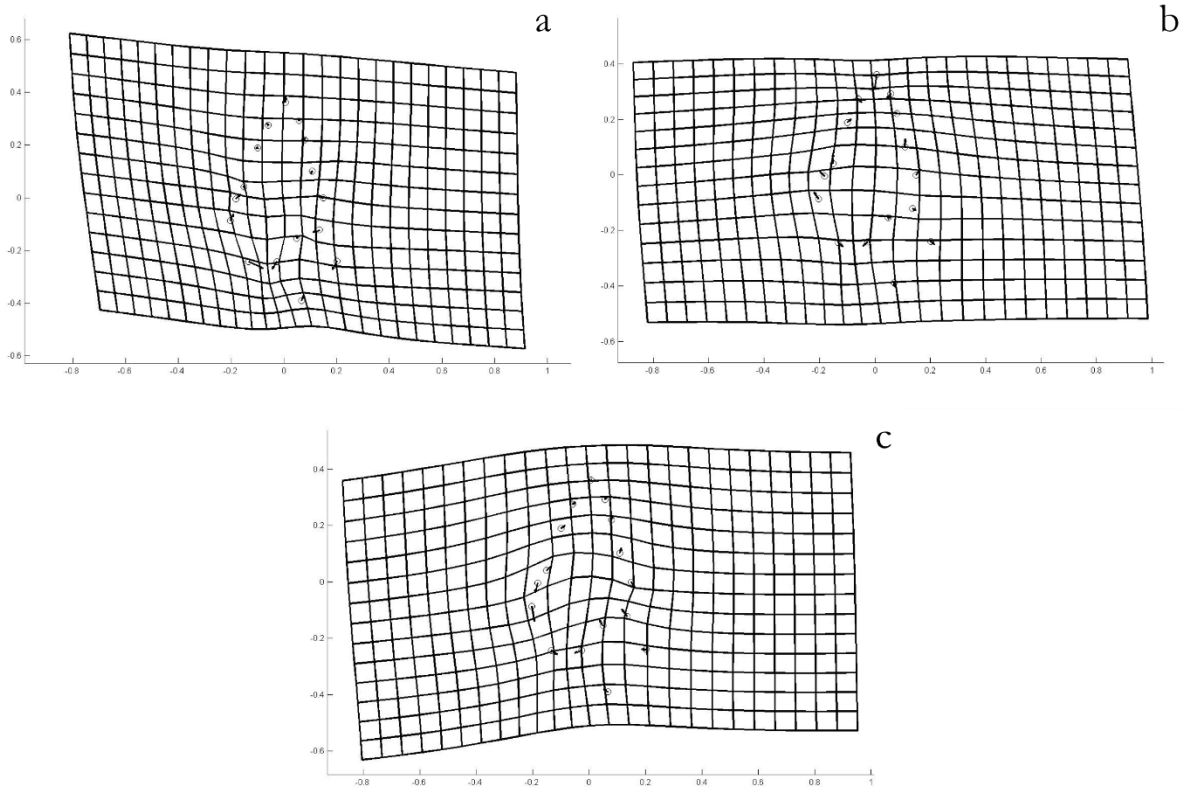

Figure 34. Deformation grids for PC 1 (a), PC 2 (b) and PC 3 (c) of the parental, the F1 and the F2 generations of the populations Alcabideque and Póvoas village. Grids show deformation from specimens with lowest (circle) to those with highest values (arrowhead).

Reaction norms

Regarding the effects of haplotype and population, snails of the F1 generation which were born and grew up in the 23°C and 19°C climate cabinets, were generally smaller than the snails of the 15°C climate cabinet (Figure 35). Snails possessing haplotype t always showed longer shells and larger centroid sizes compared to snails with haplotype z. Most of the populations (eight out of eleven), including every population possessing haplotype z, grew the longest shells under the coldest circumstances, in the 15°C climate cabinet when comparing the size patterns within the populations. Different from the snails of the populations Hohensprenz, Filzingen and J008 which had their longest shells in the 19°C medium climate cabinet (Hohensprenz) and in the 23°C warmest climate cabinet (Filzingen, J008). The development of the shortest shells showed a less clear pattern: the snails of the 19°C climate cabinet grew the shortest shells in five of the eleven populations. Looking at the pairwise statistical comparisons of slopes, we detected 17 significant cases altogether. Between the haplotypes, we found 15 of the 17 significant cases and one case per

haplotype (Table 21). In conclusion, both haplotypes presented the shortest shells at 19°C and longer shells at 15°C and 23°C. Still, we observed some variation among the populations.

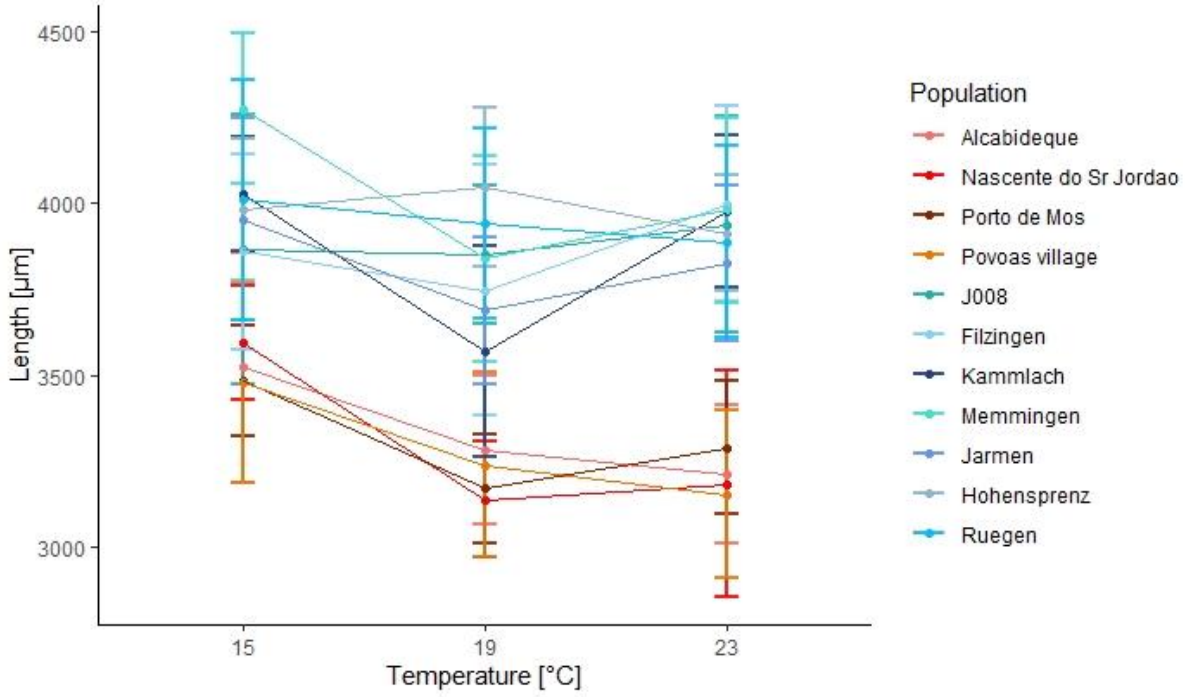

Figure 35. Reaction norms of shell lengths over the three temperatures within generation F1. Populations with haplotype t are displayed with bluish line colour, populations with haplotype z reddish.

Table 21. Comparison of reaction norm slopes of length across populations of the F1 generation using ANOVA and the calculation of the least square means. Significant differences ($p < 0.05$) are written in bold.

Length	Filzingen	Hohensprenz	J008	Jarmen	Kammlach	Memmingen	Nascente do Sr. Jordão	Porto de Mós	Póvoas village	Rügen
Alcabideque	**<.0001**	0.5300	**0.0002**	0.7802	**<.0001**	0.9985	0.4607	0.9472	1.0000	0.6907
Filzingen		0.1931	0.9938	0.1620	1.0000	**0.0123**	**<.0001**	**0.0013**	**<.0001**	0.1352
Hohensprenz			0.7347	1.0000	0.1932	0.9947	**0.0044**	0.9963	0.4270	1.0000
J008				0.6526	0.9814	0.1279	**<.0001**	**0.0346**	**0.0001**	0.6214
Jarmen					0.1599	0.9995	**0.0191**	0.9998	0.6841	1.0000
Kammlach						0.0155	**<.0001**	**0.0033**	**<.0001**	0.1389
Memmingen							0.2046	1.0000	0.9936	0.9989
Nascente do Sr. Jordão								**0.0227**	0.6542	**0.0100**
Porto de Mós									0.8858	0.9995
Póvoas village										0.5847

With centroid size, we detected a comparable, yet not indistinguishable pattern. Just as with length, the smallest shells could be found with the snails from the 19°C climate cabinet (Figure 36). We found the largest shells at 15°C, in the coldest climate cabinet, with four out of eleven populations, which is on the contrary to length: Jarmen, Nascente do Sr. Jordão, Porto de Mós and Póvoas village. Only the snails of one population possessing haplotype z, Alcabideque, had the largest centroid size at 23°C, the warmest climate cabinet temperature. Also Rügen, J008 and the three populations from Southern Germany grew the largest shells with regard to centroid size at 23°C. Only the snails from one population, Hohensprenz, developed the largest shells at 19°C. Eleven pairwise comparisons of the slopes for centroid size showed a significant difference, nine among haplotypes and each one in every haplotype (Table 22). Therefore, we had a rather distinct picture with centroid size: the shells of the snails of the 15°C and the 23°C climate cabinet were about the same size, while the smallest shells were measured at 19°C

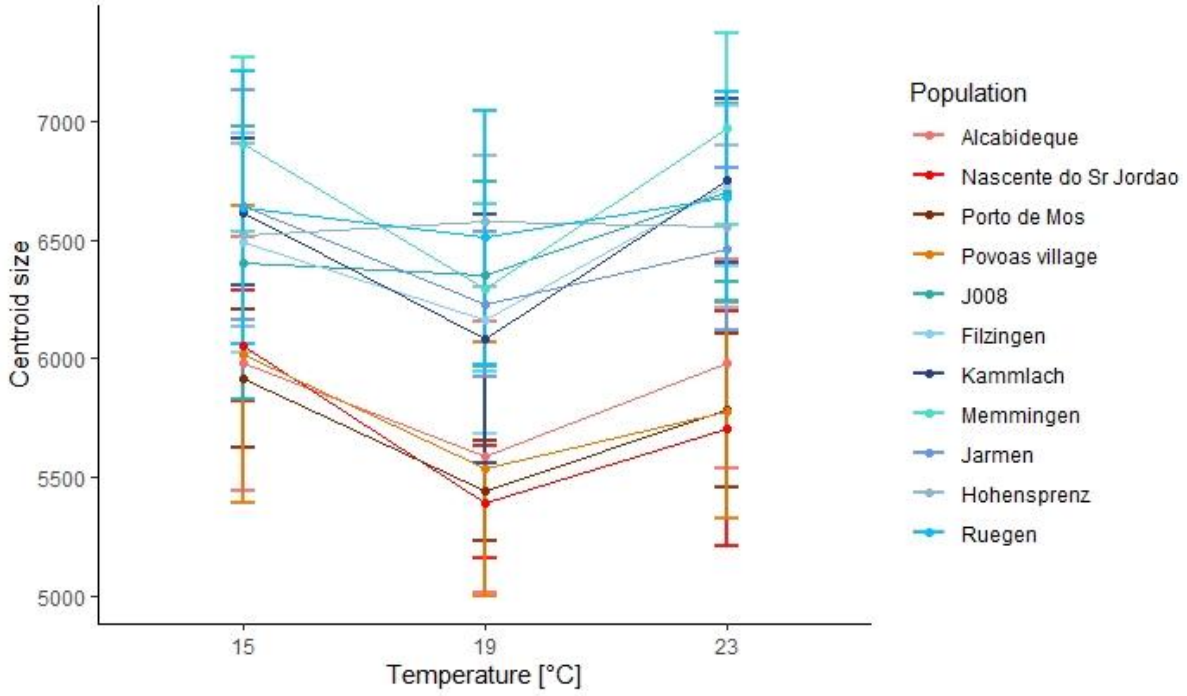

Figure 36. Reaction norm of the mean centroid size over the three temperatures within generation F1. Populations with haplotype t are displayed with bluish line colour, populations with haplotype z reddish.

Table 22. Comparison of reaction norm slopes of centroid size across populations of the F1 generation using ANOVA and the calculation of the least square means. Significant differences ($p < 0.05$) are written in bold.

Centroid size	Filzingen	Hohensprenz	J008	Jarmen	Kammlach	Memmingen	Nascente do Sr. Jordão	Porto de Mós	Póvoas village	Rügen
Alcabideque	0.8179	1.0000	0.7410	0.9571	0.2557	0.9872	**0.0089**	0.9662	0.7309	1.0000
Filzingen		0.8674	1.0000	0.1910	0.9954	1.0000	**<.0001**	0.1041	**0.0259**	0.9831
Hohensprenz			0.8205	0.9904	0.3485	0.9875	0.0552	0.9963	0.9240	1.0000
J008				0.1409	0.9946	1.0000	**<.0001**	0.0583	**0.0123**	0.9738
Jarmen					**0.0292**	0.5387	0.6985	1.0000	1.0000	0.9295
Kammlach						0.9831	**<.0001**	**0.0111**	**0.0024**	0.6321
Memmingen							**0.0016**	0.5035	0.2285	0.9996
Nascente do Sr. Jordão								0.2295	0.6399	**0.0176**
Porto de Mós									0.9999	0.9458
Póvoas village										0.7207

Figure 37 shows the reaction norms for shape of PC 1-3. We visualized the alterations of shape along the PC 1-3 in the F1 generation using deformation grids (Figure 38). On Figure 16, one can see that the aperture and the bottom components of the last whorl became narrower along PC 1. The body whorl grew bigger, and the spire became more compressed along PC 2. Along PC 3, the body whorl developed more elevated. Snails having haplotype t showed bigger values for PC 1 and PC 3 than snails with haplotype z.

Snails from the 19°C climate cabinet had the lowest PC 1 values. In every population except for Póvoas village and Filzingen, this results in the bottom components of the last whorls and the apertures being more globular. The snails from the 15°C climate cabinet held the highest values in four and the snails from the 23°C climate cabinet in five populations. The only population which had the highest values at 19°C was Filzingen (Figure 37).

All populations possessing haplotype z showed growing values with growing temperatures along PC 2. Contrary to populations with haplotype z, populations with haplotype t had the highest values at 19°C or 23°C except for Jarmen. Those values had the tendency to resemble the exception of Kammlach (Figure 37).

Along PC 3, we got a less homogenous picture. The highest values of six populations with haplotype t could be found at 15°C, five of them had their lowest values at 23°C. Across the three temperatures, the snails from the population Filzingen barely varied at all. The Spanish population J008 had the maximum values at 19°C. The highest scores of all the four populations with haplotype z were calculated at 19°C. Still, those populations showed similarly high scores at 15°C. Their lowest values were at 23°C (Figure 37).

In comparison to size, the changes in shape were in general decent differing significantly less in slopes (Tables 23-25). We detected only three significant pairwise differences for PC 1: one amidst haplotypes and two within haplotype t. With PC 2, we found seven significant differences between haplotypes and two between populations with haplotype z which was more instructive than with PC 1. Less so with PC 3: there was only one significant difference within haplotype t. Principally, the snails possessing haplotype z grew squatter shells than snails with haplotype t. In return, snails with haplotype t had the tendency to develop a wider aperture and base of the final whorl at 19°C. Additionally, with increasing temperatures, the spire grew shorter and the body whorl larger and especially higher. The changes of shape within snails possessing haplotype z were rather similar in comparison to snails with haplotype t, but more noticeable. The bottom components of the final whorl and the apertures developed narrower. The spire grew shorter and the body whorl higher and larger.

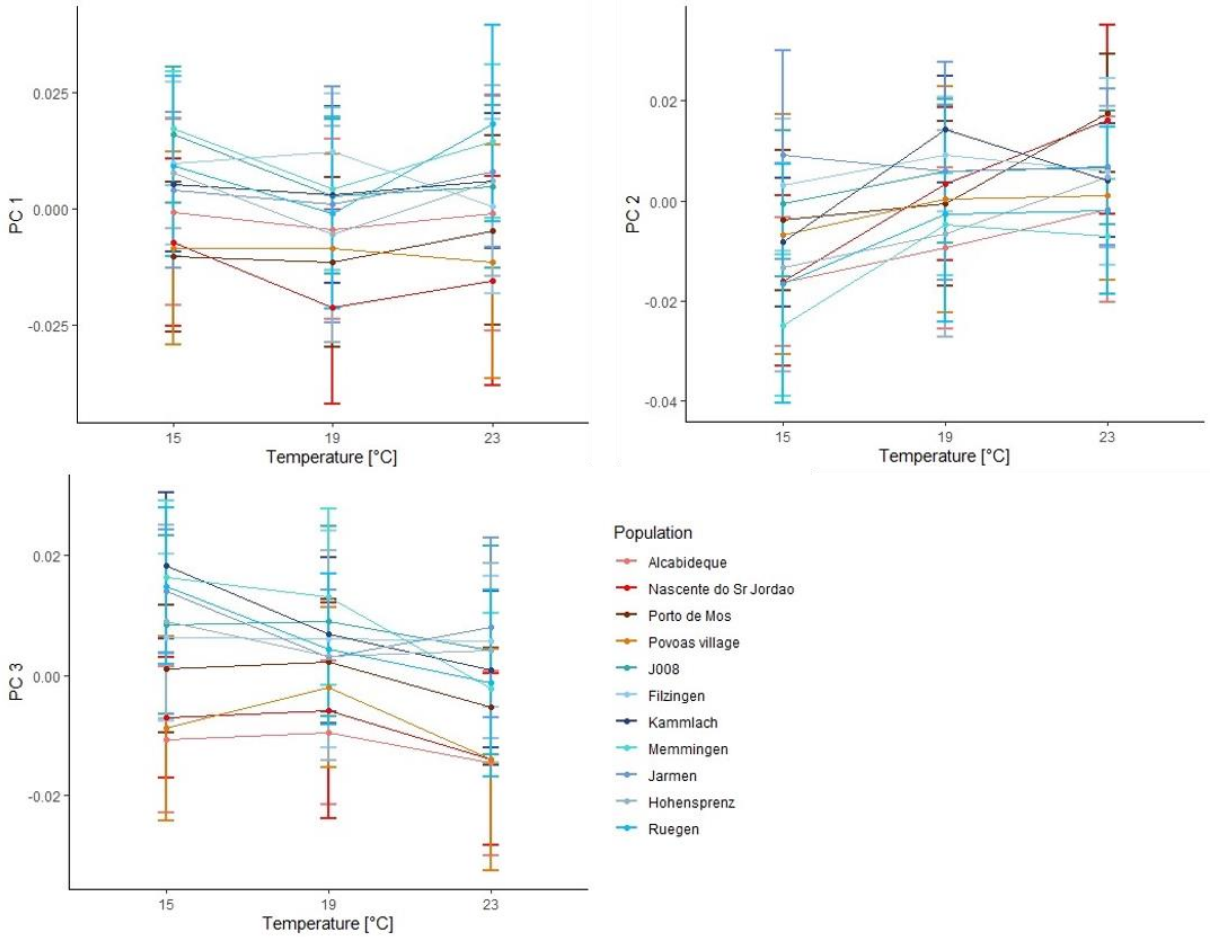

Figure 37. Reaction norms of PC 1, 2 and 3 across the three temperatures within generation F1. Populations with haplotype t with bluish, populations with haplotype z reddish line colour.

Table 23. Comparison of reaction norm slopes of PC 1 for shape across populations of the F1 generation using ANOVA and the calculation of the least square means. Significant differences ($p < 0.05$) are written in bold.

PC 1	Filzingen	Hohensprenz	J008	Jarmen	Kammlach	Memmingen	Nascente do Sr. Jordão	Porto de Mós	Póvoas village	Rügen
Alcabideque	0.8259	1.0000	0.6879	1.0000	1.0000	1.0000	0.7604	0.9973	1.0000	0.6213
Filzingen		0.8000	1.0000	0.7007	0.8329	0.9369	1.0000	0.2448	0.9903	**0.0283**
Hohensprenz			0.6845	1.0000	1.0000	1.0000	0.7309	1.0000	0.9994	0.8976
J008				0.5781	0.7329	0.8829	1.0000	0.1272	0.9704	**0.0121**
Jarmen					1.0000	1.0000	0.6283	1.0000	0.9955	0.9763
Kammlach						1.0000	0.7666	1.0000	0.9996	0.9153
Memmingen							0.8921	0.9998	1.0000	0.8429
Nascente do Sr. Jordão								0.2253	0.9732	**0.0273**
Porto de Mós									0.9177	0.9750
Póvoas village										0.3129

Table 24. Comparison of reaction norm slopes of PC 2 for shape across populations of the F1 generation using ANOVA and the calculation of the least square means. Significant differences ($p < 0.05$) are written in bold.

PC 2	Filzingen	Hohensprenz	J008	Jarmen	Kammlach	Memmingen	Nascente do Sr. Jordão	Porto de Mós	Póvoas village	Rügen
Alcabideque	0.3745	0.9997	0.9140	0.1685	0.7143	1.0000	**0.0369**	0.9453	0.9477	1.0000
Filzingen		0.1657	0.9974	0.9998	1.0000	0.6842	**<.0001**	**0.0095**	0.9958	0.8592
Hohensprenz			0.6220	0.0678	0.4047	0.9999	0.4470	1.0000	0.6940	0.9951
J008				0.9101	0.9999	0.9811	**0.0003**	0.1173	1.0000	0.9987
Jarmen					0.9994	0.3866	**<.0001**	**0.0045**	0.8941	0.5685
Kammlach						0.8759	**0.0003**	0.0787	0.9998	0.9644
Memmingen							0.1612	0.9891	0.9889	1.0000
Nascente do Sr. Jordão								0.5494	**0.0005**	**0.0437**
Porto de Mós									0.1693	0.8852
Póvoas village										0.9994

Table 25. Comparison of reaction norm slopes of PC 3 for shape across populations of the F1 generation using ANOVA and the calculation of the least square means. Significant differences ($p < 0.05$) are written in bold.

PC 3	Filzingen	Hohensprenz	J008	Jarmen	Kammlach	Memmingen	Nascente do Sr. Jordão	Porto de Mós	Póvoas village	Rügen
Alcabideque	0.9991	1.0000	1.0000	1.0000	0.4609	0.0801	1.0000	1.0000	1.0000	0.4796
Filzingen		0.9999	0.9992	0.9969	0.1400	**0.0144**	0.9988	0.9676	0.9719	0.1456
Hohensprenz			1.0000	1.0000	0.5697	0.1445	1.0000	1.0000	1.0000	0.5921
J008				1.0000	0.4760	0.0864	1.0000	1.0000	1.0000	0.4954
Jarmen					0.8484	0.3789	1.0000	1.0000	1.0000	0.8664
Kammlach						0.9996	0.7157	0.7416	0.7886	1.0000
Memmingen							0.2310	0.2108	0.2593	0.9991
Nascente do Sr. Jordão								1.0000	1.0000	0.7382
Porto de Mós									1.0000	0.7637
Póvoas village										0.8096

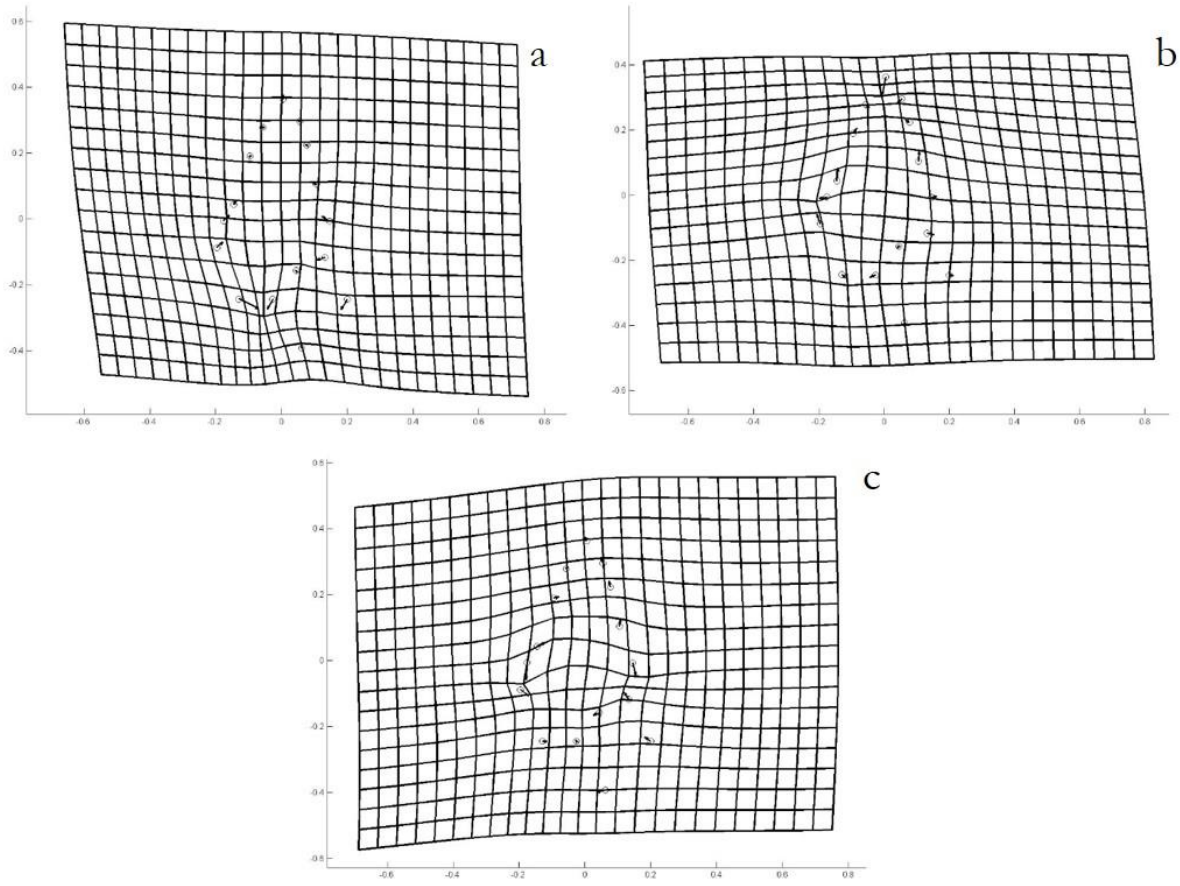

Figure 38. Deformation grids for PC 1 (a), PC 2 (b) and PC 3 (c) of the F1 generation. Grids show deformation from specimens with lowest (circle) to those with highest values (arrowhead).

Heritability

Tables 26, 27 and 28 display the values of heritability and the coefficients of genetic variance and I_A for the three distinct temperatures of the F1 generation. For the two size parameters, length and centroid size, the heritabilities showed considerably high values from 0.49 to 0.80. Therefore, genetic differentiation mainly explained the overall detected variation. The values at 19°C and at 23°C were higher than at 15°C. In general, the heritability estimates for size were bigger than for shape. Still, the values for heritability for shape showed higher values at 15°C and smaller values at 23°C.

In general, the CV_A values for both size parameters were in a rather short span from 0.39% to 1.13%. In comparison to length, the CV_A values for centroid size were somewhat smaller. In combination with the according low I_A values, this shows a very small capability to react to selection, for example low evolvability, especially at 15°C, the coldest temperature.

Table 26. Genetic variance at 15°C in the F1 generation. Overall genetic variance (V_A), residual variance (V_{res}), and broad-sense heritability (H^2) are displayed for every size and shape parameter. Furthermore, the coefficient of genetic variation (CV_A) and its square (I_A) for the size parameters are shown. Significant *p* values are written in bold.

15°C	V_A	V_{res}	H^2	CV_A	I_A	*p*
Length	88032.95	47518.18	0.65	0.0066	4.37e-09	**< 0.01**
Centroid size	148960.2	158173.8	0.49	0.0039	1.49e-09	**< 0.01**
PC 1	0.000161	0.000225	0.42	-	-	**< 0.01**
PC 2	0.000223	0.000174	0.56	-	-	**< 0.01**
PC 3	0.000121	0.000139	0.47	-	-	**< 0.01**

Table 27. Genetic variance at 19°C in the F1 generation. Overall genetic variance (V_A), residual variance (V_{res}), and broad-sense heritability (H^2) are displayed for every size and shape parameter. Furthermore, the coefficient of genetic variation (CV_A) and its square (I_A) for the size parameters are shown. Significant *p* values are written in bold.

19°C	V_A	V_{res}	H^2	CV_A	I_A	*p*
Length	141414.41	34722.71	0.80	0.0106	1.13e-08	**< 0.01**
Centroid size	254133.2	124710.2	0.67	0.0066	4.33e-09	**< 0.01**
PC 1	0.00012	0.000322	0.27	-	-	**< 0.01**
PC 2	0.000133	0.000212	0.39	-	-	**< 0.01**
PC 3	0.000075	0.000172	0.30	-	-	**< 0.01**

Table 28. Genetic variance at 23°C in the F1 generation. Overall genetic variance (V_A), residual variance (V_{res}), and broad-sense heritability (H^2) are displayed for every size and shape parameter. Furthermore, the coefficient of genetic variation (CV_A) and its square (I_A) for the size parameters are shown. Significant *p* values are written in bold.

23°C	V_A	V_{res}	H^2	CV_A	I_A	*p*
Length	150940.89	41638.51	0.78	0.0113	1.29e-08	**< 0.01**
Centroid size	233094	113647.5	0.67	0.0060	3.64e-09	**< 0.01**
PC 1	0.000168	0.00033	0.34	-	-	**< 0.01**
PC 2	0.000117	0.00016	0.42	-	-	**< 0.01**
PC 3	0.000129	0.000148	0.47	-	-	**< 0.01**

Additionally, we calculated the heritabilities for each haplotype individually, to test if distinctions across the two haplotypes determined the fairly high heritability values to a great extent. As we already suspected, the haplotype-specific heritability values in general were remarkably lower (Tables 29-34). Considering haplotype z, the heritability values now span from 0.24-0.37, whereas in haplotype t, they ranged from 0.29-0.59. We did not observe any significant changes across temperatures with haplotype z. The lowest values with haplotype t were calculated at 23°C. The CV_A scores went below 1%. Except for that we found the lowest heritability values with shape at 19°C, shape resulted a similar picture as size.

Table 29. Genetic variance in haplotype t of the F1 generation at 15°C. Overall genetic variance (V_A), residual variance (V_{res}), and broad-sense heritability (H^2) are displayed for every size and shape parameter. Furthermore, the coefficient of genetic variation (CV_A) and its square (I_A) for the size parameters are shown. Significant *p* values are written in bold.

Haplotype t, 15°C						
	V_A	V_{res}	H_2	CV_A	I_A	*p*
Length	58477.77	56286.2	0.51	0.0038	1.47e-09	**< 0.01**
Centroid size	86618.51	172421.92	0.33	0.0020	4.09e-10	**< 0.01**
PC 1	0.000065	0.000194	0.25	-	-	**< 0.01**
PC 2	0.000247	0.000155	0.61	-	-	**< 0.01**
PC 3	0.000052	0.000149	0.26	-	-	**< 0.01**

Table 30. Genetic variance in haplotype t of the F1 generation at 19°C. Overall genetic variance (V_A), residual variance (V_{res}), and broad-sense heritability (H^2) are displayed for every size and shape parameter. Furthermore, the coefficient of genetic variation (CV_A) and its square (I_A) for the size parameters are shown. Significant *p* values are written in bold.

Haplotype t, 19°C						
	V_A	V_{res}	H_2	CV_A	I_A	*p*
Length	60845.55	41509.81	0.59	0.004	1.60e-09	**< 0.01**
Centroid size	101464.7	119119.5	0.46	0.0024	5.61e-10	**< 0.01**
PC 1	0.000083	0.000298	0.22	-	-	**< 0.01**
PC 2	0.000148	0.000174	0.46	-	-	**< 0.01**
PC 3	0.000051	0.00018	0.22	-	-	**< 0.01**

Table 31. Genetic variance in haplotype t of the F1 generation at 23°C. Overall genetic variance (V_A), residual variance (V_{res}), and broad-sense heritability (H^2) are displayed for every size and shape parameter. Furthermore, the coefficient of genetic variation (CV_A) and its square (I_A) for the size parameters are shown. Significant *p* values are written in bold.

Haplotype t, 23°C						
	V_A	V_{res}	H_2	CV_A	I_A	*p*
Length	25144.97	45184.48	0.36	0.0017	2.73e-10	**< 0.01**
Centroid size	45672.2	112898.1	0.29	0.0011	1.14e-10	**< 0.01**
PC 1	0.000067	0.000297	0.18	-	-	**< 0.01**
PC 2	0.00009	0.000142	0.39	-	-	**< 0.01**
PC 3	0.000069	0.000148	0.32	-	-	**< 0.01**

Table 32. Genetic variance in haplotype z of the F1 generation at 15°C. Overall genetic variance (V_A), residual variance (V_{res}), and broad-sense heritability (H^2) are displayed for every size and shape parameter. Furthermore, the coefficient of genetic variation (CV_A) and its square (I_A) for the size parameters are shown. Significant *p* values are written in bold.

Haplotype z, 15°C						
	V_A	V_{res}	H_2	CV_A	I_A	*p*
Length	21593.71	38882.8	0.36	0.002	3.86e-10	**< 0.01**
Centroid size	45368.73	142690.28	0.24	0.0014	1.84e-10	**< 0.01**
PC 1	0.000108	0.000252	0.30	-	-	**< 0.01**
PC 2	0.000179	0.000191	0.48	-	-	**< 0.01**
PC 3	0.000034	0.000130	0.21	-	-	**< 0.01**

Table 33. Genetic variance in haplotype z of the F1 generation at 19°C. Overall genetic variance (V_A), residual variance (V_{res}), and broad-sense heritability (H^2) are displayed for every size and shape parameter. Furthermore, the coefficient of genetic variation (CV_A) and its square (I_A) for the size parameters are shown. Significant *p* values are written in bold.

Haplotype z, 19°C						
	V_A	V_{res}	H_2	CV_A	I_A	*p*
Length	15601.36	26220.15	0.37	0.0014	2.01e-10	**< 0.01**
Centroid size	41061.25	130756.52	0.24	0.0012	1.51e-10	**< 0.01**
PC 1	0.000074	0.000346	0.18	-	-	**< 0.01**
PC 2	0.000111	0.000248	0.31	-	-	**< 0.01**
PC 3	0.000039	0.000165	0.19	-	-	**< 0.01**

Table 34. Genetic variance in haplotype z of the F1 generation at 23°C. Overall genetic variance (V_A), residual variance (V_{res}), and broad-sense heritability (H^2) are displayed for every size and shape parameter. Furthermore, the coefficient of genetic variation (CV_A) and its square (I_A) for the size parameters are shown. Significant *p* values are written in bold.

Haplotype z, 23°C						
	V_A	V_{res}	H_2	CV_A	I_A	*p*
Length	18380.78	35619.87	0.34	0.0017	2.79e-10	**< 0.01**
Centroid size	67846.27	115495.07	0.37	0.0020	4.12e-10	**< 0.01**
PC 1	0.000206	0.000389	0.35	-	-	**< 0.01**
PC 2	0.000159	0.000189	0.46	-	-	**< 0.01**
PC 3	0.000101	0.000148	0.40	-	-	**< 0.01**

In Tables 35, 36 and 37, the values of heritability and the coefficients of genetic variance and I_A for the three distinct temperatures of the F2 generation are shown. In comparison to the heritability values from the F1 generation, the values for the F2 generation decreased noticeably for both, size and shape. The heritabilities of the two size parameters, length and centroid size, we estimated values from 0.0026 to 0.69. The genetic differentiation could partly or not at all explain the overall detected variation. The values at 15°C and at 23°C were higher than at 19°C. Unfortunately, the heritability values for PC 1 and 2 at 15°C could not be calculated properly as they ranged around 0. The low number of individuals also complicated the estimation of the 19°C values of the heritabilities in size which may be a reason for the non-significant p-values. Except for the 19°C climate cabinet, the heritability estimates for size were bigger than for shape, just as in the F1 generation. The heritability values for shape were higher at 23°C. This can be explained

with the difficulties due to values ranging around 0 and a low sample size, which we faced when estimating the heritability values of 15°C and 19°C.

Compared to the F1 generation, CV_A and I_A values became lower in the F2 generation. The CV_A values for both size parameters ranged from 0.000324% to 0.76% and therefore were below 0 across all temperatures. Except for the 23°C, the CV_A values for centroid size were somewhat smaller than the values for length. The very low I_A values indicate an even smaller capability to react to selection than in the F1 generation, for example low evolvability, especially at 19°C.

Table 35. Genetic variance at 15°C in the two Portuguese populations, Alcabideque and Póvoas village, in the F2 generation. Overall genetic variance (V_A), residual variance (V_{res}), and broad-sense heritability (H^2) are displayed for every size and shape parameter. Furthermore, the coefficient of genetic variation (CV_A) and its square (I_A) for the size parameters are shown. Significant *p* values are written in bold.

15°C						
	V_A	V_{res}	H^2	CV_A	I_A	*p*
Length	67048.81	29968.47	0.69	0.0061	3.75e-09	**< 0.01**
Centroid size	40412.13	67341.23	0.38	0.00097	9.56e-11	**0.0032**
PC 1	0.00	0.000174	0	-	-	-
PC 2	0.00	0.000205	0	-	-	**< 0.01**
PC 3	2.53e-05	1.69e-04	0.13	-	-	0.0787

Table 36. Genetic variance at 19°C in the two Portuguese populations, Alcabideque and Póvoas village, in the F2 generation. Overall genetic variance (V_A), residual variance (V_{res}), and broad-sense heritability (H^2) are displayed for every size and shape parameter. Furthermore, the coefficient of genetic variation (CV_A) and its square (I_A) for the size parameters are shown. Significant *p* values are written in bold.

19°C						
	V_A	V_{res}	H^2	CV_A	I_A	*p*
Length	70.78	27474.75	0.0026	6.47e-06	4.18e-15	0.3081
Centroid size	133.83	33297.696	0.0040	3.24e-06	1.05e-15	0.3106
PC 1	8.88e-05	3.27e-04	0.21	-	-	0.06
PC 2	2.36e-05	4.45e-04	0.05	-	-	0.3281
PC 3	2.17e-05	1.48e-04	0.13	-	-	0.2285

Table 37. Genetic variance at 23°C in the two Portuguese populations, Alcabideque and Póvoas village, in the F2 generation. Overall genetic variance (V_A), residual variance (V_{res}), and broad-sense heritability (H^2) are displayed for every size and shape parameter. Furthermore, the coefficient of genetic variation (CV_A) and its square (I_A) for the size parameters are shown. Significant *p* values are written in bold.

23°C						
	V_A	V_{res}	H^2	CV_A	I_A	*p*
Length	34553.12	48191.01	0.42	0.003156	9.96e-10	**< 0.01**
Centroid size	317304.1	163484.2	0.66	0.007676	5.89e-09	**< 0.01**
PC 1	0.000241	0.000457	0.35	-	-	**< 0.01**
PC 2	0.000205	0.000352	0.37	-	-	**< 0.01**
PC 3	0.000111	0.000227	0.33	-	-	**< 0.01**

Life history

We found a significant difference across haplotypes in the reproductive rates of the parental generations (Kruskal-Wallis chi-squared = 45.311, df = 24, p < 0.01). A high correlation was indicated by temperature as well as by temperature difference, the difference in water temperature between the habitat at collection time and climate cabinet. The generalized linear model which was the most appropriate contained haplotype and the climate cabinet temperature as well as their interaction as fixed factors (Table 38). The parental snails possessing haplotype t demonstrated a slower reproductive rate than mother snails with haplotype z across all temperatures. Furthermore, they terminated their reproduction approximately 99 days later than individuals with haplotype z. The average of the reproductive rate of mother snails with haplotype t was 566.28 days. Mother snails with haplotype t were significantly affected by temperature (Kruskal-Wallis chi-squared = 11.929, df = 2, p < 0.01). Solely the mother snails from the 15°C and the 23°C climate cabinet varied significantly within haplotype t. In contrast, temperature did not have any significant influence on mother snails with haplotype z (Figure 39). With 467.71 days, snails with haplotype t had a lower reproductive rate than snails with haplotype z.

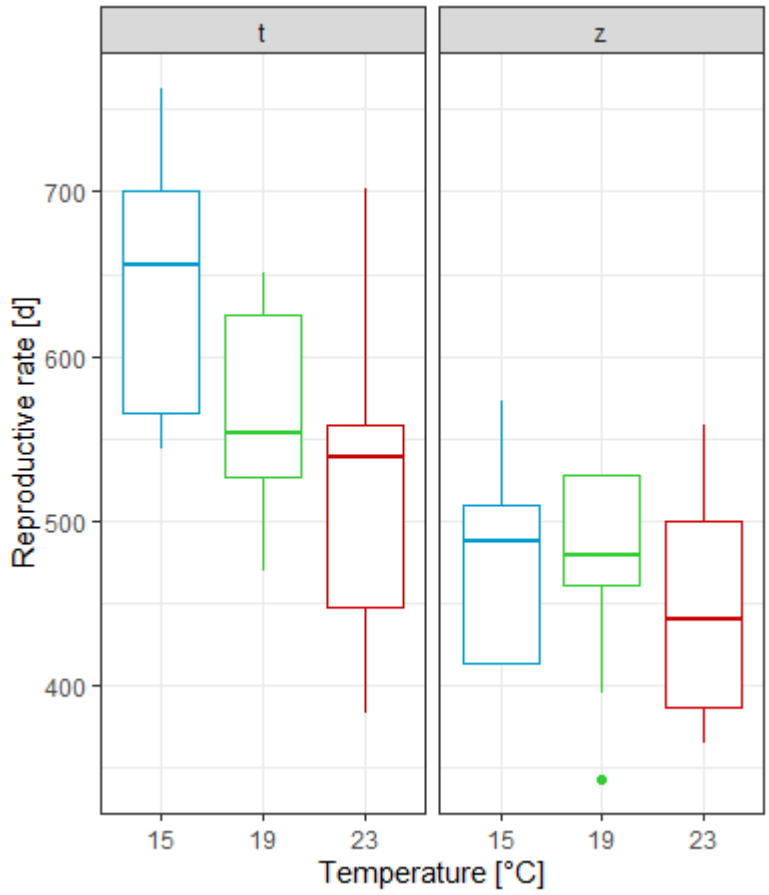

Figure 39. Reproductive rate of the parental generation depending on climate cabinet temperature and haplotype. Only the mother snails possessing haplotype t of the 15°C and the 23 °C climate cabinets showed a significant difference.

Table 38. Generalized linear model of the reproductive rate including the fixed factors, the temperature in the climate cabinets, haplotype and the temperature/haplotype interaction in the parental generation. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

	Estimate	**Standard error**	**t-value**	**p-value**
Intercept	861.827	67.374	12.792	< 2e-16 ***
Temperature	-14.834	3.336	-4.447	3.05e-05 ***
Haplotype z	-321.039	93.939	-3.418	0.00104 **
Temperature x Haplotype z	10.988	4.759	2.309	0.02378 *

Methylation patterns in molluscs

DNA methyltransferases distribution in molluscs

The homologues of all three DNMTs were detected in the genomes and transcriptomes of all eight mollusc classes (Supplemental Table S2). DNTM2 which is not further referred to in the thesis is also displayed in Supplemental Table S2. We did not detect both DNMT1 and DNMT3 in nine of the 140 examined species. Those nine species were only vaguely akin to each other and from distinct mollusc classes: (i) *Eurhomalea rufa*, *Mytilus galloprovincialis*, *Mytilus trossulus* and *Septifer virgata* from the class Bivavlia; (ii) *Sepia pharaonis* and *Sepioloidea lineolata* from the family Cephalopoda; and (iii) the gastropods *Haliotis fulgens*, *Plakobranchus ocellatus* and *Siphonaria pectinata*. We found a DNMT1 homologue in all investigated mollusc species except for the following three: (i) the cephalopod *Sepiella maindroni*, in (ii) *Margaritifera margaritifera* and (iii) *Placopecten magellanicus* from the bivalves. Contrary to the DNMT1 homologues, we continuously did not discover DNMT3 homologues in every species of specific taxonomic groups which are the following: (i) the order Oegopsida within Cephalopoda with seven species from five distinct families (*Chiroteuthis calyx*, *Dosidicus gigas* and *Stenoteuthis oualaniensis*, *Octopoteuthis deletron*, *Onychoteuthis banksii*, *Pterygioteuthis hoylei* and *Watasenia scintillans*). Furthermore, (ii) the subclass Heterobranchia within the Gastropoda with 21 not closely related species (*Melibe leonina*, *Phylliroe bucephala*, *Aplysia californica*, *Limacina antarctica*, *L. helicina*, *L. retroversa*, *Clione limacina*, *Elysia chlorotica*, *E. cornigera*, *E. timida*, *P. ocellatus*, *Siphonaria pectinata*, *B. glabrata*, *B. pfeifferi*, *Lymnaea stagnalis*, *Physella acuta*, *Radix auricularia*, *Achatina fulica*, *Arion vulgaris*, *Bradybaena similaris* and *Cepaea nemoralis*). Regarding DNTM3, DNMT3 was also not found in single representatives of distinct taxonomic groups: further twelve bivalves, eight caenogastropods, one caudofoveate, one additional decapodiform cephalopod, one patellogastropod, more two polyplacophorans, two scaphopods, three solenogastres and three vetigastropods. The presence and absence of DNMT1 to 3 of every investigated species can be viewed in Supplemental Table S2.

We conducted GLMs with binary response variable (presence/absence) and logit link function which undoubtedly indicated a direct connection between the presence/absence of DNMTs and the quality scores for the genomes and transcriptomes. Complete BUSCOs, present BUSCOs and total sequence length influenced the finding of DNMT1 [$G = 14.476$, $p = 0.00014$ (complete BUSCOs); $G = 17.821$, $p < 0.00005$ (present BUSCOs); $G = 6.718$, $p = 0.00954$ (total sequence length)]. In contrary, the contigs number did not show any significant influence ($G = 2.725$, $p = 0.09882$). Complete BUSCOs and present BUSCOs had a significant effect on the presence/absence of DNMT3 [$G = 11.47$, $p = 0.00071$ (complete BUSCOs); $G = 8.048$, $p = 0.00456$ (present BUSCOs)], whereas the contigs number and the total sequence length did

not show any significance [G = 0.016, p = 0.90095 (contigs number); G = 1.297, p = 0.25477 (total sequence length)]. Figure 40 displays the dispersion of DNMTs and BUSCO integrity across classes.

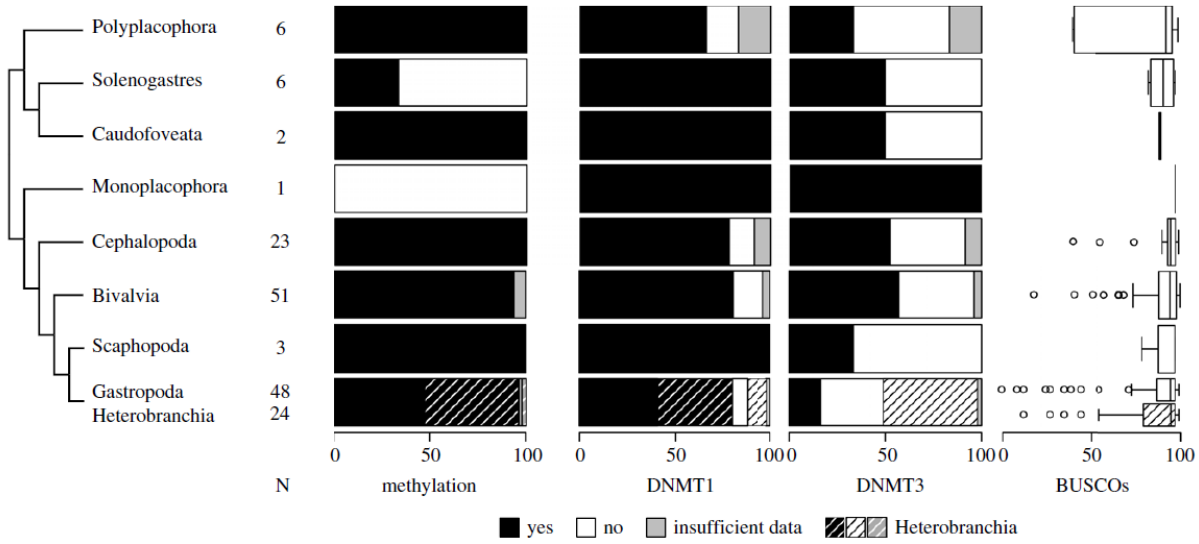

Figure 40. Methylation in mollusc classes. From left to right: Topology showing the relationships across the eight mollusc classes and the sub-class Heterobranchia; number of examined species; proportion of species exhibiting methylation inferred from CpG o/e analysis in %; proportion of species displaying the presence of DNMT1 and DNMT3 in %; boxplot of present BUSCO percentages (present = 100 – missing; $N_{metazoa\_odb10}$ = 954). The dashed boxplots of Heterobranchia are presented as subgroups stacked on the boxplots of Gastropoda. Within the gastropods, the top white boxplot represents the dispersion across all examined Gastropoda, while the bottom dashed boxplot represents the dispersion inside the Heterobranchia.

CpG observed/expected distributions

The CpG o/e values of protein-coding sequences with 140 species representing all mollusc classes were generated to conclude the presence of DNA methylation in molluscs. We inferred DNA methylation in seven out of eight mollusc classes. Therefore, we think that DNA methylation is common in the phylum. The only class in which we were not able to detect any prove of DNA methylation was the class of the Monoplacophora. However, we could only investigate one monoplacophoran species, *Laevipilina hyalina*. We additionally could not find any evidence of DNA methylation in the following four out of six representatives of the Solenogastres: *Greenland neomeniomorph*, *Neomeniomorpha* sp1, *P. custodiens* and *Wirenia argentea* (Figure 40, Supplemental Table S2).

Both "Bimodal depleted" and "unimodal, indicative of methylation" CpG o/e distribution patterns were detected in molluscs. In Bivalvia and Cephalopoda, we mainly found bimodal CpG o/e distribution patterns. Apart from *Chaetoderma nitidulum*, we only found unimodal CpG o/e distribution patterns in Aplacophora (Caudofoveata and Solenogastres). Except for *Littorina saxatilis* and *P. antipodarum* which displayed "unimodal, indicative of methylation" CpG o/e distribution patterns, all gastropods showed bimodal CpG o/e distribution patterns. We detected the two patterns in Polyplacophora and Scaphopoda. Further information can be seen in Supplemental Table S2.

In comparison to other mollusc classes, except for Monoplacophora, we found greater total mean CpG o/e values in Solenogastres (> 0.9) in protein-coding sequences, indicating a reduced mean DNA methylation in the germline. In the monoplacophorans, the mean CpG o/e value was almost 0.9 and they did not display any signature of cytosine DNA methylation. The mean CpG o/e values of the other molluscan classes were as followed: Caudofoveata 0.66, Plyplcophora 0.7, in the majority of Cephalopoda and Bivalvia between 0.58 and 0.62, Scaphopoda 0.73 and approximately 0.57 in Gastropoda. However, we detected two exceptions to Cephalopoda and Bivalvia. First, a CpG depletion was shown by *Nautilus pompilius* from the Cephalopoda having a mean CpG o/e value of 0.45. Second, *M. margaritifera* and *Mytilus edulis* from the class Bivalvia exhibited mean CpG o/e values of 0.41 and 0.43 accordingly which refers to a high CpG depletion. For two other bivalvian representatives, *Dreissena rostriformes* and *D. polymorpha*, we calculated quite high mean CpG o/e values: 0.74 and 0.8, respectively. The NZMS, *P. antipodarum* was a deviation within the Gastropoda and showed a mean CpG o/e value of 0.78. This refers to a poorer cytosine DNA methylation. On the contrary, *S. pectinata* and *B. glabrata*, also from Gastropoda, had CpG o/e values of 0.39 and 0.45, accordingly which indicates a massive CpG depletion. The mean CpG o/e values of all eight mollusc classes can be seen in Figure 41.

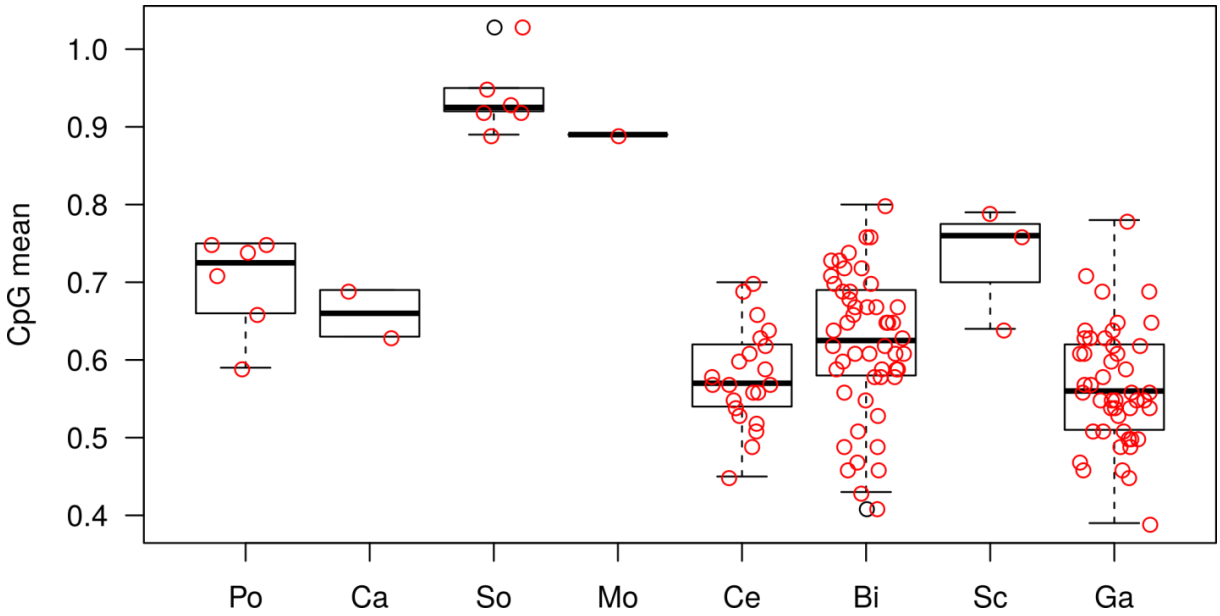

Figure 41. Mean CpG o/e values and distribution of the data points of the eight mollusc classes arranged in accordance with the tree shown in Figure 4. Outliners are displayed as black dots, the distribution of the data points as red dots. Bi, Bivalvia; Ca, Caudofoveata, Ce, Cephalopoda; Ga, Gastropoda; Mo, Monoplacophora; Po, Polyplacophora; Sc, Scaphopoda; So, Solenogastres.

V. DISCUSSION

Differentiation of genetic adaptation and phenotypic plasticity in the morphologically variable shell of the NZMS

To disentangle genetic and environmental effects on the variability of the shell morphology of the New Zealand mud snail (NZMS) and to calculate the heritability of its shell size and shape, we executed a common garden experiment. We kept parental snails and reared and raised two daughter generations from eleven European NZMS populations under three different temperatures. We were able to differentiate shell size and shell shape using the geometric morphometrics approach (Männer et al., 2022). When colonizing Europe as an invasive species, the NZMS most likely went through a genetic bottleneck. The first populations had a low genetic variation. The colonization event took place only 180 to 360 generations ago and therefore it should be unlikely that populations nowadays already recovered its genetic variance (Ponder, 1988; Verhaegen et al., 2021). However, European NZMS populations show a noticeable variation in their shell morphology. In the face of climate change and the rising temperatures that we are experiencing all over the world, we had a closer look on the genetic and phenotypically plastic responses to different temperature conditions (Masson-Delmotte et al., 2021).

Morphological traits

We observed a high variability in the shell morphology of the parental generation. Population explained the variation observed in shell size and shape, whereas the haplotype only explained the variation in shape. Just like in our study in the parental generation, Kistner & Dybdahl (2013) encountered a considerable shell shape variation in invasive NZMS. The variation in the shell morphology of the parental generation in our experiment was mainly population-specific – probably due to phenotypic plasticity.

The snails of the F1 and the F2 generation had smaller shells than the parental generation. Furthermore, as predicted in the hypothesis, the shell morphology of the F1 and the F2 generation became more similar and under same environmental conditions and across the three temperatures. In the sea snail, *Monetaria annulus*, the variation in size measured between wild populations decreased in the offspring generation which was reared in a common garden experiment (Irie & Morimoto, 2008). Our results also agreed with those of Kistner & Dybdahl (2013). They collected NZMS from three geographically distinct populations from North-America and kept them in a

common garden setting. The snails of the daughter generation which were reared in the common garden experiment became more similar to each other and possessed smaller shells than the individuals from the parental generation. However, our results concerning the shell shape of the F1 generation did not match Kistner & Dybdahls (2013) results. The variation in shell size in the North-American offspring was population-dependent, just as in the parental generation. The shell shape variation in the European F1 generation however, was temperature and haplotype-dependent. Between 42% and 55% of the shell size variation in the F1 generation could be described by temperature and haplotype. Interestingly, we detected the population-specific size difference in the Portuguese F2 generation in our experiment. Differences in the shell morphology of the F2 generation were mainly temperature and population-dependent which described between 32% and 47% of the shell size variation.

The smaller shell size in the F1 and the F2 generations might also be influenced by other factors, such as missing water flow or the water depth (Jokela, Lively, Dybdahl, et al., 1997; Verhaegen, McElroy, et al., 2018). Liess & Lange (2011) excluded the food quality to have a high impact on the growth rate of the NZMS. Of course, it is impossible to exactly replicate the conditions experienced by the snail in its natural habitat in the common garden experiment (de Villemereuil et al., 2016; Verhaegen, McElroy, et al., 2018). Still, we did our best to create an artificial environment that is as similar as possible to the natural habitat. The lack of water current or the water depth, however, may have had an impact on the shell growth. NZMS in open waters and in water bodies with a stronger current tend to possess a larger shell than individuals from swamps and seepages. A larger shell normally has a larger shell opening for the snail´s foot which enables the snail to possess a larger area to attach to the surface. Therefore, a larger foot may prevent the snail from dislodgement and resist a stronger water current (Haase, 2003). Since the snail in our experiment did not experience any water current, it may have influenced the shell growth of the offspring generations.

The shell size of the daughter generations F1 and F2 was generally bigger in the coldest temperature at 15°C which is consistent with our hypothesis that snails which were reared under lower temperatures would evolve larger shells. On the other hand, the body whorl enlarged in comparison to the spire with augmenting temperatures in both haplotypes. The body whorl contains the distal genitalia where the embryos are incubated. The enlargement of the body whorl was more noticeable in mother snails with haplotype z than in mother snails with haplotype t. This disagrees with our hypothesis that snails reared under colder temperatures would evolve more globular shells. The reaction of size followed the temperature-size-rule. The reaction of shape may be a compensation for the decrease in size in snails which were reared in the warmest temperature

to supply more room for embryos. The temperature-size-rule explains the thermal plasticity which we observed in the snails of the daughter generations. The temperature-size-rule states that organism invest more energy in the growth period and therefore start later with their reproduction since they mature at a larger body size. This can be observed with many ectotherm taxa (Atkinson, 1994, 1995; Atkinson et al., 2003; Angilletta et al., 2004). The limited genetic variation restricting the phenotypically plastic reaction may be the reason of the subtle response. This reaction may be explained with the frozen-phenotype variation model (FPV) (Jokela, Lively, Dybdahl, et al., 1997) although investigations have to be done to clarify the variation of the phenotypically plastic response of shape for a higher amount of clones. The frozen niche-variation (FNV) hypothesis proposes that clones arbitrarily collect and "freeze" the genotypes of their ancestor sexual populations. This way, only a part of the detected complete niche-use variation of the sexual population is shown by each clone possibly resulting in a selection for ecological specialization and the concurrence of clones (Vrijenhoek, 1979, 1984). The FPV model is a common form of the FNV hypothesis suggesting that the same conclusions apply to other traits which have essential effects on fitness, such as life-history traits, still, are not directly correlate with niche use.

We observed that the parental generation kept in the 15°C climate cabinet reproduced later and slower than snails which were kept in warmer temperatures. This is in accordance with the observations of Gust et al. (2011) who observed that the exposure temperature has a significant impact on the reproduction in the NZMS. They observed faster reproduction kinetics with snails held at warmer temperatures. The however also detected that snails which were kept at the medium temperature (16°C), had more embryos in their brood poach than snails kept at lower (8°C) and higher temperatures (24°C). In contrary, we noticed during the experiment that the mother snails from the 23°C climate cabinet produced more offspring than snails from the 15°C or the 19°C climate cabinets. Still, those results match our hypothesis that snails held under colder temperatures, have lower reproductive rates. Although snails with a larger shell have more space for their brood pouch and therefore for more embryos, the snails from the F1 generation which were reared in the 15°C climate cabinet did not reproduce more offspring than the snails from the warmer temperatures (Verhaegen, McElroy, et al., 2018). In general, we observed snails with haplotype z reproducing faster and more successfully with more offspring than snails with haplotype t. Further experiments may clarify if our observations may due to an interaction of reproductive traits caused by various components (Angilletta et al., 2004).

Haplotypes primarily explained the variation in the reaction norm slopes of the F1 generation. We detected 15 of the significant pairwise comparisons between haplotypes and only one each within haplotypes. Concerning the thermal plasticity response of size, this means that the

variation in size was mainly caused by the genetic differentiation and haplotype-specific. Both haplotypes show a crossover genotype-by-environment interaction (GxE) and exhibit diverse plastic responses which produce the most beneficial genotype to adapt to different environmental conditions (Kusmec et al., 2018). A population-specific variation was only recognisable to a limited extent (Pigliucci, 2005). Since Dybdahl & Kane (2005) also found a strong effect of temperature on size, but no population-specific variation, their results match our results of the common garden experiment. In contrast to our experimental set-up, they investigated three populations from North America and they set themselves a time limit for their experiment. We wanted the snails of our experiment to reach their adult size to investigate the morphological effects of temperature. This is why we used the geometric morphometrics approach to be able to clearly distinguish between size and shape. Environmental conditions may elicit phenotypic variation, still, the capacity to react has a genetic footing (Pazzaglia et al., 2021). Recent research indicates that epigenetic variations may partially cause this capacity (Douhovnikoff & Dodd, 2015). Also Kistner & Dybdahl (2013) suggest that the variation in the shell morphology of the NZMS may be influenced by an interplay of phenotypic plasticity and evolution.

Snails of the F1 generation differed in size between the two haplotypes. Just like Verhaegen, McElroy, et al. (2018), we observed snails possessing haplotype z being squatter than the snails possessing haplotype t. Comparing all populations between the parental and the F1 generation, we estimated lower PC 1 and 2 values and higher PC 3 values in the parental generation. Snails of the F1 generation therefore possess more slender shells and a comparatively smaller body whorl than their mothers (Figure 18). The comparison between all three generations of the two Portuguese populations, Alcabideque and Póvoas village, reveals that the parental and the F1 generation have slightly higher PC 1 values than the F2 generation. Snails of the F2 generation thus possess a shell which is broader at the bottom and has a broader aperture. The two daughter generations have lower PC 2 and higher PC 3 values resulting in narrower shell with a smaller aperture. In comparison with the parental generation, snails of the F1 generation became more similar in size and in shape across all eleven populations. This was more visible with size than with shape. The daughter generations F1 and F2 from Alcabideque and Póvoas village showed more variation in size than the parental generation. However, this result might be tested by further experiments because we only investigated a low sample size of two populations that possess the same haplotype. Although some influence on the variation in shape could be observed across the three generations of all populations, the fixed factors explained little of the total variation in shape: haplotype only explained 12% of the variation in PC 1, haplotype, population and temperature 18% of the variation in PC 2 and haplotype and temperature 21% of the variation in PC 3. This may lead to the conclusion that the main variation in shape is caused by genetic differentiation. Additionally,

the outcomes of the reaction norms point in the same direction explaining less variation in three significant pairwise comparisons across the populations in PC 1, nine in PC 2 and one in PC 3. This is why we observed more plastic responses in size than in shape. In some species, the lack of phenotypic plasticity in shape might be associated with a higher tolerance to changes in temperature (Albarrán-Mélzer et al., 2020).

Unfortunately, so far there have been very few studies on plastic reactions of shell morphology of freshwater snails to temperature although we face climate change and global warming on a daily basis (Whelan, 2021). In contrast to the two daughter generations of our common garden experiment, the north-American snail *Physa virgata* developed more slender shells at colder temperatures (Britton & McMahon, 2004). The trumpet snail *Tarebia granifera* which is invasive to southern-Mexican lakes, did hardly show any shell shape variation across different temperatures. In comparison, but in accordance with our results, *Pyrgophorus coronatus*, a mud snail which is native to the same southern-Mexican lakes, showed more globular shells at colder temperatures and a higher shell shape plasticity. Therefore, not all invasive species tend to have a high phenotypic plasticity (Albarrán-Mélzer et al., 2020). In contrast to the NZMS, the apple snail, *Pomacea canaliculata*, showed higher growth rates at higher temperatures resulting in larger and thinner shells possessing broader apertures (Tamburi et al., 2018). As diverse as molluscs are, as diverse are their responses to changing temperatures. Since molluscs live in a variety of different habitats with various phenotypes, the origins and evolution of their diverse phenotypes has not yet been clarified (Haszprunar & Wanninger, 2012; Wanninger & Wollesen, 2019). Different factors and combinations of different components presumably contributed to the high variation in phenotypically plastic reactions. Experimental set ups including the investigation of a combination of different components may help to shed light on the origins and causes of the diverse phenotypically plastic responses in gastropods.

Maternal ID

The exact definition of the maternal effect and its importance is still not clarified completely, even though it has already been described more than half a century ago by quantitative geneticists. Following the definition of Wolf & Wade (2009), we understand maternal effects as the causal impact of the genotype or phenotype of the mother on the phenotype of the offspring. Changes in the phenotype of the offspring are often adaptive maternal effects as response to changing environmental conditions (Marshall et al., 2008). Although maternal effects are thought to be "adaptive" as a result for the fitness of the offspring (Mousseau & Fox, 1998), they may as well be

considered as "shared phenotype" which has an concurrent effect on the maternal and the offspring fitness (Marshall & Uller, 2007). Still, selection normally increases the maternal and not the offspring fitness, especially in species with no post-natal maternal care like the NZMS (Smith & Fretwell, 1974; Bernardo, 1996). Smaller and more offspring are produced by the mother, since selection acts to increase maternal fitness. The fitness of the individual offspring is thereby sacrificed for the maternal fitness (Einum & Fleming, 2000).

In the F1 generation, the maternal effects of the size parameter, length and centroid size, explained approximately 20% of the variation. We also calculated the maternal effect by setting the "maternal ID" as random factor in our models. In the F2 generation, less than 20% of the variation was explained by the maternal effect in length. In centroid size however, 31% of the variance was due to the maternal effect (Table 17). Dybdahl & Kane (2005) detected maternal effects for size and age at first reproduction in the NZMS populations from North America. In the F1 generation, the maternal effect explained about 20% of variance in PC 1 and 3. In PC 2, 31% of the variance was caused by the maternal effect. In the F2 generation, maternal effect described between 12% and 16% of the variation on the shape parameter. Maternal effects for shell shape could not be reported by Kistner & Dybdahl (2013). Still, Smithson et al. (2020) detected that habitat-specific adaptive shell shape in different environments was comparatively stable across one generation. Nevertheless, those investigations applied other experimental ways to estimate shape. Nevertheless, part of the variation in the shell morphology of the NZMS may be due to maternal effects.

In our common garden experiment, the offspring were kept in the same jar as the mother snail until fixation. Therefore, we cannot differentiate the impact of the mother snail from the conditions in the respective jar. In the course of our experiment, we aimed at maintaining equal conditions across the jars in all three climate cabinets (apart from temperature). Still, it was impossible to eliminate all potential inequalities. Due to limited space capacities, we could not separate offspring snails and rear them in individual jars. Instead, we kept them together with their mother snail in the same jar. Since our mother snails reproduced at different times and different amounts of offspring, the snail densities in the individual jars clearly varied. When the offspring grew up, we tried to limit the number of offspring per jar to 15 offspring. It is however known that population densities in the NZMS have an impact on life history traits (Cope & Winterbourn, 2004; Neiman et al., 2013; Zachar & Neiman, 2013). Furthermore, we were not able to adapt the quantity of food algae which we fed the snails to the number of individuals in one jar because of the constant reproduction of some mother snails, it was hard to determine the exact number of offspring in a jar. The feeding rates in the NZMS also differ depending on the genetic variation (Jacobsen &

Forbes, 1997). The density of food has furthermore an effect on life history traits (Neiman et al., 2013). We also observed too much algal food having a negative impact on the water quality in the jar. When feeding to much algae, the water quality decreased. We could also not control the quantity and the formation of different epiphytic algae which were growing on the shells of the parental generation and which were brought into the jars. Feeding too much algal food probably interacted with the epiphytic algae on the shells of the parental snails and disturbed the water purity.

We were not able to measure a potential maternal effect on the reaction norms, despite being able to estimate a small but existent maternal effect in the mixed models. Thus, the differences which we saw in the reaction norms might be partially due to a maternal effect. The following arguments provide an indication why the shell morphologies were presumably not influenced by real maternal effects. Firstly, we already observed a significant difference mainly in size between parental and F1 generation. Furthermore, although the mother snails grew up and lived in different habitats, the snails from the F1 generation became more similar to each other. Lastly, the outcomes of the F2 generation did not show any large differences from the ones of the F1 generation although we were not able to compare across all populations in the F2 generation. Thus, if there is any true maternal effect present, heritable epigenetic modifications, for example, must have made it persistent across more than one generation (Youngson & Whitelaw, 2008; Ho & Burggren, 2010; Heard & Martienssen, 2014). Epigenetic mechanisms, such as DNA methylation can silence genes over generations without any alteration in the respective gene sequence. Additionally, the environmental impacts of our experiment must have been bigger than the maternal effect. To estimate a true maternal effect, further experiments across various generations would be necessary. Also, the knowledge of epigenetic mechanisms in molluscs remains rather scarce until now (Fallet et al., 2020). Investigating epigenetic mechanisms, such as DNA methylation, in molluscs, would help to understand the processes underlying a potential maternal effect.

Heritability

The heritability values for size ranged between 0.49 – 0.80 in the eleven populations of the F1 generation. In comparison to other studies about the NZMS, those values were rather high (Dybdahl & Kane, 2005). Still, Dybdahl & Kane (2005) estimated heritability values for life history traits, whereas we calculated the heritabilities for morphological traits. Also, when comparing our results to other species (Fischer et al., 2021), we calculated higher heritability values in the shell morphology parameter of the NZMS. This means that a high percentage of the variation in the shell morphology of the F1 generation across all temperatures can be explained genetically. Generally, it is known that morphological traits have higher heritabilities than life history traits.

This indicates that high heritabilities are kept within wild populations, also for traits which are supposed to be under strong selection. Furthermore, the heritability of morphological traits is higher in ectothermic than in endothermic species, probably because of the high correlation between life history and body size which can be observed in many ectotherms (Mousseau & Roff, 1987). This can also be seen in the NZMS, since a correlation between the shell morphology and the number of embryos which it carries in its brood pouch, could be detected (McKenzie et al., 2013; Verhaegen, McElroy, et al., 2018).

The snails of the F1 generation with haplotype t generally possessed higher heritability values than F1 snails with haplotype z. Additionally, they were more susceptible to the three different temperatures – although all heritability values showed a susceptibility across temperatures. Thus, the variation in shell morphology in snails with haplotype t is more genetically and less environmentally based than the variation in snails with haplotype z. However, the variation in shell morphology is influenced both by genetic components and environmental components. Snails from different populations were living in habitats with diverse environmental conditions and presumably possessing a differing genetic diversity. The snails from the different populations therefore also respond in a different way to changing environmental conditions, such as temperature, since the genetic basis is likely different.

In our hypothesis, we expected a low broad-sense heritability, because we assumed that the variability of the phenotypic traits could mainly be explained by the environment. In contrast to our hypothesis, we calculated high heritability values in both shell size and shell shape. We indeed found out that environmental conditions caused part of the phenotypic variation. This can also be seen in the heritabilities which we estimated across temperature and haplotype showing lower values which are also more commonly represented in the literature (Fischer et al., 2021). However, we also saw that the haplotype has a strong impact on the variation in the shell morphology of the F1 generation. The ability to react to environmental changes seems to have a genetic basis (Pazzaglia et al., 2021). As Verhaegen, McElroy, et al. (2018) already showed, an interplay of different environmental parameters influences the variable shell morphology of the NZMS in Europe. Therefore, temperature alone may not show a strong influence on the variation in shell shape and size, but maybe the combination and interplay of different environmental parameters.

The heritability values of the two Portuguese populations in the F2 generation were lower than the heritability values from the F1 generation. The heritability values in size for the F2 generation ranged between zero and 0.69 across all temperatures. The heritability values across size were generally larger than across shape with the 15°C and the 23°C snails. Only the 19°C snails had higher heritability values in shape than in size. Due to low sample sizes in snails from the 15°C

and the 19°C, we estimated values like zero or values close to zero which are not reliable. Furthermore, the p-values of some heritability values in the F2 generation were not significant. For the calculation of the heritability values in the F2 generation, we only used snails from two Portuguese populations due to the low number of snails in the other populations. Consequently, we were not able to compare the heritabilities between the two haplotypes t and z leading to lower heritability values. Since the heritability estimation of the F1 generation was done across all eleven populations and across the two haplotypes, more variability was given. To compare the values of the F1 and the F2 generation, a higher sample size of the F2 generation has to be provided, because an interplay of different variables may determine the variation in the shell morphology of the NZMS. Furthermore, the heritability estimates of the two daughter generations have to be calculated across the same populations and haplotypes.

Since heritability is not a suitable way to estimate and contrast evolvabilities, we calculated the coefficient of genetic variation CV_A and its square I_A. Evolvability is better estimated on the basis of genetic variation normalized by the trait means and not on the basis of the complete phenotypic variation (Houle, 1992; Hansen et al., 2011; Garcia-Gonzalez et al., 2012). Because of the negative values of the shape parameters PC 1-3, it was not possible for us to calculate the CV_A and the I_A for shape. The resulting values would have been too high to provide any reliable comparison. The values of both, CV_A and I_A, are higher in the F1 generation than in the F2 generation. Still, the comparison of the two daughter generations might be misleading due to the unequal population input for the calculations. Except for the values at 23°C, the CV_A and the I_A values of the F2 generation were mostly also smaller than the F1 generation values which were calculated individually for each haplotype. Except for the values at 23°C in the F2 generation, the values for both daughter generations were higher in length than in shape. Therefore, size seems to have a higher evolvability than shape in general. The smallest values were found at 15°C in the F1 generation an at 19°C in the F2 generation. However, the p-values for the values at 19°C in the F2 generation were not significant suggesting that the estimated numbers do not allow any trustworthy conclusion. CV_A and I_A values in the F1 generation were commonly higher in haplotype t than in haplotype z across all temperatures indicating a higher evolvability in snails with haplotype t. The additive generic variance was displaced with clonal repeatability since invasive NZMS populations are parthenogenetic. The values of all our calculations of both daughter generations were exceedingly low suggesting that the NZMS populations from Europe are genetically diminished. After the invasion of the NZMS in Europe, little variation has built up indicating a reduced ability to react to selection.

Shape had generally lower heritability values than size – with the exception of the heritability values from the 19°C snails of the F2 generation. As already mentioned above, due to the not significant p-values and the very low sample numbers, the values of the 19°C in the F2 generation are not representative. The way of calculating heritability may have caused the difference between the size and the shape values. Over 50% of the variation could not be justified with the factors which our models consisted of. The maternal ID only described between 21% - 31% of the variance. Still, the unexplained 50% of the variation form part of the complete phenotypic variation. This might explain the small values of heritability in shape which we estimated. In general, this illustrates the difficulties that appear when comparing heritability values (Houle, 1992; Hansen et al., 2011; Garcia-Gonzalez et al., 2012).

Life history

To investigate the influence of temperature on life history traits, we calculated the reproductive rate of the parental generation. The reproductive rate is the time until the needed number of offspring of the respective mother snail was reached. The reproductive rate was principally influenced by the haplotype and the temperature of the climate cabinets. The temperature difference between the water temperatures which was measured in at the collection site and the temperature in the climate cabinet did not have a strong influence on the reproductive rate. Possible maternal effects seemed to have been reduced or removed due to the time before the experiment in which the snails of the parental generation could become acclimatized. In contrast to previous studies, the reproductive rate is not influenced by the genetic variation of different populations (Jacobsen & Forbes, 1997).

The reproduction of mother snails with haplotype z was faster compared to mother snails with haplotype t. The temperature of the climate cabinets had a stronger impact on the reproductive rate of mother snails with haplotype t. Snails from the 23°C climate cabinet reproduced faster than snails from the colder temperatures. Thus, snails with haplotype z appear to be more tolerant for changes in temperature than snails possessing haplotype t. Snails with haplotype t were bigger in size than snails with haplotype z reinforcing a negative correlation between growth and the age at reproductive maturity (Larkin et al., 2016). We did not measure the growth rate of the snails in our experiment. Just like the snails with haplotype t in our common garden experiment, other investigations detected growth rates similar to an optimum curve. The growth rates accelerate with temperature and have their limit at approximately 24°C before decreasing again (Dybdahl & Kane, 2005; Bennett et al., 2015). Despite finding out that

temperature influences shell size and shape, we could not detect a clear pattern across the three temperatures. Snails investing their energy in rapid growth appear to need more time until a certain number of offspring is produced.

Since the reproductive rate was mainly influenced by haplotype and temperature, it appears to show a changeable genetic basis, but could be adjusted by phenotypic plasticity. The genetic circumstances may determine the dimensions of the phenotypic plasticity. Phenotypic plasticity plays an important role in life history traits of the NZMS (Negovetic & Jokela, 2001; Dybdahl & Kane, 2005; Kistner & Dybdahl, 2013; McKenzie et al., 2013; Bennett et al., 2015). Verhaegen et al. (2021) observed that the three northern German populations possessing haplotype t which were also part of the common garden experiment, varied in their reproductive traits depending on environmental conditions, such as salinity and water current and temperature. However, the investigation was done on NZMS living and reproducing in their natural habitats. The mother snails in the common garden setting produced their offspring under artificial conditions.

The frozen-niche variation (FNV) hypothesis proposes that clones arbitrarily collect their ancestral sexual populations´ genotype and "freeze" it. This means that an individual clone only shows a part of the complete variation in niche-use which can be seen in sexually reproducing populations resulting in selection for ecological specialization and the concurrence of clones (Vrijenhoek, 1979, 1984). The more generalized form of the model is the frozen-phenotype variation (FPV) model. The FPV model indicates that the conditions of the FNV hypothesis are also applicable on life-history traits. In accordance with the FPV model, restrictions to phenotypic plasticity would be anticipated in clonal organisms (Jokela, Lively, Fox, et al., 1997). In fact, Jokela, Lively, Fox, et al. (1997) showed flat reaction norms in the NZMS for several life-history traits and morphology.

Other factors may have an impact on the reproduction of the NZMS which we were not able to control during our experiment. The number of other parthenogenetic, present NZMS has a negative impact on the production of embryos in parthenogenetic females (Neiman, 2006). At the beginning of our experiment, we isolated the mother snails and placed the individually in small glass jars. However, we were not able to control for density after the mother snails started to produce offspring, since mother and daughter snails were kept in the same glass jar due to limited resources. Some mother snails were producing much more offspring (> 100 offspring/glass jar) than others and therefore there was a higher snail density in some jars. This may have had an impact on the reproductive rate of the mother snails. Tibbets (2009) reported that the feeding of algae with a low phosphorus content reduces the fecundity of the NZMS. Although the snails of our experiment were fed with the same food algae, the food spectrum was reduced in comparison to

the food variability in the natural habitat. Even within the same habitat, clones of the NZMS differ in certain life-history phenotypes (Fox et al., 1996; Jokela, Lively, Fox, et al., 1997). Still, excluding every factor which may influence the reproductivity of the NZMS is impossible. Since we reduced eventual external biases as far as possible, we are convinced that their impact is negligible.

Methylation patterns in molluscs

The investigations of epigenetic mechanisms in invertebrate non-model organisms are rather scarce (Aliaga et al., 2019). Various investigations of the last years have taken on the task to shed light on the genetics of non-model organisms of various phyla and classes and conducted advanced whole genome approaches. The results of the analyses indicate that animals use epigenetic processes to deal with and adapt to rapidly changing environmental conditions. Species which are taking advantage of this procedure are among others asexual populations and invaders, such as the NZMS (Vogt, 2022). Recent research started to investigate epigenetic processes, such as DNA methylation, in non-model arthropod species (Bewick et al., 2017; Provataris et al., 2018; Lewis et al., 2020). Other invertebrate groups, such as molluscs, are still rather underrepresented in the epigenetic research area despite their variety and diversity (Ponder et al., 2002; Haszprunar & Wanninger, 2012; Rosenberg, 2014). Although molluscs represent the second largest phylum, methylation was only investigated in 17 species (Fallet et al., 2020; Venkataraman et al., 2020). To investigate the DNA methylation in molluscs and other taxa, large-scale analyses, such as the presence/absence investigation of DNMTs and the calculation of the CpG o/e distribution are necessary. Aliaga et al. (2019) investigated the CpG o/e distribution in two molluscan species and Fallet et al. (2020) summarized the present expertise about DNA methylation and histone modification in molluscs. Zhang et al., (2020) published a review about DNA methylation in the growth and development in molluscs. Still, Männer et al. (2021) presented the first analyses on the DNA methylation patterns in 140 molluscan species from all eight molluscs classes including the detection of DNMTs and the estimation of the normalized CpG dinucleotide ratio.

DNA methyltransferases distribution in molluscs

We detected DNMTs in the major part of all 140 species. Furthermore, we also found DNTMs in all eight mollusc classes indicating that the enzymatic machinery for DNA methylation was already present in the last common ancestor of molluscs. This result corresponds with the findings of Aliaga et al. (2019). This outcome was supported with the GLMs indicating that the condition of the sequencing data has a high impact on the finding of DNMTs. High BUSCO values, and therefore a high BUSCO completeness, increased the chance to detect DNMTs. The absence of DNMTs in genomes with an incomplete BUSCO does not necessarily mean that that DNMTs are really absent. In contrast, the presence of DNMTs is less vague and very likely indicates the real presence of DNTMs in genome. Low BUSCO values in species in which we were not able to detect DNMTs can be misleading and the bad quality of the sequenced data may have caused problems

in finding DNMTs. Species with high BUSCO values which lack DNMTs probably lost them due to evolutionary circumstances. This suspicion is confirmed in closely related species, both of which cannot exhibit DNMTs. The last common ancestor of those species may have lost DNMTs. To confirm this, the DNMT status of more species of the same clade would have to be investigated.

DNMT1 and DNMT3 paralogues have been gained and lost in many animal genomes in the course of evolutionary history. Despite the conservational character of DNMT genes, investigations showed a sophisticated and dynamic evolution of the DNMT family. In *Caenorhabditis elegans*, for example, the entire DNMT family disappeared from its genome (Bewick et al., 2017; Lyko, 2018). Other invertebrate species lost or gained DNMTs to their genome, such as *Apis mellifera*, which build up an expansion of DNMT1. *Drosophila melanogaster* on the other hand lost both DNMTs 1 and 3 from its genome. In insects, the DNMT toolkit is not conserved in the genome (Provataris et al., 2018). We think that this might also apply to the DNMT toolkit of molluscs.

In general, twelve of the 140 species did not show any DNMT1 homologues in the examined sequenced data. However, just five of the twelve species which lacked DNMT1, had a BUSCO completeness which was higher than 80%. Nine of them furthermore also did not have DNMT3. Since the data of some species had low BUSCO, the majority of the DNMT1 deficiency was probably due to the poor data quality. We were not able to find DNMT3 in 69 species. Of all eight mollusc classes, mostly species from the Gastropoda missed the DNMT3. To examine the presence or absence of DNMTs, we used transcriptomic data. Nevertheless, transcriptomic data does not represent the whole genome, but rather illustrates a snapshot of the currently transcribed genes at the moment of fixation of the respective individual. This means that despite not detecting a certain DNMT or DNMTs in general, it is still possible that DNMTs are present in the non-transcribed part of the genome. Also, mRNA is degenerated very quickly and some sequences containing DNMTs were already degraded. Some regions of genome are only transcribed under certain external or internal conditions and therefore always only part the genes are expressed at certain times (Gygi et al., 1999; Pascal et al., 2008; Yeung et al., 2011). We still think that the twelve species possess the required DNMT machinery because all of them show the presence of DNA methylation (Supplemental Table S2). In mammals, the absence of DNMT1 implies a serious and direct loss of methylation (Li et al., 1992; Chen et al., 2007). Although the DNA methylation in invertebrates cannot be compared to the methylation in mammals, this indicates that DNMT1 could be present in the majority, if not all of the investigated molluscs.

The possibly severe consequences of the loss of DNMT1, suggests that DNMT3 is used for the long-term robustness of DNA methylation patterns in the genome (Chédin, 2011). DNMT1

could be more conserved in the genomes of molluscs than DNMT3 and the loss of DNMT3 less serious than the loss of DNMT1. Some scientists assume that DNMT1 or another enzyme may be able balance and to adopt the function of DNMT3 (Glastad et al., 2011; Maleszka, 2016; Provataris et al., 2018). In contrast, gaining DNMT3 are rather improbable events for insects and therefore maybe also unlikely for molluscs (Bewick et al., 2017). In contrast to the lack of DNMT1, the absence of DNMT3 followed a systematic arrangement among the investigated molluscs in our analysis. The following mollusc species did not show any DNMT3 homologues: both bivalve species of Dreissena, all gastropodan species of Littorina, all 21 gastropodan species of the Heterobranchia which were relatively distantly related and the seven cephalopodan species of Oegopsida. The high BUSCO values indicate a high BUSCO completeness and support our finding that DNMT3 is absent in the genomes of those species. The last common ancestor of those taxa probably already did not have any DNMT3 homologues. Only the data of six species of the Heterobranchia and one species of the Oegopsida had BUSCO values below 85%. The genome data of seven species of the Heterobranchia supported those results.

Since the groups which lacked DNMT3, were not closely related, it is possible that DNTM3 has been frequently lost in independent events over evolutionary time. The DNMT3 absence despite having DNA methylation in two species of the Heterbranchia, *B. glabrata* and *A. californica*, confirmed the results of Adema et al. (2017) and Geyer et al. (2017). Although the different DNTM3 homologues of mammals are highly important for the long-term stability of DNA methylation patterns in the genome, the loss of DNMT3 homologues in invertebrates appears to be evolutionary less problematic (Chédin, 2011; Bewick et al., 2017; Provataris et al., 2018). It apparently does not have any negative effect on the methylation status of the affected species. As stated above, the functions of the DNMT3 may be easily taken over by other enzymes. The function of the DNMT3, in mammals the *de novo* methylation, may have been taken over by the DNMT1 in the molluscan groups which lack DNMT3. This theory is also backed by the fact that all heterobranchian species (Gastropoda) revealed a bimodal CpG o/e distribution indicating that they have DNA methylation in their exons although they do not possess DNMT3.

CpG observed/expected distributions

To analyse the DNA methylation status in molluscs, we estimated the CpG o/e ratio in protein-coding sequences and therefore in gene bodies, particularly in exons, because in invertebrates DNA methylation can mainly be found in gene bodies. Until now, little is known about the process of gene regulation by gene body methylation. Still, there seems to be an evident connection between

gene body methylation and gene expression (Wang et al., 2013; Glastad et al., 2016; Gatzmann et al., 2018; Li et al., 2018). This connection has also been seen in some mollusc species, such as *B. glabrata*, *C. gigas* and *C. virginica* (Gavery & Roberts, 2013; Geyer et al., 2017; Song et al., 2017; Venkataraman et al., 2020). This means that the investigation of the DNA methylation status of different species using the CpG o/e ratio results in a relevant and phylogenetically comprehensive review on the basis of already published genomic and transcriptomic data. The outcomings of these investigations may give an insight into common patterns and pave the way for following studies.

Based on our results, we can conclude that DNA methylation is common in molluscs, apart for some exceptions. The majority of the species of the different mollusc classes revealed a bimodal CpG o/e distribution pattern. Only within the classes of the Solenogastres and the Monoplacophora, all the species had a unimodal DNA methylation pattern indicating less or no DNA methylation in the protein-coding sequences. Within the Solenogastres, although possessing DNMTs, four of the six species did not have any sign of methylation. The same accounts for the only monoplacophoran species which also did not have any methylation signature. Also, species of the Caudofoveata, Polyplacophora and Scaphopoda regularly showed unimodal levels of CpG o/e ratios. Therefore, no or a very sparse DNA methylation pattern can be found in the protein-coding sequences of the species which are part of these two groups. This is also in accordance with the increasing epigenetic analyses in insects which reveal highly diverse methylation patterns from no methylation at all to "mosaic" methylation patterns (Glastad et al., 2011; Bewick et al., 2017). Holometabolous insects, just like Monoplacophora and Solenogastres, possibly have a reduced or no DNA methylation in their exons (Provataris et al., 2018). Glastad et al. (2019) reported about investigations showing that there is few DNA methylation attached to the exons of protein-coding genes in Holometabola. This could also be the case in the molluscan classes in which some species showed a comparable DNA methylation level. Still, more molluscan species from the Caudofoveata, the Monoplacophora, the Polyplacophora, the Scaphopoda and the Solenogastres have to be sequenced and investigated to confirm this theory. Even nowadays with advancing and evolving sequencing technologies, molluscs represent a highly underrepresented taxon with regard to the genetic resources mainly caused by problems occurring during the different sequencing or preparation steps (Aoki & Koshihara, 1972; Sokolov, 2000).

Nearly all species of the other molluscan groups, Bivalvia, Cephalopoda and Gastropoda, had a bimodal CpG o/e distribution with small mean CpG o/e values pointing to a higher amount of DNA methylation in the protein-coding sequences. Pointing out the parallel with insects again, similarly low CpG o/e values can be observed in hemimetabolous insects indicating that the last common ancestor of the insects showed high levels of DNA methylation. In holometabolous

species, the DNA methylation could have been lost again over time (Bewick et al., 2017; Provataris et al., 2018). The high methylation in the majority of Bivalves, Cephalopods and Gastropods which we detected in our calculations, has also been experimentally verified in the bivalves *Chlamys farreri*, *C. gigas*, *C. virginica*, *Pinctada fucata*, *Saccostrea glomerata*, in the cephalopod *Octopus vulgaris* and in the gastropods *Haliotis discus hannai*, *A. californica*, *B. glabrata* and *Lymnaea* sp. (Fallet et al., 2020; Venkataraman et al., 2020). Projecting the conclusions of the insects to the molluscs, it may be possible that the last common ancestor of the molluscs had a high amount of DNA methylation in its exons. The amount of DNA methylation was probably decreased over time in certain mollusc classes, such as Monoplacophora and Solenogastres. Since it has already been shown that some invertebrate species have very repetitive intronic regions methylated, it would be interesting to have a closer look at the methylation level at the introns of molluscs – especially mollusc classes which showed a low or no DNA methylation in exons (Geyer et al., 2011). However, the low number of species which we were able to analyse in some classes makes such conclusions premature and doubtable. Although the genetic and epigenetic analysis and genome sequencing of different mollusc species increases, many classes are still underrepresented and it is impossible to observe any common pattern.

VI. CONCLUSION

The outcomes of our common garden analysis showed that the NZMS has a high potential to adapt morphologically to temperature. Both factors, but especially size were sensitive to changing temperature conditions. Across the three temperatures, size acted according to the temperature-size rule (Angilletta et al., 2004). It may be possible that shape changes counterbalanced the changes in size due to the space in the shell which is taken up by embryos. Even within one generation, we were able to detect the differences in the shell morphology of the NZMS. Generally, 50% of the total variation in size was defined by the interaction of temperature and haplotype.

Those results confirm that the shell morphology of the NZMS is phenotypically plastic. However, the shell morphology is also influenced by the haplotypes of European NZMS. Furthermore, the calculated heritability values were comparably high indicating that a big part of shell size and shape is influenced by the genetics of the snail. Snails with haplotype t had higher heritability values than snails with haplotype z indicating that snails possessing haplotype t have a higher genetic variability with regard to temperature and morphology and react more genetically. The variation in the shell morphology appears to have a genetic basis but with plastic adaptational capacities provoked by environmental cues. The reaction norms backed this theory revealing genotype by environment (GxE) interactions, especially strong in size. Genotype-by-environment interactions (GxE) as we observed in the reaction norms of the snails across all populations and haplotypes, means that phenotypic plasticity represents an attribute of an individual genotype when variation for plasticity is present in a population. Both haplotypes show a crossover genotype-by-environment interaction (GxE) and show diverse plastic responses which make the best genotype to adapt to different environmental conditions (Kusmec et al., 2018).

Epigenetic mechanisms, such as DNA methylation might act as a "motor" for phenotypic plasticity, also in the NZMS (Roberts & Gavery, 2012; Duncan et al., 2022). The NZMS possesses a unimodal DNA methylation pointing to a low but still present methylation status. We were also able to detect both DNMT1 and DNMT3 in the NZMS which is yet another indication of DNA methylation being present in the genome. Thorson et al. (2017) found differing DNA methylation patterns across two different habitats, lakes and rivers, in North-American NZMS descending from one clonal lineage. The snails also showed differences in their shell shape which was in accordance with the water current speed at the respective habitat. Thorson et al. (2019) investigated the variation in methylation in NZMS populations from lakes with different environmental histories in disturbances and contamination. The site-specific variations in methylation which they detected, suggested that the different environmental conditions in those habitats led to varying epigenetic responses in the snails. This indicates that environmentally induced epigenetics, such as DNA

methylation, might be a mechanism for the development of adaptive variations in shell shape across different habitats. DNA methylation and other epigenetic processes might explain the potential of asexual NZMS to successfully colonize new habitats by adaptive evolution or phenotypic plasticity (Thorson et al., 2017, 2019).

We clearly found adaptive potential among the young NZMS generations in Europe. There is genetic variation among the European populations even though no recombination is possible. Although we observed some differentiation in the reaction norms of the F1 generation, the low CV_A values in size lead to the conclusion that European populations still have a reduced genetic variation including a poor ability to react to selection. The NZMS invaded Europe approximately 180-360 generations ago. Apparently, this time has not been long enough to acquire sufficient genetic variation which is important for morphological adaptation. Despite the reduced genetic variation, the NZMS was still able to successfully colonize new habitats and even continents. Thus, the genetic variation which augments the heterogeneity in the shell morphology, was not essential to invade Europe. One factor which hay have facilitated the invasion of Europe and reduced the selective pressure acting on the recently invaded NZMS, might be the missing predators and competitors. The selection pressure on invasive species would increase if potential predator or competitors are present in the new habitat (Lee, 2002). In New Zealand, the NZMS can get infected with trematode parasites which castrate the infected snail (Winterbourn, 1974; Hechinger, 2012). In Europe, however, the amount of infected NZMS is low (e.g. Żbikowski & Żbikowska, 2009; Gérard et al., 2017; Verhaegen, McElroy, et al., 2018; Verhaegen et al., 2021). Also the low predation frequency outside of the native range, for example by fish (e.g. Bersine et al., 2008; Rakauskas et al., 2016) and the ability of the NZMS to survive the gut passage of predators (Bruce et al., 2009; Naser & Son, 2009; Butkus & Rakauskas, 2020) increases the population growth and reduces the selection pressure. Missing predators and parasites helped the NZMS to become and still be a very successful invasive species (Geist et al., 2022).

Our experiment illustrated that temperature has an effect on the shell morphology of the NZMS. Obviously, NZMS experience many other fluctuating environmental factors that may influence the shell morphology and other parameter and processes of the NZMS. Physiological processes, for example, which have an impact on the reproduction, might be more easily impacted by temperature than the shell morphology (Winterbourn, 1970; Quinn et al., 1994; Dybdahl & Kane, 2005). Since more than one factor probably influences the shell morphology and the adaptive potential of the NZMS, temperature alone will not limit or enhance the population density and spreading in the face of increasing temperatures due to climate change in Europe and other countries.

To compare the results of our common garden experiment and understand the complex mechanisms of adaptation and survival in the face on climate change, more studies and investigations are necessary – not only in the NZMS, but in other species from different taxa across the world. Especially experimental data from non-model species of different taxa will help to understand adaptation responses in species (Hoffmann & Sgrò, 2011). DNA methylation is suggested to add to adaptation by phenotypic plasticity and heritable variation – which is especially important for clonally reproducing species because they are not or only marginally able to react genetically (Pérez et al., 2006; Prentis et al., 2008). To better understand the complex nature of invasive species, more experimental and epigenetic investigations should be combined. Connecting methods such as common garden experiments and the analysis of the resulting epigenetic changes enables scientists to shed light on the roles that phenotypic plasticity and genetic adaptation play in invasion events and to have a deeper look at the running epigenetic processes (Parker et al., 2003; Dybdahl & Kane, 2005; Kistner & Dybdahl, 2013). Furthermore, more research helps to predict reactions and adaptive responses of (invasive) species in the face of climate change providing fundamental knowledge which can be support and shape future conservation techniques (Hawes et al., 2018).

Especially about molluscs, fairly less is known. The majority of investigations is focussed on model-organisms and vertebrate species. Even genetic kits, databases and processes for bioinformatic analyses are mainly designed for model-organisms and vertebrates. Despite molluscs representing a highly diverse taxon, our knowledge is scarce and especially genetic and epigenetic investigations are often very complicated (Gavery & Roberts, 2014; Fallet et al., 2020). The polysaccharides in the mucus of molluscs are supposed to intervene with and disturb enzymes in reactions of DNA extractions, sequencing libraries and different sequencing techniques (Aoki & Koshihara, 1972; Sokolov, 2000). Despite all the difficulties, it is important to increase the genetic and epigenetic investigations in molluscs. This is why we summarized and investigated the methylation status in 140 species of eight mollusc classes.

We observed molluscs to possess substantial levels of DNA methylation in gene bodies indicating that the last common ancestor of the molluscs had DNA methylation and DNMT(s). The minority of species which did not have any DNA methylation or DNMT(s) was spread over the mollusc tree suggesting convergent evolution. Those classes and species may have lost or reduced the levels of DNA methylation. Smaller classes, such as the Monoplacophora are less investigated and thus we were not able to draw any reliable conclusion in those classes. Recent investigations found evidence that some DNA methylation patterns are conserved and inherited from parent to offspring generation in *C. gigas* (Li et al., 2022). Furthermore, they found differences

in the DNA methylation patterns between male and female somatic tissue suggesting that DNA methylation is an important factor in the sex differentiation in the somatic tissues of *C. gigas*. Epigenetic mechanisms, such as DNA methylation seem to play an important role in biological processes about which we still know too little about. More research needs to be done to find out if this may be the case in several molluscs and other invertebrate taxa.

Since protocols and genetic and epigenetic processes are still mainly attuned to vertebrates and model organisms, the procedure to investigate the methylome or genome, or even annotate the genome of a mollusc is complicated and difficult and requires a lot of expertise. This could be why some of the data had a low quality which in the end hampered also our analyses. To gain a deeper knowledge of evolution and development in molluscs, it is highly important to increase the investigations of the epigenetics in molluscs regardless of technical difficulties. We want to encourage the companies which are providing sequencing material to adjust and provide techniques which are suitable for non-model and other underrepresented taxa. This would clearly help to facilitate the investigations of highly interesting and diverse organisms whose genetic resources are still underrepresented compared to model organisms.

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VIII. LIST OF ABBREVIATIONS

Abbreviation	Meaning/Explanation
ADD_DNMT	A domain of the DMNT3
AIC	Akaike information criterion, evaluating errors and thus, the relative model quality
ANOVA	Analysis of variance, composure of statistical models and their accompanying estimation process, investigating the distinctions amongst means
BUSCO	Benchmarking Universal Single-Copy Orthologs, instrument to evaluate the thoroughness of genome assembly, gene set and transcriptome (Simão et al., 2015)
CDS	Predicted coding sequences
Cl_2	Symbol of the chemical element chlorine
CpG	CpG sites, DNA regions where a guanine nucleotide goes after a cytosine nucleotide
CV_A	Coefficient of genetic variation, mean-standardized index of a trait´s evolvability (Houle, 1992; Hill, 2010)
df	Degrees of freedom, evaluation of the size of independent classes in a statistical analysis or experiment
DNMT	DNA methyltransferase, family of enzymes that attach a methyl group to the DNA
DNMT1-RFD	A domain of the DMNT1
F1	First daughter generation, offspring generation that was born by the parental generation
F2	Second daughter generation, offspring generation that was born by the first daughter generation F1
FNV	Frozen niche-variation (FNV) hypothesis, proposes that clones arbitrarily collect and "freeze" the genotypes of their ancestor sexual populations (Vrijenhoek, 1979, 1984)
FPV	Frozen-phenotype variation model, common form of the FNV hypothesis suggesting that the same conclusions apply to other traits which have essential effects on fitness, such as life-history traits, still, are not directly correlate with niche use (Jokela, Lively, Dybdahl, et al., 1997)

GH	"Gesamthärte" or general hardness of the water, explains all alkaline earth salts dissolved in water (e.g., magnesium, calcium) in German degrees of hardness (°dH)
GLM	Generalized linear model, adaptable generalization of common linear regression
GpC	GpC sites, DNA regions where a cytosine nucleotide goes after a guanine nucleotide
GxE	Genotype-environment interaction
H_2	Broad-sense heritability, proportion of total genetic variation to total phenotypic variation, contribution of all genetic effects
b_2	Narrow-sense heritability, proportion of additive genetic variation to total phenotypic variation, contribution of one genetic effect
I_A	Appropriate measure for evolvability, square of the coefficient of genetic variation (CV_A) (Houle, 1992; Hansen et al., 2011)
J008	Snail population from Tiscar, Spain
KH	"Karbonathärte" or carbonate hardness, part of the general hardness (GH) of the water, part of alkaline earth ions which are in water and bonded to carbonates and hydrogen carbonates
LRT	Likelihood ratio test, statistical test that belongs to the common tests for hypotheses in parametric models
MEK	Methyl ethyl ketone or butanone, organic compound and denaturant which is admixed to chemicals in order to add a bitter taste that should prevent from drinking the chemical
N	Number of study animals or observation involved in a study
NO_3	Nitrate, chemical compound
NZMS	New Zealand mud snail, *Potamopyrgus antipodarum*
$P_{CpG}/P_C/P_G$	Amount of 5'-CpG-3 dinucleotides/C dinucleotides/G dinucleotides
PCR	Polymerase chain reaction, technique to multiply a certain DNA sample or part of the DNA
pH	"Potential of hydrogen", range that determines the acidity or basicity of an aqueous solution
pHMM	Profile Hidden Markov Models, probabilistic models which describe the variety of biological sequences

RAPD	Randomly amplified polymorphic DNA, special form of the PCR
SNP	Single nucleotide polymorphism, variation of a single base pair in a complementary DNA double strand
TE	Transposable element, DNA fragments which can change their location within the genome (Bourque et al., 2018)
TIF files	Tagged imaged file format, data format to store image data
TPS files	"Test procedure specification" file, format to store data which were recorded with the TiePie Multi Channel software
V_A	Overall genetic variance, describing variation of the breeding value of the populations´ individuals
VIF	Variance inflation factor, describes the dimension of correlation between two predictors in a model
V_{res}	Variance within the clones

IX. TABLE OF FIGURES

X. LIST OF TABLES

XI. SUPPLEMENTAL MATERIAL

We downloaded genomic (predicted coding sequences (CDS) and proteins of structural annotations) and transcriptomic data publicly accessible at the beginning of the year 2020 from different sources (Supplemental Table S1). More in-depth explanations of the analyses of the genomic and transcriptomic data can be found in the Material and Methods section and in Männer et al. (2021). The results of the CpG o/e and DNMT estimation along with quality control (BUSCO) can be found in Supplemental Table S2.

Supplemental Table S1. Genomic and transcriptomic data resources.

Species name	NCBI taxonomy	Data source	Data-base	NCBI BioProject	Submitted by
Abdopus aculeatus	Mollusca; Cephalooda; Coleoidea; Neocoleoidea; Octopodformes; Octopoda; Incirrata; Octopodidae; Abdopus	transcriptome	NCBI	PRJNA188569	Caruana NJ, Cooke IR, Faou P, Finn P, Hall NE et al. 2016. A combined proteomic and transcriptomic analysis of slime secreted by the southern bottletail squid, *Sepiadarium austrinum* (Cephalopoda). Journal of Proteomics 148: 170-82.
Acanthochitona crinita	Mollusca; Polplacophora; Neoloricata; Chitonida; Acanthochitonina; Acanthochitonidae; Acanthochitona	transcriptome	NCBI	PRJNA357466	De Oliveira AL, Wollesen T, Kristof A, Scherholz M, Redl E et al. 2016. Comparative transcriptomics enlarges the toolkit of known developmental genes in mollusks. BMC Genomics 17: 905.
Acantochitona rubrolineata	Mollusca; Polplacophora; Neoloricata; Chitonida; Acanthochitonina; Acanthochitonidae; Acanthochitona	transcriptome - assembled by ourselves	NCBI	PRJNA515172	Institute of Oceanology, Chinese Academy of Sciences, Huan P, 2019.

Achatina fulica	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Eupulmonata; Stylommatophora; Helicina; Achatinoidea; Achatinidae; Lissachatina	genome	GigaDB	PRJNA511624	Guo Y, Zhang Y, Liu Q, Huang Y, Mao G et al. 2019. A chromosomal-level genome assembly for the giant African snail *Achatina fulica*. GigaScience 8: 1-8.
Adamussium colbecki	Mollusca; Bivalvia; Pteriomorphia; Pectinoida; Pectinoidea; Pectinidae; Adamussium	transcriptome	NCBI	PRJNA379393	University of Trieste, Gerdol M, 2017.
Amblema plicata	Mollusca; Bivalvia; Palaeoheterodonta; Unionida; Unionoidea; Unionidae; Ambleminae; Amblema	transcriptome	NCBI	PRJNA436349	The Ohio State University, Roznere I, 2018.
Antalis entalis	Mollusca; Scaphopoda; Dentaliida; Dentaliidae; Antalis	transcriptome	NCBI	part of PRJNA357466	De Oliveira AL, Wollesen T, Kristof A, Scherholz M, Redl E et al. 2016. Comparative transcriptomics enlarges the toolkit of known developmental genes in mollusks. BMC Genomics 17: 905.
Antalis entalis	Mollusca; Scaphopoda; Dentaliida; Dentaliidae; Antalis	transcriptome	NCBI	part of PRJNA72139	Smith SA, Wilson NG, Goetz FE, Fehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.
Antalis entails 2	Mollusca; Scaphopoda; Dentaliida; Dentaliidae; Antalis	transcriptome - assembled by ourselves	NCBI	PRJNA506080	Harvard University, Lemer S, 2018.
Aplysia californica	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Euopisthobranchia; Aplysiida;	genome	NCBI	PRJNA13635	Knudsen B, Kohn AB, Nahir B, McFadden CS & Moroz LL 2006. Complete DNA sequence of the mitochondrial genome of the sea-slug, *Aplysia californica*: Conservation of the gene order in Euthyneura. Molecular Phylogenetics and Evolution 38: 459-469.

	Aplysioidea; Aplysiidae; Aplysia				
Aplysia californica	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Euopisthobranchia; Aplysiida; Aplysioidea; Aplysiidae; Aplysia	transcriptome	NCBI	part of PRJNA77701	The Broad Institute, 2011.
Aplysia californica 2	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Euopisthobranchia; Aplysiida; Aplysioidea; Aplysiidae; Aplysia	transcriptome	NCBI	part of PRJNA77701	The Broad Institute, 2011.
Aplysia californica 3	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Euopisthobranchia; Aplysiida; Aplysioidea; Aplysiidae; Aplysia	transcriptome	NCBI	part of PRJNA77701	The Broad Institute, 2011.
Aplysia californica 4	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Euopisthobranchia; Aplysiida; Aplysioidea; Aplysiidae; Aplysia	transcriptome	NCBI	part of PRJNA77701	The Broad Institute, 2011.
Aplysia californica 5	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Euopisthobranchia; Aplysiida; Aplysioidea; Aplysiidae; Aplysia	transcriptome	NCBI	part of PRJNA77701	The Broad Institute, 2011.

Aplysia californica 6	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Euopisthobranchia; Aplysiida; Aplysioidea; Aplysiidae; Aplysia	transcriptome	NCBI	part of PRJNA77701	The Broad Institute, 2011.
Aplysia californica 7	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Euopisthobranchia; Aplysiida; Aplysioidea; Aplysiidae; Aplysia	transcriptome	NCBI	part of PRJNA77701	The Broad Institute, 2011.
Aplysia californica 8	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Euopisthobranchia; Aplysiida; Aplysioidea; Aplysiidae; Aplysia	transcriptome	NCBI	part of PRJNA77701	The Broad Institute, 2011.
Argopecten purpuratus	Mollusca; Bivalvia; Pteriomorphia; Pectinoida; Pectinoidea; Pectinidae	genome	GigaDB	PRJNA418203	Li C, Liu X, Liu B, Ma B, Liu F et al. 2018. Draft genome of the Peruvian scallop *Argopecten purpuratus*. GigaScience 7: 1-6.
Arion vulgaris	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Eupulmonata; Stylommatophora; Sigmurethra; Arionoidea; Arionidae; Arion	transcriptome	NCBI	PRJEB7891	Lubec Laboratory, 2015.
Bathyberthella antarctica	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Pleurobranchomorpha;	transcriptome	NCBI	PRJNA391256	The University of Alabama, Kocot K, 2017.

	Pleurobranchoidea; Pleurobranchidae; Bathyberthella.				
Bathymodiolus platifrons	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Bathymodiolinae; Bathymodiolus	genome	DRYAD	PRJNA328542	Sun J, Zhang Y, Xu T, Zhang Y, Mu H et al. 2017. Adaptation to deep-sea chemosynthetic environments as revealed by mussel genomes. Nature Ecology & Evolution 1: 0121.
Bathymodiolus platifrons	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Bathymodiolinae; Bathymodiolus	transcriptome	NCBI	PRJNA235357	Hong Kong Baptist University, Sun J, 2014.
Biomphalaria glabrata	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Hygrophila; Lymnaeoidea; Planorbidae; Biomphalaria	genome	NCBI	PRJNA290623	Yoshino TP, Dinguirard N, Kunert J & Hokke CH 2008. Molecular and functional characterization of a tandem-repeat galectin from the freshwater snail *Biomphalaria glabrata*, intermediate host of the human blood fluke *Schistosoma mansoni*. Gene, 411: 46-58; Zhang S-M, Nian H, Zeng Y & DeJong RJ 2008. Fibrinogen-bearing protein genes in the snail *Biomphalaria glabrata*: Characterization of two novel genes and expression studies during ontogenesis and trematode infection. Developmental & Comparative Immunology 32: 1119-1130.
Biomphalaria glabrata	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Hygrophila; Lymnaeoidea; Planorbidae; Biomphalaria	transcriptome	NCBI	part of PRJNA264063	Oregon State University, Tennessen J, 2014.
Biomphalaria glabrata 2	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Hygrophila;	transcriptome	NCBI	part of PRJNA264063	Oregon State University, Tennessen J, 2014.

	Lymnaeoidea; Planorbidae; Biomphalaria				
Biomphalaria glabrata 3	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Hygrophila; Lymnaeoidea; Planorbidae; Biomphalaria	transcriptome	NCBI	part of PRJNA264063	Oregon State University, Tennessen J, 2014.
Biomphalaria pfeifferi	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Hygrophila; Lymnaeoidea; Planorbidae; Biomphalaria	transcriptome	NCBI	PRJNA383396	University of New Mexico, Buddenborg SK, 2017.
Bithynia siamensis goniomphalus	Mollusca; Gastropoda; Caenogastropoda; Littorinimorpha; Truncatelloidea; Bithyniidae; Bithynia	transcriptome	NCBI	part of PRJNA190834	Prasopdee S, Sotillo J, Tesana S, Laha T, Kulsantiwong J et al. 2014. RNA-Seq Reveals Infection-Induced Gene Expression Changes in the Snail Intermediate Host of the Carcinogenic Liver Fluke, *Opisthorchis viverrini*. PLoS Neglegted Tropical Diseases 8: e2765; Cantacessi C, Prasopdee S, Sotillo J, Mulvenna J, Tesana S et al. 2013. Coming out of the shell: building the molecular infrastructure for research on parasite-harbouring snails. PLoS Neglegted Tropical Diseases 7: e2284.
Bradybaena similaris	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Eupulmonata; Stylommatophora; Sigmurethra; Helicoidea; Bradybaenidae	transcriptome	NCBI	PRJNA495927	Noda T, Satoh N & Asami T 2019. Heterochirality results from reduction of maternal *diaph* expression in a terrestrial pulmonate snail. Zoological Letters 5: 2.
Cadulus tolmiei	Mollusca; Scaphopoda; Gadilida; Gadilidae	transcriptome	DRYAD		Smith SA, Wilson NG, Goetz FE, Feehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.

Calyptogena marissinica / Archivesica marissinica	Mollusca; Bivalvia; Heterodonta; Euheterodonta; Veneroida; Glossoidea; Vesicomyidae	transcriptome	NCBI	part of PRJNA471131	Lan Y, Sun J, Zhang W, Xu T, Zhang Y et al. 2019. Host–Symbiont Interactions in Deep-Sea Chemosymbiotic Vesicomyid Clams: Insights From Transcriptome Sequencing. Frontiers in Marine Science 6: 680.
Cepaea nemoralis	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Eupulmonata; Stylommatophora; Sigmurethra; Helicoidea; Helicidae; Cepaea	transcriptome	NCBI	PRJNA377398	University of Applied Science Leiden, Kerkvliet J, 2017.
Chaetoderma nitidulum	Mollusca; Aplacophora; Caudofoveata; Chaetodermatida; Chaetodermatidae; Chaetoderma	transcriptome - assembled by ourselves	NCBI	PRJNA379065	The University of Alabama, Kocot K, 2017.
Chaetopleura apiculata	Mollusca; Polyplacophora; Neoloricata; Chitonida; Chitonina; Chaetopleuridae; Chaetopleura	transcriptome	DRYAD	part of PRJNA72139	Caruana NJ, Cooke IR, Faou P, Finn P, Hall NE et al. 2016. A combined proteomic and transcriptomic analysis of slime secreted by the southern bottletail squid, *Sepiadarium austrinum* (Cephalopoda). Journal of Proteomics 148: 170-82.
Chiroteuthis calyx	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Teuthida; Oegopsina; Chiroteuthisdae; Chiroteuthis	transcriptome	NCBI	PRJNA342927	Francis WR, Christianson LM & Haddock SHD 2017. Symplectin evolved from multiple duplications in bioluminescent squid. PeerJ 5: e3633.
Chiton olivaceus	Mollusca; Polyplacophora; Neoloricata; Chitonida; Chitonina; Chitonidae;	transcriptome - assembled by ourselves	NCBI	PRJNA181256	Riesgo A, Andrade SCS, Sharma PP, Novo M, Pérez-Porro AR et al. 2012. Comparative description of ten transcriptomes of newly sequenced invertebrates and

	Chitoninae; Chiton; Chiton olivaceus				efficiency estimation of genomic sampling in non-model taxa. Frontiers in Zoology 9: 33.
Chlamys farreri	Mollusca; Bivalvia; Pteriomorphia; Pectinoida; Pectinoidea; Pectinidae	genome	NCBI	PRJNA185465	Li Y, Sun X, Hu X, Xun X, Zhang J et al. 2017. Scallop genome reveals molecular adaptations to semi-sessile life and neurotoxins. Nature Communications 8: 1721.
Chrysomallon squamiferum	Mollusca; Gastropoda; Neomphalina; Neomphaloidea; Peltospiridae; Chrysomallon	genome	DRYAD	PRJNA523081	Sun J, Chen C, Miyamoto N, Li R, Sigwart JD et al. 2020 The Scaly-foot Snail genome and implications for the origins of biomineralised armour. Nature Communications 11: 1657.
Cipangopaludina cathayensis	Mollusca; Gastropoda; Caenogastropoda; Architaenioglossa; Viviparoidea; Viviparidae; Cipangopaludina	transcriptome	NCBI	PRJNA264140	Pearl River Fisheries Research Institute.CAFS, Xidong M, 2014.
Clione limacina	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Euopisthobranchia; Pteropoda; Gymnosomata; Clionidae; Clione	transcriptome	NCBI	PRJNA314884	Woods Hole Oceanographic Institution, Tarrant A, 2016.
Crassostrea angulata	Mollusca; Bivalvia; Pteriomorphia; Ostreoida; Ostreoidea; Ostreidae; Crassostrea	transcriptome	NCBI	part of PRJNA415283	Ocean University of China, Tu K, 2017.
Crassostrea gigas	Mollusca; Bivalvia; Pteriomorphia; Ostreoida; Ostreoidea; Ostreidae; Crassostrea	genome	NCBI, Ensembl, GigaDB	PRJNA70283	Zhang G, Fang X, Guo X, Li L, Luo R et al. 2012. The oyster genome reveals stress adaptation and complexity of shell formation. Nature 490: 49-54.
Crassostrea gigas 2	Mollusca; Bivalvia; Pteriomorphia; Ostreoida; Ostreoidea; Ostreidae; Crassostrea	transcriptome	NCBI	PRJNA298285	University of Brest, Harney E, 2018.

Crassostrea gigas 3	Mollusca; Bivalvia; Pteriomorphia; Ostreoida; Ostreoidea; Ostreidae; Crassostrea	transcriptome	NCBI	PRJNA301543	Lim H-J, Lim J-S, Lee J-S, Choi B-S, Kim D-I et al. 2016. Transcriptome profiling of the Pacific oyster *Crassostrea gigas* by Illumina RNA-seq. Genes & Genomics 38: 359-365.
Crassostrea hongkongensis	Mollusca; Bivalvia; Pteriomorphia; Ostreoida; Ostreoidea; Ostreidae; Crassostrea	transcriptome	NCBI	part of PRJNA415283	Ocean University of China, Tu K, 2017.
Crassostrea nippona	Mollusca; Bivalvia; Pteriomorphia; Ostreoida; Ostreoidea; Ostreidae; Crassostrea	transcriptome	NCBI	PRJNA482778	Key laboratory of mariculture, Gong J, 2019.
Crassostrea virginica	Mollusca; Bivalvia; Pteriomorphia; Ostreoida; Ostreoidea; Ostreidae; Crassostrea	genome	NCBI	PRJNA376014	Gómez-Chiarri M, Warren WC, Guo X & Proestou D 2015. Developing tools for the study of molluscan immunity: The sequencing of the genome of the eastern oyster, *Crassostrea virginica*. Fish & Shellfish Immunology 46: 2-4.
Crassostrea virginica	Mollusca; Bivalvia; Pteriomorphia; Ostreoida; Ostreoidea; Ostreidae; Crassostrea	transcriptome	NCBI	PRJNA517955	Center for Scientific Research and Higher Education of Ensenada, Galindo C, 2019.
Crassostrea virginica 2	Mollusca; Bivalvia; Pteriomorphia; Ostreoida; Ostreoidea; Ostreidae; Crassostrea	transcriptome	NCBI	PRJNA474514	Center for Scientific Research and Higher Education of Ensenada, Galindo C, 2018.
Crepidula atrasolea	Mollusca; Gastropoda; Caenogastropoda; Littorinimorpha; Calyptraeoidea; Calyptraeidae; Crepidula	transcriptome	NCBI	PRJNA397078	Henry JQ, Lesoway MP, Perry KJ, Osborne CC, Shankland M et al. 2017. Beyond the sea: *Crepidula atrasolea* as a spiralian model system. The International Journal of Developmental Biology 61: 479-493.
Crepidula navicella	Mollusca; Gastropoda; Caenogastropoda; Lit-	transcriptome	NCBI	PRJNA294193	Lesoway MP, Abouheif E & Collin R 2016. Comparative Transcriptomics of Alternative Developmental Phenotypes in a Marine Gastropod. Journal of Experimental Zoology

	torinimorpha; Calyptraeoidea; Calyptraeidae; Crepidula				Part B: Molecular and Developmental Evolution 326: 151-67; Lesoway MP, Abouheif E & Collin R 2014. The development of viable and nutritive embryos in the direct developing gastropod *Crepidula navicella*. The International Journal of Developmental Biology 58: 601-611.
Dosidicus gigas	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Teuthida; Oegopsina; Ommastrephidae; Dosidicus	transcriptome	NCBI	part of PRJNA534469	da Fonseca RR, Couto A, Machado AM, Brejova B, Albertin CB et al. 2020. A draft genome sequence of the elusive giant squid, *Architeuthis dux*. GigaScience 9: giz152.
Dosidicus gigas 2	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Teuthida; Oegopsina; Ommastrephidae; Dosidicus	transcriptome	NCBI	part of PRJNA342927	Francis WR, Christianson LM & Haddock SHD 2017. Symplectin evolved from multiple duplications in bioluminescent squid. PeerJ 5: e3633.
Dosidiscus gigas 3	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Teuthida; Oegopsina; Ommastrephidae; Dosidicus	transcriptome	NCBI	PRJNA181271	Caruana NJ, Cooke IR, Faou P, Finn P, Hall NE et al. 2016. A combined proteomic and transcriptomic analysis of slime secreted by the southern bottletail squid, *Sepiadarium austrinum* (Cephalopoda). Journal of Proteomics 148: 170-82.
Dreissena polymorpha	Mollusca; Bivalvia; Heterodonta; Euheterodonta; Veneroida; Dreissenoidea; Dreissenidae; Dreissena	transcriptome	NCBI	part of PRJNA507340	Péden R, Poupin P, Sohm B, Flayac J, Giambérini L et al. 2019. Environmental transcriptomes of invasive dreissena, a model species in ecotoxicology and invasion biology. Scientific Data 6: 234.

Dreissena rostriformis	Mollusca; Bivalvia; Heterodonta; Euheterodonta; Veneroida; Dreissenoidea; Dreissenidae; Dreissena	genome - annotated by ourselves	bioRxiv	PRJNA550352	University of Vienna, Calcino A, 2019.
Dreissena rostriformis	Mollusca; Bivalvia; Heterodonta; Euheterodonta; Veneroida; Dreissenoidea; Dreissenidae; Dreissena	transcriptome	NCBI	PRJNA551098	University of Vienna, Calcino A, 2019.
Dreissena rostriformis bugensis	Mollusca; Bivalvia; Heterodonta; Euheterodonta; Veneroida; Dreissenoidea; Dreissenidae; Dreissena	transcriptome	NCBI	part of PRJNA507340	Péden R, Poupin P, Sohm B, Flayac J, Giambérini L et al. 2019. Environmental transcriptomes of invasive dreissena, a model species in ecotoxicology and invasion biology. Scientific Data 6: 234.
Elysia chlorotica	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Sacoglossa; Placobranchoidea; Placobranchidae; Elysia	genome	NCBI	PRJNA484060	Cai H, Li Q, Fang X, Li J, Curtis NE et al. 2019. A draft genome assembly of the solar-powered sea slug *Elysia chlorotica*. Scientific Data 6: 190022.
Elysia cornigera	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Sacoglossa; Placobranchoidea; Placobranchidae; Elysia	transcriptome	NCBI	part of PRJNA261510	de Vries J, Woehle C, Christa G, Wägele H, Tielens AGM et al. 2015. Comparison of sister species identifies factors underpinning plastid compatibility in green sea slugs. Proceedings of the Royal Society B 282: 20142519.
Elysia timida	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Sacoglossa; Placobranchoidea; Placobranchidae; Elysia	transcriptome	NCBI	part of PRJNA261510	de Vries J, Woehle C, Christa G, Wägele H, Tielens AGM et al. 2015. Comparison of sister species identifies factors underpinning plastid compatibility in green sea slugs. Proceedings of the Royal Society B 282: 20142519.

Ennucula tenuis	Mollusca; Bivalvia; Protobranchia; Nuculoida; Nuculidae; Ennucula	transcriptome - assembled by ourselves	NCBI	part of PRJNA72139	Smith SA, Wilson NG, Goetz FE, Fehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.
Euhadra quaesita	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Eupulmonata; Stylommatophora; Sigmurethra; Helicoidea; Bradybaenidae; Euhadra	transcriptome	NCBI	PRJDB6927	Shimizu K, Kimura K, Isowa Y, Oshima K, Ishikawa M et al. 2019. Insights into the Evolution of Shells and Love Darts of Land Snails Revealed from Their Matrix Proteins. Genome Biology and Evolution 11: 380-397.
Euprymna scolopes	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Sepiolida; Sepiolidae; Euprymna	genome - annotated by ourselves	NCBI	PRJNA470951	Belcaid M, Casaburi G, McAnulty SJ, Schmidbaur H, Suria AM et al. 2019. Symbiotic organs shaped by distinct modes of genome evolution in cephalopods. Proceedings of the National Academy of Sciences of the United States of America 116: 3030–3035.
Euprymna scolopes	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Sepiolida; Sepiolidae; Euprymna	transcriptome	NCBI	PRJNA320238	University of Hawaii, McFall-Ngai M, 2016.
Euprymna scolopes 2	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Sepiolida; Sepiolidae; Euprymna	transcriptome	NCBI	PRJNA282884	Collins AJ, Schleicher TR, Rader BA & Nyholm SV 2012. Understanding the role of host hemocytes in a squid/ *Vibrio* symbiosis using transcriptomics and proteomics. Frontiers in Immunology 3: 91.
Euprymna scolopes 3	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Sepiolida; Sepiolidae; Euprymna	transcriptome	NCBI	PRJNA253571	Collins AJ, Schleicher TR, Rader BA & Nyholm SV 2012. Understanding the role of host hemocytes in a squid/ *Vibrio* symbiosis using transcriptomics and proteomics. Frontiers in Immunology 3: 91.

Euprymna tasmanica	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Sepiolida; Sepiolidae; Euprymna	transcriptome	NCBI	part of PRJNA337893	Caruana NJ, Cooke IR, Faou P, Finn P, Hall NE et al. 2016. A combined proteomic and transcriptomic analysis of slime secreted by the southern bottletail squid, *Sepiadarium austrinum* (Cephalopoda). Journal of Proteomics 148: 170-82.
Eurhomalea rufa	Mollusca; Bivalvia; Heterodonta; Euheterodonta; Veneroida; Veneroidea; Veneridae; Eurhomalea	transcriptome	NCBI	PRJNA305471	Institute of Oceanology PAN, Lubosny M, 2015.
Gadila tolmiei	Mollusca; Scaphopoda; Dentaliida; Gadilinidae	transcriptome - assembled by ourselves	NCBI	part of PRJNA72139	Smith SA, Wilson NG, Goetz FE, Fehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.
Glossaulax didyma / Neverita didyma	Mollusca; Gastropoda; Caenogastropoda; Littorinimorpha; Naticoidea; Naticidae	transcriptome	NCBI	PRJNA515540	Shandong Vocational Animal Science and Veterinary College, Wang Q, 2019.
Greenland neomeniomorph	Mollusca; Aplacophora; Solenogastres; unclassified Neomeniomorpha	transcriptome	NCBI	part of PRJNA72139	Smith SA, Wilson NG, Goetz FE, Fehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.
Gymnomenia pellucida	Mollusca; Aplacophora; Solenogastres; Pholidoskepia; Gymnomeniidae; Gymnomenia	transcriptome	NCBI	part of PRJNA357466	De Oliveira AL, Wollesen T, Kristof A, Scherholz M, Redl E et al. 2016. Comparative transcriptomics enlarges the toolkit of known developmental genes in mollusks. BMC Genomics 17: 905.
Haliotis discus hannai	Mollusca; Gastropoda; Vetigastropoda; Lepetellida; Haliotoidea; Haliotidae	transcriptome	NCBI	PRJNA522620	Mokpo National University, Lim H, 2019.

Haliotis discus hannai 2	Mollusca; Gastropoda; Vetigastropoda; Lepetellida; Haliotoidea; Haliotidae	transcriptome	NCBI	PRJNA277901	Institute of Oceanology, Chinese Academy of Sciences, Xu F, 2015.
Haliotis discus hannai 3	Mollusca; Gastropoda; Vetigastropoda; Lepetellida; Haliotoidea; Haliotidae	transcriptome	NCBI	PRJNA301895	Korea Polar Research Institute, Park H, 2015.
Haliotis fulgens	Mollusca; Gastropoda; Vetigastropoda; Lepetellida; Haliotoidea; Haliotidae; Haliotis	transcriptome	NCBI	PRJNA453554	Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Tripp-Valdez M, 2018.
Haliotis laevigata	Mollusca; Gastropoda; Vetigastropoda; Lepetellida; Haliotoidea; Haliotidae; Haliotis	genome - annotated by ourselves	NCBI	PRJNA433241	Botwright NA, Zhao M, Wang T, McWilliam S, Colgrave ML et al. 2019. Greenlip Abalone (*Haliotis laevigata*) Genome and Protein Analysis Provides Insights into Maturation and Spawning. G3: Genes, Genomes, Genetics 9: 3067-3078.
Haliotis laevigata	Mollusca; Gastropoda; Vetigastropoda; Lepetellida; Haliotoidea; Haliotidae; Haliotis	transcriptome	NCBI	PRJNA286263	Shiel BP, Hall NE, Cooke IR, Robinson NA & Strugnell JM 2015. De Novo Characterisation of the Greenlip Abalone Transcriptome (*Haliotis laevigata*) with a Focus on the Heat Shock Protein 70 (HSP70) Family. Marine Biotechnology 17: 23-32.
Haliotis midae	Mollusca; Gastropoda; Vetigastropoda; Lepetellida; Haliotoidea; Haliotidae; Haliotis	transcriptome	NCBI	PRJNA79815	Franchini P, van der Merwe M & Roodt-Wilding R 2011. Transcriptome characterization of the South African abalone *Haliotis midae* using sequencing-by-synthesis. BMC Research Notes 4: 59; Reinhold SW, Reichle A, Leiminger S, Bergler T, Hoffmann U et al. 2011. Renal function during rofecoxib therapy in patients with metastatic cancer: retrospective analysis of a prospective phase II trial. BMC Research Notes 4: 2.
Haliotis midae 2	Mollusca; Gastropoda; Vetigastropoda; Lepetellida; Haliotoidea; Haliotidae; Haliotis	transcriptome	NCBI	PRJNA257776	University of Stellenbosch, Rhode C, 2014.

Haliotis tuberculata	Mollusca; Gastropoda; Vetigastropoda; Lepetellida; Haliotoidea; Haliotidae; Haliotis	transcriptome	NCBI	part of PRJNA297974	University of Brest, Harney E, 2018.
Haliotis tuberculata 2	Mollusca; Gastropoda; Vetigastropoda; Lepetellida; Haliotoidea; Haliotidae; Haliotis	transcriptome	NCBI	part of PRJNA297974	University of Brest, Harney E, 2018.
Hapalochlaena maculosa	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Octopodiformes; Octopoda; Incirrata; Octopodidae; Hapalochlaena	transcriptome	NCBI	part of PRJNA337893	Caruana NJ, Cooke IR, Faou P, Finn P, Hall NE et al. 2016. A combined proteomic and transcriptomic analysis of slime secreted by the southern bottletail squid, *Sepiadarium austrinum* (Cephalopoda). Journal of Proteomics 148: 170-82.
Hapalochlaena maculosa 2	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Octopodiformes; Octopoda; Incirrata; Octopodidae; Hapalochlaena	transcriptome	NCBI	PRJNA188571	University of Queensland, Fry B, 2013.
Helix aspersa	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Eupulmonata; Stylommatophora; Sigmurethra; Helicoidea; Helicidae; Helix	transcriptome	NCBI	PRJNA375987	University of Athens Molecular Ecology Lab, Parmakelis A, 2017.
Idiosepius notoides 2	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Sepiolida; Idiosepiidae; Idiosepius	transcriptome	NCBI	part of PRJNA357466	De Oliveira AL, Wollesen T, Kristof A, Scherholz M, Redl E et al. 2016. Comparative transcriptomics enlarges the toolkit of known developmental genes in mollusks. BMC Genomics 17: 905.

Idiosepius notoides	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Sepiolida; Idiosepiidae; Idiosepius	transcriptome	NCBI	part of PRJNA337893	Caruana NJ, Cooke IR, Faou P, Finn P, Hall NE et al. 2016. A combined proteomic and transcriptomic analysis of slime secreted by the southern bottletail squid, *Sepiadarium austrinum* (Cephalopoda). Journal of Proteomics 148: 170-82.
Laevipilina hyalina	Mollusca; Monoplacophora; Tryblidiida; Neopilinidae; Laevipilina	transcriptome - assembled by ourselves	NCBI	part of PRJNA253054	Smith SA, Wilson NG, Goetz FE, Fehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.
Laevipilina hyalina 2	Mollusca; Monoplacophora; Tryblidiida; Neopilinidae; Laevipilina	transcriptome	NCBI	part of PRJNA253054	Zapata F, Wilson NG, Howison M, Andrande SCS, Jörger KM et al. 2014. Phylogenomic analyses of deep gastropod relationships reject Orthogastropoda. Proceedings of the Royal Society B 281: 20141739.
Laevipilina antarctica	Mollusca; Monoplacophora; Tryblidiida; Neopilinidae; Laevipilina	genome	NCBI	PRJNA427216	Kocot KM, Poustka AJ, Stöger I, Halanych KM & Schrödl M 2020. New data from Monoplacophora and a carefully-curated dataset resolve molluscan relationships. Scientific Reports 10: 101.
Lanistes nyassanus	Mollusca; Gastropoda; Caenogastropoda; Architaenioglossa; Ampullarioidea; Ampullariidae; Lanistes	transcriptome	DRYAD	PRJNA523095	Sun J, Mu H, Ip JCH, Li R, Xu T et al. 2019. Signatures of Divergence, Invasiveness, and Terrestrialization Revealed by Four Apple Snail Genomes. Molecular Biology and Evolution 36: 1507–1520.
Lepidopleurus cajetanus	Mollusca; Polyplacophora; Neoloricata; Lepidopleurida; Lepidopleuridae; Lepidopleurus	transcriptome - assembled by ourselves	NCBI	PRJNA507028	Harvard University, Lemer S, 2018.
Leptochiton rugatus	Mollusca; Polyplacophora; Neoloricata; Lepidopleurida; Lepidopleuridae; Leptochiton	transcriptome - assembled by ourselves	NCBI	part of PRJNA263418	Halanych KM & Kocot KM 2014. Repurposed Transcriptomic Data Facilitate Discovery of Innate Immunity Toll-Like Receptor (TLR) Genes Across Lophotrochozoa. The Biological Bulletin 227: 201–209.

Limacina antarctica	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Euopisthobranchia; Pteropoda; Thecosomata; Euthecosomata; Limacinoidea; Limacinidae; Limacina	transcriptome	NCBI	PRJNA295792	University of California, Santa Barbara, Johnson K, 2015.
Limacina helicina	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Euopisthobranchia; Pteropoda; Thecosomata; Euthecosomata; Limacinoidea; Limacinidae; Limacina	transcriptome	NCBI	PRJNA386290	Bermuda Institute of Ocean Sciences, Koh H, 2013.
Limacina retroversa	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Euopisthobranchia; Pteropoda; Thecosomata; Euthecosomata; Limacinoidea; Limacinidae; Limacina	transcriptome	NCBI	PRJNA260534	Woods Hole Oceanographic Institution, Maas A, 2015.
Limecola balthica balthica	Mollusca; Bivalvia; Heterodonta; Euheterodonta; Veneroida; Tellinoidea; Tellinidae; Limecola	transcriptome	NCBI	PRJNA384460	Yurchenko AA, Katolikova N, Polev D, Shcherbakova I & Strelkov P 2018. Transcriptome of the bivalve *Limecola balthica* L. from Western Pacific: A new resource for studies of European populations. Marine Genomics 40: 58-63.
Limnoperna fortunei	Mollusca; Bivalvia; Pteriomorphia; Myti-	transcriptome	NCBI	PRJNA248620	Uliano-Silva M, Alves Americo J, Brindeiro R, Dondero F, Prosdocimi F et al. 2014. Gene Discovery through Transcriptome Sequencing for the Invasive Mussel *Limnoperna fortunei*. PLoS ONE 9(7): e102973.

	loida; Mytiloidea; Mytilidae; Mytilinae; Limnoperna				
Limnoperna fortunei	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Limnoperna	genome	GigaDB	PRJNA330677	Uliano-Silva, M. et al. (2018) A hybrid-hierarchical genome assembly strategy to sequence the invasive golden mussel, *Limnoperna fortunei*. GigaScience 7: gix128.
Lithophaga lithophaga	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Lithophaginae; Lithophaga	transcriptome	NCBI	PRJNA328907	Sivka U, Toplak N, Koren S & Jakše J 2018. *De novo* transcriptome of the pallial gland of the date mussel (*Lithophaga lithophaga*). Comparative Biochemistry Physiology Part D: Genomics and Proteomics 26: 1-9.
Littorina littorea	Mollusca; Gastropoda; Caenogastropoda; Littorinimorpha; Littorinoidea; Littorinidae; Littorina	transcriptome	NCBI	PRJNA421656	Gorbushin AM 2020. Toll-like signaling pathway in the transcriptome of *Littorina littorea*. Fish & Shellfish Immunology 106: 640-644; Gorbushin AM 2018. Immune repertoire in the transcriptome of *Littorina littorea* reveals new trends in lophotrochozoan proto-complement evolution. Developmental & Comparative Immunology 84: 250-263.
Littorina littorea	Mollusca; Gastropoda; Caenogastropoda; Littorinimorpha; Littorinoidea; Littorinidae; Littorina	transcriptome	NCBI	part of PRJNA72139	Smith SA, Wilson NG, Goetz FE, Fehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.
Littorina saxatilis	Mollusca; Gastropoda; Caenogastropoda; Littorinimorpha; Littorinoidea; Littorinidae; Littorina	transcriptome	NCBI	PRJNA550990	University of Sheffield, Chaube P, 2019.
Littorina saxatilis 2	Mollusca; Gastropoda; Caenogastropoda; Lit-	transcriptome	NCBI	PRJNA550990	University of Sheffield, Chaube P, 2019.

	torinimorpha; Littorinoidea; Littorinidae; Littorina				
Littorina saxatilis 3	Mollusca; Gastropoda; Caenogastropoda; Littorinimorpha; Littorinoidea; Littorinidae; Littorina	transcriptome	NCBI	PRJNA550990	University of Sheffield, Chaube P, 2019.
Lottia cf. *kogamogai*	Mollusca; Gastropoda; Patellogastropoda; Lottioidea; Lottiidae; Lottia	transcriptome	NCBI	part of PRJNA357466	De Oliveira AL, Wollesen T, Kristof A, Scherholz M, Redl E et al. 2016. Comparative transcriptomics enlarges the toolkit of known developmental genes in mollusks. BMC Genomics 17: 905.
Lottia gigantea	Mollusca; Gastropoda; Patellogastropoda; Lottioidea; Lottiidae; Lottia	genome	NCBI, Ensembl	PRJNA259762	Simakov O, Marletaz F, Cho S-J, Edsinger-Gonzales E, Havlak P et al. 2013. Insights into bilaterian evolution from three spiralian genomes. Nature 493: 526-531.
Lymnaea stagnalis	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Hygrophila; Lymnaeoidea; Lymnaeidae; Lymnaea	genome - annotated by ourselves	NCBI	PRJEB11470	Herlitze I, Marie B, Marin F & Jackson DJ 2018. Molecular modularity and asymmetry of the molluscan mantle revealed by a gene expression atlas. Gigascience 7: giy056.
Lymnaea stagnalis 2	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Hygrophila; Lymnaeoidea; Lymnaeidae; Lymnaea	transcriptome	NCBI	PRJDB98	Sadamoto H, Takahashi H, Okada T, Kenmoku H, Toyota M et al. 2012. *De Novo* Sequencing and Transcriptome Analysis of the Central Nervous System of Mollusc *Lymnaea stagnalis* by Deep RNA Sequencing.", PLoS ONE 7(8): e42546.
Margaritifera margaritifera	Mollusca; Bivalvia; Palaeoheterodonta; Unionida; Unionoidea; Margaritiferidae; Margaritifera	transcriptome	NCBI	PRJNA371446	Bertucci A, Pierron F, Thébault J, Klopp C, Bellec J et al. 2017. Transcriptomic responses of the endangered freshwater mussel *Margaritifera margaritifera* to trace metal contamination in the Dronne River, France.

					Environmental Science and Pollution Research 24: 27145-27159.
Marisa cornuarietis	Mollusca; Gastropoda; Caenogastropoda; Architaenioglossa; Ampullarioidea; Ampullariidae; Marisa	genome	DRYAD	PRJNA445755	Sun J, Mu H, Ip JCH, Li R, Xu T et al. 2019. Signatures of Divergence, Invasiveness, and Terrestrialization Revealed by Four Apple Snail Genomes. Molecular Biology and Evolution 36: 1507–1520.
Melibe leonina	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia; Cladobranchia; Dendronotoidea; Dendronotidae; Melibe	transcriptome	NCBI	PRJNA420367	New England College, Newcomb J, 2017.
Mimachlamys varia	Mollusca; Bivalvia; Pteriomorphia; Pectinoida; Pectinoidea; Pectinidae; Mimachlamys	transcriptome	NCBI	PRJNA427371	Centre nationale de la recherche scientifique, 2017
Modiolus philippinarum	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Modiolinae; Modiolus	genome	DRYAD	PRJNA328544	Sun J, Zhang Y, Xu T, Zhang Y, Mu H et al. 2017. Adaptation to deep-sea chemosynthetic environments as revealed by mussel genomes. Nature Ecology & Evolution 1: 0121.
Mytilus californianus	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Mytilus	transcriptome	NCBI	part of PRJNA375125	DeMartini DG, Errico JM, Sjoestroem S, Fenster A & Waite JH 2017. A cohort of new adhesive proteins identified from transcriptomic analysis of mussel foot glands. Journal of the Royal Society Interface 14: 131.
Mytilus californianus 2	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Mytilus	transcriptome	NCBI	part of PRJNA375125	DeMartini DG, Errico JM, Sjoestroem S, Fenster A & Waite JH 2017. A cohort of new adhesive proteins identified from transcriptomic analysis of mussel foot glands. Journal of the Royal Society Interface 14: 131.

Mytilus californianus 3	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Mytilus	transcriptome	NCBI	part of PRJNA375125	DeMartini DG, Errico JM, Sjoestroem S, Fenster A & Waite JH 2017. A cohort of new adhesive proteins identified from transcriptomic analysis of mussel foot glands. Journal of the Royal Society Interface 14: 131.
Mytilus edulis	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Mytilus	transcriptome	NCBI	PRJNA525607	Senckenberg Gesellschaft für Naturforschung, Stuckas H, 2019.
Mytilus edulis 2	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Mytilus	transcriptome	NCBI	PRJNA494236	British Antarctic Survey, Yarra T, 2018.
Mytilus edulis 3	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Mytilus	transcriptome	NCBI	PRJEA75259	Philipp EER, Kraemer L, Melzner F, Poustka AJ, Thieme S et al. 2012. Massively Parallel RNA Sequencing Identifies a Complex Immune Gene Repertoire in the lophotrochozoan *Mytilus edulis*. PLoS ONE 7(3): e33091.
Mytilus galloprovincialis	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Mytilus	genome - annotated by ourselves	NCBI	PRJNA262617	Murgarella M, Puiu D, Novoa B, Figueras A, Posada D et al. 2016. A First Insight into the Genome of the Filter-Feeder Mussel *Mytilus galloprovincialis*. PLoS ONE 11: e0151561.
Mytilus galloprovincialis 2	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Mytilus	transcriptome	NCBI	PRJNA484309	University of Copenhagen, Fonseca R, 2018.
Mytilus galloprovincialis 3	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Mytilus	transcriptome	NCBI	part of PRJNA167773	Universidade da Coruña, Fernandez-Tajes J, 2012.
Mytilus galloprovincialis 4	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Mytilus	transcriptome	NCBI	PRJNA525609	Senckenberg Gesellschaft für Naturforschung, Stuckas H, 2019.

Mytilus gallo-provincialis 5	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Mytilus	transcriptome	NCBI	PRJNA362681	University of Ljubljana, Sivka U, 2017.
Mytilus trossulus	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Mytilus	transcriptome	NCBI	PRJNA525608	Senckenberg Gesellschaft für Naturforschung, Stuckas H, 2019.
Nautilus pompilius	Mollusca; Cephalopoda; Nautiloidea; Nautilida; Nautilidae; Nautilus	transcriptome	NCBI	part of PRJNA72139	Smith SA, Wilson NG, Goetz FE, Fehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.
Neomenia megatrapezata	Mollusca; Aplacophora; Solenogastres; Neomeniamorpha; Neomeniidae	transcriptome - assembled by ourselves	NCBI	part of PRJNA72139	Smith SA, Wilson NG, Goetz FE, Fehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.
Neomeniomorpha sp1	Mollusca; Aplacophora; Solenogastres;	transcriptome - assembled by ourselves	NCBI	part of PRJNA72139	Smith SA, Wilson NG, Goetz FE, Fehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.
Nodipecten subnodosus	Mollusca; Bivalvia; Pteriomorphia; Pectinoida; Pectinoidea; Pectinidae; Nodipecten	transcriptome	NCBI	PRJNA399878	CIBNOR, Instituto Politecnico Nacional, Galindo Torres P, 2017.
Nodipecten subnodosus 2	Mollusca; Bivalvia; Pteriomorphia; Pectinoida; Pectinoidea; Pectinidae; Nodipecten	transcriptome	NCBI	PRJNA388311	CIBNOR, Instituto Politecnico Nacional, Galindo Torres P, 2017.
Nodipecten subnodosus 3	Mollusca; Bivalvia; Pteriomorphia; Pectinoida; Pectinoidea; Pectinidae; Nodipecten	transcriptome	NCBI	PRJNA348566	CIBNOR, Instituto Politecnico Nacional, Galindo Torres P, 2016.

Nucula expansa	Mollusca; Bivalvia; Protobranchia; Nuculoida; Nuculidae; Nucula	transcriptome	NCBI	PRJNA72139	Smith SA, Wilson NG, Goetz FE, Fehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.
Nucula tumidula	Mollusca; Bivalvia; Protobranchia; Nuculoida; Nuculidae; Nucula	transcriptome	NCBI	PRJNA357466	De Oliveira AL, Wollesen T, Kristof A, Scherholz M, Redl E et al. 2016. Comparative transcriptomics enlarges the toolkit of known developmental genes in mollusks. BMC Genomics 17: 905.
Octopoteuthis deletron	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Octopodiformes; Octopoda; Incirrata; Octopodidae; Octopus	transcriptome	NCBI	PRJNA342927	Francis WR, Christianson LM & Haddock SHD 2017. Symplectin evolved from multiple duplications in bioluminescent squid. PeerJ 5: e3633.
Octopus bimaculoides	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Octopodiformes; Octopoda; Incirrata; Octopodidae; Octopus	genome	NCBI, Ensembl	PRJNA270931	Albertin CB, Simakov O, Mitros T, Yan Wang Z, Pungor JR et al. 2015. The octopus genome and the evolution of cephalopod neural and morphological novelties. Nature 524: 220-224.
Octopus kaurna	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Octopodiformes; Octopoda; Incirrata; Octopodidae; Octopus	transcriptome	NCBI	PRJNA188658	University of Queensland, Fry B, 2013.
Octopus kaurna 2	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Octopodiformes; Octopoda; Incirrata; Octopodidae	transcriptome	NCBI	part of PRJNA337893	Caruana NJ, Cooke IR, Faou P, Finn P, Hall NE et al. 2016. A combined proteomic and transcriptomic analysis of slime secreted by the southern bottletail squid, *Sepiadarium austrinum* (Cephalopoda). Journal of Proteomics 148: 170-82.

Octopus maya	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Octopodiformes; Octopoda; Incirrata; Octopodidae	transcriptome	NCBI	PRJNA496073	Center for Scientific Research and Higher Education of Ensenada, Galindo C, 2018.
Octopus maya 2	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Octopodiformes; Octopoda; Incirrata; Octopodidae	transcriptome	NCBI	PRJNA492175	Center for Scientific Research and Higher Education of Ensenada, Galindo C, 2018.
Octopus maya 3	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Octopodiformes; Octopoda; Incirrata; Octopodidae	transcriptome	NCBI	PRJNA345483	Centro de Investigacion Cientifica y de Educacion Superior de Ensenada, Juarez O, 2016.
Octopus minor	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Octopodiformes; Octopoda; Incirrata; Octopodidae	genome	GigaDB	PRJNA421033	Fenghui L, Bian L, Ge J, Han F, Liu Z et al. 2020. Chromosome-level genome assembly of the East Asian common octopus (*Octopus sinensis*) using PacBio sequencing and Hi-C technology. Molecular Ecology Resources 00: 1–11.
Octopus sinensis	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Octopodiformes; Octopoda; Incirrata; Octopodidae	genome	figshare		Baldascino E, Di Cristina G, Tedesco P, Hobbs C, Shaw TJ et al. 2017. The Gastric Ganglion of *Octopus vulgaris*: Preliminary Characterization of Gene- and Putative Neurochemical-Complexity, and the Effect of *Aggregata octopiana* Digestive Tract Infection on Gene Expression. Frontiers in Physiology 8: 1001.
Octopus sinensis	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Octopodiformes; Octopoda; Incirrata; Octopodidae	genome	NCBI	PRJNA541812/ PRJNA551489	Yokobori S, Fukuda N, Nakamura M, Aoyama T & Oshima T 2004. Long-Term Conservation of Six Duplicated Structural Genes in Cephalopod Mitochondrial Genomes. Molecular Biology and Evolution 21: 2034-2046; Takuwa-Kuroda K, Iwakoshi-Ukena E, Kanda A & Minakata H 2003. Octopus, which owns the most advanced brain in invertebrates, has two members of

					vasopressin/oxytocin superfamily as in vertebrates. Regulatory Peptides 115: 139-149.
Octopus vulgaris	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Octopodiformes; Octopoda; Incirrata; Octopodidae	transcriptome	NCBI	part of PRJNA72139	Smith SA, Wilson NG, Goetz FE, Fehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.
Octopus vulgaris	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Octopodiformes; Octopoda; Incirrata; Octopodidae	transcriptome - assembled by ourselves	NCBI	PRJNA464423	James Cook University, Zhang J, 2018.
Onychoteuthis banksii	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Teuthida; Oegopsina; Onychoteuthidae; Onychoteuthis	transcriptome	NCBI	part of PRJNA534469	da Fonseca RR, Couto A, Machado AM, Brejova B, Albertin CB et al. 2020. A draft genome sequence of the elusive giant squid, *Architeuthis dux*. GigaScience 9: giz152.
Panopea globosa	Mollusca; Bivalvia; Heterodonta; Euheterodonta; Myoida; Hiatelloidea; Hiatellidae; Panopea	transcriptome	NCBI	PRJNA407974	Centro de Investigación Científica y de Educación Superior de Enseñada, Baja California, Juarez O, 2017.
Paphia undulata	Mollusca; Bivalvia; Heterodonta; Euheterodonta; Veneroida; Veneroidea; Veneridae; Paphia	transcriptome	NCBI	PRJNA459966	Jimei University, Deng S, 2018.
Patinopecten yessoensis	Mollusca; Bivalvia; Pteriomorphia; Pectinoida; Pectinoidea;	genome	NCBI	PRJNA259405	Wang S, Zhang J, Jiao W, Li J, Xun X et al. 2017. Scallop genome provides insights into evolution of bilaterian karyotype and development. Nature Ecology & Evolution 1, 0120.

	Pectinidae; Mizuhopecten				
Pecten maximus	Mollusca; Bivalvia; Pteriomorphia; Pectinoida; Pectinoidea; Pectinidae; Pecten	genome	figshare	PRJEB35330/ PRJEB35329	Kenny NJ, McCarthy SA, Dudchenko O, James K, Betteridge E et al. 2019. The gene-rich genome of the scallop *Pecten maximus*. GigaScience 9: giaa037.
Pecten maximus	Mollusca; Bivalvia; Pteriomorphia; Pectinoida; Pectinoidea; Pectinidae; Pecten	transcriptome	NCBI	PRJNA222492	Pauletto M, Milan M, Moreira R, Novoa B, Figueras A et al. 2014. Deep transcriptome sequencing of *Pecten maximus* hemocytes: A genomic resource for bivalve immunology. Fish & Shellfish Immunology 37: 154-165.
Pecten maximus 2	Mollusca; Bivalvia; Pteriomorphia; Pectinoida; Pectinoidea; Pectinidae; Pecten	transcriptome	NCBI	PRJNA419464	University of Padova, Pauletto M, 2017.
Pecten maximus 3	Mollusca; Bivalvia; Pteriomorphia; Pectinoida; Pectinoidea; Pectinidae; Pecten	transcriptome	NCBI	PRJNA298284	University of Brest, Harney E, 2018.
Perna canaliculus	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Perna	transcriptome	NCBI	PRJNA439164	The Australian National University, Ranjard L, 2018.
Perna viridis	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Perna	transcriptome	NCBI	PRJNA478494	BGI, Zhang X, 2018.
Perna viridis 2	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Perna	transcriptome	NCBI	PRJNA181275	Guerette PA, Hoon S, Seow Y, Raida M, Masic A et al. 2013. Accelerating the design of biomimetic materials by integrating RNA-seq with proteomics and materials science. Nature Biotechnology 31: 908-915.
Perotrochus lucaya	Mollusca; Gastropoda; Vetigastropoda; Pleu-	transcriptome	NCBI	part of PRJNA72139	Smith SA, Wilson NG, Goetz FE, Fehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.

	rotomariida; Pleurotomarioidea; Pleurotomariidae; Perotrochus				
Phacoides pectinatus	Mollusca; Bivalvia; Heterodonta; Euheterodonta; Lucinoida; Lucinoidea; Lucinidae; Phacoides	transcriptome	NCBI	PRJNA282817	University of Puerto Rico Medical Sciences Campus, Montes-Rodriguez I, 2015.
Phreagena okutanii	Mollusca; Bivalvia; Heterodonta; Euheterodonta; Veneroida; Glossoidea; Vesicomyidae	transcriptome	NCBI	part of PRJNA471131	Lan Y, Sun J, Zhang W, Xu T, Zhang Y et al. 2019. Host–Symbiont Interactions in Deep-Sea Chemosymbiotic Vesicomyid Clams: Insights From Transcriptome Sequencing. Frontiers in Marine Science 6: 680.
Phylliroe bucephala	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Nudipleura; Nudibranchia; Cladobranchia; Tritonioidea; Phylliroidae; Phylliroe	transcriptome	NCBI	PRJNA342927	Francis WR, Christianson LM & Haddock SHD 2017. Symplectin evolved from multiple duplications in bioluminescent squid. PeerJ 5: e3633.
physella acuta	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Hygrophila; Lymnaeoidea; Physidae; Physella	genome - annotated by ourselves	NCBI	PRJNA407370	University of Mexico, Schultz J, 2017.
Physella acuta	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Hygrophila; Lymnaeoidea; Physidae; Physella	transcriptome	NCBI	PRJNA497591	University of Mexico, Schultz J, 2018.

Pinctada fucata	Mollusca; Bivalvia; Pteriomorphia; Pterioida; Pterioidea; Pteriidae; Pinctada	genome	GigaDB		Du X, Fan G, Jiao Y, Zhang H, Guo X et al. 2017. The pearl oyster *Pinctada fucata martensii* genome and multi-omic analyses provide insights into biomineralization. Gigascience 6: 1-12.
Pinctada fucata 2	Mollusca; Bivalvia; Pteriomorphia; Pterioida; Pterioidea; Pteriidae; Pinctada	genome	NCBI	PRJDB2628	Takeuchi T, Koyanagi R, Gyoja F, Kanda M, Hisata K et al. 2016. Bivalve-specific gene expansion in the pearl-oyster genome: implications of adaptation to a sessile lifestyle. Zoological Letters 2: 3.
Pinctada fucata 3	Mollusca; Bivalvia; Pteriomorphia; Pterioida; Pterioidea; Pteriidae; Pinctada	transcriptome	NCBI	PRJNA315354	South China Sea Insititute of Oceanology Chinese Academy of Sciences, Guan Y, 2016.
Pinctada imbricata radiata	Mollusca; Bivalvia; Pteriomorphia; Pterioida; Pterioidea; Pteriidae; Pinctada	genome - annotated by ourselves	NCBI	PRJNA283019	Du X, Fan G, Jiao Y, Zhang H, Guo X et al. 2017. The pearl oyster *Pinctada fucata martensii* genome and multi-omic analyses provide insights into biomineralization. Gigascience 6: 1-12.
Pinctada radiata	Mollusca; Bivalvia; Pteriomorphia; Pterioida; Pterioidea; Pteriidae; Pinctada	transcriptome	NCBI	PRJDB8463	The Roslin Institute, Genetics and Genomics, The University of Edinburgh, 2019.
Plakobranchus ocellatus	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Sacoglossa; Placobranchoidea; Plakobranchidae; Plakobranchus	transcriptome	NCBI	PRJNA52099	Wägele H, Deusch O, Händeler K, Martin R, Schmitt V et al. 2011. Transcriptomic Evidence That Longevity of Acquired Plastids in the Photosynthetic Slugs *Elysia timida* and *Plakobranchus ocellatus* Does Not Entail Lateral Transfer of Algal Nuclear Genes. Molecular Biology and Evolution 28: 699-706.
Placopecten magellanicus	Mollusca; Bivalvia; Pteriomorphia; Pectinoida; Pectinoidea; Pectinidae; Placopecten	transcriptome	NCBI	PRJNA182246	Pairett AN & Serb JM 2013. *De Novo* Assembly and Characterization of Two Transcriptomes Reveal Multiple Light-Mediated Functions in the Scallop Eye (Bivalvia: Pectinidae). PLoS ONE 8(7): e69852.
Pomacea canaliculata	Mollusca; Gastropoda; Caenogastropoda;	genome	GigaDB	PRJNA427478	Liu C, Zhang Y, Ren Y, Wang H, Li S et al. 2018. The genome of the golden apple snail *Pomacea canaliculata* provides

	Architaenioglossa; Ampullarioidea; Ampullariidae; Pomacea				insight into stress tolerance and invasive adaptation. Gigascience 7: giy101.
Pomacea canaliculata	Mollusca; Gastropoda; Caenogastropoda; Architaenioglossa; Ampullarioidea; Ampullariidae; Pomacea	genome	DRYAD		Sun J, Wang M, Wang H, Zhang H, Thiyagarajan V et al. 2012. *De novo* assembly of the transcriptome of an invasive snail and its multiple ecological applications. Molecular Ecology Resources 12: 1133-1144; Sun J, Mu H, Ip JCH, Li R, Xu T et al. 2019. Signatures of divergence, invasiveness, and terrestrialization revealed by four apple snail genomes. Molecular Biology and Evolution 36: 1507-1520.
Pomacea canaliculata	Mollusca; Gastropoda; Caenogastropoda; Architaenioglossa; Ampullarioidea; Ampullariidae; Pomacea	transcriptome	NCBI	PRJNA264139	Pearl River Fisheries Research Institute.CAFS, Xidong M, 2014.
Pomacea maculata	Mollusca; Gastropoda; Caenogastropoda; Architaenioglossa; Ampullarioidea; Ampullariidae; Pomacea	genome	DRYAD	PRJNA523958	Sun J, Mu H, Ip JCH, Li R, Xu T et al. 2019. Signatures of divergence, invasiveness, and terrestrialization revealed by four apple snail genomes. Molecular Biology and Evolution 36: 1507-1520.
Potamopyrgus antipodarum	Mollusca; Gastropoda; Caenogastropoda; Littorinimorpha; Truncatelloidea; Tateidae; Potamopyrgus	transcriptome	NCBI	PRJNA423241	University of Iowa, McElroy K, 2017.
Potamopyrgus antipodarum 2	Mollusca; Gastropoda; Caenogastropoda; Littorinimorpha; Truncatelloidea; Tateidae; Potamopyrgus	transcriptome	NCBI	PRJNA381223	University of Iowa, Bankers L, 2017.

Proneomenia custodiens	Mollusca; Aplacophora; Solenogastres; Cavibelonia; Proneomeniidae	transcriptome - assembled by ourselves	NCBI	part of PRJNA263418	Halanych KM & Kocot KM 2014. Repurposed Transcriptomic Data Facilitate Discovery of Innate Immunity Toll-Like Receptor (TLR) Genes Across Lophotrochozoa. The Biological Bulletin 227: 201–209.
Pteria penguin	Mollusca; Bivalvia; Pteriomorphia; Pterioida; Pterioidea; Pteriidae; Pteria	transcriptome	NCBI	PRJNA318657	South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Li M, 2016.
Pterygioteuthis hoylei	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Teuthida; Oegopsina; Enoploteuthidae; Pterygioteuthis	transcriptome	NCBI	PRJNA342927	Francis WR, Christianson LM & Haddock SHD 2017. Symplectin evolved from multiple duplications in bioluminescent squid. PeerJ 5: e3633.
Radix auricularia	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Hygrophila; Lymnaeoidea; Lymnaeidae	genome	NCBI	PRJNA350764	Schell T, Feldmeyer B, Schmidt H, Greshake B, Tills O et al. 2017. An Annotated Draft Genome for *Radix auricularia* (Gastropoda, Mollusca). Genome Biology and Evolution 9: 585-592.
Rapana venosa	Mollusca; Gastropoda; Caenogastropoda; Neogastropoda; Muricoidea; Muricidae	transcriptome	NCBI	PRJNA400655	Institute of Oceanology, Chinese Academy of Sciences, Hao S, 2017.
Rapana venosa 2	Mollusca; Gastropoda; Caenogastropoda; Neogastropoda; Muricoidea; Muricidae; Rapana	transcriptome	NCBI	PRJNA288836	Song H, Yu Z-L, Sun L-N, Gao Y, Zhang T et al. 2016. De novo transcriptome sequencing and analysis of *Rapana venosa* from six different developmental stages using Hi-seq 2500. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 17: 48-57.
Ruditapes decussatus	Mollusca; Bivalvia; Heterodonta; Euheterodonta; Veneroida;	transcriptome	NCBI	PRJNA170478	Ghiselli F, Iannello M, Puccio G, Chang PL, Plazzi F et al. 2018. Comparative Transcriptomics in Two Bivalve Species Offers Different Perspectives on the Evolution of

	Veneroidea; Veneridae; Ruditapes				Sex-Biased Genes. Genome Biology and Evolution 10: 1389-1402; Ghiselli F, Milani L, Iannello M, Procopio E, Chang PL et al. 2017. The complete mitochondrial genome of the grooved carpet shell, *Ruditapes decussatus* (Bivalvia, Veneridae). PeerJ 5: e3692.
Ruditapes philippinarum	Mollusca; Bivalvia; Heterodonta; Euheter-odonta; Veneroida; Veneroidea; Veneridae; Ruditapes	transcriptome	NCBI	PRJNA489449	Pierron F, Gonzalez P, Bertucci A, Binias C, Mérour E et al. 2019. Transcriptome-wide analysis of wild Asari (=Manila) clams affected by the Brown Muscle Disease: Etiology and impacts of the disease. Fish & Shellfish Immunology 86: 179-185.
Ruditapes philippinarum 2	Mollusca; Bivalvia; Heterodonta; Euheter-odonta; Veneroida; Veneroidea; Veneridae; Ruditapes	transcriptome	NCBI	PRJNA426752	Jimei University, Shangguan J, 2017.
Ruditapes philippinarum 3	Mollusca; Bivalvia; Heterodonta; Euheter-odonta; Veneroida; Veneroidea; Veneridae; Ruditapes	transcriptome	NCBI	PRJNA137531	Milan M, Coppe A, Reinhardt R, Cancela LM,Leite RB et al. 2011. Transcriptome sequencing and microarray development for the Manila clam, *Ruditapes philippinarum*: genomic tools for environmental monitoring.", BMC Genomics 12: 234.
Ruditapes philippinarum 4	Mollusca; Bivalvia; Heterodonta; Euheter-odonta; Veneroida; Veneroidea; Veneridae; Ruditapes	transcriptome	NCBI	PRJNA298283	University of Brest, Harney E, 2018.
Saccostrea glomerata	Mollusca; Bivalvia; Pteriomorphia; Ostre-oida; Ostreoidea; Os-treidae; Saccostrea	genome	dbSROG	PRJNA414259	Powell D, Subramanian S, Suwansa-ard S, Zhao M, O´Con-nor W et al. 2018. The genome of the oyster *Saccostrea* offers insight into the environmental resilience of bivalves. DNA Research 25: 655-665.
Saccostrea glomerata	Mollusca; Bivalvia; Pteriomorphia; Ostre-oida; Ostreoidea; Os-treidae; Saccostrea	transcriptome	NCBI	PRJNA253158	Hook SE, Johnston EL, Nair S, Roach AC, Moncuquet P et al. 2014. Next generation sequence analysis of the transcriptome of Sydney rock oysters (*Saccostrea*

					glomerata) exposed to a range of environmental stressors. Marine Genomics 18:109-111.
Saccostrea glomerata 2	Mollusca; Bivalvia; Pteriomorphia; Ostreoida; Ostreoidea; Ostreidae; Saccostrea	transcriptome	NCBI	part of PRJNA487836	McDougall C 2018. Comparative *De Novo* transcriptome analysis of the Australian black-lip and Sydney rock oysters reveals expansion of repetitive elements in *Saccostrea* genomes. PLoS ONE 13(10): e0206417.
Saccostrea glomerata 3	Mollusca; Bivalvia; Pteriomorphia; Ostreoida; Ostreoidea; Ostreidae; Saccostrea	transcriptome	NCBI	PRJNA434403	University of the Sunshine Coast, Ertl N, 2015.
Saccostrea glomerata 4	Mollusca; Bivalvia; Pteriomorphia; Ostreoida; Ostreoidea; Ostreidae; Saccostrea	transcriptome	NCBI	PRJNA434403	University of the Sunshine Coast, Ertl N, 2015.
Saccostrea sp	Mollusca; Bivalvia; Pteriomorphia; Ostreoida; Ostreoidea; Ostreidae; Saccostrea	transcriptome	NCBI	part of PRJNA487836	McDougall C 2018. Comparative *De Novo* transcriptome analysis of the Australian black-lip and Sydney rock oysters reveals expansion of repetitive elements in *Saccostrea* genomes. PLoS ONE 13(10): e0206417.
Scapharca broughtonii	Mollusca; Bivalvia; Pteriomorphia; Arcoida; Arcoidea; Arcidae; Scapharca	genome	GigaDB	PRJNA521075	Bai C-M, Xin L-S, Rosani U, Wu B, Wang Q-C et al. 2019. Chromosomal-level assembly of the blood clam, *Scapharca (Anadara) broughtonii*, using long sequence reads and Hi-C. GigaScience 8: giz067.
Scapharca broughtonii	Mollusca; Bivalvia; Pteriomorphia; Arcoida; Arcoidea; Arcidae; Scapharca	transcriptome	NCBI	PRJNA329570	Ocean University of China, Liu T, 2016.
Scutopus ventrolineatus	Mollusca; Aplacophora; Caudofoveata; Limifossorimorpha; Limifossorida; Scutopodidae; Scutopus	transcriptome	NCBI	part of PRJNA357466	De Oliveira AL, Wollesen T, Kristof A, Scherholz M, Redl E et al. 2016. Comparative transcriptomics enlarges the toolkit of known developmental genes in mollusks. BMC Genomics 17: 905.

Semisulcospira coreana	Mollusca; Gastropoda; Caenogastropoda; Sorbeoconcha; Cerithioidea; Semisulcospiridae; Semisulcospira	transcriptome	NCBI	PRJNA453668	GWNU, shared submissions, 2018.
Sepia esculenta	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Sepiida; Sepiina; Sepiidae; Sepia	transcriptome	NCBI	PRJNA471792	Wuxi Fisheries College, Zhang JY, 2018.
Sepia esculenta 2	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Sepiida; Sepiina; Sepiidae; Sepia	transcriptome	NCBI	PRJNA252556	Whitelaw BL, Strugnell JM, Faou P, da Fonseca RR, Hall NE et al. 2016. Combined Transcriptomic and Proteomic Analysis of the Posterior Salivary Gland from the Southern Blue-Ringed Octopus and the Southern Sand Octopus. Journal of Proteome Research 15: 3284-3297; Guerette PA, Hoon S, Ding D, Amini S, Masic A et al. 2014. Nanoconfined β-sheets Mechanically Reinforce the Supra-Biomolecular Network of Robust Squid Sucker Ring Teeth. ACS Nano 8: 7170-7179.
Sepia latimanus	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Sepiida; Sepiina; Sepiidae; Sepia	transcriptome	NCBI	PRJNA188659	Whitelaw BL, Strugnell JM, Faou P, da Fonseca RR, Hall NE et al. 2016. Combined Transcriptomic and Proteomic Analysis of the Posterior Salivary Gland from the Southern Blue-Ringed Octopus and the Southern Sand Octopus. Journal of Proteome Research 15: 3284-3297.
Sepia pharaonis	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Sepiida; Sepiina; Sepiidae; Sepia	transcriptome	NCBI	PRJNA305947	Guangxi Academy of Fisheries Science, Zhou Y, 2015.
Sepia pharaonis 2	Mollusca; Cephalopoda; Coleoidea; Neo-	transcriptome	NCBI	PRJNA305947	Guangxi Academy of Fisheries Science, Zhou Y, 2015.

	coleoidea; Decapodiformes; Sepiida; Sepiina; Sepiidae; Sepia				
Sepiella maindroni	Mollusca; Cephalopoda; oleoidea; Neocoleoidea; Decapodiformes; Sepiida; Sepiina; Sepiidae; Sepiella	transcriptome	NCBI	PRJNA379668	Tian K, Lou F, Gao T, Zhou Y, Miao Z et al. 2017. *De novo* assembly and annotation of the whole transcriptome of *Sepiella maindroni*. Marine Genomics 38: 13-16.
Sepioloidea linealoata	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Sepiolida; Sepiolidae; Sepioloidea	transcriptome	NCBI	part of PRJNA337893	Caruana NJ et al. 2017. A combined proteomic and transcriptomic analysis of slime secreted by the southern bottletail squid, *Sepiadarium austrinum* (Cephalopoda). J Proteomics 4; 148: 170-82.
Septifer virgatus	Mollusca; Bivalvia; Pteriomorphia; Mytiloida; Mytiloidea; Mytilidae; Mytilinae; Septifer	transcriptome	NCBI	PRJNA361571	Gerdol M, Fujii Y, Hasan I, Koike T, Shimojo S et al. 2017. The purplish bifurcate mussel *Mytilisepta virgata* gene expression atlas reveals a remarkable tissue functional specialization. BMC Genomics 18: 590.
Sinonovacula constricta	Mollusca; Bivalvia; Heterodonta; Euheterodonta; Veneroida; Tellinoidea; Solecurtidae; Sinonovacula	genome - annotated by ourselves	DRYAD	PRJNA508451	Ran Z, Li Z, Yan X, Liao K, Kong F et al. 2019. Chromosome-level genome assembly of the razor clam *Sinonovacula constricta* (Lamarck, 1818). Molecular Ecology Resources 19: 1647-1658.
Sinonovacula constricta	Mollusca; Bivalvia; Heterodonta; Euheterodonta; Veneroida; Tellinoidea; Solecurtidae; Sinonovacula	transcriptome	NCBI	PRJNA182703	Niu D, Wang L, Sun F, Liu Z & Li J 2013. Development of Molecular Resources for an Intertidal Clam, *Sinonovacula constricta*, Using 454 Transcriptome Sequencing. PLoS ONE 8(7): e67456.
Siphonaria pectinata	Mollusca; Gastropoda; Heterobranchia; Euthyneura; Panpulmonata; Siphonarimorpha;	transcriptome	NCBI	part of PRJNA72139	Smith SA, Wilson NG, Goetz FE, Fehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.

	Siphonariida; Siphonarioidea; Siphonariidae; Siphonaria				
Solemya velum	Mollusca; Bivalvia; Protobranchia; Solemyoida; Solemyoidea; Solemyidae; Solemya	transcriptome - assembled by ourselves	NCBI	part of PRJNA72139	Smith SA, Wilson NG, Goetz FE, Fehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.
Stenoteuthis oualaniensis	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Teuthida; Oegopsina; Ommastrephidae; Sthenoteuthis	transcriptome	NCBI	part of PRJNA534469	da Fonseca RR, Couto A, Machado AM, Brejova B, Albertin CB et al. 2020. A draft genome sequence of the elusive giant squid, *Architeuthis dux*. GigaScience 9: giz152.
Tegula atra	Mollusca; Gastropoda; Vetigastropoda; Trochida; Trochoidea; Tegulidae; Tegula	transcriptome	NCBI	PRJNA348830	Universidad de Concepcion, Gallardo C, 2016.
Vampyroteuthis infernalis	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Octopodiformes; Vampyromorpha; Vampyroteuthidae; Vampyroteuthis	transcriptome	NCBI	PRJNA342927	Francis WR, Christianson LM & Haddock SHD 2017. Symplectin evolved from multiple duplications in bioluminescent squid. PeerJ 5: e3633; Francis WR, Christianson LM, Kiko R, Powers ML, Shaner NC et al. 2013. A comparison across non-model animals suggests an optimal sequencing depth for *de novo* transcriptome assembly. BMC Genomics 14: 167.
Watasenia scintillans	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Teuthida; Oegopsina; Enoploteuthidae; Watasenia	transcriptome	NCBI	part of PRJNA303268	Gimenez G, Metcalf P, Paterson NG & Sharpe ML 2016. Mass spectrometry analysis and transcriptome sequencing reveal glowing squid crystal proteins are in the same superfamily as firefly luciferase. Scientific Reports 6: 27638.

Watasenia scintillans 2	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Teuthida; Oegopsina; Enoploteuthidae; Watasenia	transcriptome	NCBI	part of PRJNA303268	Gimenez G, Metcalf P, Paterson NG & Sharpe ML 2016. Mass spectrometry analysis and transcriptome sequencing reveal glowing squid crystal proteins are in the same superfamily as firefly luciferase. Scientific Reports 6: 27638.
Watasenia scintillans 3	Mollusca; Cephalopoda; Coleoidea; Neocoleoidea; Decapodiformes; Teuthida; Oegopsina; Enoploteuthidae; Watasenia	transcriptome	NCBI	part of PRJNA303268	Gimenez G, Metcalf P, Paterson NG & Sharpe ML 2016. Mass spectrometry analysis and transcriptome sequencing reveal glowing squid crystal proteins are in the same superfamily as firefly luciferase. Scientific Reports 6: 27638.
Wirenia argentea	Mollusca; Aplacophora; Solenogastres; Pholidoskepia; Gymnomeniidae; Wirenia	transcriptome	NCBI	part of PRJNA357466	De Oliveira AL, Wollesen T, Kristof A, Scherholz M, Redl E et al. 2016. Comparative transcriptomics enlarges the toolkit of known developmental genes in mollusks. BMC Genomics 17: 905.
Yoldia limatula	Mollusca; Bivalvia; Protobranchia; Nuculanoida; Yoldiidae; Yoldia	transcriptome	NCBI	part of PRJNA72139	Smith SA, Wilson NG, Goetz FE, Fehery C, Andrade SCS et al. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480: 364-367.

Supplemental Table S2. Outcomes of CpG o/e and DNMT analyses along with quality control (BUSCO). The completeness of the genome or the transcriptome is described in percentage as complete (C), fragmented (F) and missing (M) according to the BUSCO notation.

Species	Taxonomy used in tree (Fig. 1)	G, Genome T, Transcriptome	CpG o/e distribution	Inferred methylation	DNMT1	DNMT2	DNMT3	BUSCO NMetazoa_odb10=954				
								C (%)	F (%)	M (%)	# of contigs	Total length
Bivalvia												
Scapharca broughtonii	Arcoida	G/T	bimodal depleted	yes	yes	yes	yes	93.7	1.9	4.4	116264	86839770
Calyptogena marissinica	Imparidentia	T	unimodal, indicative of DNA methylation	yes	yes	yes	yes	91.4	3.9	4.7	25522	37385531
Corbicula fluminea	Imparidentia	T	bimodal depleted	yes	yes	yes	yes	98.3	0.4	1.3	58280	98508513
Dreissena polymorpha	Imparidentia	T	bimodal depleted	yes	yes	no	no	92.4	0.9	6.7	44529	103026865
Dreissena rostriformis	Imparidentia	T	bimodal depleted	yes	yes	yes	no	93.4	0.9	5.7	49672	97969969
Eurhomalea rufa	Imparidentia	T	bimodal depleted	yes	no	yes	no	41.9	24.5	33.6	39372	23265730
Limecola balthica	Imparidentia	T	bimodal depleted	yes	insufficient data			54.6	11.1	34.3	11248	15098209
Panopea globosa	Imparidentia	T	bimodal depleted	yes	yes	no	no	70.1	17.9	12.0	288526	158895095
Paphia undulata	Imparidentia	T	bimodal depleted	yes	yes	yes	no	88.7	4.2	7.1	56002	50259335
Phacoides pectinatus	Imparidentia	T	bimodal depleted	yes	yes	yes	no	33.6	40.0	26.4	182988	71107771
Phreagena okutanii	Imparidentia	T	unimodal, indicative of DNA methylation	yes	yes	yes	no	85.2	6.5	8.3	25249	28184488
Ruditapes decussatus	Imparidentia	T	bimodal depleted	yes	yes	yes	yes	86.1	5.2	8.7	39459	43706522
Ruditapes philippinarum	Imparidentia	T	bimodal depleted	yes	yes	yes	yes	73.5	18.1	8.4	106786	58239801
Sinonovacula constricta	Imparidentia	G/T	bimodal depleted	yes	yes	yes	yes	93.5	1.2	5.3	51197	64160199
Bathymodiolus platifrons	Mytiloida	G/T	bimodal depleted	yes	yes	no	no	79.2	10.2	10.6	33584	52252203
Limnoperna fortunei	Mytiloida	G/T	bimodal depleted	yes	yes	yes	no	32.6	25.1	42.3	60717	55668684

Lithophaga lithophaga	Mytiloida	T	bimodal depleted	yes	**insufficient data**			15.9	35.0	49.1	62490	25280655
Modiolus philippinarum	Mytiloida	G	bimodal depleted	yes	yes	yes	no	67.2	15.7	17.1	36549	56330006
Mytilus californianus	Mytiloida	T	bimodal depleted	yes	no	yes	no	60.7	22.6	16.7	59027	30295976
Mytilus edulis	Mytiloida	T	bimodal depleted	yes	yes	yes	yes	95.2	3.7	1.1	353272	244451632
Mytilus galloprovincialis	Mytiloida	G/T	bimodal depleted	yes	yes	yes	yes	96.9	1.9	1.2	290192	223843395
Mytilus trossulus	Mytiloida	T	bimodal depleted	yes	no	yes	no	92.0	5.9	2.1	437716	268720177
Perna canaliculus	Mytiloida	T	bimodal depleted	yes	yes	yes	yes	99.0	0.7	0.3	536672	445328692
Perna viridis	Mytiloida	T	bimodal depleted	yes	yes	yes	no	68.3	20.4	11.3	73264	43974515
Septifer virgata	Mytiloida	T	bimodal depleted	yes	no	no	no	59.6	9.3	31.1	49498	33594482
Crassostrea angulata	Ostreoida	T	**insufficient data**		yes	yes	yes	93.1	4.4	2.5	94218	86341091
Crassostrea gigas	Ostreoida	G/T	bimodal depleted	yes	yes	yes	yes	96.3	1.8	1.9	41369	58003320
Crassostrea hongkongensis	Ostreoida	T	**insufficient data**		yes	yes	yes	94.9	2.6	2.5	113757	106892789
Crassostrea nippona	Ostreoida	T	bimodal depleted	yes	yes	yes	yes	97.4	0.6	2.0	66353	72052376
Crassostrea virginica	Ostreoida	G/T	bimodal depleted	yes	yes	yes	yes	97.4	0.3	2.3	60213	118005401
Saccostrea glomerata	Ostreoida	G/T	bimodal depleted	yes	yes	yes	yes	92.6	6.0	1.4	374992	266506034
Saccostrea_sp_non-mordax_lineage	Ostreoida	T	bimodal depleted	yes	yes	yes	yes	92.6	6.0	1.4	374992	266506034
Amblema plicata	Palaeoheterodonta	T	bimodal depleted	yes	yes	yes	no	79.4	11.4	9.2	264027	190222260
Margaritifera margaritifera	Palaeoheterodonta	T	bimodal depleted	yes	no	yes	yes	95.3	0.3	4.4	51353	113185668
Adamussium colbecki	Pectinoida	T	bimodal depleted	yes	yes	yes	yes	93.0	5.3	1.7	239776	176062928
Argopecten purpuratus	Pectinoida	G	bimodal depleted	yes	yes	yes	yes	88.3	4.3	7.4	26256	37238646
Chlamys farreri	Pectinoida	G	bimodal depleted	yes	yes	yes	yes	88.9	4.0	7.1	28602	40468841
Mimachlamys varia	Pectinoida	T	**insufficient data**		yes	yes	yes	97.1	1.9	1.0	333012	353061882
Nodipecten subnodosus	Pectinoida	T	bimodal depleted	yes	yes	yes	yes	88.5	7.5	4.0	80250	74950749

Patinopecten yessoensis	Pectinoida	G	bimodal depleted	yes	yes	yes	no	97.8	0.4	1.8	41567	81917079
Pecten maximus	Pectinoida	G/T	bimodal depleted	yes	yes	yes	yes	97.2	0.5	2.3	39918	72445869
Placopecten magellanicus	Pectinoida	T	bimodal depleted	yes	no	yes	yes	25.4	15.2	59.4	25687	19950093
Ennucula tenuis	Protobranchia	T	bimodal depleted	yes	yes	yes	yes	90.1	7.3	2.6	252473	191520797
Nucula tumidula	Protobranchia	T	bimodal depleted	yes	yes	yes	no	91.5	5.6	2.9	273272	378309195
Nucula_expansa	Protobranchia	T	bimodal depleted	yes	**insufficient data**			59.3	18.4	22.3	174068	112138893
Solemya velum	Protobranchia	T	bimodal depleted	yes	yes	yes	yes	98.6	0.4	1.0	109533	132147566
Yoldia limatula	Protobranchia	T	bimodal depleted	yes	**insufficient data**			4.9	13.1	82.0	14726	5788804
Pinctada fucata	Pterioida	G/T	bimodal depleted	yes	yes	yes	yes	67.8	8.0	24.2	30815	47471558
Pinctada imbricata	Pterioida	G	bimodal depleted	yes	yes	yes	yes	84.3	4.1	11.6	51752	66518079
Pinctada radiata	Pterioida	T	bimodal depleted	yes	yes	yes	yes	97.5	0.5	2.0	68930	136928509
Pteria penguin	Pterioida	T	bimodal depleted	yes	yes	yes	no	65.3	22.6	12.1	76785	53804762
Caudofoveata												
Chaetoderma nitidulum	Chaetodermatidae	T	bimodal depleted	yes	yes	yes	yes	72.6	15.5	11.9	226582	139057750
Scutopus ventrolineatus	Limifossaridae	T	unimodal, indicative of DNA methylation	yes	yes	yes	no	73.8	15.2	11.0	220258	253037497
Cephalopoda												
Euprymna scolopes	other Decapodiformes	T	bimodal depleted	yes	yes	yes	yes	83.2	10.0	6.8	53705	46879491
Euprymna tasmannica	other Decapodiformes	T	bimodal depleted	yes	yes	yes	no	88.5	5.9	5.6	102403	73039107
Idiosepius notoides	other Decapodiformes	T	bimodal depleted	yes	yes	yes	yes	89.1	6.7	4.2	108831	69910037
Sepia esculenta	other Decapodiformes	T	bimodal depleted	yes	yes	yes	yes	97.1	0.9	2.0	176390	197511471
Sepia pharaonis	other Decapodiformes	T	bimodal depleted	yes	no	yes	no	91.1	3.9	5.0	73298	65783467
Sepiella maindroni	other Decapodiformes	T	bimodal depleted	yes	no	yes	yes	89.1	3.6	7.3	58163	45531710

Sepioloidea lineolata	other Decapodiformes	T	bimodal depleted	yes	no	no	no	80.7	9.2	10.1	116459	71585051
Nautilus pompilius	Nautiloidea	T	bimodal depleted	yes	**insufficient data**			10.8	29.1	60.1	31771	14493825
Hapalochlaena maculosa	Octopoda	T	bimodal depleted	yes	yes	yes	yes	98.1	0.9	1.0	196889	185561668
Octopus bimaculoides	Octopoda	G	bimodal depleted	yes	yes	yes	yes	89.3	6.4	4.3	38556	39567255
Octopus kaurna	Octopoda	T	bimodal depleted	yes	yes	yes	yes	86.1	7.5	6.4	86314	59855196
Octopus maya	Octopoda	T	bimodal depleted	yes	yes	yes	yes	90.5	6.0	3.5	85223	53825609
Octopus minor	Octopoda	G	bimodal depleted	yes	yes	yes	yes	60.0	14.7	25.3	30010	35243297
Octopus sinensis	Octopoda	G	bimodal depleted	yes	yes	yes	yes	98.0	0.7	1.3	25656	46570204
Octopus vulgaris	Octopoda	T	bimodal depleted	yes	yes	yes	yes	82.4	10.4	7.2	118474	79943713
Chiroteuthis calyx	Oegopsida	T	bimodal depleted	yes	yes	yes	no	82.8	8.5	8.7	78425	54474438
Dosidicus gigas	Oegopsida	T	bimodal depleted	yes	yes	yes	no	85.7	7.5	6.8	81540	71045954
Octopoteuthis deletron	Oegopsida	T	bimodal depleted	yes	yes	yes	no	95.6	2.2	2.2	122671	114222634
Onychoteuthis banksii	Oegopsida	T	bimodal depleted	yes	yes	yes	no	97.3	1.2	1.5	133923	106736130
Pterygioteuthis hoylei	Oegopsida	T	bimodal depleted	yes	yes	yes	no	92.8	4.2	3.0	93186	112234068
Stenoteuthis oualaniensis	Oegopsida	T	bimodal depleted	yes	**insufficient data**			30.9	24.8	44.3	32652	15937850
Watasenia scintillans	Oegopsida	T	bimodal depleted	yes	yes	yes	no	97.3	1.4	1.3	216307	239630561
Vampyroteuthis infernalis	Vampyromorpha	T	bimodal depleted	yes	yes	no	yes	86.1	8.6	5.3	149900	105426544
Gastropoda												
Bithynia siamensis goniomphalus	Caenogastropoda	T	bimodal depleted	yes	yes	no	no	71.7	15.3	13.0	167642	105479132
Cipangopaludina cathayensis	Caenogastropoda	T	bimodal depleted	yes	yes	yes	no	83.5	9.6	6.9	150998	97482859
Crepidula atrasolea	Caenogastropoda	T	bimodal depleted	yes	yes	yes	yes	88.6	4.8	6.6	400489	276076045
Crepidula navicella	Caenogastropoda	T	bimodal depleted	yes	yes	yes	no	96.1	2.2	1.7	285307	220642386

Littorina littorea	Caenogastropoda	T	bimodal depleted	yes	yes	yes	no	97.1	1.2	1.7	77038	127718725
Littorina saxatilis	Caenogastropoda	T	unimodal, indicative of DNA methylation	yes	yes	yes	no	93.4	1.3	5.3	36595	77144400
Lanistes nyassanus	Caenogastropoda	G	bimodal depleted	yes	yes	yes	yes	93.1	3.9	3.0	20938	63465499
Marisa cornuarietis	Caenogastropoda	G	bimodal depleted	yes	yes	yes	no	92.6	3.4	4.0	23827	70611737
Neverita didyma	Caenogastropoda	T	bimodal depleted	yes	**insufficient data**			76.9	3.2	19.9	14374	23861775
Pomacea canaliculata	Caenogastropoda	G/T	bimodal depleted	yes	yes	yes	yes	88.3	7.8	3.9	76080	82567644
Pomacea maculate	Caenogastropoda	G	bimodal depleted	yes	yes	yes	yes	93.6	2.9	3.5	23475	49106907
Potamopyrgus antipodarum	Caenogastropoda	T	unimodal, indicative of DNA methylation	yes	yes	yes	yes	94.4	1.9	3.7	62862	62782566
Rapana venosa	Caenogastropoda	T	bimodal depleted	yes	yes	yes	no	95.4	2.5	2.1	246627	169741352
Semisulcospira coreana	Caenogastropoda	T	bimodal depleted	yes	yes	yes	no	82.7	10.0	7.3	134402	93326634
Aplysia californica	Euopisthobranchia	G/T	bimodal depleted	yes	yes	yes	no	92.2	4.9	2.9	157989	134670333
Clione limacine	Euopisthobranchia	T	bimodal depleted	yes	yes	yes	no	87.7	9.1	3.2	300994	181817793
Limacina retroversa	Euopisthobranchia	T	bimodal depleted	yes	yes	yes	no	88.9	7.2	3.9	116910	64809087
Limacina antarctica	Euopisthobranchia	T	bimodal depleted	yes	yes	yes	no	87.5	7.9	4.6	81226	59791880
Limacina helicina	Euopisthobranchia	T	bimodal depleted	yes	yes	yes	no	73.4	17.0	9.6	83458	46671785
Achatina fulica	Eupulmonata	G	bimodal depleted	yes	yes	yes	no	88.4	3.9	7.7	23726	37012266
Arion vulgaris	Eupulmonata	T	bimodal depleted	yes	yes	yes	no	88.7	6.6	4.7	136407	91533740
Bradybaena similaris	Eupulmonata	T	bimodal depleted	yes	yes	yes	no	69.0	2.7	28.3	295262	197603403
Cepaea nemoralis	Eupulmonata	T	bimodal depleted	yes	yes	yes	no	92.7	2.9	4.4	147397	115481543
Euhadra quaesita	Eupulmonata	T	bimodal depleted	yes	**insufficient data**			9.3	17.7	73.0	59618	14956220
Helix aspersa	Eupulmonata	T	bimodal depleted	yes	**insufficient data**			5.8	6.3	87.9	9445	3398980

Biomphalaria glabrata	Hygrophila	G/T	bimodal depleted	yes	yes	yes	no	88.0	6.4	5.6	36675	54426120
Biomphalaria pfeifferi	Hygrophila	T	**insufficient data**		yes	yes	no	98.6	0.6	0.8	186204	121848955
Lymnaea stagnalis	Hygrophila	G/T	bimodal depleted	yes	yes	yes	no	97.8	1.0	1.2	88056	188170745
Physella acuta	Hygrophila	G/T	bimodal depleted	yes	yes	yes	no	94.7	2.0	3.3	325524	250092673
Radix auricularia	Hygrophila	G	bimodal depleted	yes	yes	yes	no	83.3	6.8	9.9	17338	25297842
Chrysomallon squamiferum	Neomphaliones	G	bimodal depleted	yes	yes	yes	yes	86.0	4.1	9.9	16917	30710173
Bathyberthella antarctica	Nudipleura	T	bimodal depleted	yes	**insufficient data**			39.1	15.6	45.3	69122	41616777
Melibe leonine	Nudipleura	T	bimodal depleted	yes	yes	yes	no	93.5	4.2	2.3	167742	139197820
Phylliroe bucephala	Nudipleura	T	bimodal depleted	yes	yes	no	no	77.3	10.2	12.5	66529	45080555
Lottia gigantean	Patellogastropoda	G	bimodal depleted	yes	yes	yes	yes	95.8	1.7	2.5	23340	26846418
Lottia kogamogai	Patellogastropoda	T	bimodal depleted	yes	yes	yes	no	67.8	20.4	11.8	34794	25737707
Elysia chlorotica	Sacoglossa	G	bimodal depleted	yes	yes	yes	no	90.9	3.4	5.7	23871	31102552
Elysia timida	Sacoglossa	T	bimodal depleted	yes	yes	yes	no	96.1	2.3	1.6	149629	201769247
Elysia cornigera	Sacoglossa	T	bimodal depleted	yes	yes	yes	no	97.0	1.5	1.5	249813	221967966
Plakobranchus ocellatus	Sacoglossa	T	bimodal depleted	yes	no	yes	no	21.7	22.6	55.7	77648	49209194
Siphonaria pectinata	Siphonarimorpha	T	bimodal depleted	yes	no	yes	no	7.9	27.1	65.0	33154	14345522
Haliotis discus hannai	Vetigastropoda	T	bimodal depleted	yes	yes	yes	no	86.9	7.7	5.4	89388	64966738
Haliotis fulgens	Vetigastropoda	T	bimodal depleted	yes	no	yes	no	68.2	4.7	27.1	95036	66962539
Haliotis laevigata	Vetigastropoda	T	bimodal depleted	yes	yes	yes	yes	97.8	1.4	0.8	217815	300581213
Haliotis midae	Vetigastropoda	T	bimodal depleted	yes	**insufficient data**			2.8	5.5	91.7	30876	12056660
Haliotis tuberculata	Vetigastropoda	T	bimodal depleted	yes	yes	yes	no	94.7	3.9	1.4	328519	270947680
Perotrochus lucaya	Vetigastropoda	T	bimodal depleted	yes	**insufficient data**			7.2	17.4	75.4	22008	9036838

Tegula atra	Vetigastropoda	T	bimodal depleted	yes	yes	no	no	10.3	28.5	61.2	43045	18525961
Monoplacophora												
Laevipilina hyalina	Monoplacophora	T	unimodal, not indicative of DNA methylation	no	yes	yes	yes	90.0	7.1	2.9	200452	174817214
Polyplacophora												
Acanthochitona crinita	Acanthochitonina	T	bimodal depleted	yes	yes	yes	yes	84.4	11.0	4.6	364800	689247497
Acantochitona rubrolineata	Acanthochitonina	T	bimodal depleted	yes	yes	yes	no	96.0	2.7	1.3	331583	278289731
Chaetopleura apiculata	Chitonina	T	bimodal depleted	yes	**insufficient data**			14.0	25.1	60.9	24362	10226341
Chiton olivaceus	Chitonina	T	unimodal, indicative of DNA methylation	yes	yes	yes	yes	74.4	17.2	8.4	300737	192695052
Lepidopleurus cajetanus	Lepidopleurida	T	bimodal depleted	yes	**insufficient data**			14.1	26.1	59.8	101173	43775967
Leptochiton rugatus	Lepidopleurida	T	unimodal, indicative of DNA methylation	yes	yes	yes	no	81.5	10.7	7.8	118131	100562468
Scaphopoda												
Antalis entalis	Dentaliida	T	bimodal depleted	yes	yes	yes	yes	91.7	5.2	3.1	351943	369111329
Gadila tolmiei	Dentaliida	T	unimodal, indicative of DNA methylation	yes	yes	yes	no	91.0	6.0	3.0	350427	193530161
Cadulus tolmiei	Gadilida	T	bimodal depleted	yes	yes	no	no	61.2	17.3	21.5	184402	72081010
Solenogastres												
Greenland neomeniomorph	indet	T	unimodal, not indicative of DNA methylation	no	yes	yes	no	65.6	18.1	16.3	282377	154561407
Neomeniomorpha sp1	indet	T	unimodal, not indicative of DNA methylation	no	yes	yes	yes	94.3	2.8	2.9	324411	230260009
Gymnomenia pellucida	Gymnomeniidae	T	unimodal, indicative of DNA methylation	yes	yes	yes	yes	90.1	6.0	3.9	228678	408484174

Wirenia argentea	Gymnomeniidae	T	unimodal, not indicative of DNA methylation	no	yes	yes	no	64.6	21.9	13.5	394251	495209150
Neomenia megatrapezata	Neomeniidae	T	unimodal, indicative of DNA methylation	yes	yes	yes	yes	87.4	7.3	5.3	102378	96112257
Proneomenia custodiens	Proneomeniidae	T	unimodal, not indicative of DNA methylation	no	yes	yes	no	68.8	13.2	18.0	171358	116945130

Transcriptome Assembly

We downloaded RNAseq data (Supplemental Table S3) and mapped the RNAseq reads to the particular genome assembly. While downloading, reads were filtered using the SRA Toolkit´s fastq-dump 2.10.8 with the options "--split-spot --clip --minReadLen 50 --skip-technical --split-e". We conducted the transcriptome assemblies using Trinity 2.11.0 (Grabherr et al., 2011) combined with Jellyfish 2.3.0 (Marçais & Kingsford, 2011), Salmon 0.14.1 (Patro et al., 2017), Bowtie2 2.4.1 (Langmead & Salzberg, 2012), Samtools 1.10 (Li et al., 2009), Python 3.7.4 and Java 1.8.0_221. We ran Trinity with the options "--seqType fq --max_memory 995G --CPU 64 --full_cleanup". To estimate the entirety of the transcriptome assemblies, we performed BUSCO 4.1.4 (Simão et al., 2015) combined with the tools BLAST 2.2.31+ (Camacho et al., 2009), Augustus 3.3.3 (Stanke et al., 2004) and HMMER 3.3.1 (http://eddylab.org/software/hmmer/hmmer.org) together with the metazoa_odb10 set and the options "-c 8 -m tran --offline". The outcome of the transcriptome assembly can be seen in Supplemental Table S4.

Supplemental Table S3. Accession numbers of *de novo* transcriptome assemblies.

Species	**Accession number**
Acanthochitona rubrolineata	SRR8442642
Antalis entalis	SRR8217848
Chaetoderma nitidulum	SRR5341487
Chiton olivaceus	SRR618506
Ennucula tenuis	SRR331123
Gadila tolmiei	SRR331897
Laevipilina hyalina	SRR1505115
Lepidopleurus cajetanus	SRR8245857
Leptochiton rugatus	SRR1611558
Neomenia megatrapezata	SRR331899
Neomeniomorpha sp. 1 SS-2011	SRR331902
Octopus vulgaris	SRR331946
Proneomenia custodiens *(Proneomenia sp. KMK-2014)*	SRR1611561
Solemya velum	SRR330465

Supplemental Table S4. Overview of the transcriptome assemblies, the respective number of contigs, total length in bp and BUSCO value. The completeness of the transcriptome assemblies is described in percentage as complete (C), complete and single-copy (S), complete and duplicated (D), fragmented (F) and missing (M) according to the BUSCO notation.

Species	**Number of contigs**	**Total length [bp]**	**BUSCO $N_{Metazoa\_odb10}$ = 954**
Acanthochitona rubrolineata	331583	278289731	C: 96.0% [S: 22.6%, D: 73.4%], F:2.7%, M: 1.3%
Antalis entalis	368288	146101824	C: 53.2% [S: 30.2%, D: 23.0%], F: 23.2%, M: 23.6%
Chaetoderma nitidulum	226582	139057750	C: 72.6% [S: 50.6%, D: 22.0%], F: 15.5%, M: 11.9%
Chiton olivaceus	300737	192695052	C: 74.4% [S: 32.1%, D: 42.3%], F: 17.2%, M: 8.4%
Ennucula tenuis	252473	191520797	C: 90.1% [S: 43.1%, D: 47.0%], F: 7.3%, M: 2.6%
Gadila tolmiei	350427	193530161	C: 91.0% [S: 22.4%, D: 68.6%], F: 6.0%, M: 3.0%
Laevipilina hyalina	200452	174817214	C: 90.0% [S: 36.3%, D: 53.7%], F: 7.1%, M: 2.9%
Lepidopleurus cajetanus	101173	43775967	C: 14.1% [S: 10.2%, D: 3.9%], F: 26.1%, M: 59.8%
Leptochiton rugatus	118131	100562468	C: 81.5% [S: 27.0%, D: 54.5%], F: 10.7%, M: 7.8%
Neomenia megatrapezata	102378	96112257	C: 87.4% [S: 56.8%, D: 30.6%], F: 7.3%, M: 5.3%
Neomeniomorpha sp. 1 SS-2011	324411	230260009	C: 94.3% [S: 31.8%, D: 62.5%], F: 2.8%, M: 2.9%
Octopus vulgaris	118474	79943713	C: 82.4% [S: 59.5%, D: 22.9%], F: 10.4%, M: 7.2%
Proneomenia custodiens (*Proneomenia sp.* KMK-2014)	171358	116945130	C: 68.8% [S: 29.0%, D: 39.8%], F: 13.2%, M: 18.0%
Solemya velum	109533	132147566	C: 98.6% [S: 63.5%, D: 35.1%], F: 0.4%, M: 1.0%

Structural annotation of existing genome assemblies

Publicly accessible genome assemblies of eight mollusc species were structurally annotated using GeMoMa 1.6.4 (Keilwagen et al., 2016, 2018). We downloaded and filtered RNAseq data (Supplemental Table S6). Then, we used HISAT 2.1.0 (Kim et al., 2019) to map the RNAseq reads to the particular genome assembly. Afterwards they were sorted via samtools sort. We used GeMoMa CLI ERE with the additional parameter "c=true" to obtain RNAseq evidence. We then filtered the created gff file with GeMoMa CLI DenoiseIntrons, defining the "coverage.bedgraph" file as input for parameter "coverage_unstranded" and adjusting the maximum intron length to the largest value of the introns.gff file.

The GeMoMa pipeline was run as CLI GeMoMaPipeline using the options "threads=96 tblastn=false r=EXTRACTED introns=denoised_introns.gff coverage_unstranded=coverage.bedgraph GeMoMa.Score=ReAlign AnnotationFinalizer.r=NO o=true" and the maximum intron size of the denoised_introns.gff as values for the options DenoiseIntrons.m and GeMoMa.m. We used a group of reference annotations in GeMoMaPipeline for each of the eight genome assemblies that should be annotated (see Supplemental Table S5 for sources and Supplemental Table S7 for combinations). We predominantly used species from the same class as references. We additionally used two bivalves and two gastropods for *Euprymna scolopes*, since only two *Octopus* annotations were obtainable. The annotations which were originally coming from the references were merged by GeMoMa Annotation Filter per "to-be-annotated" species with CLI GAF and specifying a two-letter abbreviation per reference as prefix (option "p").

The outcoming filtered_predictions.gff file was worked through using GeMoMa CLI AnnotationFinalizer with the options "a=filtered_predictions.gff u=YES c=UNSTRANDED coverage_unstranded=coverage.bedgraph i=denoised_introns.gff" and an prefix (option "p") and then with CLI AnnotationEvidence and the options "a=filtered_predictions.gff i=denoised_introns.gff c=UNSTRANDED coverage_unstranded=coverage.bedgraph ao=true". We used GeMoMa CLI Extractor with the options "a=annotation_with_attributes.gff c=true p=true" to obtain predicted CDS and protein sequences. We determined the statistics of the annotation_with_attributes.gff files with an in-house script and performed BUSCO 4.1.4 as above to assess the quality of the final annotation protein and CDS fasta files. Supplemental Table S8 displays the overview of the outcome of the structural annotations.

Supplemental Table S5. Sources of reference genomes and their according annotations.

Reference species	**Source (if any)**	**Accession number**
Bathymodiolus platifrons	Sun et al. (2017)	GCA_002080005.1
Crassostrea gigas	Zhang et al. (2012)	GCF_902806645.1
Crassostrea virginica	-	GCF_002022765.2
Mizuhopecten yessoensis	Wang et al. (2017)	GCF_002113885.1
Modiolus philippinarum	Sun et al. (2017)	GCA_002080025.1
Mytilus galloprovincialis	Murgarella et al. (2016)	GCA_001676915.1
Pecten maximus	Kenny et al. (2020)	GCF_902652985.1
Octopus bimaculoides	Albertin et al. (2015)	GCF_001194135.1
Octopus sinensis	Li et al. (2020)	GCF_006345805.1
Aplysia californica	-	GCF_000002075.1
Biomphalaria glabrata	Adema et al. (2017)	GCF_000457365.1
Chrysomallon squamiferum	Sun et al. (2020)	GCA_012295275.1
Elysia chlorotica	Cai et al. (2019)	GCA_003991915.1
Lottia gigantea	Simakov et al. (2013)	GCF_000327385.1
Pomacea canaliculata	Liu et al. (2018)	GCF_003073045.1
Radix auricularia	Schell et al. (2017)	GCA_002072015.1

Supplemental Table S6. Accession numbers of the to-be-annotated assemblies and the RNAseq reads which were used.

	Bivalvia				Cephalopoda	Gastropoda		
Species	*Dreissena rostriformis*	*Sinonovacula constricta*	*Mytilus galloprovincialis*	*Pinctada imbricata radiata*	*Euprymna scolopes*	*Lymnaea stagnalis*	*Physella acuta*	*Haliotis laevigata*
Genome assembly accesstion number	GCA_007657795.1	GCA_007844125.1	GCA_001676915.1	GCA_002216045.1	GCA_004765925.1	GCA_900036025.1	GCA_004329575.1	GCA_008038995.1
RNAseq accession numbers	DRR174576 DRR174577 DRR174578 SRR8354726 SRR8354727 SRR8354730 SRR8354731 SRR8354734 SRR8354743 SRR8354744 SRR8354745 SRR8354748 SRR8354749 SRR8354750	SRR10097413 SRR10097414 SRR10097415 SRR10097416 SRR10097417 SRR10097418 SRR10097419 SRR10097420 SRR10097421 SRR10097422 SRR10097423 SRR10097424 SRR2012394 SRR2012396	SRR7138608 SRR7138609 SRR7138610 SRR7138611 SRR7138612 SRR7138613 SRR7138614 SRR7138615 SRR7138616 SRR7138617 SRR7138618 SRR7138619 SRR7138620 SRR7138621	SRR830491 SRR8357272 SRR8357273 SRR8379433 SRR8379434 SRR8379435 SRR8379436 SRR8379437 SRR8379438 SRR8379439 SRR8379440 SRR8379441 SRR8379442	SRR3472306 SRR3493755 SRR3493756 SRR3493757 SRR3493758 SRR3493851 SRR3493852 SRR3495043 SRR3495044 SRR3495045 SRR3495046 SRR3495047 SRR3495048 SRR3495103	DRR086866 DRR086867 DRR086868 DRR086869 DRR086870 DRR086871 SRR3469744 SRR6336932 SRR6336933 SRR6832917 SRR6832918 SRR6832919 SRR6832920 SRR6832921	ERR2179419 SRR8080746	SRR3384033 SRR3384034 SRR3384035 SRR3384036 SRR3384037 SRR3384038 SRR3384039 SRR3384040 SRR3384041 SRR3384042 SRR3384043 SRR3384044 SRR3384045 SRR3384046

	SRR8354751	SRR2012706	SRR7138622		SRR3495104	SRR6832922		SRR3384047
	SRR8354752	SRR2012747	SRR7138623		SRR3495105	SRR6832923		SRR3384048
	SRR8354753	SRR2162883	SRR7138624		SRR3495106	SRR6832924		SRR3384049
	SRR8354754	SRR2162887	SRR7138625		SRR3495107			SRR3384050
	SRR8354755	SRR2162892	SRR7145578		SRR3495108			SRR3384051
	SRR8354756	SRR2162895	SRR7145579		SRR5121903			SRR3384052
	SRR8354757	SRR2162898	SRR7145580		SRR5121904			SRR3384053
	SRR8354758	SRR2162902	SRR7145581		SRR5121905			SRR3384054
	SRR8354776	SRR6893872	SRR7145582		SRR5121906			SRR3384055
	SRR8354777	SRR6893873	SRR7145583		SRR5121907			SRR3384056
	SRR8354778	SRR6893874	SRR7145584		SRR5121908			SRR3384057
	SRR8354779	SRR6893875	SRR7145585		SRR5121909			SRR3384058
	SRR8354780	SRR6893876	SRR7145586		SRR5121910			SRR3384059
	SRR8354781	SRR6893877	SRR7145587		SRR5121911			SRR3384060
	SRR8354782	SRR6893878	SRR7145588		SRR5121912			SRR3384061
	SRR8354783	SRR6893879	SRR7145589		SRR5121913			SRR3384062
	SRR8354784	SRR6893880	SRR7145590		SRR5121914			SRR6677998
	SRR8354785	SRR8325910	SRR7145591		SRR5121915			SRR6677999
	SRR8354786	SRR8325911	SRR7145592		SRR5121916			SRR6678000
	SRR8354788	SRR8325912	SRR7640805		SRR5121917			SRR6678001
	SRR8354790	SRR8325913	SRR7640806		SRR5121918			SRR6678002

	SRR8354791	SRR8325914	SRR7640807		SRR5121919			SRR6678003
	SRR8354792	SRR8325915	SRR7640808		SRR5121920			SRR6678004
	SRR9620651	SRR8325916	SRR7640809		SRR5121921			SRR6678005
	SRR9620652	SRR8861911	SRR7640810		SRR5121922			SRR6678006
	SRR9620653	SRR8861912	SRR7640811		SRR5121923			SRR6678007
	SRR9620654	SRR8861913	SRR7640812		SRR5121924			SRR6678008
	SRR9620659	SRR8861914	SRR7640813		SRR5121925			
	SRR9620660	SRR8861915	SRR7640814		SRR5121926			
	SRR9620661	SRR8861916	SRR7640815		SRR5121927			
	SRR9620662	SRR8861917	SRR7640816		SRR5121928			
	SRR9620663	SRR8861918	SRR7640817		SRR5121929			
	SRR9620664	SRR8861919	SRR7640818		SRR5121930			
	SRR9620665	SRR8861920	SRR7640819		SRR5121931			
	SRR9620666	SRR8861921	SRR7640820		SRR5121932			
	SRR9620667	SRR8861922	SRR7640821		SRR5121933			
	SRR9620668	SRR8861923	SRR7725722		SRR5121934			
	SRR9620669	SRR8861924	SRR7725723		SRR5121935			
	SRR9620670	SRR8861925	SRR7725724		SRR5121936			
	SRR9620671	SRR8861926	SRR7725725		SRR5121937			
	SRR9620672	SRR8861927	SRR7725726		SRR5121938			
		SRR8861928	SRR7725727		SRR5121939			

		SRR8861929	SRR7725728		SRR5121940			
		SRR8861930	SRR7725729		SRR5121941			
		SRR8861931	SRR8707268		SRR5121942			
		SRR8861932	SRR8713540		SRR5121943			
		SRR8861933	SRR8713541		SRR5121944			
		SRR8861934	SRR8713542		SRR5121945			
		SRR8861935	SRR8713543		SRR5121946			
		SRR8861936	SRR8713544		SRR5121947			
		SRR8861937	SRR8713545		SRR5121948			
		SRR8861938	SRR8713546		SRR5121949			
		SRR8861939	SRR8713547		SRR5121950			
		SRR8861940	SRR9984957		SRR5121954			
		SRR9937008	SRR9984958		SRR5121958			
		SRR9937009	SRR9984959		SRR5121963			
		SRR9937010	SRR9984960		SRR5121967			
		SRR9937011	SRR9984961		SRR5121971			
		SRR9937012	SRR9984962		SRR5121980			
		SRR9937013	SRR9984963		SRR5121982			
		SRR9943679	SRR9984964		SRR5121983			
		SRR9943680	SRR9984965		SRR5121984			
		SRR9943681	SRR9984966		SRR5121985			

		SRR9943682	SRR9984967		SRR5121986			
		SRR9943683	SRR9984968		SRR5121987			
		SRR9943684	SRR9984975		SRR5121988			
		SRR9943685	SRR9984976		SRR5121989			
		SRR9943686	SRR9984977		SRR5121990			
		SRR9943687	SRR9984978		SRR5121991			
		SRR9943688	SRR9984979		SRR5121992			
		SRR9943689	SRR9984980		SRR5121993			
		SRR9943690	SRR9984981		SRR5121994			
		SRR9959746	SRR9984982		SRR5121995			
		SRR9959747	SRR9984983		SRR5121996			
		SRR9959748	SRR9984984		SRR5121997			
		SRR9959749	SRR9984985		SRR5121998			
		SRR9959750	SRR9984986		SRR5121999			
		SRR9959751	SRR9984987		SRR5122000			
		SRR9959752	SRR9984988		SRR5122001			
		SRR9959753	SRR9984989		SRR5122002			
		SRR9959754	SRR9984991		SRR5122003			
			SRR9984992		SRR5122004			
			SRR9984993		SRR5122005			
			SRR9984994		SRR5122006			

			SRR9984995 SRR9984996 SRR1042397 SRR1045900 SRR1046115 SRR1046116 SRR1046117 SRR1046118 SRR1046119 SRR2392495 SRR2392762 SRR2409049 SRR442031 SRR442032 SRR442033 SRR442034 SRR442035 SRR442036 SRR6051613 SRR6238445 SRR6238446		SRR5122007 SRR5122009 SRR5122021 SRR5122111 SRR5122114 SRR5122119 SRR5122125 SRR5122131 SRR5122135 SRR5122140 SRR5122145 SRR5122149 SRR5122152 SRR5122156 SRR5122161 SRR5122171 SRR5122177 SRR5122180 SRR5122184 SRR5122189 SRR5122193			

			SRR6238447 SRR6238448 SRR6238449 SRR6238450 SRR6238451 SRR6238452 SRR6238453 SRR6238454 SRR6238455 SRR6238456 SRR7044533 SRR7138626 SRR7138627 SRR7138628 SRR7138629 SRR7138630 SRR7138631		SRR5122198 SRR5122204 SRR5122208 SRR5122212 SRR5122217 SRR5122221 SRR5122225 SRR5122229 SRR5122233 SRR5122236 SRR5122240 SRR5122246 SRR5122247 SRR5122248 SRR5122249 SRR5122250 SRR5122251 SRR5122252 SRR5122253 SRR5122254 SRR5122255			

					SRR5122256 SRR5122257 SRR5122258 SRR5122259 SRR5122260 SRR5122261 SRR5122262 SRR5122263 SRR5122264 SRR5122265 SRR5122266 SRR5122267 SRR5122268 SRR5122269 SRR5122270 SRR5122271 SRR5122272 SRR5122273 SRR5122274 SRR5122275 SRR5122276			

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					SRR5122298 SRR5122299 SRR5122300 SRR5122301 SRR5122302 SRR5122303 SRR5122304 SRR5122305 SRR5122306 SRR5122307 SRR5122308 SRR5122309 SRR5122310 SRR5122311 SRR5122312 SRR5122313 SRR5122314 SRR5122315 SRR5122316 SRR5122317 SRR7686318			

					SRR7686320 SRR7686323 SRR8159233 SRR8159234 SRR8159235 SRR8159236 SRR8159237 SRR8159238 SRR8159239 SRR8159240 SRR8159241 SRR8159242 SRR8159243 SRR8159244 SRR8159245 SRR8159246 SRR8159247 SRR8159248 SRR8159249 SRR8159250 SRR8172521			

					SRR8172522 SRR8172523 SRR8172524 SRR8172525 SRR8172526 SRR8172527 SRR8172528 SRR8172529 SRR8172530 SRR8172531 SRR8172532 SRR8172533 SRR8172534 SRR8172535 SRR8172536 SRR8172537 SRR8172538 SRR8172539 SRR8172540 SRR8172541 SRR8172542			

					SRR8172543 SRR8172544 SRR8172545 SRR8172546 SRR8172547 SRR8172548 SRR8172549 SRR8172550 SRR8172551 SRR8172552 SRR8172553 SRR8172554 SRR8172556 SRR8172557 SRR8172558 SRR8172559 SRR8172560 SRR8172561 SRR8172562 SRR8172563 SRR8172564			

					SRR8172565 SRR8172566 SRR8172567 SRR8172568			

Supplemental Table S7. Combination of the to-be-annotated assemblies and the utilized references.

To be annotated	**Bivalvia**				**Cephalopoda**	**Gastropoda**		
Reference	*Dreissena rostriformis*	*Sinonovacula constricta*	*Mytilus galloprovin-cialis*	*Pinctada imbricata radiata*	*Euprymna scolopes*	*Lymnaea stagnalis*	*Physella acuta*	*Haliotis laevigata*
Bivalvia								
Bathymodiolus platifrons	X	X	X	X				
Crassostrea gigas	X	X	X	X	X			
Crassostrea virginica	X	X	X	X				
Mizuhopecten yessoensis	X	X	X	X	X			
Modiolus philippinarum	X	X	X	X				
Mytilus galloprovincialis	X	X	X	X				
Pecten maximus	X	X	X	X				
Cephalopoda								
Octopus bimaculoides					X			

Octopus sinensis					X			
Gastropoda								
Aplysia californica					X	X	X	X
Biomphalaria glabrata						X	X	X
Chrysomallon squamiferum						X	X	X
Elysia chlorotica						X	X	X
Lottia gigantea						X	X	X
Pomacea canaliculata					X	X	X	X
Radix auricularia						X	X	X

Supplemental Table S8. Summary of the structural annotations. BUSCO results stem from a group of annotated proteins.

	Bivalvia				**Cephalopoda**	**Gastropoda**		
	Dreissena rostriformis	*Mytilus galloprovincialis*	*Pinctada imbricata radiata*	*Sinonovacula constricta*	*Euprymna scolopes*	*Haliotis laevigata*	*Lymnaea stagnalis*	*Physella acuta*
Number								
Gene	34867	37068	17258	34867	25686	28941	35449	37258
mRNA	51212	45348	21696	51212	33297	37866	52350	50677
CDS	312840	131915	89815	312840	161483	210636	324316	252363
Mean								
mRNAs/gene	1.46878	1.22337	1.25716	1.46878	1.29631	1.30839	1.47677	1.36016
CDSs/mRNA	6.10872	2.90895	4.1397	6.10872	4.84978	5.56267	6.19515	4.97983
Median length								
Gene	11864	1355	1483	11864	2539	9520	9089	7019
mRNA	15249,5	1692	1787	15249,5	3443	11171	12098	8877
CDS	134	156	132	134	131	129	133	135
Total space								
Gene	867915626	80635350	36546875	867915626	104166367	480964264	722007352	540560203
mRNA	867915626	80635350	36546875	867915626	104166367	480964264	722007352	540560203
CDS	43722739	27217549	13974581	43722739	25184008	29833477	46531198	38624210
Single								

CDS mRNA	8421	10997	3525	8421	5592	5090	8905	8926
BUSCO [%] (N=954)								
C	73.5	13.1	85.2	94.1	83.0	77.2	72.5	51.5
S	58.1	9.7	59.1	62.1	67.6	57.5	51.3	38.7
D	15.4	3.4	26.1	32.0	15.4	19.7	21.2	12.8
F	8.6	15.7	4.5	1.7	4.9	9.2	7.8	14.5
M	17.9	71.2	10.3	4.2	12.1	13.6	19.7	34.0

XII. EIGENSTÄNDIGKEITSERKLÄRUNG

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum
Zwecke der Promotion eingereicht wurde.
Ferner erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe.

_____ _____

Lisa Männer Datum

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