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Incidence du processus de vieillissement et des traits d'histoire de vie sur l'évolution de l'ADN mitochondrial chez les bivalves

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PAR

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RÉSUMÉ

Les bivalves sont de bons modèles biologiques pour l'étude du vieillissement cellulaire en raison de leurs grandes variations de longévités. L'objectif principal de la maîtrise était de comprendre le mode d'évolution de l'ADN mitochondrial (ADNmt) en lien avec le vieillissement cellulaire chez les bivalves. Pour répondre à cet objectif, deux études ont été réalisées. La première étude a permis de décrire les génomes mitochondriaux de neuf espèces de la sous-classe des Heterodonta, de reconstituer la phylogénie des Heterodonta et d'étudier la répartition génétique de l'espèce *Arctica islandica*. La deuxième étude a permis d'étudier le mode d'évolution de l'ADNmt par rapport à trois traits d'histoire de vie (longévité, temps de génération et température maximale létale). La composition des génomes mitochondriaux des neuf espèces d'Heterodonta correspond à celle retrouvée généralement chez les animaux : 13 gènes codant pour les protéines, 2 gènes ribonucléiques ribosomiques et 22 gènes ribonucléiques de transfert ; même si quelques particularités propres aux bivalves ont été observées (duplication du gène COX2, addition de gènes tRNA-Met, absence du gène ATP8 et configuration du gène CYTB en deux cadres de lecture). Les phylogénies des Heterodonta réalisées à partir de la concaténation des séquences nucléotidiques de 12 gènes mitochondriaux codant pour les protéines ont permis de confirmer les précédentes phylogénies et d'établir une relation de parenté solide entre les espèces *Arctica islandica* et *Corbicula fluminea*. L'étude sur la répartition génétique de l'espèce *Arctica islandica* montre que la population du golfe du Saint-Laurent est génétiquement plus proche de la population de la mer Baltique, que des populations de la mer du Nord et d'Islande. Le vieillissement, représenté par la longévité dans la deuxième étude, est expliqué à 68% par le temps de génération et à 22% par la température. Le taux de substitution synonyme est expliqué en partie (entre 9 et 30%) par la longévité. Les autres traits d'histoire de vie étudiés (temps de génération et température maximale létale) n'influencent pas l'évolution de l'ADNmt chez les bivalves. Nous présentons ici, la première étude sur l'impacte de la longévité sur le mode d'évolution de l'ADNmt chez des invertébrés.

Mots clés : vieillissement, génome mitochondrial, bivalve, phylogénie Bayésienne, évolution des traits d'histoire de vie, taux de substitution, Arctica islandica.

ABSTRACT

Bivalves are good model for studying evolutionary processes related to aging as they exhibit great variations in longevities among species. The aim of this Master was to understand how mitochondrial DNA (mtDNA) has evolved in relation to aging in bivalves. Two studies were performed to answer this question. The first chapter examines the description of the mitochondrial genomes of nine species of the subclass Heterodonta, provides a new Heterodonta phylogeny, and examines intraspecific variation in *Arctica islandica* using the full mitochondria genomes. The second study focus on the evolution of mtDNA relative to three life-history traits (longevity, generation time, and maximum lethal temperature). Mitochondrial genome cartography for the nine Heterodonta species corresponds to what is generally found in animals: 13 protein-coding genes, 2 ribosomal RNA genes and 22 transfer RNA genes. Some characters specific to bivalves were observed (duplication of COX2 gene, addition of tRNA-Met genes, lack of ATP8 gene and two reading frames for CYTB gene). Heterodonta phylogenies performed from the concatenation of 12 protein-coding genes nucleotide sequences confirmed previous phylogenies and established a strong relationship between the species *Arctica islandica* and *Corbicula fluminea*. Results on genetics repartition of *Arctica islandica* showed that the population from the Gulf of St. Lawrence was genetically closer to Baltic Sea population than to populations from the North Sea and Iceland. Aging, represented by longevity in the second chapter, was explained by generation time (68%) and by temperature (22%). The synonymous substitution rate is explained in part (between 9 and 30%) by longevity. Other life history traits studied (generation time and maximum lethal temperature) did not influence mtDNA evolution in bivalves. We present here, the first study on the evolution of mtDNA relative to longevity in invertebrates.

Keywords: *aging, mitochondrial genome, bivalve, Bayesian phylogeny, life-history evolution, substitution rates, Arctica islandica*.

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Contexte et problématique

L'étude du vieillissement

Plus de 300 théories ont été émises afin d'expliquer le vieillissement des cellules animales (Medvedev 1990). Plusieurs explications ont été proposées telles que le raccourcissement des télomères, la perte de la réponse immunitaire, l'accumulation de lipofuscine, etc. (Viña et al. 2007). Malgré des décennies d'études, le mécanisme du vieillissement cellulaire n'est toujours pas résolu. La théorie des radicaux libres proposée par Harman (1956) a été longtemps privilégiée. Harman (1956) a suggéré que les radicaux libres (également appelés espèces réactives de l'oxygène (ERO)) générés au cours de l'activité métabolique des cellules sont responsables de l'oxydation progressive des composants cellulaires (protéines, lipides, acide désoxyribonucléique (ADN), etc.). Le stress cellulaire ainsi engendré est appelé stress oxydant. Il entraîne entre autre une déficience énergétique conduisant à un déclin de la performance de reproduction et de survie de l'organisme: c'est le principe de sénescence (Harman 1956; Michalakis et al. 2010).

Les ERO sont majoritairement produites dans les mitochondries. Les mitochondries sont des organites cellulaires qui génèrent de l'énergie sous forme d'ATP (Adénosine Triphosphate). La chaîne respiratoire mitochondriale permet la réduction des molécules d'oxygène en eau. Les ERO sont créées lors de la production d'énergie par la chaîne respiratoire mitochondriale lors de la réduction partielle de l'oxygène (Pamplona and Barja 2011). La réduction de l'oxygène est induite par des complexes protéiques de la chaîne respiratoire qui sont codés par l'ADN mitochondrial (présent dans les mitochondries, ADNmt) et l'ADN nucléaire (présent dans le noyau des cellules). Une coévolution des

deux types d'ADN (et coadaptation de leurs produits) est indispensable afin d'assurer le bon fonctionnement des mitochondries et limiter le stress oxydant (Blier et al. 2001).

De nombreuses études sur le mode d'évolution des espèces se sont intéressées au lien entre la génétique et le phénotype afin de comprendre le processus de vieillissement chez les animaux. L'ADN des gènes impliqués dans la production de ERO, évolue rapidement au cours du temps par des mutations telles que des substitutions, des insertions et des délétions (Hu et al. 2014). Ces mutations sont provoquées par des facteurs environnementaux (Davies et al. 2004) mais peuvent également être liées aux traits d'histoire de vie des espèces (Bromham et al. 1996). La longévité est un trait d'histoire de vie très étudié, entre autre parce qu'il semble étroitement lié aux stratégies de reproduction. Par exemple, une espèce à maturation tardive, vivra plus longtemps qu'une espèce à maturation précoce (Ridgway et al. 2011a). Galtier et al. (2009a), Lartillot et Poujol (2011) et Lartillot (2013) ont montré que la longévité était négativement corrélée aux taux de mutations de l'ADN mitochondrial et de l'ADN nucléaire chez les mammifères. Un programme d'inférence bayésienne utilisant une méthode de Monte Carlo par chaîne de Markov a permis d'obtenir ces corrélations (Lartillot and Poujol 2011). Ce programme permet d'estimer et de corréler le temps de divergence, les taux de substitution et les traits d'histoire de vie entre différentes espèces. Ce type de modèle est notamment utilisé afin d'identifier les adaptations génétiques potentielles permettant des ajustements de la modulation du stress oxydant (Nabholz et al. 2008). Trois paramètres sont nécessaires au bon fonctionnement de ce modèle (Lartillot and Poujol 2011) : (1) une matrice de traits d'histoire de vie, (2) un alignement de séquences ADN et (3) un arbre phylogénétique retraçant l'évolution génétique des espèces les unes par rapport aux autres.

Les bivalves

Depuis quelques années, les bivalves se sont imposés comme un outil très puissant pour étudier les mécanismes liés au processus du vieillissement (Abele et al. 2009; Bodnar 2009; Philipp and Abele 2010). Les bivalves sont des ectothermes de faibles complexités

représentés par plus de 20 000 espèces, et possédant des caractéristiques phénotypiques extrêmement variées (Abele et al. 2009) :

(1) Ils présentent une vaste étendue de longévité pouvant aller de 1-2 ans (*Argopecten irradians, Musculista senhousia*) jusqu'à 507 ans (*Arctica islandica*) (Mistri 2002; Guo 2009; Butler et al. 2012). *Arctica islandica*, communément appelé quahog nordique, est l'animal non-colonial qui possède la plus grande longévité maximale connue. Plusieurs autres espèces possèdent une durée de vie maximale supérieure à 150 ans, telles que *Panopea abrupta* et *Margaritifera margaritifera*. Celles-ci affichent des longévités potentielles maximales de 163 et 210 ans respectivement (Ziuganov et al. 2000; Bureau et al. 2002). L'âge des bivalves est facilement mesurable et peut être déterminé en comptant le nombre d'anneaux de croissance présent sur la face interne de sa coquille, sachant qu'un anneau de croissance se forme chaque année (Richardson 2001; Wanamaker et al. 2008). De plus, de nombreuses études sur le taux métabolique des bivalves ont mis en avant des caractéristiques spécifiques aux espèces longévives. Les espèces de longue durée de vie ont un plus bas taux métabolique et produisent moins de ERO que les bivalves de courte durée de vie (Philipp et al. 2005b; Munro et al. 2013). Elles semblent également caractérisées par une meilleure résistance de leur macromolécules (protéines, lipides et ADN) aux dommages oxydatifs sans disposer toutefois d'une défense accrue en antioxydant (Munro and Blier 2012; Munro and Blier 2014).

(2) Leur temps de génération est corrélé à leur longévité suggérant que leur développement et leur vieillissement sont liés (Ridgway et al. 2011a). Une espèce longévive atteindra la maturité sexuelle plus tard qu'une espèce de courte durée de vie.

(3) Ils sont répartis sur la totalité du globe terrestre des pôles jusqu'aux tropiques (Abele et al. 2009). En tant qu'ectothermes, les bivalves sont thermo-dépendants à leur environnement. Leur croissance et leur taux métabolique sont ainsi gouvernés par la température environnementale. Les populations des eaux froides ont une croissance plus lente, une maturation et une reproduction tardives, un taux métabolique plus lent et une plus longue durée de vie, comparées aux populations d'eaux plus chaudes (Philipp et al.

2005a; Philipp et al. 2005b; Philipp et al. 2006). Les différentes conditions environnementales rencontrées par ces espèces pourraient influencer leurs modes de vieillissement.

(4) Ils possèdent différents modes de vie et vivent dans des milieux variés (Taylor et al. 2007; Abele et al. 2009). Les bivalves sont présents autant en milieu marin, qu'en eau saumâtre ou en eau douce. La plupart des espèces sont dites endobenthiques ; elles vivent de manière sessile, enfouies dans le sédiment où elles sont à l'abri des prédateurs. Les bivalves endobenthiques sont caractérisés par un environnement où des conditions hypoxiques sont fréquentes, pouvant favoriser une longue durée de vie (Taylor 1976; Philipp and Abele 2010). Ils semblent également tolérer de plus hauts niveaux de dommages cellulaires et d'accumulation de déchets cellulaires comparé aux bivalves mobiles (Philipp et al. 2005a; Philipp et al. 2006). De plus, les bivalves utilisent différentes sources de nourriture. La majorité des espèces se nourrissent en filtrant l'eau de leur milieu tandis que d'autres vont être détritivores ou même carnivores (Taylor et al. 2007).

À ma connaissance aucune étude à ce jour ne s'est intéressée à étudier le mode d'évolution du génome mitochondrial des bivalves en lien avec leur phénotype. Le génome mitochondrial est situé à proximité de la production des ERO et est ainsi plus susceptible d'être confronté aux dommages oxydatifs que l'ADN nucléaire (Barja and Herrero 2000; Philipp and Abele 2010). L'ADN mitochondrial a la particularité d'évoluer plus rapidement que l'ADN nucléaire (Nabholz et al. 2008).

ADN mitochondrial

L'ADN mitochondrial a généralement une forme circulaire et mesure entre 14 et 47 Kb chez les animaux (Boore 1999; Liu et al. 2013). Il est habituellement composé de 37 gènes : 13 gènes codant pour les protéines (PCGs), 2 gènes ribonucléiques ribosomiques (rRNA), 22 gènes ribonucléiques de transfert (tRNA) et une large région non-codante (contenant les éléments de réplication et de transcription) (Boore 1999; Blier et al. 2001;

Gissi et al. 2008). Les gènes sont codés sur un ou sur les deux brins de l'ADNmt et peuvent avoir différentes directions de transcription.

La taille, la composition et l'organisation des gènes sur le génome mitochondrial sont généralement invariantes chez les vertébrés (Gissi et al. 2008). En revanche, ces caractéristiques sont extrêmement variables chez les mollusques, et en particulier chez les bivalves. Cette variabilité peut même être observée au niveau intragénérique chez les bivalves. La duplication de gènes codant pour les protéines, la perte du gène ATP8, la séparation en deux parties distinctes ou la duplication d'un gène rRNA et l'addition ou la perte d'un gène tRNA sont des caractères courants chez les bivalves (Hoffmann et al. 1992; Milbury and Gaffney 2005; Gissi et al. 2008).

Un autre caractère génétique est unique aux bivalves : la double transmission uniparentale (Breton et al. 2007). L'ADN mitochondrial est strictement d'origine maternelle chez les animaux, à l'exception de certaines espèces de bivalve où il est issu des deux parents. Chez ces espèces, l'ADNmt des cellules germinales des mâles peut être d'origine paternelle alors que celui de leurs cellules somatiques est d'origine maternelle (Garrido-Ramos et al. 1998). Malgré ces particularités, l'ADNmt des bivalves est considéré, et en particulier l'ADN mitochondrial maternel, comme un bon marqueur de l'évolution des espèces (Gissi et al. 2008; Yuan et al. 2012b). Il peut aider à résoudre le statut taxonomique des espèces et à élaborer des hypothèses phylogénétiques.

Plusieurs arbres phylogénétiques ont déjà été réalisés afin d'établir les liens de parenté entre les différentes espèces de bivalves (Cope 1996; Adamkewicz et al. 1997; Giribet and Wheeler 2002; Serb and Lydeard 2003; Doucet-Beaupré et al. 2010; Plazzi et al. 2011). Deux types de reconstructions phylogénétiques sont possibles à partir du génome mitochondrial : (1) des séquences ADN et (2) de l'arrangement des gènes. La majorité de ces reconstructions sont conçues à partir des séquences ADN de un à trois gènes. Il est cependant souhaitable de réaliser ces reconstructions sur un maximum de gènes afin d'obtenir la reconstruction phylogénétique la plus proche de la réalité (Russo et al. 1996; Zardoya and Meyer 1996). Les reconstructions phylogénétiques réalisées à partir de

l'arrangement des gènes sont plus rares. Les modèles mis en place pour réaliser ces phylogénies sont plus difficiles à concevoir et rencontrent quelques problèmes (modèles simplistes, faible précision, peu robuste, manque d'estimation statistique, etc.) (Hu et al. 2014). Hu et al. (2014) ont développé un nouveau modèle basé sur le principe de vraisemblance afin de contrer les problèmes préalablement rencontrés. Ce modèle a l'avantage d'avoir une meilleure précision et flexibilité par rapport aux anciennes méthodes et de comprendre une inférence statistique. Malgré les problèmes rencontrés, les deux sources de données de l'ADNmt (séquence d'ADN et ordre des gènes) apparaissent comme des outils efficaces et complémentaires afin de clarifier la phylogénie des bivalves (Boore and Brown 1998; Serb and Lydeard 2003; Xu et al. 2012; Yuan et al. 2012a,b). D'après les récentes reconstructions phylogénétiques, les bivalves sont répartis en cinq sous-classes : (1) Protobranchia, (2) Palaeoheterodonta, (3) Anomalodesmata, (4) Heterodonta et (5) Pteriomorphia (Plazzi et al. 2011). La majorité des bivalves appartiennent à trois sous-classes : les Paleoheterodonta, les Heterodonta et les Pteriomorphia.

Dans cette étude, l'ADN mitochondrial de onze espèces a été séquencés afin de comprendre le mode d'évolution des bivalves. Ces espèces ont été choisies parce qu'elles affichent de larges variations de longévité et de température environnementales recontrées et aucune information sur leurs génomes mitochondriaux complet n'était disponible au début de l'étude. Neuf de ces espèces font parties de la sous-classe des Heterodonta : *Anomalocardia brasiliana, Arctica islandica, Corbicula fluminea, Macoma constricta, Mactromeris polynyma, Mercenaria mercenaria, Mya arenaria, Spisula solidissima* et *Tivela mactroides* ; les deux autres appartiennent à la sous-classe des Palaeoheterodonta : *Elliptio* sp. et *Margaritifera margaritifera*. Ces onze espèces sont endobentiques et filtreuses. Elles possèdent une étendue de longévité de 2,5 à 507 ans (Butler et al. 2012; Turra et al. 2015), de maturité sexuelle de 0,42 à 32 ans (Thorarinsdottir and Steingrimsson 2000; Turra et al. 2015) et un gradient de température maximale tolérée de 17 à 35°C (Roegner and Mann 1991; Witbaard and Bergman 2003) (Table 1). Les Palaeoheterodonta sont présentes en eau douce tandis que les Heterodonta sont principalement présentes en

milieu marin. Dans notre étude, seulement un Heterodonta, *Corbicula fluminea* vit en eau douce ou en eau saumâtre.

Table 1. Traits d'histoire de vie des onze espèces sélectionnées

	Longévité (ans)	Maturité sexuelle (ans)	Température maximale tolérée* (°C)	Milieu de vie
Anomalocardia brasiliana	3	0,5	-	Milieu marin, climat tropical
Arctica islandica	507	32	17	Milieu marin, climat polaire et tempéré
Corbicula fluminea	5	0,75	30	Eau douce et saumâtre, climat tempéré et tropical
Elliptio sp.	-	-	-	Eau douce, climat tempéré
Macoma constricta	-	-	-	Milieu marin, climat tropical
Mactromeris polynyma	92	12	-	Milieu marin, climat polaire et tempéré
Margaritifera margaritifera	210	20	28	Eau douce, climat polaire et tempéré
Mercenaria mercenaria	106	-	35	Milieu marin, climat tempéré
Mya arenaria	28	1,6	32,5	Milieu marin, climat polaire et tempéré
Spisula solidissima	37	4	28	Milieu marin, climat polaire et tempéré
Tivela mactroides	2,5	0,42	-	Milieu marin, climat tropical

*correspondant à la température maximale atteinte avant la mort, mesurée en laboratoire/Références des données en Annexe XII.

Notre étude s'insère également dans le cadre des études récentes sur l'impact physiologique et évolutif des changements climatiques. Les modifications apportées aux milieux de vie des ectothermes dans les prochaines années, en raison du changement climatique, pourraient entraîner un déclin dans la performance de reproduction et de la

survie des espèces. Grâce à leur grande étendue de longévité et de maturité sexuelle, leur vaste distribution et leur mode de vie divergents, les bivalves apparaissent comme un excellent modèle animal pour étudier le vieillissement.

Intérêt du projet

L'intérêt du projet de maîtrise est de comprendre l'évolution moléculaire d'espèces de bivalves possédant différentes longévités. Nous supportons l'hypothèse « du stress oxydant mitochondrial du vieillissement cellulaire », selon laquelle les dysfonctions mitochondriales seraient induites par les ERO. La gestion du stress oxydant est en partie assumée par le système de transports des électrions encodés en partie par le génome mitochondrial. Dans ces conditions, l'ADNmt devrait subir des pressions sélectives importantes de façon à minimiser la production de ERO. Nous suppposons donc que des traces de sélection associées à la longévité devrait se retrouver dans l'ADN mitochondrial des espèces. Le projet a été séparé en deux parties comprenant chacune différentes étapes de réalisation :

Objectif 1 : Proposer une nouvelle reconstruction phylogénétique robuste et informative des Heterodonta et comparer les génomes mitochondriaux de quatre populations de l'espèce *Arctica islandica*.

a) Établir la cartographie de neuf nouveaux génomes mitochondriaux d'Heterodonta. Décrire et comparer la composition et l'ordre des gènes, la composition en nucléotides, l'usage des codons, les régions répétées et les régions non-codantes présents dans les neuf nouveaux génomes mitochondriaux.

c) Décrire l'arrangement des gènes sur les génomes mitochondriaux d'Heterodonta et estimer leurs états de caractères ancestraux.

d) Réaliser et comparer plusieurs phylogénies de la sous-classe des Heterodonta à partir de la concaténation des séquences nucléotidiques de 12 gènes codant pour les protéines.

e) Comparer l'annotation du génome mitochondrial de cinq individus de l'espèce *Arctica islandica* et réaliser une étude phylogénétique à partir de la concaténation des séquences nucléotidiques mitochondriales de 12 gènes codant pour les protéines de quatre populations de l'espèce *Arctica islandica*.

Objectif 2 : Clarifier les relations évolutives de différents groupes taxonomiques et étudier le mode d'évolution de l'ADN mitochondrial en lien avec trois traits d'histoire de vie chez les bivalves.

a) Déterminer les valeurs des trois traits d'histoire de vie (longévité, maturité sexuelle et température) à partir de données existantes pour 76 espèces de bivalves.

b) Appliquer les données de traits d'histoire de vie et la concaténation des séquences en acides aminés de 12 gènes mitochondriaux codant pour les protéines à un modèle de covariance Bayésien déjà existant.

Ces deux objectifs sont présentés sous forme de deux articles scientifiques au chapitre I et au chapitre II. Le chapitre I est un préalable au chapitre II. En effet, il est indispensable de connaître le patron mitochondrial et la phylogénie des bivalves (chapitre I) afin d'étudier leur mode d'évolution mitochondrial (chapitre II). Une conclusion générale clôturera ce mémoire afin de mettre en évidence la portée de l'étude, de soulever les problèmes rencontrés et d'y proposer des solutions.

CHAPITRE 1
NOUVELLES PHYLOGÉNIES D'HETERODONTA (MOLLUSCA : BIVALVIA) BASÉES SUR L'ADN ET L'ORDRE DES GÈNES MITOCHONDRIAUX

Aurore Levivier, France Dufresne, Marcelo V. Kitahara, Alvaro E. Migotto, Pierre U. Blier

1.1. RÉSUMÉ EN FRANÇAIS DU PREMIER ARTICLE

Les Heterodonta sont une sous-classe des bivalves, largement distribués, comprenant une espèce très particulière, *Arctica islandica*, qui est connue pour être l'animal complexe vivant le plus longtemps sur Terre. Démêler les relations évolutives entre les espèces de ce groupe permettra de mieux comprendre l'évolution de la durée de vie et des traits d'histoire de vie associés. Dans cette étude, nous avons séquencé le génome mitochondrial (mitogénome) de neuf espèces d'Heterodonta en utilisant la technique de séquençage Illumina MiSeq. Le génome mitochondrial complet a été séquencé pour *Arctica islandica, Corbicula fluminea, Macoma constricta, Mactromeris polynyma, Mya arenaria* et *Spisula solidissima* alors qu'un mitogénome partiel a été retrouvé pour *Anomalocardia brasiliana, Mercenaria mercenaria* et *Tivela mactroides*. Les mitogénomes mesuraient entre 14658 et 23210 pb. Treize gènes codant pour les protéines (PCGs), 2 gènes ribonucléiques ribosomiques (rRNA) et 22 gènes ribonucléiques de transfert (tRNA) codaient sur le brin principal et ont été globalement trouvés pour les neuf espèces. Quelques exceptions étaient présentes : absence des gènes 16S-rRNA et tRNA-Cys pour *A. brasiliana* (génome partiel), présence de deux cadres de lecture pour le gène CYTB chez *A. islandica*, présence de deux gènes COX2 et de cinq gènes tRNA-Met pour *M. polynyma*, et absence du gène ATP8 et présence de deux gènes tRNA-Met pour *M. arenaria*.

Afin d'établir les relations évolutives entre les espèces de la sous-classe des Heterodonta, 28 autres mitogénomes de 18 genres ont été ajoutés. Un extrême

réarrangement des gènes a été trouvé et plusieurs associations de gènes sont apparues caractéristiques des Heterodonta : 12S-COX3, 16S-ATP6 et ATP8-ND4-HES, et une autre association, CYTB-16S, est considérée comme un caractère ancestral des bivalves. Deux phylogénies ont été réalisées (1) une phylogénie en Maximum de vraisemblance et (2) une phylogénie Bayésienne sur les séquences nucléotidiques de 12 PCGs (le gène ATP8 étant exclu). La seule différence observée entre ces deux phylogénies était la position de l'espèce *Hiatella arctica*. *Arctica islandica* et *Corbicula fluminea* ont été clairement établis comme étant des « groupes sœurs ».

Nous nous concentrons sur la relation génétique mitochondriale entre quatre populations d'*Arctica islandica*. Une phylogénie à partir de la méthode de l'estimation du maximum de vraisemblance a été réalisée sur les séquences nucléotidiques de 12 PCGs. Notre étude a trouvé que la population du golfe du St Laurent (Îles de la Madeleine) était génétiquement plus proche de la population de la mer Baltique que de deux populations de la mer du Nord et de l'Atlantique du Nord (Islande).

Notre étude est l'une des études mitogénomiques la plus complète des Heterodonta et permet d'améliorer nos connaissances de l'ordre des gènes et de la composition des génomes de ce groupe. Des efforts supplémentaires de séquençage de mitogénomes permettront d'obtenir une image complète de la phylogénie des Heterodonta.

Ce premier chapitre, intitulé *New phylogenies of Heterodonta (Mollusca: Bivalvia) based on mitochondrial DNA and gene order*, fut et sera corédigé par moi-même ainsi que par les professeurs France Dufresne, Marcelo Kitahara, Alvaro Migotto et Pierre Blier. Il sera prochainement soumis à la revue *Molecular Phylogenetics and Evolution* pour publication. En tant que premier auteur, j'ai réalisé la recherche documentaire sur l'état de l'art, une partie de l'échantillonnage, les expériences en laboratoire, le traitement des données et la rédaction de l'article. Les professeurs Marcelo Kitahara et Alvaro Migotto, troisième et quatrième auteurs, m'ont accueilli au Brésil. Ils m'ont permis d'accéder à leur laboratoire, ils ont mis à ma disposition le matériel nécessaire à mes expériences et m'ont également aidé à organiser l'échantillonnage. Le professeur Pierre Blier, cinquième auteur,

a fourni l'idée originale. Le professeur France Dufresne, deuxième auteur, ainsi que le professeur Pierre Blier, ont aidé à la recherche sur l'état de l'art, au développement de la méthode et à la révision de l'article.

Mots-clés : Heterodonta, génome mitochondrial, arrangement des gènes, phylogénie à partir de l'ordre des gènes, phylogénie Bayésienne, mitogénomiques, Arctica islandica.

1.2. NEW PHYLOGENIES OF HETERODONTA (MOLLUSCA: BIVALVIA) BASED ON MITOCHONDRIAL DNA AND GENE ORDER

1.2.1. Abstract

Heterodonta is a widely distributed subclass of bivalves including a very peculiar species, *Arctica islandica*, which is known to be the longest living complex animal on earth. Unraveling evolutionary relationships among species of this group is thus timely to better understand the evolution of lifespan and associated life history traits. In this study, we sequenced the mitochondrial genomes (mitogenomes) of nine Heterodonta species using Illumina MiSeq. The entire mitochondrial genomes were sequenced from *Arctica islandica, Corbicula fluminea, Macoma constricta, Mactromeris polynyma, Mya arenaria* and *Spisula solidissima* whereas a partial mitogenome was recovered for *Anomalocardia brasiliana, Mercenaria mercenaria* and *Tivela mactroides*. The mitogenomes varied between 14658 and 23210 bp. Thirteen protein-coding (PCGs) genes, 2 ribosomal RNA (rRNA) genes and 22 transfer RNA (tRNA) genes encoded on the heavy strand were globally found for the nine species. Some exceptions were however present: no 16S-rRNA and tRNA-Cys genes were found in the partial genome of *A. brasiliana*, two reading frames were found for the CYTB gene in *A. islandica*, two COX2 genes and five tRNA-Met genes were found in *M. polynyma*, and no ATP8 gene and two tRNA-Met genes were found in *M. arenaria*.

In order to establish evolutionary relationships among Heterodonta species, 28 other mitogenomes from 18 genera were added from GenBank (Benson et al. 2013). Extreme gene rearrangements were found and some gene associations appeared characteristic of

Heterodonta: 12S-COX3; 16S-ATP6 and ATP8-ND4-HES and another CYTB-16S was considered as bivalve ancestral state. Two phylogenies were performed (1) a Maximum likelihood phylogeny and (2) a Bayesian phylogeny on 12 PCGs (ATP8 gene was excluded) nucleotide sequences. Only one difference was observed between these two phylogenies, the position of the species *Hiatella arctica*. *Arctica islandica* and *Corbicula fluminea* were clearly established such as « sister groups ».

Secondly, we also explored mitochondrial genetic relationship between four populations of *Arctica islandica*. A Maximum likelihood phylogeny was performed on 12 PCGs nucleotide sequences. Our study found that a population from the Gulf of St. Lawrence (Îles de la Madeleine) was genetically closer to Baltic Sea population than to two populations from the North Sea and the North Atlantic (Iceland).

Our study is one of the most complete mitogenomic studies in Heterodonta and improves our knowledge of gene orders and genome composition in this group. Additional sequencing efforts of mitogenomes would obtain a complete picture of Heterodonta phylogeny.

Keywords: Heterodonta, mitochondrial genome, gene arrangement, phylogeny from gene order data, Bayesian phylogeny, mitogenomics, Arctica islandica.

1.2.2. Introduction

Phylogenies realized with molecular data are commonly used to establish evolutionary relationships among species and therefore validate taxonomy (e.g. Mikkelsen et al. 2006). Mitochondrial DNA (mtDNA) has proven to be a good phylogenetic marker with the capacity to discriminate closely related species due to absence of recombination and rapid evolutionary dynamics (Gissi et al. 2008). Standard molecular phylogenies are often performed with only few genes (Yuan et al. 2012b) while the whole mitochondrial genome (mitogenome) is more informative for determination of phylogenetic relationships allowing increased resolution and higher statistical confidence (Russo et al. 1996; Zardoya and

Meyer 1996; Gissi et al. 2008; Yuan et al. 2012b). Furthermore, mitochondrial phylogenies are mainly built using DNA substitutions but gene arrangement can also provide a powerful character for reconstructing phylogenies (Boore and Brown 1998; Serb and Lydeard 2003). Indeed, mitogenome gene rearrangement often corresponds to phylogeny structure based on DNA (Xu et al. 2012; Yuan et al. 2012b).

The metazoan mitochondrial genome is relatively conserved in terms of gene content and organization (Boore 1999). Most animal mitochondrial genomes are closed-circular containing 37 genes with 13 protein-coding genes (PCGs), 2 ribosomal RNAs (rRNAs), 22 transfer RNAs (tRNAs) and a large non-coding region (NCR; containing elements for regulation of replication and transcription) (Boore 1999; Blier et al. 2001; Gissi et al. 2008). The size of mitogenomes ranges from 14 to 47 kb (Boore 1999; Liu et al. 2013). Although gene content, organization, and size are almost invariant in vertebrates, extreme gene rearrangement is commonplace in mollusks and particularly in bivalves, even among species from the same genera (Gissi et al. 2008). Several features in bivalve mitochondrial DNAs deviate from common metazoan mitogenomes such as additional or losses of tRNA genes (e.g. addition of tRNA-Met gene), split or duplication of rRNA genes, presence of CYTB translational frameshift, loss of ATP8 gene, etc (Hoffmann et al. 1992; Milbury and Gaffney 2005; Gissi et al. 2008).

Bivalves include five subclasses (Taylor et al. 2007; Plazzi et al. 2011). The most diverse subclass is Heterodonta. Heterodonts are grouped into two extant orders: Veneroida and Myoida (Giribet and Wheeler 2002). The large order Veneroida comprises 18 superfamilies and the Myoida order contains four superfamilies. They have a worldwide distribution from the poles to the tropics (e.g. Silva-Cavalcanti and Costa 2011 and Butler et al. 2012). Most of them occupy marine environments but others are found in brackish or freshwater habitat (Taylor et al. 2007). They modulate significantly community structure as well as trophic resource abundance and diversity (e.g. Lewis et al. 2007). Heterodonta and particularly Veneroida order include some families of economical importance such as Cardiidae, Tellinidae, Veneridae and Mactridae (Taylor et al. 2007). Heterodonta taxonomy is not

resolved yet (Mikkelsen et al. 2006; Yuan et al. 2012b). For example, the Hiatellidae family was excluded from Myoida order by molecular phylogenetic analyses that placed this taxon closer to Veneroida than Myoida species (Taylor et al. 2007; Yuan et al. 2012b).

Currently, the complete mitochondrial genomes of 37 Heterodonta species from nine superfamilies are found in GenBank. One of these species recently attracted a particular interest: *Arctica islandica*, the ocean quahog, which has been found to live up to 507 years old, making it the longest-lived non-colonial metazoan (Butler et al. 2012). This heterodont species belongs to the order Veneroida and the family Arcticidae (Glöckner et al. 2013). It is widely distributed over North Atlantic Boreo-Arctic shelves regions in western (North America) and eastern (Europe) parts. It occupies a muddy/silty habitat over 15 to 256 m water depth with most abundance at 30 to 60 m (Dahlgren et al. 2000). *A. islandica* is also an important commercial fisheries species (Dahlgren et al. 2000).

The aim of this study was to improve the extant phylogenetic knowledge of heterodont subclass to ensure proper evolutionary inferences in future comparative studies of heterodonts. For this purpose, we describe the gene content and organization, codon usage, repeated regions, AT content and non-coding regions for nine new complete mitochondrial genomes of heterodont bivalves. Eight of them belong to the order Veneroida: *Anomalocardia brasiliana, Arctica islandica, Corbicula fluminea, Macoma constricta, Mactromeris polynyma, Mercenaria mercenaria, Spisula solidissima* and *Tivela mactroides*; and one belong to the order Myoida, *Mya arenaria*. Additionally, gene arrangement comparison and two phylogenic analyses were performed on these nine newly determined sequences together with 28 other heterodont mitogenomes available in GenBank. The two phylogenies included: (1) a maximum likelihood phylogeny, and (2) a Bayesian phylogeny on 12 PCGs (ATP8 gene was excluded) nucleotide sequences. Furthermore, a mitogenomic comparison of four populations: Gulf of St. Lawrence (Îles de la Madeleine), North Atlantic (Iceland), North Sea, and Baltic Sea of *Arctica islandica* was performed to improve our knowledge of intraspecific genetic structure in this species.

1.2.3. Materials and methods

1.2.3.1. Sample collection

Arctica islandica and *Spisula solidissima* were collected in the Îles-de-la-Madeleine (47°22'N, 61°58'W, Québec, Canada); *Mactromeris polynyma* in Portneuf sur mer (48°36'N, 69°05'W, Québec, Canada); *Mercenaria mercenaria* in Neguac (47°15'N, 65°03'W, New Brunswick, Canada); and *Mya arenaria* in Bic (48°23'N, 68°40'W, Québec, Canada) in 2009. *Anomalocardia brasiliana* and *Macoma constricta* were collected in São Sebastião (23°48'S, 45°24'W, São Paulo, Brazil); *Corbicula fluminea* in Ribeirão Preto (21°06'S, 47°45'O, São Paulo, Brazil); and *Tivela mactroides* in Caraguatatuba (23°34'S, 45°18'W, São Paulo, Brazil) in 2013. Samples were frozen at -80°C (*A. islandica*, *S. solidissima*, *M. polynyma*, *M. mercenaria*, *M. arenaria* and *C. fluminea*) or preserved in 95% ethanol (*A. brasiliana*, *M. constricta* and *T. mactroides*).

1.2.3.2. Isolation of mitochondria and DNA extraction

The protocol for the isolation of mitochondria was adapted from Munro and Blier (2012). The isolation of mitochondria was performed using about 5g of fresh or frozen adductor muscle, mantle, and gill tissues for the following species *A. islandica, C. fluminea, M. polynyma, M. mercenaria, M. arenaria* and *S. solidissima*. The final pellet was dried and conserved at -80°C until DNA extraction. The mitochondrial DNA was extracted using the Mollusc DNA kit (Omega Bio-tek, Inc., USA) following the manufacter's protocol (see Table 2 for more details).

For *Anomalocardia brasiliana, Macoma constricta* and *Tivela mactroides*, total DNA extractions were performed using the same extraction kit as above.

1.2.3.3. Quantification of DNA and sequencing by Illumina MiSeq method

The double-stranded DNA quantity was measured using Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen™, Canada) following the manufacter's protocol. The library preparation and paired-end sequencing were performed on the Illumina MiSeq platform at

the “Plateforme d’analyses génomiques” of the Institute for integrative and systems biology (IBIS, Université Laval, Québec, Canada).

1.2.3.4. Mitogenome assemblies, annotation and analyses

CLC Genomics Workbench v.7.0 (CLC bio, a QIAGEN company, Aarhus C, Denmark) was used for stringently trimming the reads and for de novo assembly. A BLAST (Altschul et al. 1990) of each assembled mitochondrial genome was performed to confirm the identification at the species level.

Mitochondrial genomes were annotated using Geneious v.8.0.3 (Biomatters, Auckland, New Zealand; (Kearse et al. 2012)). Protein coding genes were analyzed by NCBI ORF Finder (NCBI website) and Geneious v.8.0.3 using the invertebrate mitochondrial code. Initiation and termination codons were verified by eyes after translating the sequence in amino acid. The ends of the two rRNAs were assumed comparing MITOS WebServer (Bernt et al. 2012), BLAST results and the border with adjacent genes. The validation of the position of each PCG and rRNA were obtained with MITOS WebServer using the invertebrate genetic code and mitogenome alignments of other veneroid species already present on GenBank (Benson et al. 2013) with Geneious v.8.0.3. tRNA annotation was performed comparing tRNAcan-SE v.1.21 (Lowe and Eddy 1997), ARWEN (Laslett and Canbäck 2008) and MITOS WebServer results.

Base composition was calculated with Geneious v.8.0.3. The formulas of Perna and Kocher (1995) (AT skew = (A-T)/(A+T) and GC skew = (G-C)/(G+C)) were used to measure asymmetric nucleotide composition. Codon usage was determined using MEGA v.5.2 (Tamura et al. 2011). Amino acid divergence among species was calculated using p-distance model with MEGA v.5.2. Repeated regions were identified using Tandem Repeat Finder v.4.07b (Benson 1999).

1.2.3.5. Gene order comparison and DNA phylogenies of Heterodonta

Complete mitochondrial genomes sequences of 28 heterodont species in GenBank (Benson et al. 2013) and our nine newly sequenced mitogenomes were used for phylogenetic

reconstructions. Sequences from GenBank were downloaded in November 2014 with their annotations (accession numbers are in Annex I).

Bayesian and maximum likelihood phylogenetic reconstructions from nucleotide concatenations of 12 PCGs (ATP8 gene was excluded) were performed. The 12 PCG nucleotide sequences were aligned separately with MUSCLE software v.3.8.31 (Edgar 2004), the poorly aligned positions were stringently removed using Gblocks v.0.91b (Castresana 2000). After concatenation, 7734 nucleotides were left for phylogenetic analyses. A Bayesian inference using Monte Carlo Markov Chain (MCMC) algorithm phylogenetic tree following GTR and DGAM 4 models was constructed in PhyloBayes v.3.3f (Lartillot et al. 2009). GTR (for General Time Reversible matrix) is a model where exchange rates are free parameters and with prior distribution, a product of independent exponential distributions of means 1. A DGAM 4 option allowed using discrete gamma distribution with four categories (Lartillot et al. 2009). Maximum likelihood (ML) phylogeny was performed using HKY model and Gamma substitution model with TOPALi v2.5 (Milne et al. 2004). HKY (for Hasegawa, Kishino and Yano) is a model where base frequencies are variable and that use one transition rate and one transversion rate (Hasegawa et al. 1985). Gamma option uses the common gamma model with 4 rate categories. To estimate the reliability of branches in the ML trees, 100 bootstrap trees were generated. The protobranch *Solemya velum* was used as outgroup. Amino acid divergence was calculated using p-distance model with MEGA v.5.2.

Gene order comparison (with PCGs, rRNA and tRNA) was realized following maximum likelihood phylogeny hierarchy.

Maximum likelihood phylogenetic reconstructions from gene order data (PCGs, rRNA and tRNA) were performed on the geneorder.org web-server (Lin et al. 2013) (Annex II). This phylogeny had a low statistic resolution, so it will be not considered in this paper.

1.2.3.6. Intraspecific genetic relationships in *Arctica islandica*

Two mitogenomes of two individuals of *A. islandica* from Îles-de-la-Madeleine (Québec, Canada) were sequenced in our study. These two mitochondrial genomes were obtained by (1) isolation of mitochondria, DNA extraction and Illumina MiSeq sequencing (see above) and (2) primer-walking and Sanger sequencing (Annex III). Sanger method was used to validate mitochondrial genome sequences obtained by Illumina technology.

Three other mitogenomes of *A. islandica* were obtained from GenBank (accession numbers: KC197241 from Iceland population; KF363951 from Baltic Sea; and KF363952 from North Sea (Glöckner et al. 2013)).

A comparison of these five mitogenomes (this study and Glöckner et al. (2013)) was done. Two phylogenies were performed on the concatenation of 11 004 nucleotides: (1) Maximum likelihood, and (2) Neighbor joining using Kimura 2-parameter model in MEGA v.5.2.. *Corbicula fluminea* was used as an outgroup. Amino acid and nucleotide divergences were calculated using p-distance model using MEGA v.5.2.

1.2.4. Results

1.2.4.1. Illumina MiSeq technology performance

DNA quantities for the nine species, before the sequencing, ranged from 93 ng/µL (*T. mactroides*) to 561 ng/µL (*A. brasiliana*; Table 2). The Illumina MiSeq sequencing resulted in approximately 3 million paired-end reads of 290 nucleotides for each species. After trimming, 2 562 992 paired-end reads of 245 nucleotides resulted and were used for de novo assembly (Annex IV). The complete mitochondrial genomes of *Macoma constricta* and *Mya arenaria* were respectively obtained in a single contig. For *Arctica islandica, Corbicula fluminea, Mactromeris polynyma* and *Spisula solidissima*, an alignment of some contigs with ClustalW (Thompson et al. 1994) allowed us to identify their whole mitogenomes. A partial mitogenome was obtained for *Anomalocardia brasiliana, Mercenaria mercenaria* and *Tivela mactroides*. The coverage of mitochondrial contigs for

these three species was 16X, 18X and 19X respectively, whereas for the other species the coverage ranged from 50X to 313X (Table 2).

1.2.4.2. Genome compositions

The whole closed-circular mitochondrial genome sizes ranged from 16308 (*M. constricta*) to 23210 bp (*M. polynyma*; Table 3). Globally, 13 PCGs, 2 rRNAs and 22 tRNAs encoded on the heavy strand were found for the nine species. Detailed annotations for each species are presented in Tables 5, 6 and Annex V (Tables S1-S8).

1.2.4.2.1. Protein-coding genes

Thirteen PCGs were generally found for the nine species (Table 3, Annex V, Tables S1-S8). However some exceptions were present:

- Two COX2 copies were found in *M. polynyma*. The first one is 2058 bp, which is longer than the typical size whereas the other is 1311 bp, closer to actual size (Table 3). Moreover, the alignment of these two COX2 with the COX2 genes of others species revealed that the second one had a better alignment. This sequence was therefore kept for the analyses.

- *A. islandica* CYTB gene was separated in two reading frames.

- The ATP8 gene was found in eight out of the nine species studied. Only *M. arenaria* did not have the ATP8 gene. The first six amino acids at the N-terminus (MPQFAP or MPQFSP), which are indicative of ATP8 gene in bivalves, were used to identify this gene (Table 4).

Table 2. Method used, DNA concentration and assembling result for nine species

Species	Method used	Number individus/tube	DNA concentration (ng/µL)	Assembling result			Final size
				Contigs [1]	Total reads	Coverage	
Anomalocardia brasiliana	DNA extraction	1	561.575	14658	903	16	14658
Arctica islandica	Isolation mito + DNA extraction	1	478.53	18304	7204	104.14	18304
Corbicula fluminea	Isolation mito + DNA extraction	4	97.1	16828	20997	312.91	16687
Macoma constricta	DNA extraction	1	116	16308	6647	98.96	16308
Mactromeris polynyma	Isolation mito + DNA extraction	1	210.44	23027	4734	49.73	23210
Mercenaria mercenaria	Isolation mito + DNA extraction	1	138.8	16901	1172	17.85	17038
Mya arenaria	Isolation mito + DNA extraction	1	102.11	17936	6573	95.27	17936
Spisula solidissima	Isolation mito + DNA extraction	1	324.26	18747	6392	82.32	18747
Tivela mactroides	DNA extraction	1	93.551	18641	1534	19.36	18851

[1] Larger contig size

Start (ATG, ATA, ATC, ATT, GTG, TTG) and stop (TAA and TAG) codons of invertebrate mtDNA genetic code were used to annotate PCGs (Table 3). One degenerate stop codon was found for COX3 in *M. mercenaria*. All genes were characterized by at least two start or stop codons in heterodont species analyzed in this studies.

Concerning amino acid divergences (Annex VI, Tables S1-S5), COX1 was the most conserved gene among the nine species with the maximum distance between two species of 40% (between *M. constricta* and *T. mactroides*). ND3 and ND6 were the least conserved, with the maximum amino-acid distances between two species of 89.2 % (between

A. brasiliana and *T. mactroides*) and 85.2% (between *M. constricta* and *M. arenaria*) respectively.

1.2.4.2.2. Ribosomal and transfer RNA genes

Two ribosomal RNA genes are usually present in bivalves: 12S and 16S (Table 3). For the nine newly sequenced mtDNAs, the size of the 12S gene varied between 850 and 1197 bp. The 16S gene was not found for one partial mitogenome (*A. brasiliana*), for the eight other species, its size varied between 1192 and 1505 bp.

22 tRNA genes were usually found, with sizes ranging from 61 to 73 bp. Some exceptions were found. Generally only one tRNA-Met gene is present in bivalve mitogenomes. Here, five tRNA-Met genes were found in the mitogenome of *M. polynyma* and two in the mitogenome of *M. arenaria*. No tRNA-Cys gene was found in the partial mitogenome of *A. brasiliana*. The majority of tRNA was folded into a typical cloverleaf secondary structure with four arms (Annex VII, Figures S1-S9). However, tRNA-Tyr in *A. brasiliana* and *C. fluminea*, tRNA-Ser (TCT) in *A. islandica, C. fluminea, M. constricta, M. polynyma, M. mercenaria* and *S. solidissima*, tRNA-Ser (TGA) in *A. brasiliana, C. fluminea, M. polynyma, M. mercenaria, M. arenaria* and *S. solidissima*, and tRNA-Ser (CGA) in *A. islandica* lacked the DHU arm. tRNA-Arg in *A. brasiliana* lacked the TΨC arm, and tRNA-Arg and tRNA-Gly in *M. polynyma* had no terminal TΨC loop. Few others mismatched base pairs were also observed.

Table 3. Region sizes and start/stop codons for PCGs

	A. brasiliana	***A. Islandica***	***C. fluminea***	***M. constricta***	***M. polynyma***	***M. mercenaria***	***M. arenaria***	***S. solidissima***	***T. mactroides***
Size (bp)	14658 (P)	18304	16687	16308	23210	17038 (P)	17936	18747	18851 (P)
COX 1	1587 (ATG/TAA)	1671 (ATG/TAA)	1617 (ATA/TAA)	1704 (ATA/TAG)	1743 (ATA/TAA)	1563 (ATA/TAA)	1791 (ATT/TAA)	1707 (TTG/TAG)	1683 (ATG/TAG)
COX 2	1050 (ATG/TAA)	1023 (ATG/TAA)	1320 (ATT/TAA)	867 (ATG/TAA)	2058/1311 (ATG/TAA)	891 (TTG/TAG)	1434 (ATG/TAA)	1374 (ATG/TAG)	1053 (GTG/TAG)
COX 3	846 (ATT/TAG)	846 (TTG/TAA)	876 (TTG/TAA)	885 (GTG/TAG)	1068 (TTG/TAG)	850 (ATG/T**)	861 (ATA/TAG)	1113 (ATG/TAA)	888 (GTG/TAA)
CYTB	1143 (ATT/TAG)	1158 (ATG/TAA)	1161 (TTG/TAA)	1233 (GTG/TAG)	1203 (ATG/TAA)	1155 (ATA/TAG)	1143 (GTG/TAA)	1197 (ATG/TAA)	1161 (GTG/TAA)
ND1	918 (ATA/TAA)	912 (ATG/TAA)	912 (GTG/TAG)	924 (GTG/TAG)	912 (ATG/TAA)	918 (ATA/TAA)	912 (ATG/TAA)	915 (ATG/TAG)	918 (ATG/TAA)
ND2	1008 (GTG/TAG)	1023 (ATG/TAA)	1023 (GTG/TAG)	1062 (ATG/TAG)	1101 (GTG/TAA)	1023 (TTG/TAA)	1065 (ATG/TAA)	1044 (ATT/TAA)	1071 (ATT/TAA)
ND3	312 (TTG/TAG)	411 (ATG/TAG)	423 (ATT/TAG)	363 (GTG/TAA)	438 (ATC/TAA)	405 (ATT/TAG)	423 (ATG/TAG)	414 (ATG/TAG)	435 (ATG/TAG)
ND4	990 (TTG/TAG)	1359 (TTG/TAA)	1359 (ATT/TAA)	1332 (ATG/TAG)	1353 (GTG/TAG)	1011 (ATT/TAA)	1032 (ATT/TAA)	1356 (ATG/TAG)	1362 (ATG/TAA)
ND4L	300 (ATT/TAA)	285 (ATA/TAG)	288 (ATA/TAG)	294 (GTG/TAG)	306 (ATA/TAG)	252 (TTG/TAA)	384 (ATA/TAA)	297 (ATT/TAA)	303 (ATT/TAG)
ND5	1710 (TTG/TAA)	1725 (ATA/TAG)	1707 (TTG/TAA)	1728 (ATA/TAG)	1833 (ATG/TAA)	1698 (ATT/TAA)	1707 (TTG/TAA)	1824 (ATA/TAG)	1746 (ATT/TAA)
ND6	483 (ATA/TAA)	504 (ATT/TAA)	471 (ATG/TAG)	540 (GTG/TAA)	486 (GTG/TAG)	483 (ATA/TAG)	549 (ATT/TAG)	492 (ATT/TAG)	597 (ATG/TAA)
ATP 6	741 (ATT/TAA)	744 (ATG/TAA)	741 (GTG/TAG)	738 (ATG/TAG)	780 (GTG/TAG)	756 (ATA/TAA)	729 (ATG/TAA)	747 (GTG/TAG)	828 (ATA/TAA)
ATP 8	114 (ATG/TAG)	114 (ATG /TAA)	114 (ATG/TAA)	126 (ATG/TAG)	114 (ATG/TAA)	114 (ATG/TAA)	/	120 (ATG/TAA)	117 (ATG/TAG)
12S	946	950	850	902	922	1005	981	1197	975
16S	/	1289	1192	1245	1342	1505	1396	1240	1317
MNR	370…	1095/1244	1027	849	2630/739	348/927…	470/838	582/269/269	815/1195…
NCR	1177…	2867	1206	935	4567	2008…	2028	2296	2963…

NOTE. (P), partial genome; MNR, major non-coding region, and NCR, non-coding region

Table 4. Amino acid sequences at the N-terminus version of the ATP8 gene

Species	Start ATP8
Anomalocardia brasiliana	MPQFAPMFS
Arctica islandica	MPQFSPSYS
Corbicula fluminea	MPQMAPTAS
Macoma constricta	MPQMAPLYW
Mactromeris polynyma	MVMFSPIFV
Mercenaria mercenaria	MPQFAPMFS
Mya arenaria	/
Spisula solidissima	MMMFSPVHA
Tivela mactroides	MPQFAPIYS

1.2.4.2.3. Non-coding regions (NCRs) and tandem repeat regions

Total size of NCRs ranged from 935 (*M. constricta*) to 4567 bp (*M. polynyma*; Table 3). NCRs with a size greater than 250 bp were qualified as major non-coding regions (MNR).

Repeated regions were found in different locations on the mitogenomes (Annex VIII). *A. islandica* mtDNA possessed repeat regions in both MNR and ND4L genes. *C. fluminea* and *M. constricta* mtDNA had repeat regions in MNR. *M. polynyma* mtDNA had repeat regions in one MNR and in five tRNA-Met genes. *M. arenaria* mtDNA possessed one repeat region in the COX2 gene. *S. solidissima* mtDNA had repeat regions in COX1 and in two MNRs. *T. mactroides* mtDNA had one repeat region in the 16S-rRNA gene. *A. brasiliana* and *M. mercenaria* mtDNA did not possess repeat regions. Repeat regions were unique to each species.

1.2.4.2.4. Base composition and codon usage

The nine newly sequenced mitochondrial genomes were poor in GC (Annex IX). *M. polynyma, S. solidissima* and *M. constricta* had the highest composition in GC (around

40%) whereas *A. islandica, C. fluminea* and *M. mercenaria* owned the lowest composition in GC (around 30%). The nine species had a nucleotide composition in A in favor of T (-0.286 – -0.131) and in C in favor of G (0.249 – 0.410; Annex IX).

Four species had their highest composition in GC for COX1 (*M. mercenaria, A. islandica*, *C. fluminea* and *M. constricta*) (Annex IX). Other species possessed their highest composition in GC for different PCGs: COX3 (*A. brasiliana*), ND1 (*M. polynyma*), ND4 (*M. arenaria*), COX2 (*S. solidissima*) and CYTB (*T. mactroides*). The majority of the species (except *M. polynyma*) had their lowest composition in GC for the same PCG, i.e. ND4L. MNRs were more AT rich compared to PCGs or to the entire mitogenome for the nine species (Annex IX).

The most frequent codons were UUU (Phenylalanine) and UUA (Leucine) and the least used codon was CGC (Arginine) for the majority of the nine species studied (Annex X, Tables S1-S9).

1.2.4.3. Gene order comparison and DNA-based phylogenies of Heterodonta

28 mitogenomes of Heterodonta from GenBank were added to previous nine mitogenomes to resolve the relationships among Heterodonta families.

1.2.4.3.1. Gene arrangement in Heterodonta

A summary of genome annotations for each family or superfamily is presented in Figure 1. In general, Tellinoidea and Lucinidae possessed the most conserved annotation whereas Hiatellidae and Cardiidae had the least preserved annotation. tRNA genes were the most relocated elements. Some gene associations (without considering tRNA genes) were present in several species: CYTB-16S; 12S-COX3; ATP8-ND4 and 16S-ATP6. CYTB-16S was present in Veneroida (except Tellinoidea), Solenidae and *Panopea* spp.. 12S-COX3 was present in *Acanthocardia tuberculata*, Corbiculidae, Arcticidae, Mactridae, *Meretrix* spp., *Tivela mactroides*, Solenoidea and *Panopea* spp.. ATP8-ND4 was present in Veneridae, Mactridae, Cardiidae, Corbiculidae, Arcticidae, Pharidae and *Panopea* spp.. 16S-ATP6 was present in *Paphia* spp., *Ruditapes philippinarum*, Arcticidae, *Panopea* spp.,

Solenidae, Tellinoidea and *Mya arenaria*. Considering tRNA annotation, association of Histidine (H), Glutamate (E) and Serine (S) was conserved for Veneridae, Corbiculidae, Arcticidae, Mactridae, Tellinoidea and *Panopea* spp. and was often found following the association of ATP8-ND4. A translocation of ATP8-ND4-HES was present between 16S and ATP6 in *Meretrix* spp., *Tivela mactroides*, Mactridae, and Corbiculidae.

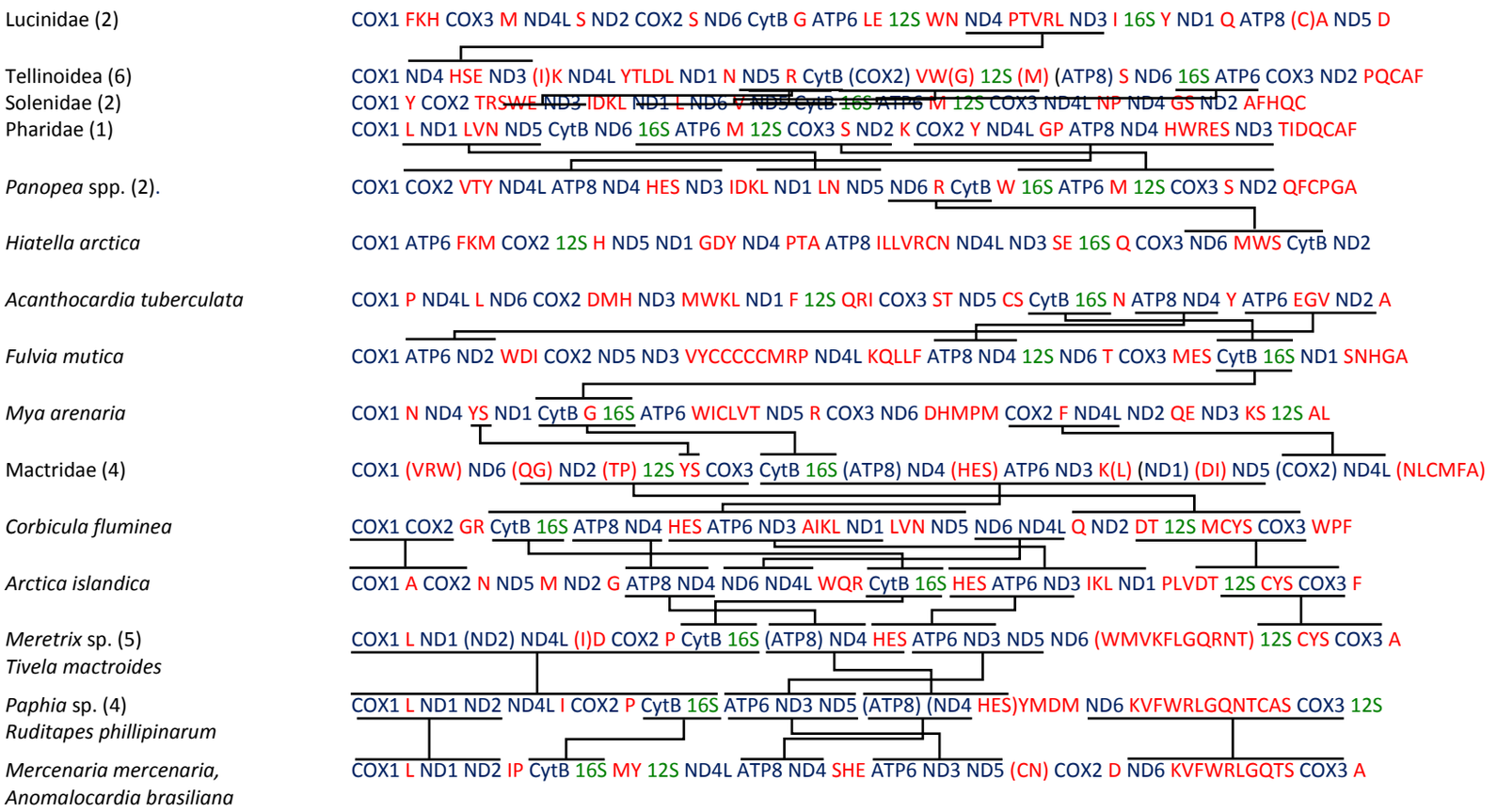


Figure 1. Gene order comparison of Heterodonta mitochondrial genomes. As the standard convention for metazoan mt genomes, COX1 has been designated the start point for all genomes. All genes are transcribed from left-to-right. Different types of genes are represented in different colors: PCGs in blue, rRNA in green and tRNA in red. tRNA are characterized by one letter. The bars show identical gene blocks. Number in parentheses indicates the species number in each family or superfamily. The non-coding regions are not presented and gene segments are not drawn to scale.

1.2.4.3.2. Comparison of Bayesian and ML phylogenies

Only one difference was present between phylogenies (Figures 2,3), the position of *Hiatella arctica*. In ML phylogeny, *H. arctica* was closest to Cardioidae species whereas in Bayesian phylogeny, it was close to Solenoidea. Bootstrap values were lower for the ML (27 to 100) than for the Bayesian (63 to 100) phylogeny.

Our nine sequenced species had the same position in both phylogenies (Figures 2,3). *A. brasiliana* was the closest relative to *M. mercenaria* with similar gene arrangements (Figure 1) and showed 11.9% of difference in amino acid composition (results not shown). *T. mactroides* was genetically close to *Meretrix* spp. (similar gene arrangement and 16% of amino acid difference). *C. fluminea* was clustered with *A. islandica* (similar gene arrangement and 20.6% of amino acid difference). *M. polynyma* and *S. solidissima* were part of the Mactridae cluster and were closest neighbours (similar gene arrangement and 24.9% of amino acid difference). *M. arenaria* was located in basal position of Veneridae, Arcticidae, Corbiculidae and Mactridae. *M. constricta* was part of Tellinoidea. Tellinoidea gene annotation was highly conserved so it was difficult to attribute the closest neighbour to *M. constricta*. *M. constricta* was closest to *Moerella iridescens* with 9% of amino acid difference.

Major superfamilies such as Tellinoidea, Mactroidea, Veneridae, Cardioidae, Solenoidea and Lucinidae were monophyletic in both phylogenies.

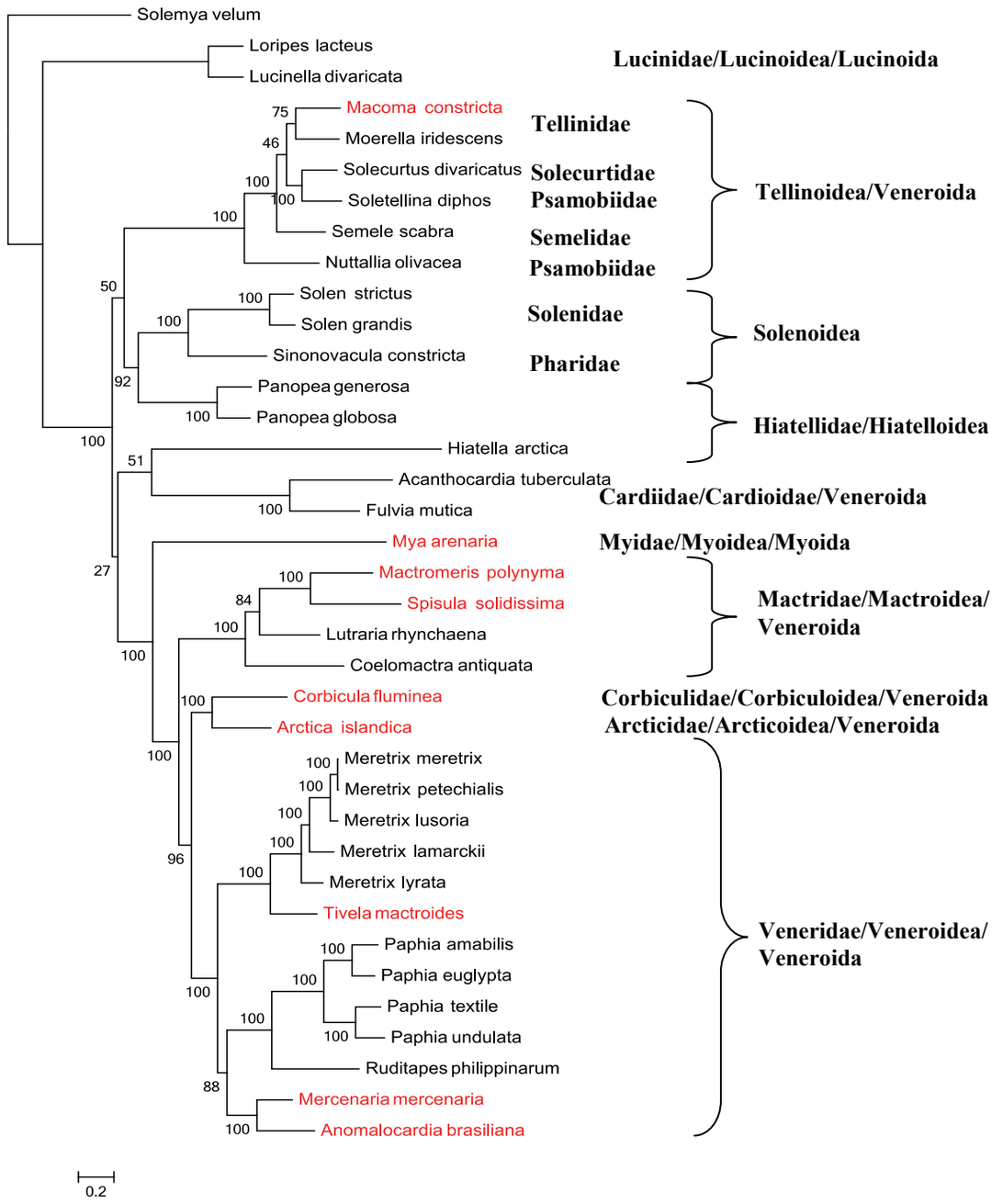


Figure 2. Maximum likelihood phylogenetic tree of heterodont bivalves based on the concatenated nucleotide sequences of 12 protein-coding genes (except ATP8 gene). Node numbers correspond to Maximum likelihood bootstrap proportions. Heterodonta taxonomy is presented as follows, family/superfamily/order. Species in red correspond to the nine newly sequenced species.

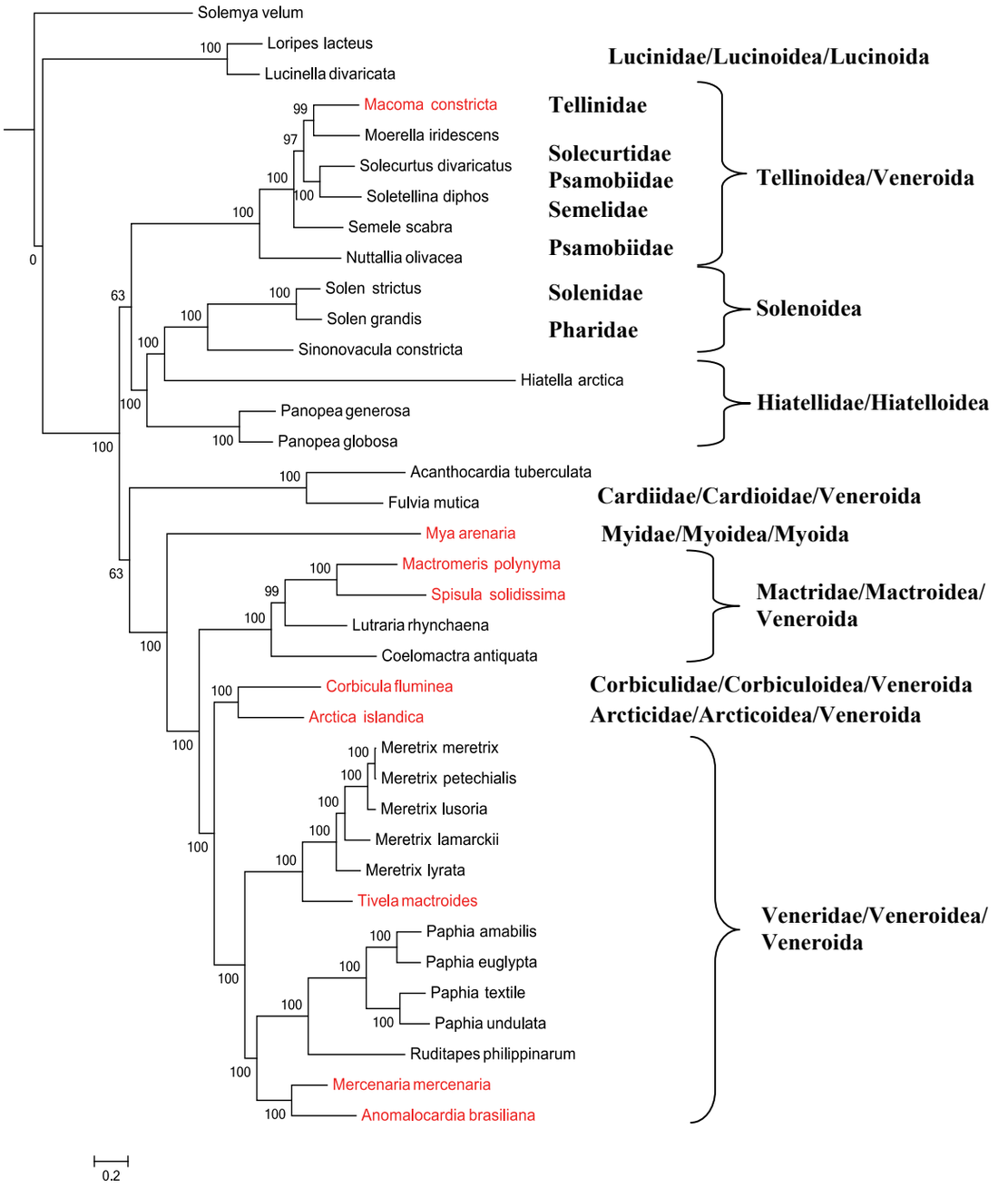


Figure 3. Bayesian phylogenetic tree of heterodont bivalves based on the concatenated nucleotide sequences of 12 protein-coding genes (except ATP8 gene). Node numbers correspond to Bayesian posterior probabilities. The tree was rooted using *Solemya velum*. Heterodonta taxonomy is presented as follows, family/superfamily/order. Species in red correspond to the nine newly sequenced species.

1.2.4.4. Intraspecific genetic relationships in *Arctica islandica*

1.2.4.4.1. Five mitogenomes of *Arctica islandica*: genome sizes and annotation comparison

Two mitogenomes of *A. islandica* from Gulf of St Lawrence (Îles-de-la-Madeleine, Qc, Canada) were sequenced. These two mitochondrial genomes were obtained in different ways: (1) with Illumina MiSeq sequencing and (2) with Sanger sequencing. The final size of these whole mitogenomes was 18304 bp with Illumina MiSeq method and 18244 bp with Sanger method (Tables 5, 6). The principal observed difference in the annotation was the length of ND4 gene (1119 bp for Sanger and 1359 bp for Illumina MiSeq) and ND6 gene (504 bp for Sanger and 471 bp for Illumina MiSeq). The non-coding region was greater for Sanger method (3074 bp) than for Illumina MiSeq method (2867 bp).

Three other mitogenomes of *A. islandica* were taken from GenBank. They measured 18270 bp for Iceland (but it was not complete), 18289 bp for Baltic Sea and 18267 bp for North Sea (Glöckner et al. 2013).

Some differences appeared among annotations (this study and Glöckner et al. 2013). The ATP8 gene was found in the present study (starts by MPQFSP and measures 114 bp (Tables 4, 5, 6)), while it was missing in Glöckner et al. (2013). Because of this annotation, the ND4 gene for Canadian specimens starts 58 bp after the one of Glöckner et al. (2013) specimens. The ND4 gene obtained with Sanger method was shortest and appeared truncated. ND6 gene size in Glöckner et al. (2013) was closer to what we obtained from Sanger method. CYTB gene annotations for Canadian specimens start 40 bp before Glöckner et al. (2013) specimens. In the both cases, CYTB gene was divided in two reading frames. Two tRNA-Glu were found by Glöckner et al. (2013) whereas in our study, only one tRNA-Glu was found. Only one tRNA-Ser was found by Glöckner et al. (2013) whereas here two tRNA-Ser were revealed. The second tRNA-Ser was placed before COX3 gene. COX3 gene annotation for Canadian specimens was therefore shorter, compare to Glöckner et al. (2013) specimens. Some other differences in size between both

Table 5. Mitogenome annotations of *Arctica islandica*, sequenced with Illumina Miseq

Arctica islandica Illumina (18304 bp)						
Gene	Location	Size	Start codon	Stop codon	Anti-codon	IR
COX1	1-1671	1671	ATG	TAA		42
tRNA-Ala	1714-1777	64			TGC	0
COX2	1778-2800	1023	ATG	TAA		50
tRNA-Asn	2851-2914	64			GTT	-14
ND5	2901-4625	1725	ATA	TAG		40
tRNA-Met	4666-4731	66			CAT	2
ND2	4734-5756	1023	ATG	TAA		12
tRNA-Gly	5769-5832	64			TCC	1244
*ATP8	7077-7190	114	ATG	TAA		7
*ND4	7198-8556	1359	TTG	TAA		16
*ND6	8573-9043	471	ATG	TAA		5
ND4L	9049-9333	285	ATA	TAG		58
tRNA-Trp	9392-9456	65			TCA	8
*tRNA-Gln	9465-9531	67			TTG	11
*tRNA-Arg	9543-9605	63			TCG	0
*Cytb	9606-9874-9876-10764	1158	ATG	TAA		1/0
*16S	10765-12053	1289				0
*tRNA-His	12054-12115	62			GTG	-2
tRNA-Glu	12114-12179	66			TTC	-5
tRNA-Ser	12175-12239	63			CGA	-1
ATP6	12239-12982	744	ATG	TAA		29
ND3	13012-13422	411	ATG	TAG		3
tRNA-Ile	13426-13495	70			GAT	-4
tRNA-Lys	13492-13559	68			TTT	0
*tRNA-Leu	13560-13623	64			TAA	1
ND1	13625-14536	912	ATG	TAA		15
*tRNA-Pro	14552-14613	62			TGG	1095
tRNA-Leu	15709-15771	63			TAG	2
tRNA-Val	15774-15838	65			TAC	49
tRNA-Asp	15888-15950	63			GTC	22
tRNA-Thr	15973-16035	63			TGT	0
*12S	16036-16985	950				0
tRNA-Cys	16986-17048	63			GCA	-1
tRNA-Tyr	17048-17111	64			GTA	94
*tRNA-Ser	17206-17271	66			TCT	1
*COX3	17273-18118	846	TTG	TAA		26
tRNA-Phe	18145-18210	66			GAA	34
NCR						2867

NOTE. IR, intergenic region. Yellow highlight represents differences between two Canadian mitogenomes. Asterisks (*) represent differences encounter with Glöckner et al. (2013) annotation.

Table 6. Mitogenome annotations of *Arctica islandica*, sequenced with Sanger

Arctica islandica Sanger (18244 bp)						
Gene	Location	Size	Start codon	Stop codon	Anti-codon	IR
COX1	1-1671	1671	ATG	TAA		42
tRNA-Ala	1714-1777	64			TGC	0
COX2	1778-2800	1023	ATG	TAA		50
tRNA-Asn	2851-2914	64			GTT	-14
ND5	2901-4625	1725	ATA	TAG		40
tRNA-Met	4666-4731	66			CAT	2
ND2	4734-5756	1023	ATG	TAA		12
tRNA-Gly	5769-5832	64			TCC	1198
*ATP8	7031-7144	114	ATG	TAA		7
*ND4	7152-8270	1119	TTG	TAG		225
*ND6	8496-8999	504	ATT	TAA		5
ND4L	9005-9289	285	ATA	TAG		58
tRNA-Trp	9348-9412	65			TCA	8
*tRNA-Gln	9421-9487	67			TTG	11
*tRNA-Arg	9499-9561	63			TCG	0
*Cytb	9562-9830-9832-10720	1158	ATG	TAA		1/0
*16S	10721-12009	1289				0
*tRNA-His	12010-12071	62			GTG	-2
tRNA-Glu	12070-12135	66			TTC	-4
tRNA-Ser	12132-12194	63			CGA	0
ATP6	12195-12938	744	ATG	TAA		29
ND3	12968-13378	411	ATG	TAG		3
tRNA-Ile	13382-13451	70			GAT	-4
tRNA-Lys	13448-13515	68			TTT	0
*tRNA-Leu	13516-13579	64			TAA	1
ND1	13581-14492	912	ATG	TAA		15
*tRNA-Pro	14508-14569	62			TGG	1079
tRNA-Leu	15649-15711	63			TAG	2
tRNA-Val	15714-15778	65			TAC	49
tRNA-Asp	15828-15890	63			GTC	22
tRNA-Thr	15913-15975	63			TGT	0
*12S	15976-16925	950				0
tRNA-Cys	16926-16988	63			GCA	-1
tRNA-Tyr	16988-17051	64			GTA	94
*tRNA-Ser	17146-17211	66			TCT	1
*COX3	17213-18058	846	TTG	TAA		26
tRNA-Phe	18085-18150	66			GAA	94
NCR						3074

NOTE. IR, intergenic region. Yellow highlight represents differences between two Canadian mitogenomes. Asterisks (*) represent differences encounter with Glöckner et al. (2013) annotation.

studies were observed for: tRNA-Gln, tRNA-Arg, 16S-rRNA, tRNA-His, tRNA-Leu (UAA), tRNA-Pro and 12S-rRNA.

1.2.4.4.2. Five mitogenomes of *Arctica islandica*: genetic distances and phylogenies

We calculated nucleotide and amino acid genetic distances between the five *A. islandica* sequences for each PCG. ND3 and ND5 genes were the most conserved genes between the five mitogenomes (maximum of 0.5 and 0.8% of nucleotides difference respectively; Annex XI, Tables S1-S2). ND4 and ND6 were the least conserved genes (maximum of 2.1 and 1.7% of nucleotides difference respectively). No amino acid differences were found for ATP6, CYTB, ND3, ND4L and ND5 genes (Annex XI, Tables S3-S4). ND4 gene was the least conserved gene (maximum in terms of amino acid divergence of 3.5%).

Two *A. islandica* genetic trees were performed: (1) Maximum likelihood (Figure 4) and (2) Neighbor joining phylogeny (result not shown). Two clusters were found: one containing Baltic Sea and Gulf of St Lawrence (Îles de la Madeleine) individuals and the other comprising North Sea and North Atlantic (Iceland) individuals.

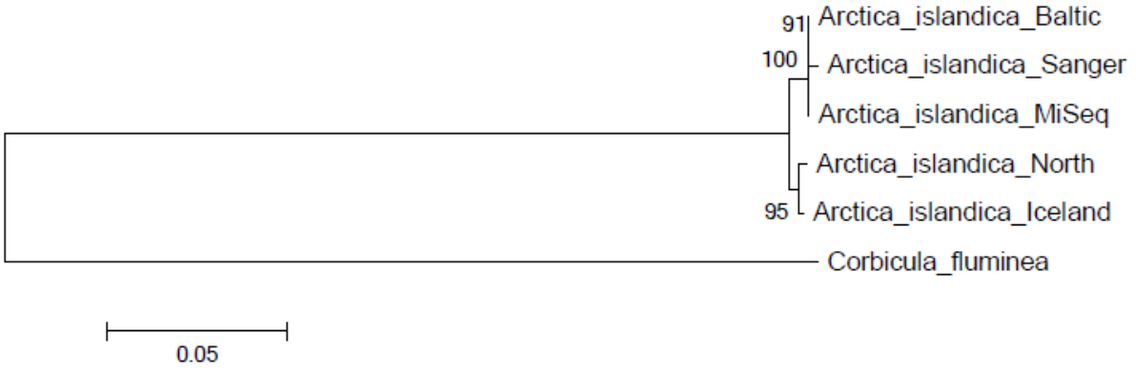


Figure 4. Phylogenetic trees of different populations of *Arctica islandica* based on maximum likelihood method. Reconstruction was realized on nucleotides sequences concatenation of 12 PCGs (ATP8 gene was excluded). Nodes numbers correspond to Maximum likelihood bootstrap proportions. *Arctica islandica* Sanger and MiSeq represent Gulf of St Lawrence (Îles de la Madeleine) population.

1.2.5. Discussion

1.2.5.1. Mitogenome structure

Mitogenome sizes varied between 14 and 23 kb, which is a common range in animals. Mitogenome size is dependent on length of NCR and also on genes duplication events (Gissi et al. 2008; Xu et al. 2010; Xu et al. 2012). *M. polynyma* mtDNA had the largest size (23210 bp) due to its NCR size (4567 bp) and its COX2 and tRNA-Met genes duplications, whereas *M. constricta* had the smallest one (16308 bp) with a NCR of 935 bp.

The nine mitogenomes had low GC content (30-40%), which is a characteristic feature of bivalves (Xu et al. 2010; Xu et al. 2012). The most frequent codon is UUU (Phenylalanine) whereas the least used is CGC (Arginine), two characteristics previously reported in bivalves (Doucet-Beaupré et al. 2010; Wang et al. 2010; He et al. 2011). The codon UUA (Leucine) is also quite frequent in our study. Similar to previous studies, the COX1 gene is the most conserved PCGs in bivalve mtDNAs (Yu et al. 2008; Wu et al. 2009; Xu et al. 2010). In our study, the ND3 and ND6 genes appear to be the least conserved genes.

Thirteen PCGs, 2 rRNAs and 22 tRNAs are usually present in bivalves (Boore 1999; Blier et al. 2001; Gissi et al. 2008). Some exceptions were however present: no 16S-rRNA and tRNA-Cys genes were found in the partial genome of *A. brasiliana*, two reading frames were found for the CYTB gene in *A. islandica*, two COX2 genes and five tRNA-Met genes were found in *M. polynyma*, and no ATP8 gene and two tRNA-Met genes were found in *M. arenaria*. Only one partial mitogenome did not present all mitochondrial genes known. No 16S-rRNA and tRNA-Cys were found in *A. brasiliana* partial mitogenome. A big portion of this mitogenome is missing and probably contains these two genes, which are usually present in Metazoan (Boore 1999; Blier et al. 2001). Our observation of two reading frames of the CYTB of *A. islandica* corroborate previous results from Glöckner et al. (2013) for the same species. It was also documented in other bivalve such as *Crassostrea virginica* (Milbury and Gaffney 2005). Loripes, Meretrix and Ruditapes have also two COX2 genes

(Xu et al. 2010; Stöger and Schrödl 2013) such as *M. polynyma*. tRNA genes are the most variant (duplication or losses) element in mtDNA (Gissi et al. 2008). We found five tRNA-Met genes in the mitogenome of *M. polynyma* and two in *M. arenaria*. Many bivalves have several tRNA-Met genes in their mitogenomes (e.g. Xu et al. 2012). For example, *Paphia* spp., *Ruditapes philippinarum*, *Acanthocardia tuberculata* and *Hiatella arctica* possess two tRNA-Met genes. The duplication of tRNA-Met may have been lost early in metazoan diversification and have been re-acquired independently and recently in different bivalve lineages (Gissi et al. 2008; Xu et al. 2012; Wu et al. 2014). Several tRNA's secondary structures deviate from the usual cloverleaf pattern. tRNA loss of D-arm and T-arm is frequent and are also present in other bivalves (Doucet-Beaupré et al. 2010; Saunier et al. 2014; Wu et al. 2014). ATP8 gene is the smallest and the fastest evolving mitochondrial PCG (Gissi et al. 2008; Stöger and Schrödl 2013). We found ATP8 gene for eight out of our nine species. Several authors documented the absence of ATP8 gene in bivalves (Wang et al. 2010; He et al. 2011), whereas Breton et al. (2010) and Stöger and Schrödl (2013) re-annotated mitogenomes of Heterodonta and found this gene in all Heterodonta. ATP8 is characterized by high amino acid variability, heterogeneity in size and a lack of conservation of MPQL unit at the N-terminus (Breton et al. 2010). These characteristics can explain why ATP8 gene was not detected in numerous studies. Another *M. arenaria* mitogenome has recently been sequenced by Wilson et al. (2015). The same genes composition was present (with the absence of ATP8 gene and the presence of two tRNA-Met genes).

1.2.5.2. Control region

Mitochondrial genome of most metazoans usually contains a single MNR where signals for initiating replication and transcription are present. This region is commonly called control region (Boore 1999; Groenenberg et al. 2012; Yuan et al. 2012a; Yuan et al. 2012b and references therein). Repeat regions and an extreme AT bias in MNR are typical of the control region (Gissi et al. 2008; Timmermans et al. 2010; Yuan et al. 2012a). The mtDNA of the nine Heterodonta have high content of MNRs. Stöger and Schrödl (2013) suggested

that several multiple MNR correspond to several control regions. However some MNRs do not possess repeat regions. For example, *S. solidissima* mtDNA has three MNRs with proportions of 71.0, 65.4 and 58.0% in AT respectively but only the last two possess repeat regions. The third one has a weaker composition in AT and control region is generally characterized by an extreme AT bias. It is therefore possible that the first and the last MNR do not possess any control function. A contrario, *A. islandica* mtDNA possesses two MNRs containing repeat regions with 72.3 and 72.8% in AT respectively, and in this case it is conceivable that this specie's mtDNA possess two control regions. In the case of *C. fluminea* and *M. constricta*, their mtDNAs revealed a single MNR including repeat regions with 74.9 and 66.3% in AT respectively. This high AT bias and repeat regions in MNR for these both species is typical of a control region. Other species (*A. brasiliana, M. polynyma, M. mercenaria, M. arenaria* and *T. mactroides*) do not possess repeat regions or have a weak AT composition in their MNRs, so it is difficult to conclude on the position of their control regions. These regions are usually annotated by lack of coding structure rather than direct evidence for replicative or transcriptional involvement (Lunt et al. 1998). The proper control region position in the nine studied species could not be established with certainty; further analyses are required.

Repeat regions were also present in coding regions of five species (*A. islandica, M. polynyma, M. arenaria, S. solidissima* and *T. mactroides*). For *M. polynyma*, repeat regions are associated to five tRNA-Met. Dreyer and Steiner (2006) found repeat regions in ND6 genes of *Hiatella arctica* and we found repeat regions in ND4 of *Soletellina diphos*, in CYTB of *Meretrix lamarckii*, in ND6 of *Solen grandis*, in COX3 of *Solecurtus divaricatus*, in ND3 of *Nuttallia olivacea* and in 12S-rRNA of *Lucinella divaricata* (results not shown). Repeat regions in coding regions are then present in some Heterodonta but this phenomenon is uncommon.

1.2.5.3. Heterodonta gene arrangement and phylogenies

1.2.5.3.1. Gene arrangement

Important rearrangement in genome organization, characteristic of mollusks and bivalves in particular, has repeadly been reported (Doucet-Beaupré et al. 2010; Wang et al. 2010; He et al. 2011; Wu et al. 2014). tRNA genes are the most frequently relocated element in animal mitogenomes (Gissi et al. 2008). Their secondary structure facilitates translocations (Cantatore et al. 1987). Several gene associations were noticed: CYTB-16S; 12S-COX3; ATP8-ND4 and 16S-ATP6. CYTB-16S is also present in Unionoid (Bivalvia, Palaeoheterodonta) mitogenomes (Serb and Lydeard 2003; Doucet-Beaupré et al. 2010). Serb and Lydeard (2003) postulated that this CYTB-16S association is a bivalve ancestral state. Other gene associations 12S-COX3; ATP8-ND4 and 16S-ATP6 were not found in other bivalves species (except for 12S-COX3 which was present in *Solemya velum*; (Plazzi et al. 2013)). These three gene associations seem to be common and specific characters of Heterodonta.

Here, in spite of high rearrangement, the association of three tRNA (Histidine (H), Glutamate (E) and Serine (S)) was extremely conserved in Veneroida, Tellinoidea and *Panopea* spp.. This tRNA association was not present in other bivalves species and consequently, it appears to be a characteristic of Heterodonta. This tRNA association was often found following the association of ATP8-ND4 in mtDNA of Heterodonta.

1.2.5.3.2. Phylogenies and gene rearrangements

Phylogeny using mitochondrial PCGs sequences with a high number of species of Heterodonta has been previously published (Meng et al. 2012; Yuan et al. 2012b; Glöckner et al. 2013). Furthermore phylogeny based on mitochondrial gene rearrangement has also been published (Serb and Lydeard 2003). In the present study, we used both approaches to detail Heterodonta phylogeny.

Species belonging to family Veneridae were grouped in the same clade in our trees, supporting the monophyly of Veneridae (Taylor et al. 2007; Yuan et al. 2012b). They were

separated in two clusters with one containing Anomalocardia, Mercenaria, Paphia, and Ruditapes (with Anomalocardia closest relative to Mercenaria) and the other including Meretrix and Tivela. This disposition confirmed the phylogeny published by Mikkelsen et al. (2006), based on short fragments of mtDNA.

Mactridae, Corbiculidae and Arcticidae were positioned in basal position of Veneridae. Meng et al. (2012) found the same relationship between Mactridae and Veneridae. However, their analysis did not consider Arcticidae and Corbiculidae. Phylogenies published by Giribet and Wheeler (2002) and Taylor et al. (2007), based on short fragments of nuclear and mtDNA, placed Arcticidae as a sister group to Veneridae whereas Corbiculidae was placed at basal position of these two groups and Mactridae was positioned in basal position of these three group. The relationship between Arcticidae and Veneridae was not verified in our paper. Arcticidae was a sister group of Corbiculidae in our two phylogenies. To our knowledge, this relationship between *Arctica islandica* and *Corbicula fluminea* is established for the first time, and is robust [with a bootstrap value of 100 in our two phylogenies]. In all cases, Arcticidae and Corbiculidae are close to Mactridae (Giribet and Wheeler 2002; Taylor et al. 2007). Species belonging to family Mactridae were clustered together, supporting the monophyly of Mactridae.

Mya arenaria is usually found in basal position of Veneroidae/Mactridae/Corbiculidae and Arcticidae (Giribet and Wheeler 2002; Taylor et al. 2007). Present phylogenies confirm this position even when considering important gene rearrangement in this species.

Two species belonging to family of Cardiidae, *Acanthocardia tuberculata* and *Fulvia mutica*, have an extreme gene rearrangement (Imanishi et al. 2013). Only three gene associations (when excluding tRNA) are shared by these two species: CYTB-16S, ATP8-ND4 and ATP6-ND2. Taylor et al. (2007) and Glöckner et al. (2013) postulated that Cardiidae are a sister group to Tellinoidea. This relationship was however not confirmed by our two phylogenies as well as by Plazzi et al. (2011) and Yuan et al. (2012b).

Panopea spp. were sister group of Solenoidea. This relationship between Hiatellidea and Solenoidea corroborate results from Taylor et al. (2007), Plazzi et al. (2011) and Yuan et al. (2012b). In these studies, *Hiatella arctica* (another species from Hiatellidea family such as *Panopea* spp.) was also considered as sister group of Solenoidea [as seen in Bayesian phylogeny]. In ML phylogeny, this relation was not verified; *H. arctica* was found close to Cardioidae. This relationship between *H. arctica* and Cardioidae has not already been reported by previous study. *H. arctica* mitogenomes arrangement is however completely different from *Panopea* spp. and Solenoidea, suggesting that major rearrangement can occur between closely related taxon.

Species belonging to superfamily Tellinoidea were clustered together, supporting the monophyly of the group (Taylor et al. 2007; Yuan et al. 2012b). This superfamily shows strong conservation of mitogenomes annotation among its six species. However, families of Tellinoidea such as Psammobiidae and Tellinidae are confirmed to be non-monophyletic (Yuan et al. 2012b).

In our two phylogenies, Lucinidae was placed in basal position of the tree as previously reported (Taylor et al. 2007; Plazzi et al. 2011; Meng et al. 2012; Yuan et al. 2012b; Glöckner et al. 2013). Two species of Lucinidae, *Loripes lacteus* and *Lucinella divaricata*, show extremely conserved mitogenomes.

This study consolidates the relationship already known among Heterodonta species. Our two phylogenies show a similar pattern. Major superfamilies such as Veneridae, Mactroidea, Cardioidae, Solenoidea, Tellinoidea and Lucinidae are confirmed as monophyletic as in previous studies (Taylor et al. 2007; Yuan et al. 2012b). The relationship between Arcticidae and Corbiculidae was however revealed.

1.2.5.4. Intraspecific genetic relationships in *Arctica islandica*

The two sequencing methods (Sanger and Illumina MiSeq) used to obtain the entire mitochondrial genome of *Arctica islandica* showed some differences in result (mitogenome size, gene boundaries). The Illumina MiSeq method seems to be the best sequencing

method to obtain an entire mitochondrial genome (quicker and mitogenome closer to previous mitogenome).

Some annotation differences in *A. islandica* mitogenomes were found between the present study and Glöckner et al. (2013). The major differences were the presence of ATP8 gene, 1 or 2 tRNA-Glu and 1 or 2 tRNA-Ser. An annotation of a mitochondrial genome could differ among authors caused by various technic of annotation.

Gulf of St Lawrence (Îles de la Madeleine) population grouped with the Baltic Sea population whereas the North Sea population was genetically closer to the North Atlantic (Iceland) population. These relationships were not supported in literature (Dahlgren et al. 2000; Holmes et al. 2003; Glöckner et al. 2013). Dahlgren et al. (2000) and Glöckner et al. (2013) used CYTB gene or single nucleotide polymorphism from a large part of mtDNA and different populations. Dahlgren et al. (2000) found that Iceland population was genetically closer to western North Atlantic population [USA and Nova Scotia (Canada)] than eastern North Atlantic population (North Sea). Glöckner et al. (2013) studied several individuals and found that Baltic Sea population were separated in two haplotypes with one shared with North Sea population and the other with Iceland population whereas North Sea and North Atlantic (Iceland) populations did not share any haplotypes. Another study performed a RAPD-PCR on four North Sea populations and one Nova Scotia (Canada) population (Holmes et al. 2003). Holmes et al. (2003) showed that these five populations were genetically distinct from each other.

Baltic Sea and Gulf of St Lawrence (Îles de la Madeleine) populations are the most geographically distant in our study but they are genetically the closest. Further studies are required to understand the genetic diversity and repartition of this species.

1.2.6. Conclusion

Our phylogenies allowed consolidating the extant relationship knowledge of heterodont subclass. Using complete mitochondrial genome is a good advantage, allowed to have a

greater DNA references and ensured proper evolutionary inferences. The nine newly described mitogenomes allowed to have a bigger database for phylogenies and to increase our knowledge on gene arrangement in heterodonts. For the first time, several ancestral character states, 12S-COX3, 16S-ATP6 and ATP8-ND4-HES, were established in Heterodonta and *Arctica islandica* and *Corbicula fluminea* were clearly recognized such as "sister group". Several Heterodonta families were not represented in our phylogenies. Further sequencing effort of mitogenomes is necessary to complete Heterodonta phylogenies and to verify actual Heterodonta taxonomy.

As mentioned earlier, *Arctica islandica* is an important species, which is known to be the longest living complex animal on earth. Unfolding the evolution of its populations is not an easy task and our small database could not resolve the extant relationships between its populations. Further investigation on *A. islandica* population genetics is essential to clarify the genetic repartition of this species.

CHAPITRE 2.
RECONSTRUCTION DE L'ÉVOLUTION DES TAUX DE SUBSTITUTION ET DES TRAITS D'HISTOIRE DE VIE DES GÉNOMES MITOCHONDRIAUX DE BIVALVES EN UTILISANT UN MODÈLE DE COVARIANCE PHYLOGÉNÉTIQUE

Aurore Levivier, Nicolas Lartillot, France Dufresne, Pierre Blier

2.1. RÉSUMÉ EN FRANÇAIS DU DEUXIÈME ARTICLE

Les bivalves représentent un modèle intéressant pour l'étude du vieillissement en raison de leur grande variation de longévité (de 1-2 ans à plus de 500 ans). Aucune étude n'a à ce jour examiné les relations entre des traits d'histoire de vie et l'évolution de l'ADN mitochondrial (ADNmt) chez ce groupe. Nous avons utilisé un modèle d'évolution de covariance phylogénétique Bayésien sur 12 gènes mitochondriaux codant pour des protéines chez 76 espèces de bivalves. Trois traits d'histoire de vie (longévité, temps de génération et température maximale létale) ont été corrélés avec (1) le taux de substitution synonyme (dS), (2) le ratio du taux de substitution non-synonyme sur synonyme (dN/dS), (3) le taux de remplacement conservateur en acide aminé (Ks), et (4) le ratio du taux de remplacement en acide aminé radical sur conservateur (Kr/Kc). La longévité était négativement corrélée à la température maximale létale et au dS mais positivement corrélée au temps de génération. Aucune corrélation n'a été trouvée entre les trois traits d'histoire de vie, le Kc et les ratios dN/dS et Kr/Kc. La longévité apparaît influencer (9-30%) l'évolution de l'ADNmt des bivalves, comme cela a été précédemment observé chez les oiseaux, les mammifères et les plantes. Nous suggérons que la corrélation négative entre la longévité et le dS est universelle pour les endothermes et les ectothermes (vertébrés et invertébrés).

Cette étude représente une première étape dans la compréhension des relations entre les traits d'histoire de vie et l'évolution de l'ADNmt chez les invertébrés.

Ce deuxième article, intitulé *Reconstruction of substitution rates, evolution and life-history traits in mitochondrial genomes of bivalves using a phylogenetic covariance model*, fut corédigé par moi-même ainsi que par les professeurs Nicolas Lartillot, France Dufresne et Pierre Blier. Il sera prochainement soumis à la revue *Molecular Biology and Evolution* pour publication. En tant que premier auteur, j'ai réalisé la recherche documentaire sur l'état de l'art, l'acquisition et le traitement des données et la rédaction de l'article. Le professeur Nicolas Lartillot, deuxième auteur, m'a appris à me servir du langage BioPerl et des modèles nécessaires à cette étude, m'a donné un espace virtuel de travail, et il m'a aidé dans l'interprétation des résultats. Le professeur Pierre Blier, quatrième auteur, a fourni l'idée originale. Le professeur France Dufresne, troisième auteur, ainsi que le professeur Pierre Blier, ont aidé à la recherche sur l'état de l'art et à la révision de l'article. Une version abrégée de cet article a été présentée sous forme d'une affiche scientifique à la première conférence conjointe des sociétés canadiennes d'écologie et d'évolution, de zoologie et de limnologie, *Genomes to/aux Biomes*, à Montréal (Québec, Canada) au printemps 2014. Les résultats de cet article ont également été présentés sous forme d'une affiche scientifique au congrès annuel de la société de biologie moléculaire et d'évolution à Vienne (Autriche) à l'été 2015.

Mots-clés: évolution des traits d'histoire de vie, longévité, génome mitochondrial, taux de substitution, méthode de Monte Carlo par chaîne de Markov, statistique bayésienne, bivalve.

2.2. RECONSTRUCTION OF SUBSTITUTION RATES, EVOLUTION AND LIFE-HISTORY TRAITS IN MITOCHONDRIAL GENOMES OF BIVALVES USING A PHYLOGENETIC COVARIANCE MODEL

2.2.1. Abstract

Bivalves represent an interesting animal model for aging studies given their wide variation in longevity (from 1-2 to >500 years). Surprisingly, no study has examined the existence of relationship between life-history traits and mitochondrial DNA (mtDNA) evolution pattern. We performed here an analysis with a Bayesian phylogenetic covariance model of evolution using 12 mitochondrial protein coding-genes for 76 species. Three life-history traits (longevity, generation time, and maximum lethal temperature) were tested against (1) synonymous substitution rates (dS), (2) ratios of nonsynonymous over synonymous substitution rates (dN/dS), (3) conservative amino acid replacement rates (Kc) and (4) ratios of radical over conservative amino acid replacement rates (Kr/Kc). Longevity was negatively correlated with maximum lethal temperature and dS but positively correlated with generation time. No correlation was found between any of the three life-history traits, Kc and the dN/dS or Kr/Kc ratios. Longevity appears to influence (9-30%) the evolution of mtDNA of bivalves, as previously found in birds, mammals and plants. This study supports the suspition that negative correlation between longevity and dS could be universal for endotherms and ectotherms (vertebrates and invertebrates). This study represents a first step in the understanding of the relation between life-history traits on mtDNA evolution in invertebrates.

Keywords: life-history evolution, longevity, mitochondrial genome, substitution rates, Markov chain Monte Carlo, Bayesian statistics, bivalve.

2.2.2. Introduction

Even if species evolution is complex, the history of species evolution can partly be reconstructed studying morphological and molecular divergences among species. Molecular approaches are favored because of their reliability. DNA is species-specific and evolves quickly over time owing to mutations such as substitutions, insertions and deletions (Hu et al. 2014). Ecological and environmental factors (Davies et al. 2004) and life-history traits (Bromham et al. 1996) influence DNA mutations making each individual species unique.

Longevity is an important trait in evolutionary studies (Nabholz et al. 2008; Galtier et al. 2009a; Lartillot and Poujol 2011; Lartillot and Delsuc 2012; Lartillot 2013). Longevity is negatively correlated with nuclear DNA and mitochondrial DNA (mtDNA) mutation rates in mammals and birds. This relationship is supported by natural selection and mitochondrial theory of aging (Harman 1956; Nabholz et al. 2008; Galtier et al. 2009b). Harman (1956) introduced the mitochondrial theory of aging, which states that reactive oxygen species (ROS) generated throughout the life span of an organism account for progressive degradation of cells by oxidation of proteins, DNA and other constituents of cells and cause senescence. The mitochondrial oxidative stress theory postulates that oxidative damage caused by ROS should be lower for long-lived species than for short-lived ones (Hulbert et al. 2007; Pamplona and Barja 2011; Munro and Blier 2012; Munro et al. 2013) since progressive accumulation of DNA mutation caused by ROS is involved in aging mechanism (Harman 1956; Barja 2004). To date, no one has examined if this relationship also holds for invertebrates ectotherms.

Bivalves are a good model to study longevity because (1) they have an exceptional range of life span (Guo 2009; Butler et al. 2012); (2) measuring their age is easy (Richardson 2001) and, (3) they have a worldwide distribution from the poles to the tropics (e.g. Philipp et al. 2005b; Silva-Cavalcanti and Costa 2011). Recently, a 507 year old bivalve *Arctica islandica* has been found in Iceland making it the longest-lived non-colonial animal (Butler

et al. 2012). Few other bivalves can live more than 150 years: *Margaritifera margaritifera* [210 years; (Ziuganov et al. 2000)] and *Panopea abrupta* [163 years; (Bureau et al. 2002)]. In contrast, some bivalve species live only one or two years such as *Argopecten irradians* and *Musculista senhousia* (Guo 2009; Mistri 2002). Bivalves have then a wider range of lifespan than mammals or birds which are currently used in longevity models [maximum recorded longevity inferior to 250 years, (Tacutu et al. 2013)]. Moreover, age at sexual maturity is positively correlated with their longevity (Abele and Philipp 2013; Ridgway et al. 2011a). For example, *Margaritifera margaritifera* which can live over 210 years reaches sexual maturity at 20 years (Bauer 1987) whereas *Musculista senhousia* that lives only two years reaches sexual maturity before one year old (Mistri 2002). The age of bivalves is measured from their shell (Richardson 2001). Each year a growth ring develops in the inner face of their shell. Counting the number of rings allows determining the age of an individual. Bivalves with longest lifespan live generally in cold waters whereas those with shortest longevity live in the tropics (Cardoso and Veloso 2003; Philipp et al. 2005b). As ectotherms, bivalves are thermo-dependent on their environment. In the current context of global warming, ectotherms are the first living organisms impacted. Several studies showed that an increase of the environmental temperature causes a rise in production of ROS inducing oxidative stress and aging (Samain 2011). Understanding the relation between ROS metabolism, mitochondrial DNA integrity and aging process will help predicting impact of climate changes.

To correlate molecular and phenotypic characters, Lartillot and Poujol (2011) introduced a Bayesian phylogenetic reconstruction and Markov chain Monte Carlo (MCMC) estimation method. This method jointly estimates divergence times, substitution rates, life-history traits and the correlation between them (Lartillot and Poujol 2014). Several substitution parameters are commonly measured: the synonymous substitution rate (dS), the ratio of nonsynonymous over synonymous substitution rates (dN/dS) and the ratio of radical over conservative amino acid replacement rates (Kr/Kc) (Nabholz et al. 2013). This model has already been applied to mammal and bird data (Nabholz et al. 2013; Lartillot and Delsuc 2012; Lartillot and Poujol 2011; Lartillot 2013; Nabholz et al. 2011). The main results

showed dS negatively correlated with longevity and dN/dS and Kr/Kc positively correlated with longevity both in nuclear and mitochondrial DNAs. This model has not yet been applied on bivalves. Considering the importance of mtDNA-encoded peptides in the management of ROS production as well as their thermal-sensitivity (Blier et al. 2014) we could suspect that signature of elongated lifespan or any associated life history trait could be detected in bivalve mitochondrial genomes.

We present here the first study, which attempts to qualify and quantify the relation between longevity or various life-history traits and the evolution of mitochondrial DNA in bivalves. A Bayesian framework and MCMC estimation method was performed on 12 mitochondrial protein coding-genes (PCGs) for 76 bivalves. Several substitution parameters were measured: dS, dN/dS, Kc and Kr/Kc. Three life-history traits were tested: (1) the maximum lifespan recorded considered as a proxy for longevity, (2) the age of female at sexual maturity taken as a proxy for generation time, and (3) the maximum lethal temperature measured in laboratory considered as a proxy for maximum lethal temperature in environment. We hypothesized that longevity would be positively correlated to generation time and dN/dS and negatively correlated to maximum lethal temperature and dS (Nabholz et al. 2008; Lartillot and Poujol 2011).

2.2.3. Results

2.2.3.1. Relationship between the three life-history traits

A Bayesian inference and MCMC algorithm was performed on three life-history traits: longevity, generation time and maximum lethal temperature for 76 bivalve species using the model of Lartillot and Poujol (2011). Considering only life-history traits, longevity was highly positively correlated with generation time (posterior probability, *pp*=1) and strongly negatively correlated with maximum lethal temperature (*pp*=0.01; Table 7). When the analysis was restricted to a single parameter, significance between longevity and generation time (i.e. controlled for maximum lethal temperature) and between longevity and maximum lethal temperature (i.e. controlled for generation time) remained (*pp*=1 and 0.07

respectively, results not shown). The square of the correlation coefficient between two components r^2_{lk} was measured to explain how much of the total variation of l is explained by k and vice versa (Lartillot and Poujol 2011). The longevity in bivalve was explained at 68% by the generation time and at 22% by the maximum lethal temperature.

The relationship between generation time and maximum lethal temperature was more complex. A weak marginal correlation (*pp*=0.05) was found but this correlation was not supported when evolutionary signal was lower [i.e. in partial correlation (*pp*=0.46) and when the longevity is controlled (results not shown, *pp*=0.65); Table 7]. The correlation between generation time and maximum lethal temperature seems therefore to be indirect and caused by strong correlation of both these traits with longevity.

Table 7. Covariance analysis between dS, dN/dS and life-history traits in 76 bivalve species

Marginal correlations[1]	**dN/dS**	**Longevity (years)**	**Maturity (years)**	**Maximum lethal temperature (°C)**
dS	n.a.	-0.30 (0.04)*	0.09 (0.69)	0.31 (0.92)
dN/dS	-	n.a.	n.a.	n.a.
Longevity	-	-	0.80 (1)**	-0.47 (0.01)**
Maturity	-	-	-	-0.33 (0.05)
Partial correlations[2]	**dN/dS**	**Longevity (years)**	**Maturity (years)**	**Maximum lethal temperature (°C)**
dS	n.a.	-0.57 (<0.01)**	0.58 (1)**	0.18 (0.78)
dN/dS	-	n.a.	n.a.	n.a.
Longevity	-	-	0.85 (1)**	-0.19 (0.23)
Maturity	-	-	-	-0.03 (0.46)

[1]Correlation coefficients corresponding to marginal correlations between each pair of variables.
[2]Correlation coefficients corresponding to partial correlations.
*PP>0.95 or <0.05
**PP>0.975 or <0.025

Table 8. Covariance between Kc, Kr/Kc and life-history traits in 76 bivalve species

Marginal correlations[1]	**Kr/Kc**	**Longevity (years)**	**Maturity (years)**	**Maximum lethal temperature (°C)**
Kc	0.05 (0.59)	-0.28 (0.07)	-0.05 (0.42)	0.39 (0.94)
Kr/Kc		0.17 (0.66)	0.28 (0.76)	0.23 (0.73)
Partial correlations[2]	**Kr/Kc**	**Longevity (years)**	**Maturity (years)**	**Maximum lethal temperature (°C)**
Kc	-0.11 (0.39)	-0.28 (0.14)	0.3 (0.86)	0.27 (0.82)
Kr/Kc		0.06 (0.58)	0.30 (0.80)	0.45 (0.88)

[1]Correlation coefficients corresponding to the marginal correlations between each pair of variables.
[2]Correlation coefficients corresponding to the partial correlations.
*PP>0.95 or <0.05
**PP>0.975 or <0.025

2.2.3.2. dS and life-history traits

The synonymous substitution rate was positively correlated with longevity (*pp*=0.04; Table 7). No correlation was found between the synonymous substitution rate and generation time or maximum lethal temperature in marginal correlations. The correlation between the substitution rate and longevity increased in partial correlations (i.e. when the generation time and maximum lethal temperature were controlled; *pp*=<0.01). Therefore in bivalves, 9 to 30% of species longevity differences (marginal and partial correlations result respectively) are associated with synonymous substitution rates in their mitochondrial genomes. The synonymous substitution rate was higher in Heterodonta and Pteriomorphia than in Palaeoheterodonta (Figure 5).

2.2.3.3. dN/dS, Kr/Kc and life-history traits

The ratio of nonsynonymous over synonymous substitution rates (dN/dS) analysis indicated no relationship with the life-history traits studied here, likely due to the high saturation of mitochondrial DNA substitution among species. The ratio of a radical over conservative amino acid replacement rates (Kr/Kc) has thus been used as an alternative to the dN/dS ratio (Nabholz et al. 2013). This ratio is based on amino acids, which are less saturated, in terms of substitution rates, than nucleotides (used for dN and dS). In this case, the model

was informative but no significant correlation was present between Kr/Kc and the three life-history traits (*pp*<0.80; Table 8). Higher values of Kr/Kc were found in Palaeoheterodonta species than in Anomalodesmata, Heterodonta and Pteriomorphia (Figure 6). As for the dS, two groups of bivalves emerged, Palaeoheterodonta, which evolved slowly, and Anomalodesmata, Heterodonta and Pteriomorphia, which evolved faster. The conservative amino acid replacement rate (Kc) was also considered. The correlations between Kc and the three life-history traits were weak and non-significant (*pp*<0.95 or <0.05, Table 8).

2.2.4. Discussion

We report here, for the first time, a strong negative correlation between longevity and substitution rates (i.e. there is a greater accumulation of mutation in short-lived than in long-lived bivalves) as well as a negative correlation between longevity and maximum lethal temperature and a positive relationship between longevity and generation time.

2.2.4.1. Correlation between the three life-history traits

Species displaying low maximum lethal temperature (such as *Arctica islandica*, *Laternula elliptica*), generally inhabit polar or subpolar regions (Witbaard and Bergman 2003; Rodrigues et al. 2007). Species living in polar habitats might possess some characteristics helping them to live longer [e.g., low metabolic rates, absence of reproductive senescence, self-induction of metabolic reduction (Taylor 1976; Abele et al. 2009; Bodnar 2009, and reference therein)]. The correlation between longevity and temperature obtained in this study validates the hypotheses that long-lived species live principally in polar region. Surprisingly, temperature has however a weak influence on longevity: maximum lethal temperature explains only 22% of longevity divergences. Even if temperature has a significant effect on ROS production and metabolic rates (Samain 2011; Blier et al. 2014), it is not the main factor driving longevity in bivalves. To our knowledge, this is the first study, which quantifies the effect of temperature on longevity in ectothermic invertebrates.

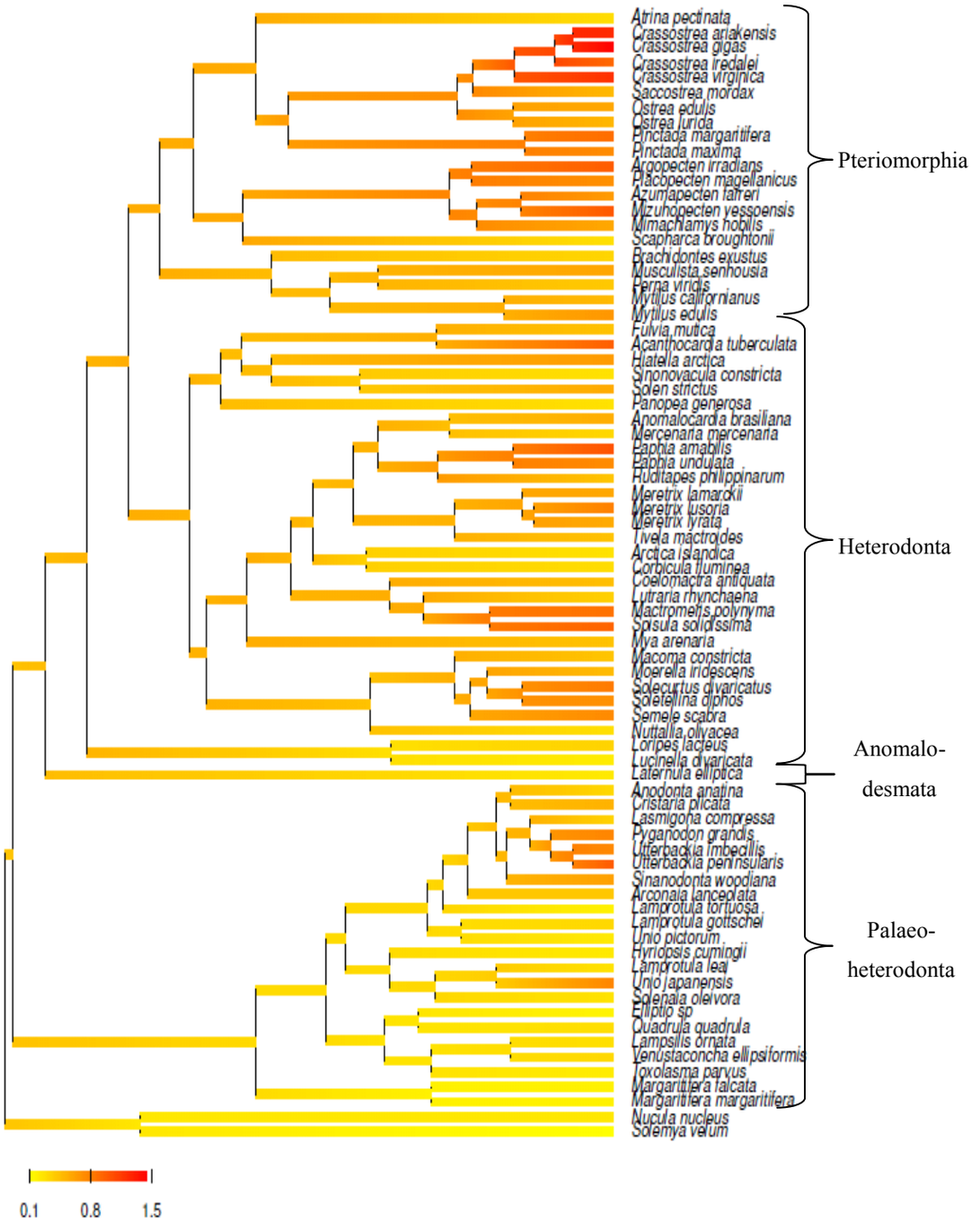


Figure 5. Posterior mean reconstruction of the evolution of dS along phylogeny of mtDNA in bivalves. The colors yellow and red correspond to low and high dS respectively.

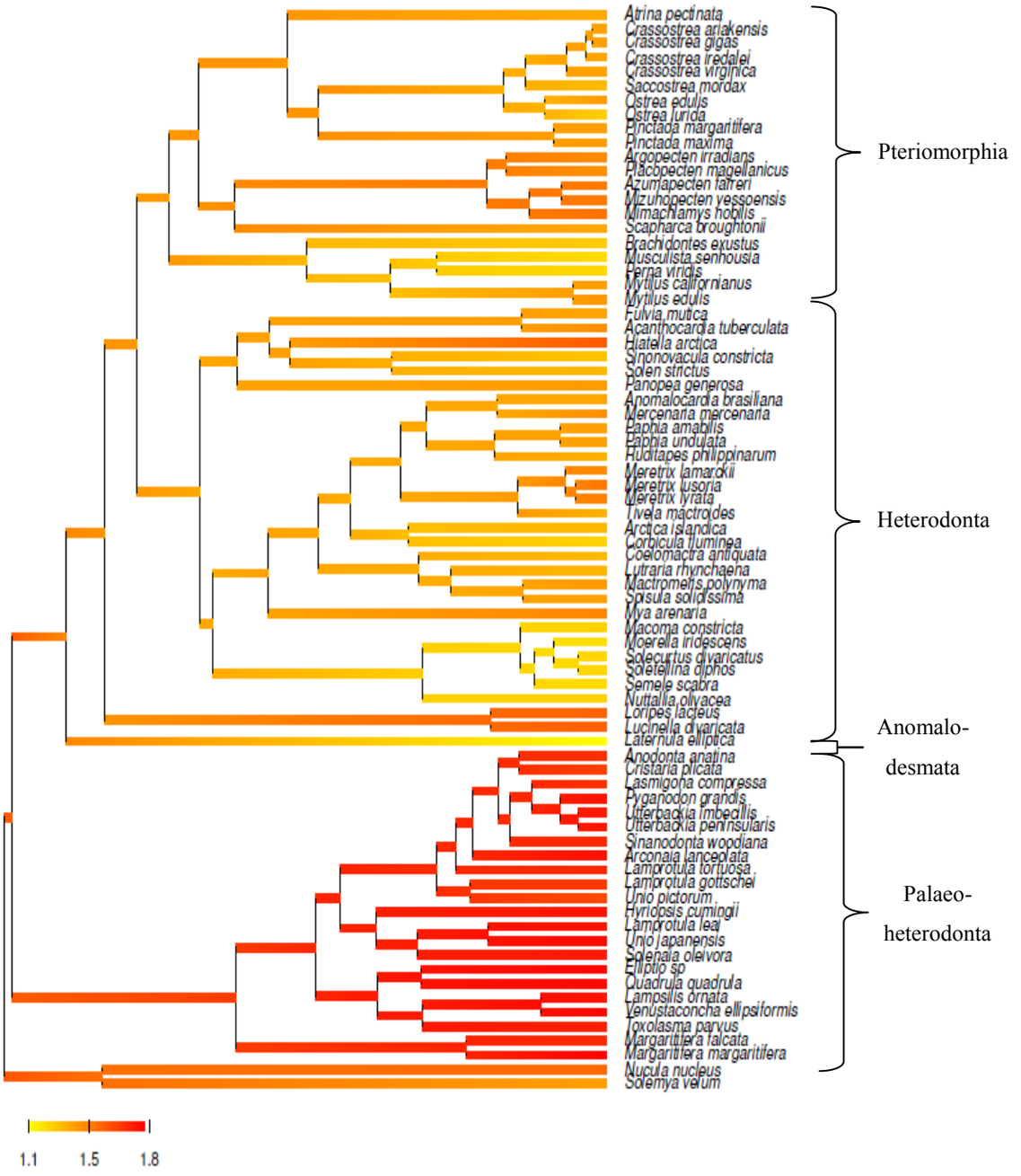


Figure 6. Posterior mean reconstruction of the evolution of Kr/Kc along phylogeny of mtDNA in bivalves. The colors yellow and red correspond to low and high Kr/Kc respectively.

Longevity is generally associated with generation time in mammals, birds and bivalves (Lartillot and Poujol 2011; Ridgway et al. 2011a; Abele and Philipp 2013). A species with late maturation will have a longer lifespan than a species with early maturation. Our result confirm the positive relationship between longevity and generation time previously found by Haag and Rypel (2011) and Ridgway et al. (2011a) in bivalves. The generation time had however a stronger influence on longevity (68%) in our study than in their studies (around 40%). These results suggest that development and aging are strongly related in bivalves.

2.2.4.2. Correlation between longevity, maximum lethal temperature and dS and dN/dS

As mentioned earlier, substitution rates and the ratio of nonsynonymous over synonymous substitution rates are important measures in molecular evolutionary studies (Laroche et al. 1997; Nabholz et al. 2008; Galtier et al. 2009a; Thomas et al. 2010; Lartillot and Poujol 2011; Lartillot and Delsuc 2012; Lartillot 2013; Nabholz et al. 2013). Our results indicate a strong negative correlation between longevity and substitution rates (i.e. there is a greater accumulation of mutation in short-lived than in long-lived species). Indeed, longevity explains 9 to 30% of dS in bivalves. This negative relationship was also identified in mammals, birds and plants (Laroche et al. 1997; Laroche and Bousquet 1999; Andreasen and Baldwin 2001; Nabholz et al. 2008; Galtier et al. 2009a; Lartillot and Poujol 2011; Lartillot and Delsuc 2012; Lartillot 2013). This correlation thus seems general for endotherms and ectotherms. Natural selection tends to decrease mutation rate in long-lived species in agreement with « mitochondrial theory of aging » (Harman 1956; Nabholz et al. 2008; Galtier et al. 2009b). This also suggests that a high selective constraint acting on the mtDNA of long-lived species.

This is one of the first studies looking at the influence of temperature on mitochondrial DNA evolution in both ectotherms and invertebrates. Surprisingly, no relationship between dS and maximum lethal temperature was found, probably caused by saturation of mtDNA. Despite their thermal dependence to their environment, dS of bivalves do not seem directly affected by temperature. Studies on Archaea, protozoan, invertebrates, fish, amphibians,

reptiles, birds, mammals and plants revealed that temperature explain a significant part of DNA mutations and substitution rates (Allen et al. 2006; Gillooly et al. 2005; Estabrook et al. 2007; Groussin and Gouy 2011; Davies et al. 2004; Wright et al. 2003). Gillooly et al. (2005) found that mitochondrial DNA evolution rate is higher for warmer-bodied endotherms than for ectothermic animals of similar size, suggesting that mitochondrial DNA evolution of ectotherms is influenced by temperature. This also suggests that considering the strong impact of temperature on metabolic rate in ectotherms and bivalves (Munro and Blier 2014; Munro et al. 2015; Samain 2011) metabolic rate *per se* could hardly explain significant part of dS divergences among bivalves.

As for mammals, no correlation between generation time and dS was found in mtDNA of bivalves (Lartillot and Poujol 2011). However, Thomas et al. (2010) established a negative relationship between generation time and dS in 143 species of invertebrates (including a few bivalves). To avoid the saturation of mtDNA, Thomas et al. (2010) removed the effect of transition substitution from the estimate of substitution rates (which are likely to reach saturation quickly) and conserved only the effect of transversions. This allowed them to establish the relationship between generation time and dS. This suggests that the correlation between generation time and dS is complex in mtDNA. The lack of correlation in the present study could be explained by high level of saturation related to long evolutionary history of studied species (the node of our phylogenetic trees being over 500 My distant).

The dN/dS ratio was usually positively correlated with longevity in mammals (Lartillot and Poujol 2011). Our model cannot validate the analysis of dN/dS for any studied life-history traits probably because of a high saturation of mtDNA in bivalves. Bivalves have diverged up to 530 millions of years ago, in the Cambrian period (Plazzi and Passamonti 2010, and reference therein). Accumulation of mutations among species over time likely caused the saturation of mtDNA and the underestimation of substitutions parameters. The very high saturation of mtDNA prevented the detection of molecular evolutionary signals (Lartillot and Poujol 2011; Nabholz et al. 2013; Galewski et al. 2006; Springer et al. 2001).

2.2.4.2.1. Correlation between longevity, maximum lethal temperature and Kc and Kr/Kc

Because of the high saturation of mtDNA, Kr/Kc might be more informative to evaluate the fixation rate of either slightly deleterious or adaptive mutations in mtDNA (Nabholz et al. 2013; Hanada et al. 2007; Popadin et al. 2007; Smith 2003; Zhang 2000). Indeed, the substitution of amino acid among mtDNAs is less saturated than the nucleotide substitution. Kr/Kc is correlated to the dN/dS ratio (Zhang 2000). It has a more reliable relationship with life-history traits than dN/dS (Nabholz et al. 2013). However, our model did not detect any correlation between Kr/Kc and the three life-history traits. A recent study on mammals found a positive relationship between Kr/Kc and longevity (Nabholz et al. 2013). Bivalves have diverged longtime before mammals so maybe more mutations among bivalve species were accumulated rending the evolutionary signal hardly detectable (Plazzi and Passamonti 2010). Analyse of Kc alone is seldom reported in the literature. One study reported a negative relationship between Kc and longevity in mammals and birds (Galtier et al. 2009a), contrary to our study where no correlation with life-history traits could be found in bivalves. Substitutions in amino acid (such as Kr/Kc or Kc) are suspected to have a stronger relationship with life-history traits than substitutions in codon (such as dN/dS or dS) (Nabholz et al. 2013). If the substitution in codon has a stronger influence on life-history traits than the substitution in amino acid, this suggests an important selection on the rate of DNA replication.

2.2.4.3. Reconstruction phylogenic of substitution parameters evolution

Numerous studies have attempted to reconstruct the phylogeny of bivalves using mtDNA and/or nuclear DNA to determine the relationship between the different subclasses (e.g. Cope 1996; Adamkewicz et al. 1997; Giribet and Wheeler 2002; Dreyer et al. 2003; Doucet-Beaupré et al. 2010; Plazzi et al. 2011). Bivalves comprise five subclasses. In all studies, the Protobranchia is considered as the most primitive group and it is placed in basal positions. The places of the other four subclasses are not well resolved yet. In our study, Pteriomorphia is placed as sister subclass of Heterodonta and Anomalodesmata and

Palaeoheterodonta is located in the same cluster but in basal position relative to precedent subclasses (Figures 5, 6). Our reconstruction of the evolution of dS (Figure 5) and Kr/Kc (Figure 6) confirm these phylogenies. With these reconstructions two groups emerge, the Paleoheterodonta, which evolves slowly and the Anomalodesmata, Heterodonta and Pteriomorphia, which evolve faster. Palaeoheterodonta seems to have diverged a long time ago comparing to others subclasses and accumulated the mutation slowly.

Palaeoheterodonta is characterized by freshwater species whereas Anomalodesmata, Heterodonta and Pteriomorphia are represented principally by marine species (Adamkewicz et al. 1997). Freshwater and marine species are supposed to have two effective population sizes different, with marine species, which have a higher effective population size. The substitution rate is known to be influenced by effective population size (Lanfear et al. 2014). Our result, slower evolution in the subclass Palaeoheterodonta, could be caused by their smaller effective population size.

Another potential explanation of this result is the relationship among the substitution rates, mitochondrial genome structure and genome rearrangement. Indeed, Palaeoheterodonta species possess their genes on two strands of mitochondrial genome and have a gene order conserved whereas the others subclasses (Anomalodesmata, Heterodonta and Pteriomorphia) possess all genes on one strand with a lot of rearrangements (Breton et al. 2006; Doucet-Beaupré et al. 2010; Smith and Snyder 2007; Levivier et al, in prep). According to Xu et al. (2006), the substitution rate could increase as a result of the increase in the genome rearrangement rate, but the causality could also go in the opposite direction, i.e., where the genome rearrangmenet rate increases as a result of the increase in mutation rate. The former hypothesis is associated with the potential disequilibrium in base composition due to a displacement of a gene to another place of the mtDNA, i.e., if a gene moves to a new location on the mtDNA, the base frequencies within this gene can be out of equilibrium with the mutational processes typical for its new position, and this will lead to a rapid burst of substitutions until equilibrium in the base frequencies is reached (Xu et al. 2006). The alternative hypothesis, i.e., where the genome rearrangement rate increases as a

result of the increase in substitution rate, might occur if the increase in substitution rate lead to an increase in the rate of recombination events by creating repeated/similarities sequences that are prone to recombination (Xu et al. 2006). The high rate of molecular evolution found in the present study and the high rate of genome rearrangements known (Gissi et al. 2008) for Anomalodesmata, Heterodonta and Pteriomorphia subclasses reinforce this potential scenario; suggesting a strong relationship among "the all-genes-on-one strand phenotype", high rate of molecular evolution and high rate of genome rearrangements in bivalve mitochondrial genomes.

2.2.5. Conclusion and perspectives

We performed the first analysis of evolutionary signals in mitochondrial DNA potentially linked to life-history traits in bivalves. Our results are the first to clearly establish a negative relationship between substitution rates and longevity in a group of invertebrates. The relation that exists between longevity and generation time or temperature does not influence this relationship. This suggests a direct link between the modulation of the mutation rate in mtDNA and longevity in bivalves. Such correlation has already been established in mammals, birds and plants (Laroche et al. 1997; Laroche and Bousquet 1999; Andreasen and Baldwin 2001; Nabholz et al. 2008; Galtier et al. 2009a; Lartillot and Poujol 2011; Lartillot and Delsuc 2012; Lartillot 2013). We therefore suggest that the negative correlation between longevity and dS is general for endotherms and ectotherms (vertebrates and invertebrates). Even if no direct correlation between longevity and dN/dS, Kc or Kr/Kc was found, our model also confirms that longevity is affected by temperature.

Bivalve phylogenies are complex. Based on reconstruction of dS (Figure 5) and Kr/Kc (Figure 6), our results suggest two groups: (1) Paleoheterodonta which evolves slowly and (2) Anomalodesmata, Heterodonta and Pteriomorphia which evolve faster. Paleoheterodonta subclasss possess different characteristics comparing to others bivalve subclasses (freshwater species, effective population size, low gene rearrangement rate, genes in two strands), which could explain this result.

The same study was run on each mtDNA gene separately but no result could be obtained probably because of a high saturation level in mtDNA substitutions (results not shown). Additional studies, on marine bivalve species only, would solve this problem and help to verify our results of substitution rates. However, sequencing of mitochondrial genomes of new species will be necessary to insure a high enough resolution.

2.2.6. Materials and Methods

2.2.6.1. Data set

Complete female mitochondrial genomes were downloaded from GenBank (Benson et al. 2013) or sequenced by the authors (n=11 species) in November 2014 for 76 bivalve species (Annex XII). The protobranchs, *Solemya velum* and *Nucula nucleus* were used as outgroup for phylogenetic analyses. Three life-history traits were tested: (1) the maximum recorded lifespan taken as a proxy for longevity, (2) the age of female at sexual maturity taken as a proxy for generation time, and (3) the maximum lethal temperature measured in laboratory taken as a proxy for maximum lethal temperature in environment. When it was possible, life-history traits data were obtained from the literature (37 datas were found for both longevity and generation time and 24 were found for maximum lethal temperature, Annex XII); otherwise the model (presented in section 2.2.6.2) estimated the values of the missing life-history traits.

2.2.6.2. Model

We used a probabilistic model to estimate divergence times, substitution rates, life-history traits and the correlation between them.

A concatenation of 12 protein coding gene sequences (PCG) (ATP8 was excluded) from the 76 bivalve mtDNAs was realized. The 12 PCG sequences were first aligned separately [MUSCLE software v.3.8.31 (Edgar 2004)], and the poorly aligned positions were stringently removed [Gblocks v.0,91b (Castresana 2000)]. After concatenating the PCGs, 1645 amino acid were left for phylogenetic analyses. A Bayesian inference, using MCMC

algorithm phylogenetic tree following CAT-GTR model, was constructed [PhyloBayes 3.3f (Lartillot et al. 2009)]. CAT-GTR model is a Dirichlet process mixture of profiles of equilibrium frequencies combined with general exchange rates (*i.e*. an infinite mixture of matrices sharing the same set of exchange rates, and differing only by their equilibrium frequencies) (Lartillot et al. 2009). Two independent runs were initiated at a same time. Program BPCOMP of PhyloBayes compared the frequency of the bipartitions obtained in the two independent chains (maximal difference, *maxdiff*<0.1). The phylogeny, life-history traits matrix and a concatenation of sequence alignment of codon dataset with a total of 6276 nucleotides were inserted in Coevol 1.4b model (Lartillot and Poujol 2011). This model consists of a Bayesian inference program using MCMC method. The synonymous substitution rate (dS) and the ratio of nonsynonymous over synonymous substitution rates (dN/dS) were estimated. Then, in replacement of codon dataset, an amino acid dataset for each species was aligned and concatenated to measure the conservative amino acid replacement rate (Kc) and the ratio of radical over conservative amino acid replacement rates (Kr/Kc) (Nabholz et al. 2013). In this study, we used the Kr/Kc model where the substitution is considered as radical when amino acids change polarity and volume. Two independent runs were performed for each analysis on Coevol 1.4b model. Convergence and mixing were assessed by measuring several key statistics (log likelihood, mean substitution rate over the tree, mean omega over the tree, entries of the covariance matrix, root age, etc.), the effective sample size, and the discrepancy between the credibility intervals obtained from the two independent runs [TRACECOMP program of Coevol (Lartillot et al. 2009; Lartillot and Poujol 2011)]. For all these statistics, an effective sample size superior to 500 and a credibility interval inferior to 0.1 was accepted. Covariances matrix, ancestral reconstructions and divergence times were obtained [READCOEVOL program of Coevol (Lartillot and Poujol 2011)]. We were able to control one or few traits of the model to clarify the results. A LaTeX program allowed us to draw phylogenies.

CONCLUSION GÉNÉRALE

Le vieillissement cellulaire est une thématique importante en évolution. Comparer le mode d'évolution génétique des espèces longévives à celui des espèces de courte durée de vie peut aider à identifier les processus cellulaires clés impliqués dans le vieillissement. Dans le contexte actuel des changements climatiques, notre intérêt concernant les mécanismes de vieillissement chez les bivalves est d'autant plus pertinent que le métabolisme de ces organismes est directement affecté par la température de leur milieu et que le métabolisme mitochondrial est suspecté être un déterminant important de la longévité.

Cartographie des génomes mitochondriaux et reconstructions phylogénétiques

Le séquençage et la cartographie de nouveaux génomes mitochondriaux sont des étapes indispensables pour comprendre le mode d'évolution mitochondrial des espèces. Onze nouveaux génomes mitochondriaux de neuf espèces de la sous-classe des Heterodonta (*Anomalocardia brasiliana, Arctica islandica, Corbicula fluminea, Macoma constricta, Mactromeris polynyma, Mercenaria mercenaria, Mya arenaria, Spisula solidissima* et *Tivela mactroides*) et de deux espèces de la sous-classe des Palaeoheterodonta (*Elliptio* sp. et *Margaritifera margaritifera* ; Annexe XIII) ont été séquencés et cartographiés pendant ce projet. Les génomes mitochondriaux de neuf de ces espèces ne sont actuellement pas connus ni publiés. La technique de séquençage de nouvelle génération Illumina MiSeq a été utilisée. Cette méthode, est à l'heure actuelle, la plus performante afin d'obtenir le génome mitochondrial complet d'une espèce. Dans la majorité des cas, elle permet d'obtenir le génome mitochondrial complet d'une espèce sans nécessiter une étape d'amplification supplémentaire après le séquençage. Huit génomes mitochondriaux complets (*Arctica islandica, Corbicula fluminea*, *Elliptio* sp.*, Macoma constricta, Mactromeris polynyma, Margaritifera margaritifera*, *Mya arenaria* et *Spisula*

solidissima) et trois génomes mitochondriaux partiels (*Anomalocardia brasiliana, Mercenaria mercenaria* et *Tivela mactroides*) ont été documentés en utilisant cette méthode.

La composition des génomes mitochondriaux est connue pour être variable chez les bivalves. La cartographie des génomes mitochondriaux des neuf espèces d'Heterodonta correspond à celle retrouvée généralement chez les animaux (Article I) avec 13 gènes codant pour les protéines, 2 gènes ribonucléiques ribosomiques et 22 gènes ribonucléiques de transfert. Les quelques particularités observées, soient la duplication du gène COX2 (*Mactromeris polynyma*), l'addition de gènes tRNA-Met (*Mactromeris polynyma, Mya arenaria*), l'absence du gène ATP8 (*Mya arenaria*) et la configuration du gène CYTB en deux cadres de lecture (*Arctica islandica*) sont des caractéristiques fréquemment retrouvées dans l'ADNmt des bivalves. Ces nouveaux génomes mitochondriaux ont permis non seulement de connaître de manière plus approfondie la génomique mitochondriale de chaque espèce mais également d'avoir une plus grande quantité de références génétiques afin de comparer plus efficacement les espèces et d'établir des relations phylogénétiques plus précises.

Des régions répétées ont été retrouvées au niveau des parties codantes des génomes mitochondriaux des Heterodonta. Elles sont généralement présentes dans les régions non-codantes des génomes mitochondriaux. Leur présence dans les parties codantes est rarement observée. Elles sont certaines fois dues à des duplications ou à des multiplications du même gène (exemple : *Mactromeris polynyma*), et d'autres fois, elles sont présentes au milieu d'un gène codant sans raison apparente (exemple : *Tivela mactroides*). Cette particularité est pour l'instant peu étudiée. Une étude plus approfondie sera nécessaire afin d'identifier la fonction et l'importance des régions répétées dans les parties codantes du génome mitochondrial chez les animaux.

L'ordre des gènes mitochondriaux sur l'ADNmt est connu pour être variable chez les bivalves. Néanmoins, nous avons été capables de mettre en évidence des états de caractères ancestraux pour la sous-classe des Heterodonta. Les associations des gènes 12S-COX3,

ATP8-ND4-HES et 16S-ATP6 sont caractéristiques des Heterodonta. Nous présentons ici, la première étude capable d'identifier des états de caractères ancestraux chez les Heterodonta.

Les reconstructions phylogénétiques des Heterodonta à partir des génomes mitochondriaux complets sont rares. Nous présentons ici la quatrième étude reconstruisant l'évolution des Heterodonta à partir de leur génome mitochondrial. Notre étude a l'avantage d'apporter huit nouvelles espèces (*Arctica islandica* étant déjà présente dans une des études précédentes). Certains liens de parenté ont été confirmés tels que les relations de parenté entre les différents genres chez les Veneridae, l'absence de relation de parenté entre les Cardiidae et les Tellinoidae, la non-monophylie des familles des Tellinoidea et la position des Mactroidea et des Lucinidae sur l'arbre. D'autres relations ont été révélées telles que la relation entre *Arctica islandica* et *Corbicula fluminea*, la potentielle non-monophylie des Hiatellidae. Les reconstructions phylogénétiques ont également permis de confirmer la monophylie des principales familles et superfamilles d'Heterodonta ; des Veneridae, des Mactroidea, des Cardioidae, des Solenoidea, des Tellinoidea et des Lucinidae. Le séquençage et la cartographie de génomes mitochondriaux de nouvelles espèces sont attendus afin de confirmer les états de caractères ancestraux, d'améliorer les phylogénies existantes et ainsi de consolider la taxonomie des Heterodonta.

Arctica islandica

Arctica islandica est une espèce importante pour l'étude du vieillissement cellulaire car elle est l'espèce animale non-coloniale qui possède la plus grande longévité maximale connue (507 ans, Butler et al, 2012). Notre étude a permis d'établir les relations phylogénétiques entre les populations de cette espèce. Cinq génomes mitochondriaux de quatre populations d'*A. islandica* étaient à notre disposition. À l'heure actuelle, quatre études décrivent la répartition génétique des différentes populations de cette espèce (Dahlgren et al. 2000; Holmes et al. 2003; Glöckner et al. 2013; et la nôtre). Aucune distribution génétique claire des populations d'*A. islandica* n'a cependant pu être établie.

Des études additionnelles sont nécessaires pour comprendre la diversité et la répartition génétique des populations de l'espèce *Arctica islandica*.

Le mode d'évolution

Le deuxième chapitre de ce mémoire présente la première étude réalisée sur le mode d'évolution de l'ADN mitochondrial des bivalves en lien avec le vieillissement cellulaire. Les onze nouveaux génomes mitochondriaux séquencés et cartographiés pendant cette maîtrise ont permis d'apporter des espèces d'une importance capitale dans l'étude du vieillissement cellulaire telles que *Arctica islandica* et *Margaritifera margaritifera* qui possèdent respectivement une longévité de 507 et 210 ans. Les divergences de longévité dans cette étude, sont expliquées à 68% de manière positive par la vitesse de développement, à 22% de manière négative par la température. Le taux de substitution synonyme (dS) est expliqué en partie (entre 9 et 30%) par la longévité. Les relations qui existent entre la longévité et la vitesse de développement ou la température n'ont pas influencé la relation observée entre la longévité et le dS. Les relations entre le vieillissement et le développement, et le vieillissement et la température sont bien connues. Elles ont fait l'objet de nombreuses études. Nous sommes néanmoins les premiers à quantifier la relation entre le vieillissement et la température chez les bivalves. La démonstration de la relation entre le vieillissement et le taux de substitution synonyme est elle aussi tout à fait nouvelle chez les invertébrés bien qu'elle ait été précédemment observé chez les oiseaux, les mammifères et les plantes. L'ADNmt des espèces de longue durée de vie subit donc une contrainte sélective importante aussi bien chez les vertébrés que chez les invertébrés. Cette relation négative entre la longévité et le taux de substitution synonyme de l'ADNmt apparaît être commune aux ectothermes et aux endothermes. Ceci valide notre hypothèse proposée dans l'introduction générale selon laquelle des traces de sélection associées à la longévité seraient présentes dans l'ADNmt des espèces.

Nous présentons la première étude qui implique les rapports de substitutions (dN/dS et Kr/Kc) et la longévité chez des ectothermes. Nous n'avons toutefois pas réussi à démontrer quelques relations que ce soit entre ces rapports de substitution et la longévité.

Nous supposons que les nombreuses mutations qui se sont accumulées dans l'ADNmt des espèces, depuis l'apparition des bivalves il y a environ 530 millions d'années, sont responsables de la saturation de l'ADNmt et donc de l'absence de résultat. Les espèces ont évolué à différentes vitesses (telles que le montrait les reconstructions phylogénétiques du dS et du Kr/Kc). Les Pteriomorphia, les Heterodonta et les Anomalodesmata semblent avoir évolués plus rapidement que les Palaeoheterodonta. Une autre explication est également possible. Les Palaeoheterodonta possèdent des caractéristiques particulières en comparaison des autres sous classes : ils sont composés par des espèces d'eau douce impliquant une plus petite taille effective de population, leurs gènes sont répartis sur les deux brins de l'ADNmt et ceux-ci ont maintenu un ordre de gènes très conservés. De précédentes études ont montrés que les taux de substitutions sont influencés par la taille effective des populations et par le taux de réarrangement des gènes.

Les autres traits d'histoire de vie étudiés (temps de génération et température maximale létale) n'influencent pas, à première vue, l'évolution de l'ADNmt des bivalves. Il serait intéressant d'étudier également le mode d'évolution génétique des bivalves en lien avec le taux de croissance, la disponibilité en nourriture, la prédation et le taux métabolique.

Notre étude sur le mode d'évolution des bivalves a révélé un taux de saturation de l'ADNmt élevé et des vitesses d'évolutions différentes entre les sous-classes. Il serait intéressant de recommencer celle-ci en étudiant uniquement le mode d'évolution d'une seule sous-classe afin de limiter la saturation de l'ADNmt. La sous-classe des Heterodonta est un bon candidat pour ces futures études. Elle est la plus vaste des sous-classes et elle présente les valeurs les plus diversifiées de longévité (1 à 507 ans), de maturité sexuelle (0,4 à 32 ans) et de température maximale (17 à 35 °C). Le séquençage de génomes mitochondriaux de nouvelles espèces de cette sous-classe sera cependant nécessaire afin d'assurer une résolution statistique suffisamment élevée.

Perspectives

De nombreuses autres études pourront être réalisées à partir des données récoltées au cours de ce projet. Deux sujets en particulier sont proposées : (1) une étude de comparaison sur les similarités et les différences morphologiques, génétiques et de traits d'histoire de vie des espèces *Arctica islandica* et *Corbicula fluminea* ; et (2) une étude phylogénétique des Palaeoheterodonta.

Du point de vue du génome mitochondrial (voir article I), *Arctica islandica* et *Corbicula fluminea* sont relativement proches. Elles sont considérées comme des « groupes sœurs ». Ce lien de parenté a également été observé lors d'une étude phylogénétique que nous avons réalisé à partir du gène COX1 pour 87 espèces d'Heterodonta. Ces deux espèces possèdent des caractéristiques phénotypiques similaires. Elles vivent enfouies dans le sédiment et elles se nourrissent majoritairement en filtrant l'eau de leur milieu. Elles possèdent cependant certaines caractéristiques très divergentes. Arctica vit en milieu marin tandis que Corbicula vit en eau saumâtre ou en eau douce. Arctica vit principalement en milieu polaire et subpolaire tandis que Corbicula est une espèce cosmopolite envahissante. Corbicula a été retrouvée au Québec au niveau du panache thermique de la centrale nucléaire de Gentilly (Bécancour, Québec, Canada). Elle vit cependant essentiellement en milieu tempéré et tropical. Leur longévités maximum sont également extrêmement divergentes. Arctica peut vivre pendant plus de 500 ans tandis que l'âge maximal de Corbicula est estimé à 5 ans. Leur similitude génétique contraste avec leurs caractéristiques phénotypiques. Une revue sur la ressemblance génétique et les caractéristiques phénotypiques d'*Arctica islandica* et de *Corbicula fluminea* serait à envisager. Cette étude serait un point de départ pour comprendre comment deux espèces d'apparences si différentes peuvent avoir un ADNmt relativement proche.

Une étude descriptive et phylogénétique à partir du génome mitochondrial des Palaeoheterodonta devrait être également envisagée. Les génomes mitochondriaux d'*Elliptio* sp. et de *Margaritifera margaritifera* (Palaeoheterodonta, Unionoida) ont été séquencés et cartographiés pendant cette maîtrise (Annexe XIII). Aucune description de

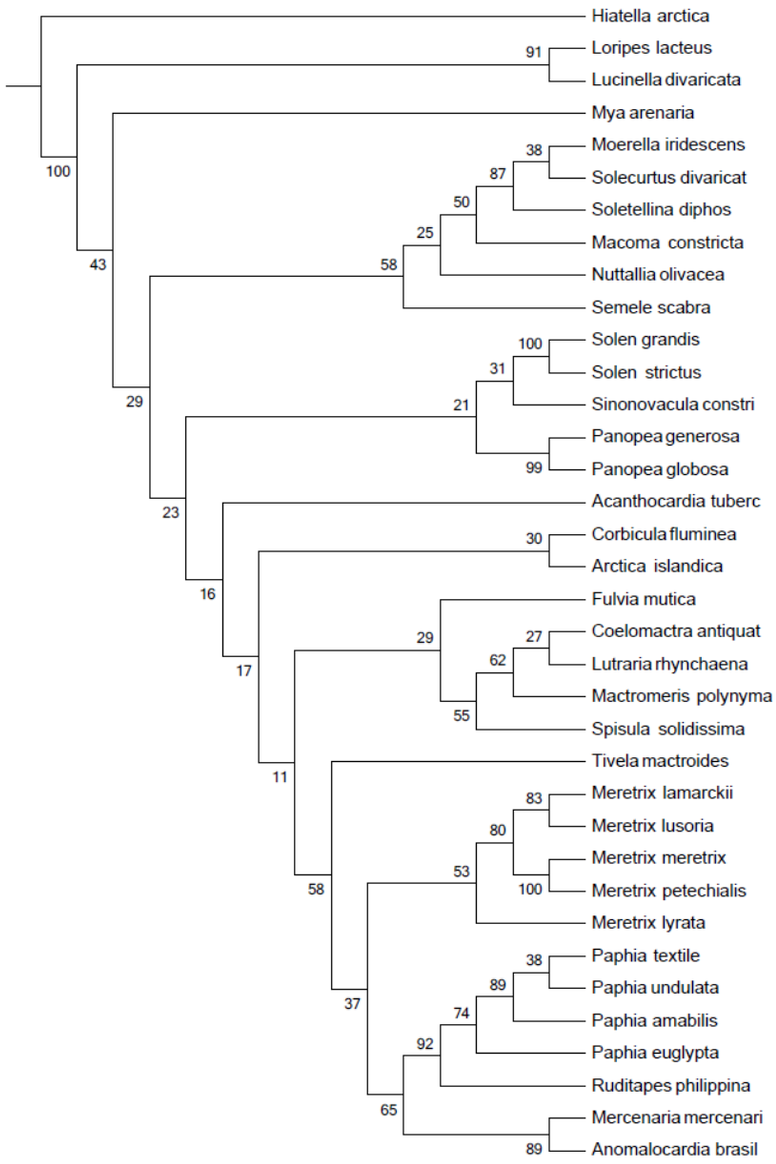
ceux-ci n'a cependant pu être réalisée dans ce mémoire. Contrairement aux Heterodonta, les espèces appartenant aux Unionoida possèdent en général, un arrangement des gènes sur leur génome mitochondrial très conservé, ce qui est également le cas pour nos deux espèces. Dans ce cas, l'histoire évolutive du réarrangement des gènes sera probablement plus simple à établir et la construction d'une nouvelle phylogénie à partir des génomes mitochondriaux complets permettra d'approfondir les connaissances actuelles sur le mode d'évolution et les relations de parenté des Palaeoheterodonta.

ANNEXE I.

GENBANK ACCESSION NUMBERS

Species	GenBank accession number/reference
Acanthocardia tuberculata	NC_008452
Coelomactra antiquata	NC_021375
Fulvia mutica	NC_022194
Hiatella arctica	NC_008451
Loripes lacteus	NC_013271
Lucinella divaricata	NC_013275
Lutraria_rhynchaena	NC_023384
Meretrix larmarckii	NC_016174
Meretrix lusoria	NC_014809
Meretrix lyrata	NC_022924
Meretrix meretrix	NC_013188
Meretrix petechialis	NC_012767
Moerella iridescens	NC_018371
Nuttalia olivacea	NC_018373
Panopea generosa	KM580067
Panopea globosa	KM580068
Paphia amabilis	NC_016889
Paphia euglypta	NC_014579
Paphia textile	NC_016890
Paphia undulata	NC_016891
Ruditapes philippinarum	NC_003354
Semele scabra	NC_018374
Sinonovacula constricta	NC_018375
Solecurtus divaricatus	NC_018376
Solemya velum	NC_017612
Solen grandis	NC_016665
Solen strictus	NC_017616
Soletellina diphos	NC_018372

ANNEXE II.
MAXIMUM LIKELIHOOD PHYLOGENETIC TREE OF HETERODONT BIVALVES BASED ON GENES ORDER



ANNEXE III.

PRIMER-WALKING PROTOCOL AND PRIMERS USED

Total genomic DNA of one individual of *Arctica islandica* was extracted from the adductor muscle using Mollusc DNA kit (Omega Bio-tek. Inc.. USA) following the manufacter's protocol. Primers from Mikkelsen et al. (2006) (GCAAYGAGAGTTGTRCTAAGGTAGC and ATAATCCAACATCGAGGTCGCAAA) was used to amplify 16S-rRNA gene; and primers from Dahlgren et al. (2000) (CCTTGGGGCAGATATCTTTTTG and GCRWAYARAAARTAYCAYTCWGG) was used to amplify CYTB gene. News sequences of CYTB and 16S-rRNA genes and the COX1 sequence (GenBank No. DQ184853.1) were then used to design species-specific long-PCR primer pairs (Table S1). Three fragments of 1815. 7424 and 9916 bp allowed obtaining whole mtDNA. The polymerase chain reaction (PCR) of 1815 bp fragment was performed in 25 µL of a solution containing ≈80 ng of total DNA. 2.5 µL of 10X buffer. 1.1 µL of $MgCl_2$ (50mM). 0.5 µL of dNTP mix (10mM). 0.8 µL of each primer (10 µM). 0.2 µL of Native Taq DNA polymerase (Invitrogen). PCR reactions were performed using a G-storm GS4 thermal cycler as follows: pre-denaturation at 94°C for 4 min; then 35 cycles of 94°C for 15 s. annealing at 55°C for 30 s. extension at 72°C for 5 min. and a final extension step at 72°C for 6 min. PCR of others fragments (7424 and 9916 bp) were performed in 50 µL of a solution containing ≈80 ng of total DNA. 5 µL of 10X high fidelity PCR buffer. 2 µL of $MgSO_4$ (50mM). 1 µL of dNTP mix (10mM). 2 µL of each primer (10 µM). 0.2 µL of Platinum Taq DNA polymerase (Invitrogen). PCR reactions were performed using a G-storm GS4 thermal cycler as follows: pre-denaturation at 94°C for 2 min; then 35 cycles of 94°C for 15 s. annealing at 55°C for 30 s. extension at 68°C for 10 min. and a final extension step at 72°C for 10 min. PCR products were checked by electrophoresis on 0.8%

agarose gel. Sequencing was performed for both strands of each fragment using the primer-walking approach on a 3730xl DNA Analyzer (Applied Biosystems) of McGill University (Montréal. Québec. Canada).

Table S1. Primers used in primer-walking approach

	Primer name	Sequence (5'-3')	Product size	Joined product size
1rst sequencing	Arc16S-F	AAAAGACGAGAAGACCCCGT	696 bp	/
	ArcCOI-R	CACAGGATCTCCAAGCCCTAC	671 bp	/
	ArcCOI-F	ACTTCTTTTGGGGTCGGGAT	729 bp	/
	ArcCytb-R	GGCTGAATGTGCAAAGGAGT	740 bp	/
	ArcCytb-F	AGTGCTGTGCCTTATGTTGG	706 bp	/
	Arc16S-R	AGGTCGCAAACTTTTCCCTC	759 bp	/
2nd sequencing	Arc16S-F2	GGGAGGTGGCGGTTATATTT	702 bp	1346 bp
	ArcCOI-R2	ACCAGCGATAAGCCCAATTA	711 bp	1332 bp
	ArcCOI-F2	TGTATTGAGCAGGGGGTTTT	657 bp	1351 bp
	ArcCytb-R2	GGCAGCCAAAAGTAGTCCAG	613 bp	1250 bp
	ArcCytb-F2	CCCATTAGCTTGTTTATGATGAG	705 bp	1339 bp
	Arc16S-R2	CCTAGAGCTTAGCCCCTAAGAA	695 bp	1444 bp
3rd sequencing	Arc16S-F3	TTTGTTGGGCTTTTATTACGG	734 bp	1948 bp
	ArcCOI-R3	TTACAGCATTATGTGCCCAAG	660 bp	1974 bp
	ArcCOI-F3	AGACCCAAAAACAGAAAATGC	672 bp	2020 bp
	ArcCytb-R3	CACCGCTTCACAAACACCTAA AND AACACCGCTTCACAAACACC	752 bp	1901 bp
	ArcCytb-F3	CTTCGCCCGTTTAACAAAAA	396 bp	1737 bp
	Arc16S-R3	CGCAATTACCCCACCTAACT	343 bp	1733 bp
4rth sequencing	Arc16S-F4	TCCGAAGCATTTGATTGTCCA	615 bp	2506 bp
	ArcCOI-R4	GGACGCCTCTTCACACTTTAG	681 bp	2672 bp
	ArcCOI-F4	GCTATCCCTGGACGAACAAA	622 bp	2659 bp
	ArcCytb-R4	CACCTGCAACCTAAAACTAAACT	731 bp	2590 bp
5th sequencing	Arc16S-F5	AGGTGCAATACGAGCTGTGG	580 bp	2944 bp
	ArcCOI-R5	GGCTCGTAGTCCCCGTATAA	637 bp	3188 bp
	ArcCOI-F5	GAAGGTCTCTGCTTTTAGGCTC	662 bp	3304 bp
	ArcCytb-R5	AACCGAAGAACCCCGTATCC	772 bp	3253 bp
6th sequencing	Arc16S-F6	AAGTTTTGGGAGCTTAAGATGC	776 bp	3693 bp
	ArcCOI-R6	TTGGCGTAAACAAAACACACT	749 bp	3977 bp
	ArcCOI-F6	GTGCTGTAGGAGAGGTAGACA	793 bp	4010 bp
	ArcCytb-R6	ATCATCATAATACGGCAGAGACA	785 bp	4025 bp

NOTE. /, unknown

Table S2. Primers used in primer-walking approach (suite 1)

	Primer name	Sequence (5'-3')	Product size	Joined product size
7th sequencing	Arc16S-F7	TGACCACACAAAAACTGAATCT	400 bp	4254 bp
	ArcCOI-R7	TGCAAAGCATCCCCTTATTT AND ACGGATGTGAGCAATTTAATCCA	719 bp	4480 bp
	ArcCOI-F7	AGTTTCATTTGGGGAAGGGG	787 bp	4776 bp
	ArcCytb-R7	CTTTACCCGTGGCCCACC	≈ 800 bp	/
8th sequencing	Arc16S-F8	CCTCTGGTATCAAGCTGGCT AND AGTGTGTTTTGTTTACGCCAA	771 bp	4899 bp
	ArcCOI-R8	GCATCTTAAGCTCCCAAAACTT	683 bp	5103 bp
	ArcCOI-F8	AGTTGAAATCTGTGCGGGAA	698 bp	5406 bp
	ArcCytb-R8	AAAACGGACCTTCTATGTGGAA	/	/
9th sequencing	Arc16S-F9	TCGAAGAGGTTGGCGGTATT	720 bp	5580 bp
	ArcCOI-R9	CCACAGCTCGTATTGCACC	/	/
	ArcCOI-F9	TTCCACATAGAAGGTCCGTTTT	748 bp	6065 bp
	ArcCytb-R9	TTCCCGCACAGATTTCAACT	/	/
10th sequencing	Arc16S-F10	ATCTTTTGTGTGGGCGGTTC	/	/
	ArcCOI-R10	AGTCCCCTCATTATATTCATGCA	/	/
	ArcCOI-F10	ATTGGTTCTGGGCTAAATTGCT	778 bp	/
	ArcCytb-R10	AGTCCCCTTCCCCAAATGAA	/	/
11th sequencing	Arc16S-F11	TGGCTGACAGAGTTTATGGTTC	/	/
	ArcCOI-R11	AGGCCGAATCATCACCCTT	/	/
	ArcCOI-F11	CAATGATTAGCTGTGGATGGAGT	/	/
	ArcCytb-R11	CATCTTGAACTCCTCCCCTCA	/	/
12th sequencing	Arc16S-F12	ACCTGGCTCTCTCTTACAAGA	/	/
	ArcCOI-R12	TCCAACGTGAAAAGCTGACG	/	/
	ArcCOI-F12	CCCTGATTGCCTATTCTTCAGT	/	/
	ArcCytb-R12	CCAATCAGTCCCAGCCAATT	/	/
13th sequencing	ArcCOI-F13	AGTAGCTTTATATTCTGGTCCCC	/	/
	ArcCytb-R13	TCCAGCTCCACACAACTCAT	/	/
14th sequencing	ArcCOI-F14	TGTGAAGCGGTGTTAGGACT	/	/
	ArcCytb-R14	GCACCAAAACCATTACTACAACA	/	/
15th sequencing	ArcCOI-F15	GTTTTCATGCTAACGGGGCT	/	/
	ArcCytb-R15	TTTCGAACCAGTCCACCAGA	/	/

NOTE. /, unknown

ANNEXE IV.

TRIM SUMMARY

Species	Number of reads	Average length (nt)	After trim		
			Number of reads	% trimmed	Average length (nt)
Anomalocardia brasiliana	2 944 388	295.0	2 676 451	90.9	259.7
Arctica islandica	3 067 192	297.3	2 731 708	89.06	251.0
Corbicula fluminea	2 604 318	297.2	2 221 229	85.29	243.5
Macoma fluminea	3 161 644	283.6	2 503 472	79.18	240.7
Mactromeris polynyma	4 027 090	285.5	3 405 373	84.56	249.1
Mercenaria mercenaria	2 551 310	281.3	2 172 079	85.14	238.1
Mya arenaria	2 793 148	285.6	2 235 863	80.05	237.0
Spisula solidissima	2 764 618	293.9	2 366 084	85.58	245.7
Tivela mactroides	3 275 682	294.1	2 754 677	84.09	244.4

ANNEXE V.
MITOCHONDRIAL GENOME ANNOTATIONS FOR EIGHT HETERODONTA SPECIES

Table S1. *Anomalocardia brasiliana* partial mitochondrial genome annotation

Anomalocardia brasiliana						
Gene	Location	Size	Start codon	Stop codon	Anti-codon	Intergenic region
tRNA-Met	132-198	67			CAT	67
tRNA-Tyr	266-328	63			GTA	0
12S-rRNA	329-1274	946				0
ND4L	1275-1574	300	ATT	TAA		56
ATP8	1631-1744	114	ATG	TAG		370
ND4	2115-3104	990	TTG	TAG		2
tRNA-Ser	3107-3171	65			TGA	10
tRNA-His	3182-3242	61			GTG	6
tRNA-Glu	3248-3311	64			TTC	45
ATP6	3357-4097	741	ATT	TAA		1
ND3	4099-4410	312	TTG	TAG		237
tRNA-Asn	4648-4709	62			GTT	0
ND5	4710-6419	1710	TTG	TAA		67
COX2	6487-7536	1050	ATG	TAA		11
tRNA-Asp	7548-7610	63			GTC	4
ND6	7615-8097	483	ATC	TAA		-1
tRNA-Lys	8097-8158	62			TTT	9
tRNA-Val	8168-8230	63			TAC	7
tRNA-Phe	8238-8304	67			GAA	-1
tRNA-Trp	8304-8365	62			TCA	-1
tRNA-Arg	8365-8426	62			TCG	0
tRNA-Leu	8427-8489	63			TAA	0
tRNA-Gly	8490-8551	62			TCC	0
tRNA-Gln	8552-8618	67			TTG	1
tRNA-Thr	8620-8689	70			TGT	1
tRNA-Ser	8691-8757	67			TCT	0
COX3	8758-9603	846	ATT	TAG		-2
tRNA-Ala	9602-9666	65			TGC	1
COX1	9668-11254	1587	ATG	TAA		3
tRNA-Leu	11258-11320	63			TAG	-3
ND1	11318-12235	918	ATA	TAA		63
ND2	12299-13306	1008	GTG	TAG		-2
tRNA-Ile	13305-13367	63			GAT	2
tRNA-Pro	13370-13432	63			TGG	31
CYTB	13464-14606	1143	ATT	TAG		183...

NOTE. 16S-rRNA and tRNA-Cys genes are absent.

Table S2. *Corbicula fluminea* mitochondrial genome annotation

Corbicula fluminea						
Gene	Location	Size	Start codon	Stop codon	Anti-codon	Intergenic region
COX1	1-1617	1617	ATA	TAA		58
COX2	1676-2995	1320	ATT	TAA		5
tRNA-Gly	3001-3063	63			TCC	2
tRNA-Arg	3066-3127	62			TCG	0
CYTB	3128-4288	1161	TTG	TAA		0
16S-rRNA	4289-5480	1192				2
ATP8	5483-5596	114	ATG	TAA		6
ND4	5603-6961	1359	ATT	TAA		-1
tRNA-His	6961-7022	62			GTG	2
tRNA-Glu	7025-7089	65			TTC	-4
tRNA-Ser	7086-7148	63			TGA	0
ATP6	7149-7889	741	GTG	TAG		21
ND3	7911-8333	423	ATT	TAG		-1
tRNA-Ala	8333-8396	64			TGC	1
tRNA-Ile	8398-8461	64			GAT	7
tRNA-Lys	8469-8534	66			TTT	0
tRNA-Leu	8535-8598	64			TAA	0
ND1	8599-9510	912	GTG	TAG		-1
tRNA-Leu	9510-9574	65			TAG	-1
tRNA-Val	9574-9639	66			TAC	-1
tRNA-Asn	9639-9701	63			GTT	0
ND5	9702-11408	1707	TTG	TAA		1
ND6	11410-11880	471	ATG	TAG		2
ND4L	11883-12170	288	ATA	TAG		2
tRNA-Gln	12173-12239	67			TTG	0
ND2	12240-13262	1023	GTG	TAG		-1
tRNA-Asp	13262-13324	63			GTC	0
tRNA-Thr	13325-13390	66			TGT	-1
12S-rRNA	13390-14239	850				12
tRNA-Met	14252-14319	68			CAT	4
tRNA-Cys	14324-14387	64			GCA	1
tRNA-Tyr	14389-14454	66			GTA	-1
tRNA-Ser	14454-14522	69			TCT	-1
COX3	14522-15397	876	TTG	TAA		-1
tRNA-Trp	15419-15480	62			TCA	2
tRNA-Pro	15483-15546	64			TGG	1027
tRNA-Phe	16574-16636	63			GAA	51

Table S3. *Macoma constricta* mitochondrial genome annotation

Macoma constricta						
Gene	Location	Size	Start codon	Stop codon	Anti-codon	Intergenic region
COX1	1-1704	1704	ATA	TAG		2
ND4	1707-3038	1332	ATG	TAG		-1
tRNA-His	3038-3102	65			GTG	0
tRNA-Ser	3103-3166	64			TGA	1
tRNA-Glu	3168-3234	67			TTC	0
ND3	3235-3597	363	GTG	TAA		1
tRNA-Ile	3599-3667	69			GAT	3
tRNA-Lys	3671-3739	69			CTT	0
ND4L	3740-4033	294	GTG	TAG		13
tRNA-Tyr	4047-4108	62			GTA	0
tRNA-Thr	4109-4174	66			TGT	0
tRNA-Leu	4175-4240	66			TAG	-1
tRNA-Asp	4240-4303	64			GTC	2
tRNA-Leu	4306-4369	64			TAA	1
ND1	4371-5294	924	GTG	TAG		24
tRNA-Asn	5319-5382	64			GTT	9
ND5	5392-7119	1728	ATA	TAG		-10
tRNA-Arg	7110-7175	66			TCG	0
CYTB	7176-8408	1233	GTG	TAG		0
COX2	8409-9275	867	ATG	TAA		0
tRNA-Val	9276-9340	65			TAC	1
tRNA-Trp	9342-9408	67			TCA	2
tRNA-Gly	9411-9476	66			TCC	2
12S-rRNA	9479-10380	902				849
tRNA-Met	11230-11296	67			CAT	14
ATP8	11311-11436	126	ATG	TAG		-2
tRNA-Ser	11435-11503	69			TCT	-1
ND6	11503-12042	540	GTG	TAA		0
16S-rRNA	12043-13287	1245				0
ATP6	13288-14025	738	ATG	TAG		3
COX3	14029-14913	885	GTG	TAG		6
ND2	14920-15981	1062	ATG	TAG		0
tRNA-Pro	15982-16050	69			TGG	2
tRNA-Gln	16053-16119	67			TTG	-1
tRNA-Cys	16119-16179	61			GCA	0
tRNA-Ala	16180-16243	64			TGC	-1
tRNA-Phe	16243-16308	66			GAA	0

Table S4. *Mactromeris polynyma* mitochondrial genome annotation

Mactromeris polynyma						
Gene	Location	Size	Start codon	Stop codon	Anti-codon	Intergenic region
COX1	1-1743	1743	ATA	TAA		43
tRNA-Val	1787-1851	65			TAC	131
tRNA-Arg	1983-2047	65			TCG	50
tRNA-Trp	2098-2162	65			TCA	6
ND6	2169-2654	486	GTG	TAG		-2
tRNA-Gln	2653-2719	67			TTG	14
tRNA-Pro	2734-2795	62			TGG	83
ND2	2879-3979	1101	GTG	TAA		8
tRNA-Thr	3988-4052	65			TGT	154
12S-rRNA	4207-5128	922				2
tRNA-Tyr	5131-5193	63			GTA	1
tRNA-Ser	5195-5258	64			TCT	1
COX3	5260-6327	1068	TTG	TAG		48
CYTB	6376-7578	1203	ATG	TAA		122
16S-rRNA	7701-9042	1342				1
ATP8	9044-9157	114	ATG	TAA		5
ND4	9163-10515	1353	GTG	TAG		1
tRNA-His	10517-10579	63			GTG	2
tRNA-Glu	10582-10646	65			TTC	-5
tRNA-Ser	10642-10704	63			TGA	0
ATP6	10705-11484	780	GTG	TAG		20
ND3	11505-11942	438	ATC	TAA		2
tRNA-Lys	11945-12008	64			TTT	4
tRNA-Leu	12013-12076	64			TAA	0
ND1	12077-12988	912	ATG	TAA		1
tRNA-Asp	12990-13052	63			GTC	46
tRNA-Gly	13099-13160	62			TCC	2630
tRNA-Ile	15791-15855	65			GAT	0
ND5	15856-17688	1833	ATG	TAA		62
COX2	17751-19808	2058/	ATG/	TAA/		151
	19960-21270	1311	ATG	TAA		739
ND4L	22010-22315	306	ATA	TAG		2
tRNA-Asn	22318-22380	63			GTT	2
tRNA-Leu	22383-22448	66			TAG	2
tRNA-Cys	22451-22514	64			GCA	7
tRNA-Met **x5**	22522-22588	66-67			CAT	33/
	22622-22687					33/
	22721-22786					33/
	22820-22885					33/
	22919-22984					4
tRNA-Phe	22989-23052	64			GAA	50
tRNA-Ala	23103-23169	67			TGC	41

Table S5. *Mercenaria mercenaria* partial mitochondrial genome annotation

Mercenaria mercenaria						
Gene	Location	Size	Start codon	Stop codon	Anti-codon	Intergenic region
ND6	296-778	483	ATA	TAG		13
tRNA-Lys	792-859	68			TTT	1
tRNA-Val	861-923	63			TAC	8
tRNA-Phe	932-995	64			GAA	-1
tRNA-Trp	995-1056	62			TCA	-1
tRNA-Arg	1056-1116	61			TCG	2
tRNA-Leu	1119-1182	64			TAA	-1
tRNA-Gly	1182-1246	65			TCC	11
tRNA-Gln	1258-1324	67			TTG	10
tRNA-Thr	1335-1400	66			TGT	6
tRNA-Ser	1407-1471	65			TCT	1
COX3	1473-2322	850	ATG	T**		3
tRNA-Ala	2326-2388	63			TGC	27
COX1	2416-3978	1563	ATA	TAA		47
tRNA-Leu	4026-4088	63			TAG	-3
ND1	4086-5003	918	ATA	TAA		65
ND2	5069-6091	1023	TTG	TAA		0
tRNA-Ile	6092-6155	64			GAT	13
tRNA-Pro	6169-6232	64			TGG	26
CYTB	6259-7413	1155	ATA	TAG		0
16S-rRNA	7414-8918	1505				0
tRNA-Met	8919-8984	66			CAT	18
tRNA-Tyr	9003-9064	62			GTA	5
12S-rRNA	9070-10074	1005				0
ND4L	10075-10326	252	TTG	TAA		78
ATP8	10405-10518	114	ATG	TAA		348
ND4	10867-11877	1011	ATT	TAA		10
tRNA-Ser	11888-11950	63			TGA	16
tRNA-His	11967-12028	62			GTG	8
tRNA-Glu	12037-12102	66			TTC	24
ATP6	12127-12882	756	ATA	TAA		59
ND3	12942-13346	405	ATT	TAG		38
ND5	13385-15082	1698	ATT	TAA		227
tRNA-Cys	15310-15371	62			GCA	8
tRNA-Asn	15380-15441	62			GTT	0
COX2	15442-16332	891	TTG	TAG		9
tRNA-Asp	16342-16406	65			GTC	927...

Table S6. *Mya arenaria* mitochondrial genome annotation

Mya arenaria						
Gene	Location	Size	Start codon	Stop codon	Anti-codon	Intergenic region
COX1	1-1791	1791	ATT	TAA		2
tRNA-Asn	1794-1859	66			GTT	470
ND4	2330-3361	1032	ATT	TAA		2
tRNA-Tyr	3364-3428	65			GTA	0
tRNA-Ser	3429-3491	63			TGA	5
ND1	3497-4408	912	ATG	TAA		96
CYTB	4505-5647	1143	GTG	TAA		12
tRNA-Gly	5660-5726	67			TCC	0
16S-rRNA	5727-7122	1396				0
ATP6	7123-7851	729	ATG	TAA		4
tRNA-Trp	7856-7922	67			TCA	6
tRNA-Ile	7929-7992	64			GAT	1
tRNA-Cys	7994-8056	63			GCA	0
tRNA-Leu	8057-8122	66			TAG	0
tRNA-Val	8123-8185	63			TAC	35
tRNA-Thr	8221-8286	66			TGT	5
ND5	8292-9998	1707	TTG	TAA		6
tRNA-Arg	10005-10069	65			TCG	0
COX3	10070-10930	861	ATA	TAG		34
ND6	10965-11513	549	ATT	TAG		15
tRNA-Asp	11529-11596	68			GTC	44
tRNA-His	11641-11705	65			GTG	-1
tRNA-Met	11705-11770	66			CAT	65
tRNA-Pro	11836-11899	64			TGG	180
tRNA-Met	12080-12148	69			CAT	838
COX2	12987-14420	1434	ATG	TAA		73
tRNA-Phe	14494-14557	64			GAA	39
ND4L	14597-14980	384	ATA	TAA		1
ND2	14982-16046	1065	ATG	TAA		7
tRNA-Gln	16054-16117	64			TTG	36
tRNA-Glu	16154-16219	66			TTC	0
ND3	16220-16642	423	ATG	TAG		21
tRNA-Lys	16664-16728	65			TTT	-6
tRNA-Ser	16723-16794	72			TCT	0
12S-rRNA	16795-17775	981				12
tRNA-Ala	17788-17851	64			TGC	19
tRNA-Leu	17871-17936	66			TAA	0

Table S7. *Spisula solidissima* mitochondrial genome annotation

Spisula solidissima						
Gene	Location	Size	Start codon	Stop codon	Anti-codon	Intergenic region
COX1	1-1707	1707	TTG	TAG		59
COX2	1767-3140	1374	ATG	TAG		32
tRNA-Asn	3173-3237	65			GTT	84
tRNA-Gln	3322-3388	67			TTG	37
tRNA-Ala	3426-3491	66			TGC	21
tRNA-Ile	3513-3578	66			GAT	159
tRNA-Gly	3738-3802	65			TCC	582
tRNA-Trp	4385-4449	65			TCA	0
ND6	4450-4941	492	ATT	TAG		105
ND2	5047-6090	1044	ATT	TAA		222
tRNA-Thr	6313-6374	62			TGT	188
tRNA-Pro	6563-6625	63			TGG	0
12S-rRNA	6626-7822	1197				-1
tRNA-Tyr	7822-7883	62			GTA	24
tRNA-Ser	7908-7971	64			TCT	1
COX3	7973-9085	1113	ATG	TAA		48
CYTB	9134-10330	1197	ATG	TAA		0
16S-rRNA	10331-11570	1240				1
ATP8	11572-11691	120	ATG	TAA		-1
ND4	11691-13046	1356	ATG	TAG		51
tRNA-Arg	13098-13159	62			TCG	-1
tRNA-Val	13159-13225	67			TAC	269
tRNA-His	13495-13556	62			GTG	0
tRNA-Glu	13557-13624	68			TTC	-5
tRNA-Ser	13620-13682	63			TGA	0
ATP6	13683-14429	747	GTG	TAG		29
ND3	14459-14872	414	ATG	TAG		8
tRNA-Lys	14881-14946	66			TTT	4
tRNA-Leu	14951-15016	66			TAA	1
ND1	15018-15932	915	ATG	TAG		6
tRNA-Asp	15938-15999	62			GTC	269
ND5	16269-18092	1824	ATA	TAG		12
ND4L	18105-18401	297	ATT	TAA		15
tRNA-Leu	18417-18483	67			TAG	9
tRNA-Cys	18493-18555	63			GCA	9
tRNA-Met	18565-18630	66			CAT	4
tRNA-Phe	18635-18700	66			GAA	47

Table S8. *Tivela mactroides* mitochondrial genome annotation

Tivela mactroides						
Gene	Location	Size	Start codon	Stop codon	Anti-codon	Intergenic region
tRNA-Trp	270-334	65			TCA	26
COX1	361-2043	1683	ATG	TAG		21
tRNA-Leu	2065-2129	65			TAG	1
ND1	2131-3048	918	ATG	TAA		102
ND4L	3151-3453	303	ATT	TAG		97
tRNA-Asp	3551-3613	63			GTC	72
COX2	3686-4738	1053	GTG	TAG		15
tRNA-Pro	4754-4817	64			TGG	46
CYTB	4864-6024	1161	GTG	TAA		1
16S-rRNA	6026-7342	1317				0
ATP8	7343-7459	117	ATG	TAG		7
ND4	7467-8828	1362	ATG	TAA		7
tRNA-His	8836-8898	63			GTG	-1
tRNA-Glu	8898-8962	65			TTC	-3
tRNA-Ser	8960-9024	65			TGA	4
ATP6	9029-9856	828	ATA	TAA		22
ND3	9879-10313	435	ATG	TAG		27
ND5	10341-12086	1746	ATT	TAA		56
ND6	12143-12739	597	ATG	TAA		40
tRNA-Val	12780-12846	67			TAC	40
tRNA-Phe	12887-12954	68			GAA	44
tRNA-Arg	12999-13063	65			TCG	73
tRNA-Lys	13137-13202	66			TTT	13
tRNA-Leu	13216-13279	64			TAA	2
tRNA-Gly	13282-13344	63			TCC	15
tRNA-Gln	13360-13427	68			TTG	13
tRNA-Asn	13441-13502	62			GTT	49
tRNA-Thr	13552-13618	67			TGT	0
12S-rRNA	13619-14593	975				0
tRNA-Met	14594-14658	65			CAT	8
tRNA-Cys	14667-14731	65			GCA	14
tRNA-Tyr	14746-14810	65			GTA	37
tRNA-Ser	14848-14920	73			TCT	-1
COX3	14920-15807	888	GTG	TAA		55
ND2	15863-16933	1071	ATT	TAA		46
tRNA-Ile	16980-17044	65			GAT	815
tRNA-Ala	17860-17924	65			TGC	1195...

ANNEXE VI.
AMINO ACID DIVERGENCES IN NINE HETERODONTA SPECIES

Table S1. Amino acid divergences in nine Heterodonta species: ATP6-8, COX1-3 and CYTB genes

Species 1	Species 2	ATP6	ATP8	COX1	COX2	COX3	CYTB
Anomalocardia_ brasiliana	*Macoma_ constricta*	0.687	0.676	0.378	0.638	0.535	0.437
Anomalocardia_ brasiliana	*Spisula_ solidissima*	0.687	0.649	0.257	0.538	0.383	0.453
Anomalocardia_ brasiliana	*Mactromeris_ polynyma*	0.687	0.649	0.248	0.512	0.395	0.317
Anomalocardia_ brasiliana	*Mya_arenaria _Qc*	0.673	-	0.308	0.612	0.578	0.445
Anomalocardia_ brasiliana	*Corbicula_ fluminea*	0.564	0.432	0.201	0.504	0.352	0.264
Anomalocardia_ brasiliana	*Tivela_ mactroides*	0.474	0.405	0.209	0.419	0.438	0.253
Anomalocardia_ brasiliana	*Arctica_islandica _MiSeq*	0.493	0.514	0.162	0.419	0.289	0.264
Anomalocardia_ brasiliana	*Mercenaria_ mercenaria*	0.142	0.216	0.041	0.192	0.090	0.136
Macoma_ constricta	*Spisula_ solidissima*	0.735	0.703	0.392	0.685	0.508	0.517
Macoma_ constricta	*Mactromeris_ polynyma*	0.777	0.676	0.365	0.681	0.551	0.445
Macoma_ constricta	*Mya_arenaria _Qc*	0.687	-	0.396	0.700	0.566	0.477
Macoma_ constricta	*Corbicula_ fluminea*	0.725	0.595	0.382	0.662	0.523	0.435
Macoma_ constricta	*Tivela_ mactroides*	0.697	0.676	0.400	0.662	0.574	0.416
Macoma_ constricta	*Arctica_islandica _MiSeq*	0.701	0.595	0.359	0.635	0.496	0.437
Macoma_ constricta	*Mercenaria_ mercenaria*	0.673	0.676	0.378	0.638	0.527	0.421

Table S2. Amino acid divergences in nine Heterodonta species: ATP6-8, COX1-3 and CYTB genes (suite 1)

Species 1	Species 2	ATP6	ATP8	COX1	COX2	COX3	CYTB
Spisula_ Solidissima	*Mactromeris_ polynyma*	0.555	0.405	0.154	0.365	0.309	0.400
Spisula_ Solidissima	*Mya_arenaria _Qc*	0.749	-	0.304	0.658	0.566	0.493
Spisula_ Solidissima	*Corbicula_ fluminea*	0.701	0.649	0.285	0.519	0.430	0.453
Spisula_ Solidissima	*Tivela_ mactroides*	0.720	0.595	0.302	0.550	0.500	0.464
Spisula_ Solidissima	*Arctica_islandica _MiSeq*	0.716	0.541	0.265	0.438	0.352	0.461
Spisula_ Solidissima	*Mercenaria_ mercenaria*	0.664	0.622	0.257	0.542	0.391	0.453
Mactromeris_ Polynyma	*Mya_arenaria _Qc*	0.773	-	0.312	0.642	0.602	0.440
Mactromeris_ Polynyma	*Corbicula_ fluminea*	0.706	0.703	0.275	0.508	0.441	0.333
Mactromeris_ Polynyma	*Tivela_ mactroides*	0.697	0.676	0.279	0.515	0.492	0.355
Mactromeris_ Polynyma	*Arctica_islandica _MiSeq*	0.678	0.595	0.230	0.450	0.375	0.347
Mactromeris_ Polynyma	*Mercenaria_ mercenaria*	0.697	0.595	0.246	0.500	0.395	0.344
Mya_arenaria _Qc	*Corbicula_ fluminea*	0.744	-	0.316	0.623	0.551	0.445
Mya_arenaria _Qc	*Tivela_ mactroides*	0.716	-	0.322	0.642	0.594	0.459
Mya_arenaria _Qc	*Arctica_islandica _MiSeq*	0.720	-	0.292	0.612	0.570	0.461
Mya_arenaria _Qc	*Mercenaria_ mercenaria*	0.682	-	0.304	0.638	0.590	0.437
Corbicula_ Fluminea	*Tivela_ mactroides*	0.607	0.514	0.267	0.527	0.477	0.275
Corbicula_ Fluminea	*Arctica_islandica _MiSeq*	0.521	0.568	0.148	0.412	0.336	0.237
Corbicula_ Fluminea	*Mercenaria_ mercenaria*	0.540	0.541	0.195	0.492	0.379	0.264
Tivela_ Mactroides	*Arctica_islandica _MiSeq*	0.573	0.514	0.236	0.442	0.484	0.272
Tivela_ Mactroides	*Mercenaria_ mercenaria*	0.498	0.459	0.193	0.419	0.469	0.227

Table S3. Amino acid divergences in nine Heterodonta species: ATP6-8, COX1-3 and CYTB genes (suite 2)

Species 1	Species 2	ATP6	ATP8	COX1	COX2	COX3	CYTB
Arctica_islandica _MiSeq	*Mercenaria_ mercenaria*	0.502	0.486	0.148	0.423	0.297	0.259

NOTE. *M. arenaria* do not possess ATP8 gene. The second COX2 (1311 bp) was used for *M. polynyma*.

Table S4. Amino acid divergences in nine Heterodonta species: ND1-6 and ND4L genes

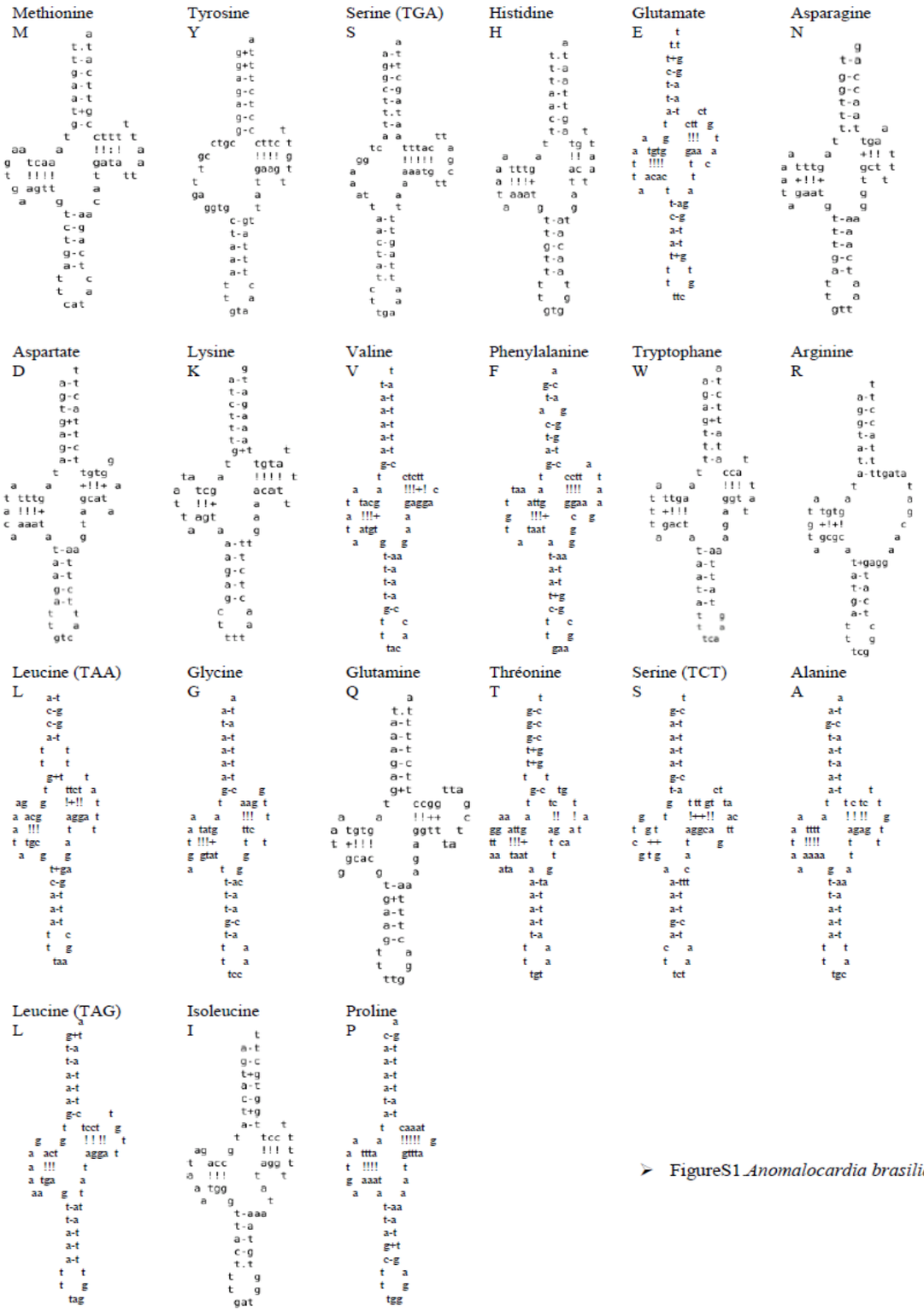
Species 1	Species 2	ND1	ND2	ND3	ND4	ND4L	ND5	ND6
Anomalocardia_ Brasiliana	*Macoma_ constricta*	0.548	0.658	0.867	0.494	0.687	0.583	0.796
Anomalocardia_ Brasiliana	*Spisula_ solidissima*	0.412	0.767	0.867	0.472	0.639	0.585	0.768
Anomalocardia_ Brasiliana	*Mactromeris_ polynyma*	0.439	0.674	0.855	0.497	0.627	0.576	0.718
Anomalocardia_ Brasiliana	*Mya_arenaria _Qc*	0.455	0.637	0.855	0.479	0.687	0.587	0.796
Anomalocardia_ Brasiliana	*Corbicula _fluminea*	0.326	0.525	0.880	0.387	0.542	0.510	0.577
Anomalocardia_ Brasiliana	*Tivela _mactroides*	0.362	0.481	0.892	0.347	0.470	0.466	0.563
Anomalocardia_ Brasiliana	*Arctica_islandica _MiSeq*	0.326	0.543	0.867	0.383	0.566	0.506	0.577
Anomalocardia_ Brasiliana	*Mercenaria_ mercenaria*	0.226	0.357	0.855	0.261	0.289	0.321	0.373
Macoma_ Constricta	*Spisula_ solidissima*	0.515	0.739	0.651	0.546	0.711	0.604	0.768
Macoma_ Constricta	*Mactromeris_ polynyma*	0.518	0.730	0.663	0.558	0.723	0.609	0.746
Macoma_ Constricta	*Mya_arenaria _Qc*	0.522	0.661	0.651	0.558	0.735	0.583	0.852
Macoma_ Constricta	*Corbicula_ fluminea*	0.515	0.658	0.578	0.506	0.627	0.567	0.761
Macoma_ Constricta	*Tivela_ mactroides*	0.505	0.646	0.590	0.506	0.651	0.583	0.761
Macoma_ Constricta	*Arctica_islandica _MiSeq*	0.502	0.640	0.566	0.546	0.639	0.583	0.803
Macoma_ Constricta	*Mercenaria_ mercenaria*	0.525	0.665	0.566	0.497	0.699	0.611	0.782
Spisula_ Solidissima	*Mactromeris_ polynyma*	0.189	0.652	0.277	0.273	0.313	0.440	0.437

Table S5. Amino acid divergences in nine Heterodonta species: ND1-6 and ND4L genes (suite)

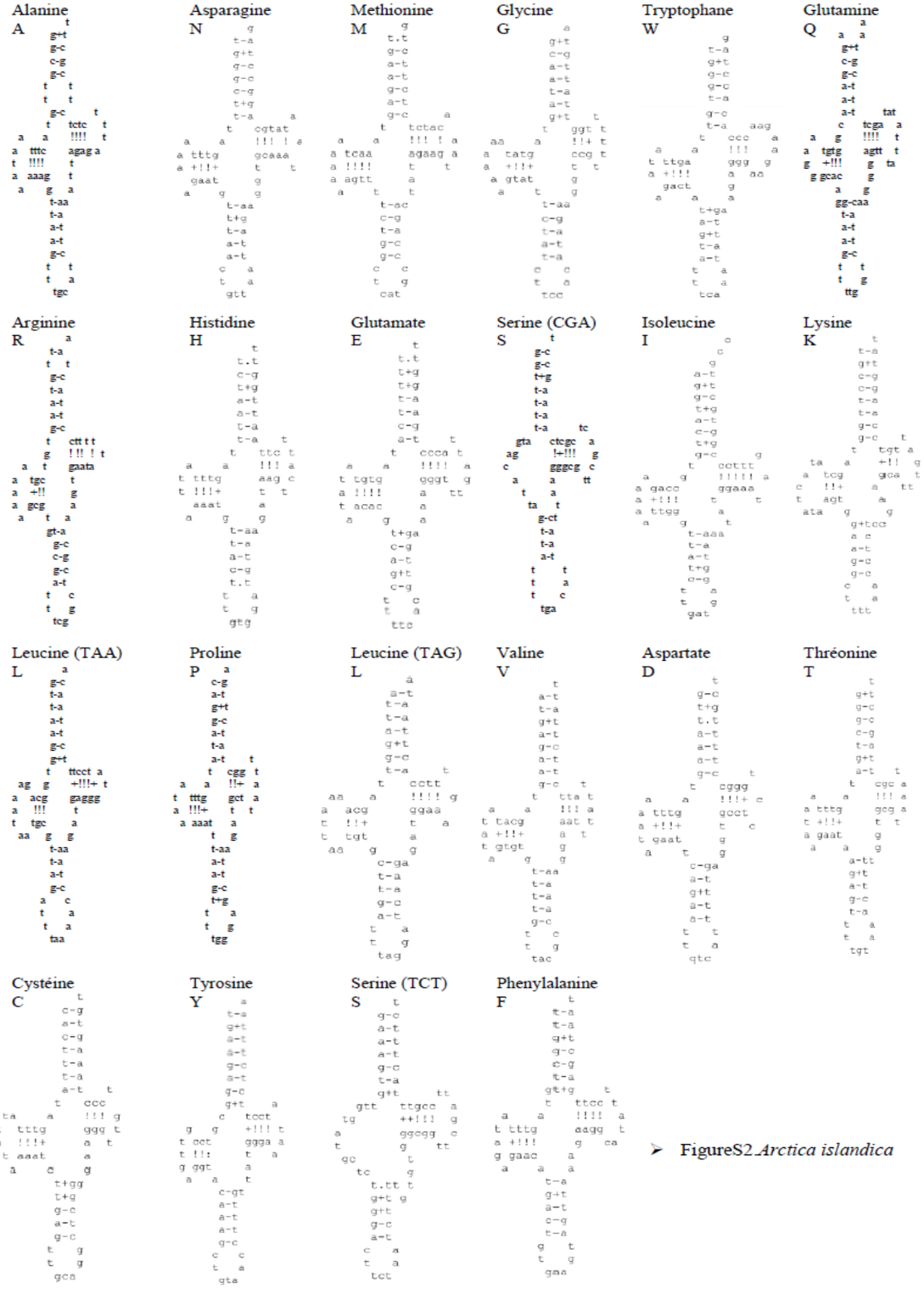
Species 1	Species 2	ND1	ND2	ND3	ND4	ND4L	ND5	ND6
Spisula_ solidissima	*Mya_arenaria _Qc*	0.465	0.776	0.578	0.521	0.651	0.620	0.796
Spisula_ solidissima	*Corbicula_ fluminea*	0.365	0.733	0.602	0.469	0.614	0.567	0.690
Spisula_ solidissima	*Tivela_ mactroides*	0.402	0.733	0.566	0.466	0.614	0.596	0.732
Spisula_ solidissima	*Arctica_islandica _MiSeq*	0.375	0.742	0.566	0.460	0.518	0.565	0.739
Spisula_ solidissima	*Mercenaria_ mercenaria*	0.402	0.767	0.590	0.475	0.602	0.572	0.761
Mactromeris_ polynyma	*Mya_arenaria _Qc*	0.485	0.711	0.578	0.546	0.663	0.607	0.768
Mactromeris_ polynyma	*Corbicula_ fluminea*	0.385	0.686	0.530	0.472	0.602	0.583	0.697
Mactromeris_ polynyma	*Tivela_ mactroides*	0.412	0.671	0.506	0.500	0.675	0.567	0.746
Mactromeris_ polynyma	*Arctica_islandica _MiSeq*	0.385	0.696	0.530	0.463	0.542	0.593	0.725
Mactromeris_ polynyma	*Mercenaria_ mercenaria*	0.405	0.680	0.590	0.472	0.614	0.563	0.732
Mya_arenaria _Qc	*Corbicula_ fluminea*	0.458	0.649	0.590	0.521	0.639	0.552	0.782
Mya_arenaria _Qc	*Tivela_ mactroides*	0.472	0.630	0.614	0.512	0.675	0.582	0.768
Mya_arenaria _Qc	*Arctica_islandica _MiSeq*	0.445	0.671	0.627	0.525	0.614	0.549	0.796
Mya_arenaria _Qc	*Mercenaria_ mercenaria*	0.432	0.624	0.614	0.482	0.735	0.613	0.796
Corbicula _fluminea	*Tivela_ mactroides*	0.349	0.556	0.458	0.371	0.651	0.539	0.599
Corbicula _fluminea	*Arctica_islandica _MiSeq*	0.239	0.522	0.325	0.347	0.518	0.406	0.549
Corbicula _fluminea	*Mercenaria_ mercenaria*	0.326	0.562	0.446	0.383	0.542	0.506	0.563
Tivela_ mactroides	*Arctica_islandica _MiSeq*	0.306	0.534	0.386	0.393	0.590	0.519	0.627
Tivela_ mactroides	*Mercenaria_ mercenaria*	0.322	0.469	0.361	0.356	0.446	0.472	0.570
Arctica_islandica _MiSeq	*Mercenaria_ mercenaria*	0.322	0.525	0.373	0.371	0.494	0.514	0.606

ANNEXE VII.

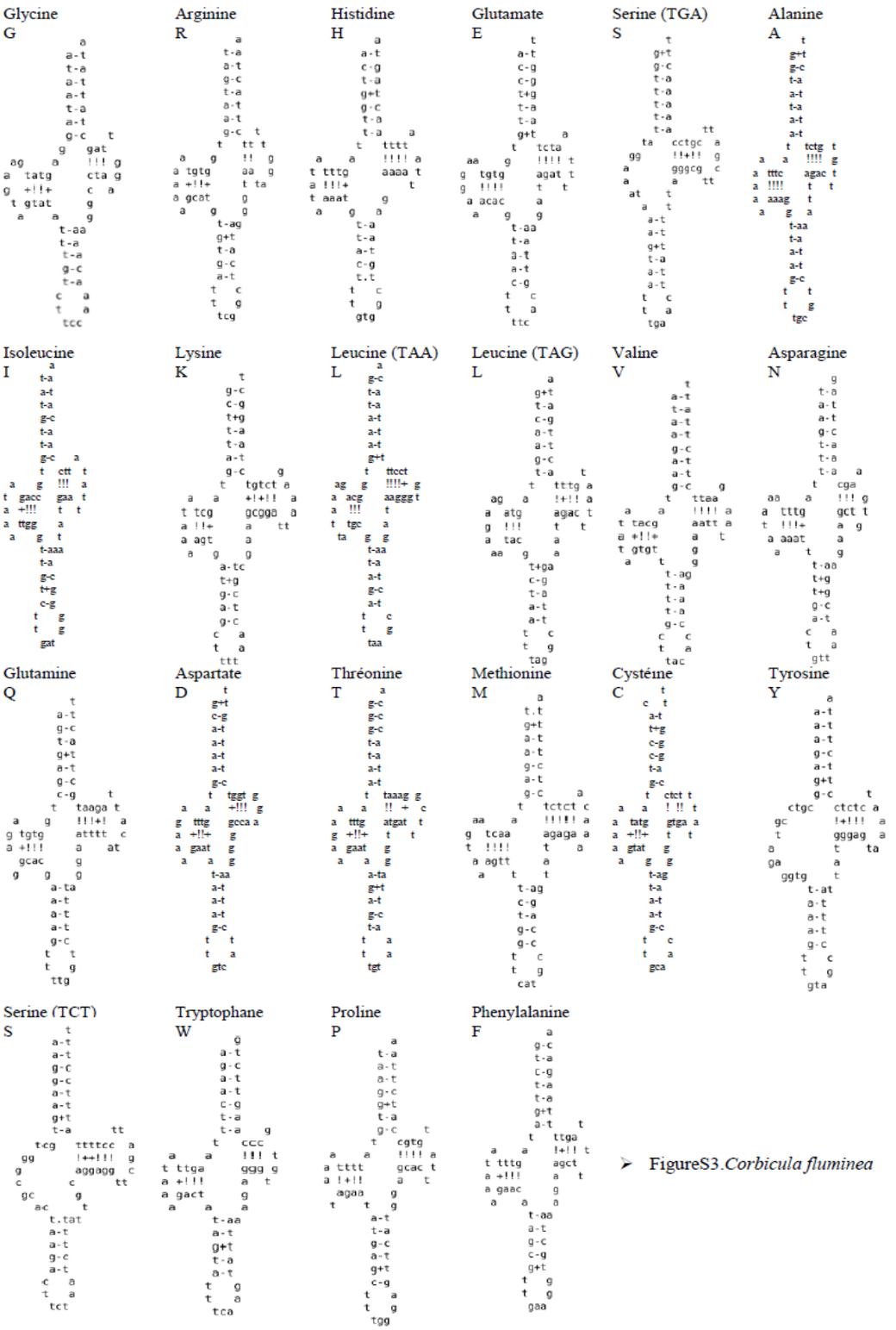
TRNA CLOVERLEAF STRUCTURES FOR NINE HETERODONTA SPECIES



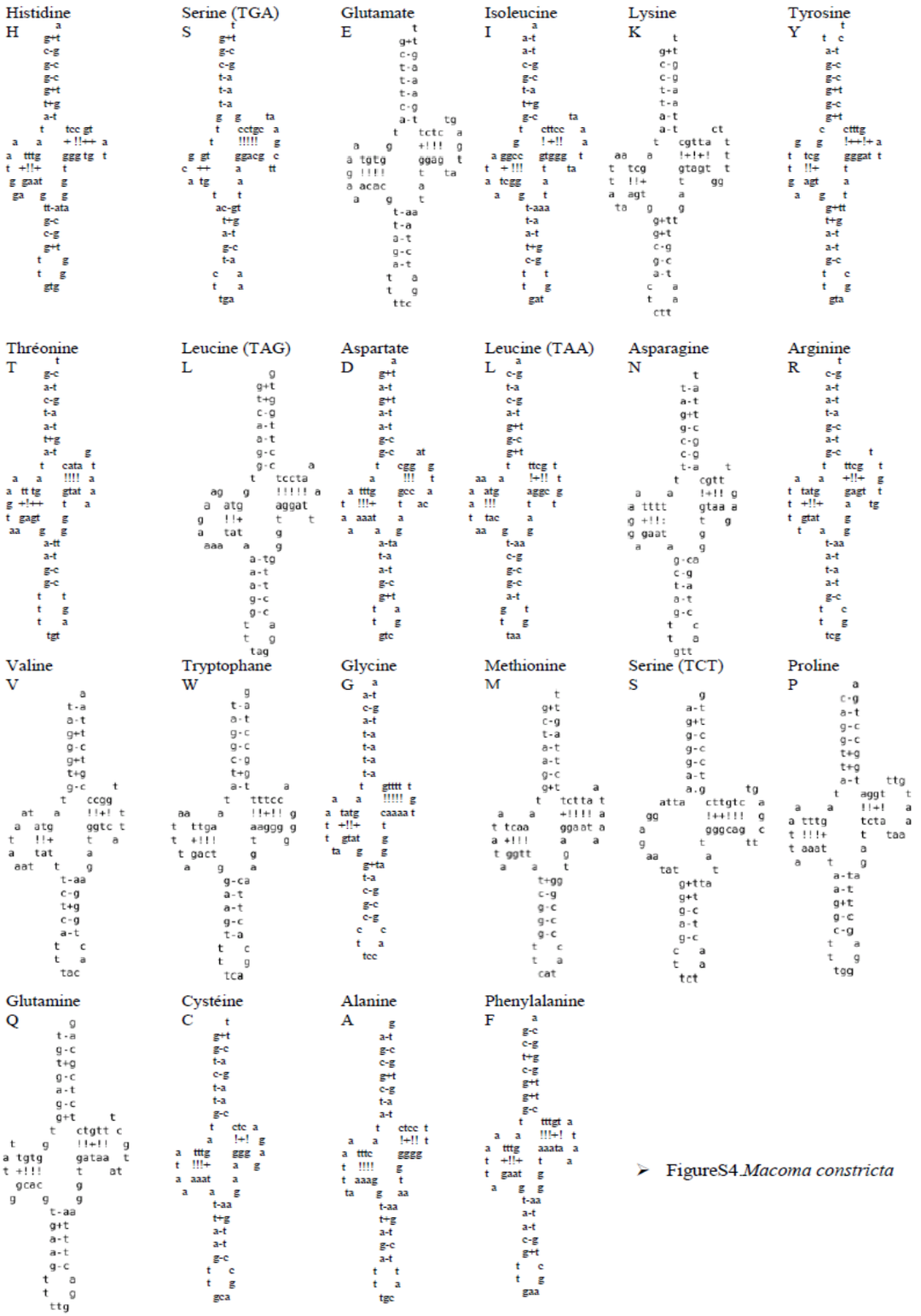
➢ FigureS1 *Anomalocardia brasiliana*



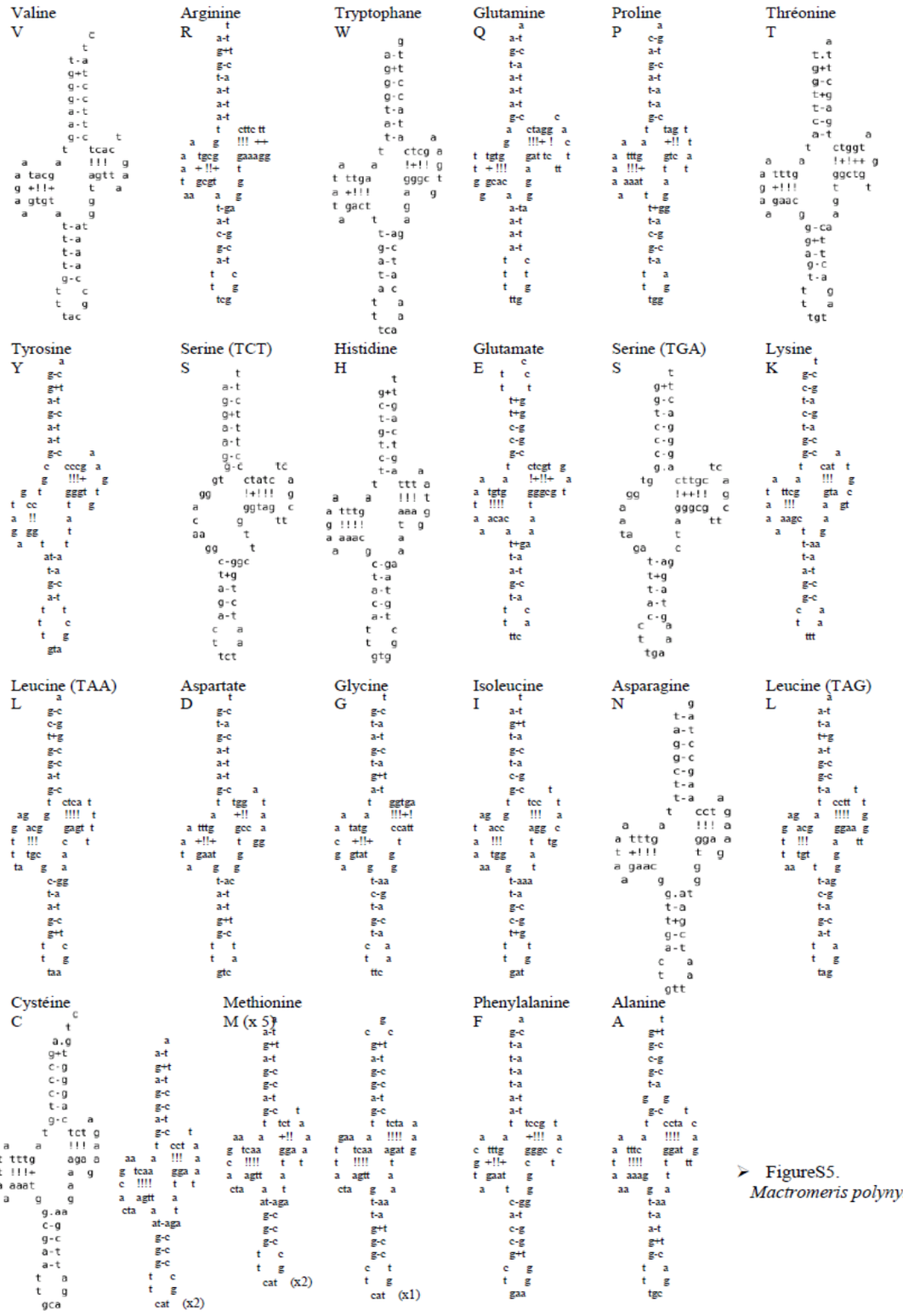
➢ FigureS2 *Arctica islandica*



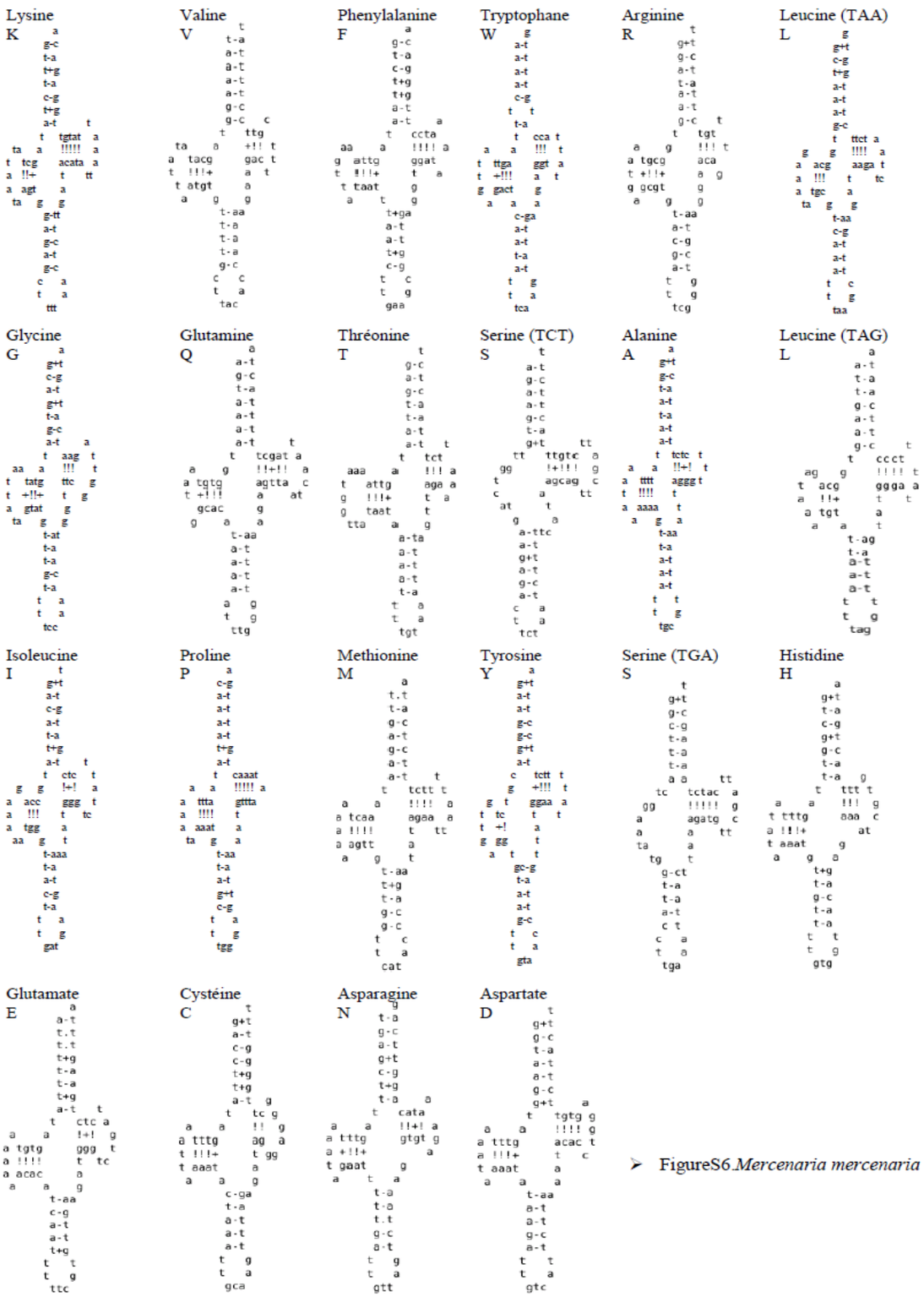
➢ FigureS3.*Corbicula fluminea*



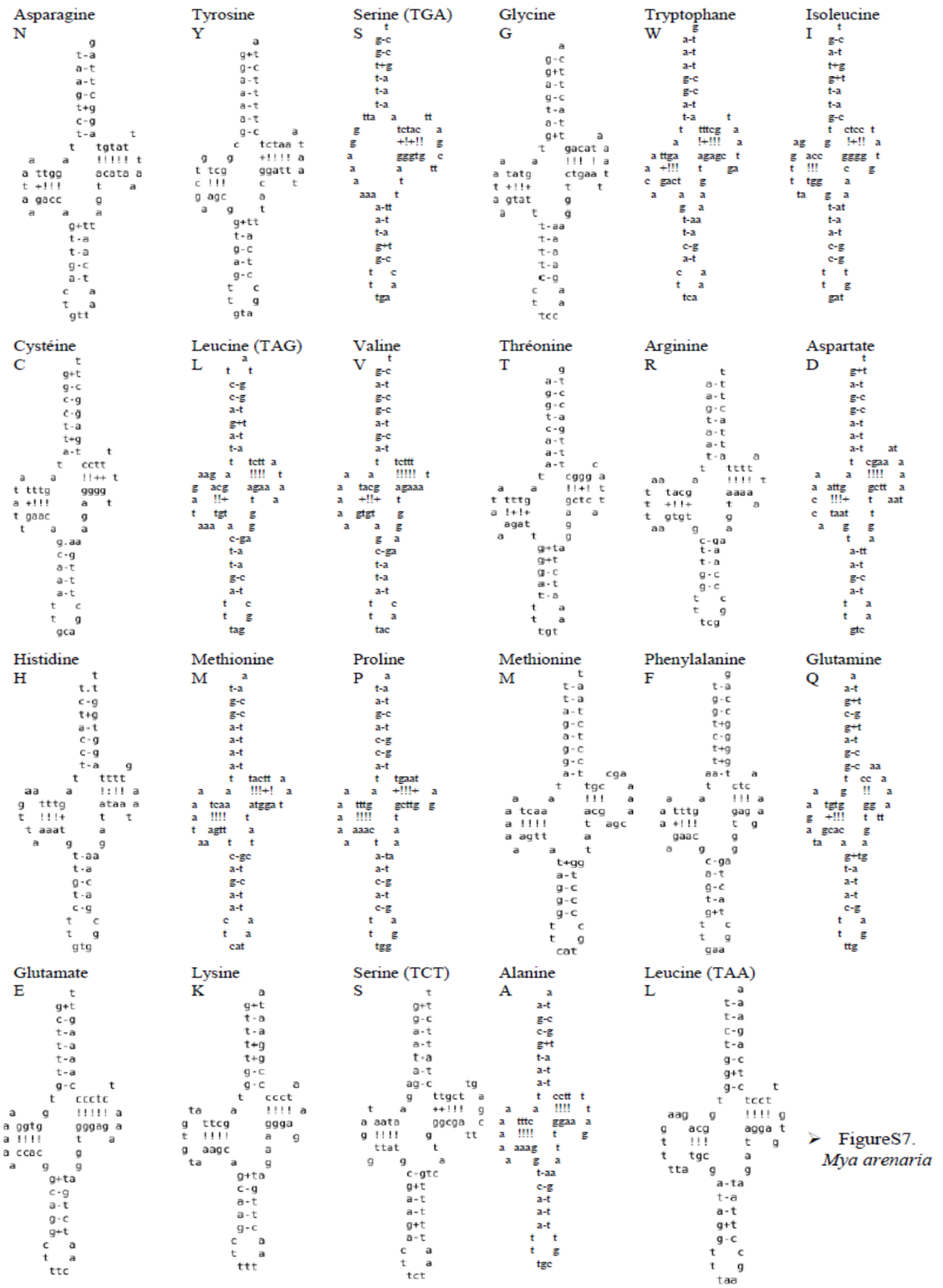
- FigureS4 *Macoma constricta*



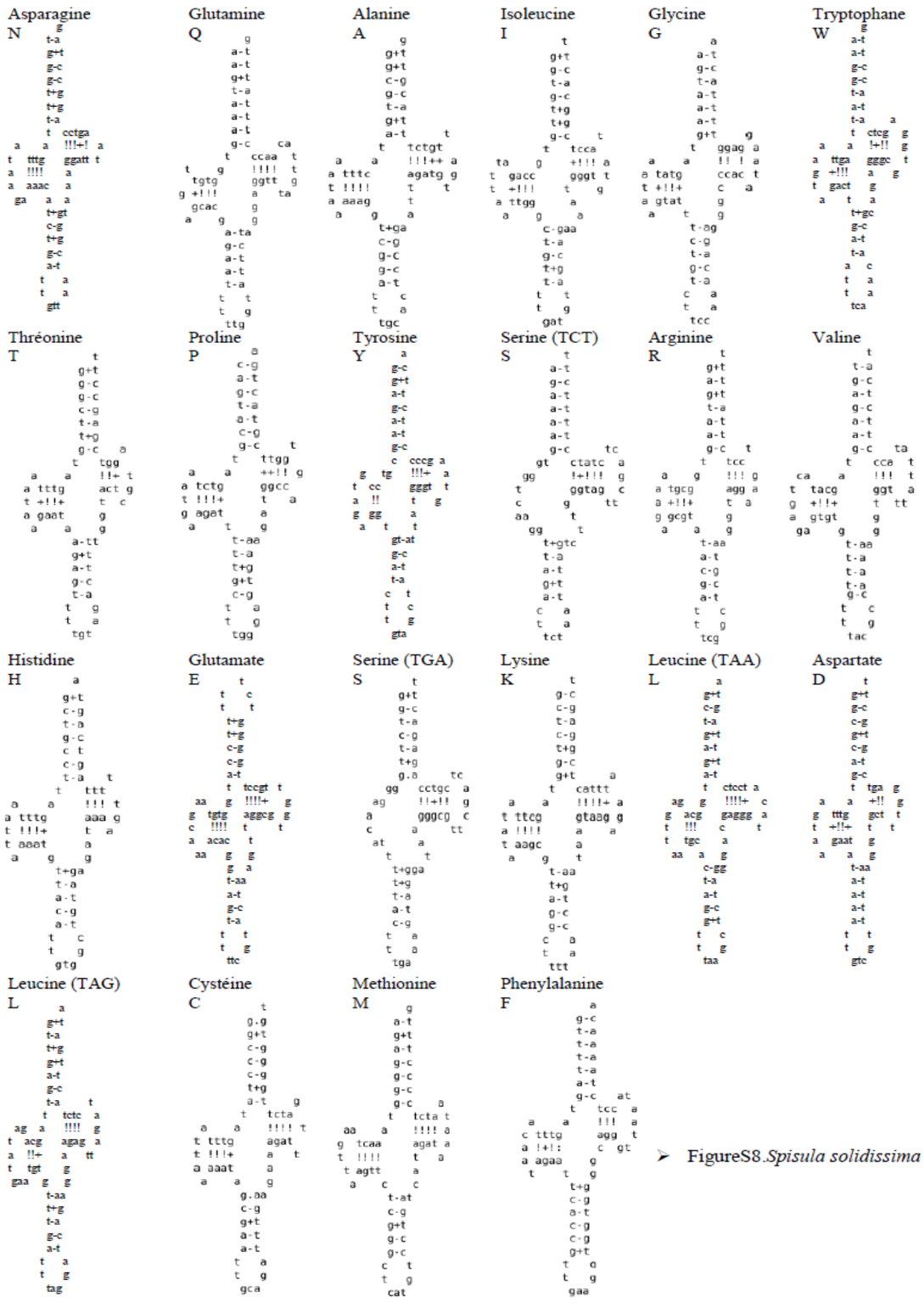
➢ FigureS5.
Mactromeris polynyma



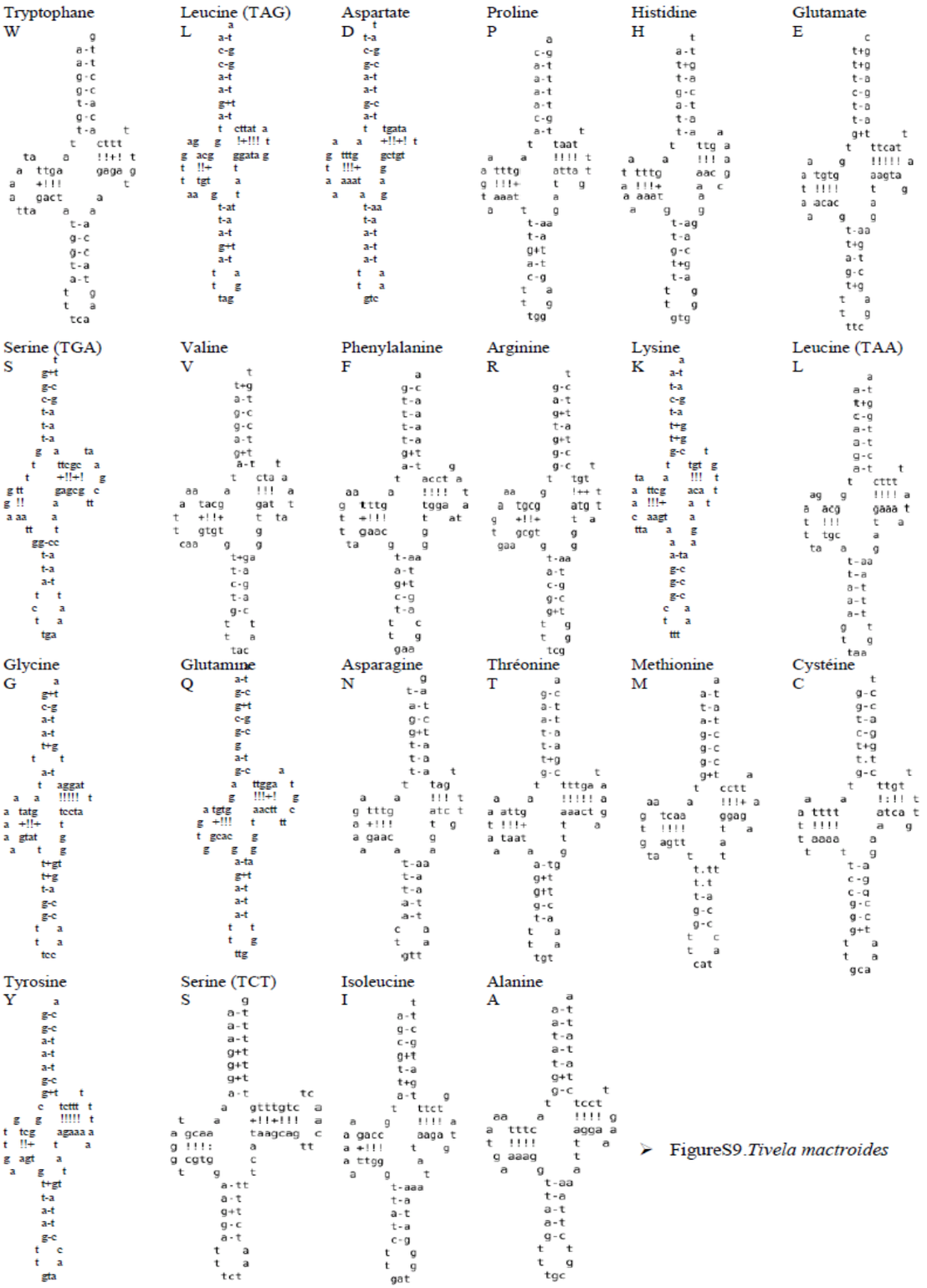
➢ FigureS6 *Mercenaria mercenaria*



➢ FigureS7. *Mya arenaria*



➢ FigureS8.*Spisula solidissima*



➢ FigureS9.*Tivela mactroides*

ANNEXE VIII.

TANDEM REPEAT REGIONS IN SEVEN HETERODONTA SPECIES

	Location	Bases number (xtime)	mtDNA region
Arctica islandica	6239-6268	15 (x2)	MNR 1
	9055-9088	16 (x2.1)*	ND4L
	15125-15167	8 (x5.4)	MNR 2
	15117-15167	24 (x2.1)*	
Corbicula fluminea	15613-15649	2 (x18.5)	MNR
	15660-15704	18 (x2.5)*	
	15675-15741	16 (x4.1)*	
	15675-15737	33 (x1.9)*	
Macoma constricta	10536-10572	14 (x2.7)	MNR
Mactromeris polynyma	13933-13989	29 (x2)	MNR 1
	13964-14029	20 (x3.3)	
	13964-14030	10 (x6.7)*	
	22591-22990	99 (x40)*	tRNA-Met 2-5
Mya arenaria	13410-13512	14 (x2.7)*	COX2
Spisula solidissima	1646-1686	12 (x3.4)*	COX1
	1646-1694	12 (x4.1)*	
	1646-1694	24 (x2)*	
	13314-13363	1 (x50)	MNR 2
	16189-16249	24 (x2.6)*	MNR 3
Tivela mactroides	6021-6083	31 (x2)	16S

NOTE. *, differences in tandem repeat. *Anomalocardia brasiliana* and *Mercenaria mercenaria* do not have any tandem repeat regions.

ANNEXE IX.
GC/AT SKEW AND GC CONTENT IN NINE HETERODONTA SPECIES

	A. brasiliana	***M. mercenaria***	***T. mactroides***	***M. polynyma***	***S. solidissima***	***A. Islandica***	***C. fluminea***	***M. arenaria***	***M. constricta***
GC skew	0.330	0.397	0.362	0.249	0.265	0.306	0.394	0.317	0.410
AT skew	-0.218	-0.226	-0.194	-0.153	-0.254	-0.164	-0.238	-0.131	-0.286
G+C % total	31.5	30.8	33.7	41.2	38.7	30.7	30.7	34.3	38.0
COX1	34.6	36.2	37.9	39.8	39.8	34.3	34.2	36.9	40.5
COX2	32.4	31.6	37.5	43.8	44.7	31.1	31.5	35.6	36.9
COX3	36.1	34.4	35.2	40.7	38.3	32.6	33.7	37.4	40.1
CYTB	34.0	33.8	38.4	39.2	39.5	32.7	30.4	34.8	38.8
ND1	32.6	33.7	36.7	44.4	40.5	32.5	33.4	37.1	39.5
ND2	30.9	29.6	34.5	40.5	39.4	28.6	31.7	34.8	37.1
ND3	30.4	31.4	32.6	40.2	38.6	28.5	30.3	32.2	38.6
ND4	33.7	31.5	34.4	40.9	40.5	31.1	32.2	38.6	38.7
ND4L	25.0	28.6	28.7	39.2	31.3	23.9	24.7	29.7	35.0
ND5	29.9	31.0	34.1	41.0	38.8	29.8	29.3	34.4	38.7
ND6	30.8	31.3	37.0	39.1	36.8	30.4	32.3	34.2	38.5
ATP6	32.1	32.0	32.4	37.6	39.2	31.9	27.9	33.6	36.3
ATP8	28.9	29.8	30.8	34.2	31.7	32.5	30.7	/	37.3
12S	30.3	28.6	31.8	39.6	38.6	33.1	33.8	33.8	36.0
16S	/	28.4	27.5	38.9	34.5	30.5	26.4	31.7	36.6
MNR	33	31.6 – 26.6	33.6 – 31.5	44.1 – 39.8	29.0 – 34.6– 42	27.2– 27.7	25.1	33.8 – 29.2	33.7

ANNEXE X.
CODON USAGE IN NINE HETERODONTA SPECIES

aa. amino acid; N. number of codon; RSCU. relative synonymous codon usage

Table S1. *Anomalocardia brasiliana* partial mitochondrial genome codon usage

Anomalocardia brasiliana (partial genome)											
Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU
UUU(F)	426	1.59	UCU(S)	72	1.22	UAU(Y)	204	1.47	UGU(C)	164	1.56
UUC(F)	110	0.41	UCC(S)	30	0.51	UAC(Y)	73	0.53	UGC(C)	46	0.44
UUA(L)	227	2.15	UCA(S)	53	0.9	UAA(*)	184	1.13	UGA(W)	80	0.85
UUG(L)	150	1.42	UCG(S)	27	0.46	UAG(*)	141	0.87	UGG(W)	109	1.15
CUU(L)	118	1.12	CCU(P)	20	1.25	CAU(H)	41	1.28	CGU(R)	26	1.51
CUC(L)	37	0.35	CCC(P)	16	1	CAC(H)	23	0.72	CGC(R)	5	0.29
CUA(L)	59	0.56	CCA(P)	16	1	CAA(Q)	34	1.19	CGA(R)	14	0.81
CUG(L)	43	0.41	CCG(P)	12	0.75	CAG(Q)	23	0.81	CGG(R)	24	1.39
AUU(I)	185	1.64	ACU(T)	66	2.05	AAU(N)	147	1.55	AGU(S)	117	1.98
AUC(I)	41	0.36	ACC(T)	15	0.47	AAC(N)	43	0.45	AGC(S)	31	0.52
AUA(M)	132	1.29	ACA(T)	30	0.93	AAA(K)	145	1.21	AGA(S)	72	1.22
AUG(M)	72	0.71	ACG(T)	18	0.56	AAG(K)	94	0.79	AGG(S)	71	1.2
GUU(V)	192	1.85	GCU(A)	45	1.88	GAU(D)	61	1.56	GGU(G)	124	1.91
GUC(V)	40	0.39	GCC(A)	11	0.46	GAC(D)	17	0.44	GGC(G)	24	0.37
GUA(V)	83	0.8	GCA(A)	17	0.71	GAA(E)	85	1.13	GGA(G)	37	0.57
GUG(V)	100	0.96	GCG(A)	23	0.96	GAG(E)	66	0.87	GGG(G)	75	1.15

NOTE. Average codons=4886

Table S2. *Arctica islandica* mitochondrial genome codon usage

Arctica islandica											
Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU
UUU(F)	537	1.67	UCU(S)	104	1.27	UAU(Y)	253	1.69	UGU(C)	155	1.51
UUC(F)	106	0.33	UCC(S)	36	0.44	UAC(Y)	46	0.31	UGC(C)	50	0.49
UUA(L)	318	2.45	UCA(S)	70	0.85	UAA(*)	226	1.17	UGA(W)	118	1.1
UUG(L)	154	1.19	UCG(S)	29	0.35	UAG(*)	160	0.83	UGG(W)	96	0.9
CUU(L)	143	1.1	CCU(P)	62	2	CAU(H)	58	1.45	CGU(R)	28	1.3
CUC(L)	40	0.31	CCC(P)	25	0.81	CAC(H)	22	0.55	CGC(R)	13	0.6
CUA(L)	69	0.53	CCA(P)	22	0.71	CAA(Q)	50	1.2	CGA(R)	21	0.98
CUG(L)	54	0.42	CCG(P)	15	0.48	CAG(Q)	33	0.8	CGG(R)	24	1.12
AUU(I)	296	1.73	ACU(T)	77	1.83	AAU(N)	181	1.59	AGU(S)	116	1.41
AUC(I)	47	0.27	ACC(T)	23	0.55	AAC(N)	46	0.41	AGC(S)	50	0.61
AUA(M)	196	1.38	ACA(T)	50	1.19	AAA(K)	210	1.29	AGA(S)	143	1.74
AUG(M)	89	0.62	ACG(T)	18	0.43	AAG(K)	115	0.71	AGG(S)	109	1.33
GUU(V)	186	1.99	GCU(A)	79	2.05	GAU(D)	98	1.46	GGU(G)	126	1.37
GUC(V)	18	0.19	GCC(A)	18	0.47	GAC(D)	36	0.54	GGC(G)	41	0.45
GUA(V)	104	1.12	GCA(A)	40	1.04	GAA(E)	105	1.24	GGA(G)	93	1.01
GUG(V)	65	0.7	GCG(A)	17	0.44	GAG(E)	65	0.76	GGG(G)	107	1.17

NOTE. Average codons=6101

Table S3. *Corbicula fluminea* mitochondrial genome codon usage

Corbicula fluminea											
Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU
UUU(F)	620	1.73	UCU(S)	72	1.02	UAU(Y)	196	1.54	UGU(C)	173	1.53
UUC(F)	98	0.27	UCC(S)	44	0.62	UAC(Y)	58	0.46	UGC(C)	53	0.47
UUA(L)	284	2.27	UCA(S)	59	0.84	UAA(*)	228	1.28	UGA(W)	121	0.89
UUG(L)	202	1.61	UCG(S)	20	0.28	UAG(*)	129	0.72	UGG(W)	152	1.11
CUU(L)	134	1.07	CCU(P)	32	1.6	CAU(H)	37	1.64	CGU(R)	24	1.28
CUC(L)	28	0.22	CCC(P)	15	0.75	CAC(H)	8	0.36	CGC(R)	3	0.16
CUA(L)	52	0.42	CCA(P)	19	0.95	CAA(Q)	27	1.23	CGA(R)	26	1.39
CUG(L)	51	0.41	CCG(P)	14	0.7	CAG(Q)	17	0.77	CGG(R)	22	1.17
AUU(I)	218	1.74	ACU(T)	57	2.09	AAU(N)	149	1.63	AGU(S)	121	1.72
AUC(I)	33	0.26	ACC(T)	13	0.48	AAC(N)	34	0.37	AGC(S)	43	0.61
AUA(M)	125	1.17	ACA(T)	26	0.95	AAA(K)	199	1.26	AGA(S)	96	1.36
AUG(M)	88	0.83	ACG(T)	13	0.48	AAG(K)	116	0.74	AGG(S)	109	1.55
GUU(V)	200	2.04	GCU(A)	71	2.22	GAU(D)	76	1.71	GGU(G)	145	1.72
GUC(V)	34	0.35	GCC(A)	19	0.59	GAC(D)	13	0.29	GGC(G)	34	0.4
GUA(V)	79	0.8	GCA(A)	18	0.56	GAA(E)	93	1.19	GGA(G)	70	0.83
GUG(V)	80	0.81	GCG(A)	20	0.63	GAG(E)	63	0.81	GGG(G)	89	1.05

NOTE. Average codons=5562

Table S4. *Macoma constricta* mitochondrial genome codon usage

Macoma constricta											
Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU
UUU(F)	364	1.69	UCU(S)	83	1.34	UAU(Y)	165	1.59	UGU(C)	153	1.51
UUC(F)	68	0.31	UCC(S)	20	0.32	UAC(Y)	43	0.41	UGC(C)	50	0.49
UUA(L)	246	1.9	UCA(S)	19	0.31	UAA(*)	94	0.95	UGA(W)	95	0.76
UUG(L)	260	2.01	UCG(S)	32	0.52	UAG(*)	103	1.05	UGG(W)	155	1.24
CUU(L)	101	0.78	CCU(P)	66	2.03	CAU(H)	72	1.64	CGU(R)	42	1.66
CUC(L)	30	0.23	CCC(P)	21	0.65	CAC(H)	16	0.36	CGC(R)	8	0.32
CUA(L)	64	0.5	CCA(P)	16	0.49	CAA(Q)	31	1.03	CGA(R)	19	0.75
CUG(L)	74	0.57	CCG(P)	27	0.83	CAG(Q)	29	0.97	CGG(R)	32	1.27
AUU(I)	210	1.73	ACU(T)	62	1.7	AAU(N)	111	1.71	AGU(S)	120	1.94
AUC(I)	33	0.27	ACC(T)	23	0.63	AAC(N)	19	0.29	AGC(S)	46	0.74
AUA(M)	144	0.94	ACA(T)	38	1.04	AAA(K)	62	0.91	AGA(S)	79	1.28
AUG(M)	164	1.06	ACG(T)	23	0.63	AAG(K)	75	1.09	AGG(S)	96	1.55
GUU(V)	268	1.79	GCU(A)	110	2.06	GAU(D)	88	1.63	GGU(G)	180	1.56
GUC(V)	49	0.33	GCC(A)	30	0.56	GAC(D)	20	0.37	GGC(G)	60	0.52
GUA(V)	115	0.77	GCA(A)	39	0.73	GAA(E)	70	0.95	GGA(G)	95	0.82
GUG(V)	168	1.12	GCG(A)	35	0.65	GAG(E)	78	1.05	GGG(G)	128	1.11

NOTE. Average codons=5436

Table S5. *Mactromeris polynyma* mitochondrial genome codon usage

Mactromeris polynyma											
Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU
UUU(F)	442	1.49	UCU(S)	141	1.27	UAU(Y)	173	1.24	UGU(C)	158	1.34
UUC(F)	153	0.51	UCC(S)	63	0.57	UAC(Y)	107	0.76	UGC(C)	77	0.66
UUA(L)	281	1.87	UCA(S)	74	0.67	UAA(*)	120	0.98	UGA(W)	135	0.93
UUG(L)	170	1.13	UCG(S)	72	0.65	UAG(*)	124	1.02	UGG(W)	155	1.07
CUU(L)	171	1.14	CCU(P)	101	1.64	CAU(H)	114	1.5	CGU(R)	52	0.9
CUC(L)	64	0.43	CCC(P)	54	0.87	CAC(H)	38	0.5	CGC(R)	35	0.61
CUA(L)	104	0.69	CCA(P)	42	0.68	CAA(Q)	52	0.83	CGA(R)	67	1.17
CUG(L)	110	0.73	CCG(P)	50	0.81	CAG(Q)	73	1.17	CGG(R)	76	1.32
AUU(I)	246	1.56	ACU(T)	98	1.41	AAU(N)	161	1.38	AGU(S)	137	1.24
AUC(I)	69	0.44	ACC(T)	49	0.71	AAC(N)	72	0.62	AGC(S)	75	0.68
AUA(M)	151	0.92	ACA(T)	64	0.92	AAA(K)	215	1.12	AGA(S)	115	1.04
AUG(M)	177	1.08	ACG(T)	67	0.96	AAG(K)	169	0.88	AGG(S)	210	1.89
GUU(V)	227	1.43	GCU(A)	143	1.63	GAU(D)	109	1.22	GGU(G)	168	1.13
GUC(V)	70	0.44	GCC(A)	80	0.91	GAC(D)	70	0.78	GGC(G)	91	0.61
GUA(V)	143	0.9	GCA(A)	63	0.72	GAA(E)	118	0.93	GGA(G)	105	0.71
GUG(V)	195	1.23	GCG(A)	65	0.74	GAG(E)	137	1.07	GGG(G)	229	1.54

NOTE. Average codons=7736

Table S6. *Mercenaria mercenaria* partial mitochondrial genome codon usage

Mercenaria mercenaria (partial genome)											
Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU
UUU(F)	517	1.67	UCU(S)	105	1.56	UAU(Y)	223	1.66	UGU(C)	160	1.62
UUC(F)	102	0.33	UCC(S)	21	0.31	UAC(Y)	45	0.34	UGC(C)	38	0.38
UUA(L)	308	2.48	UCA(S)	38	0.57	UAA(*)	151	1.16	UGA(W)	98	0.93
UUG(L)	211	1.7	UCG(S)	19	0.28	UAG(*)	109	0.84	UGG(W)	113	1.07
CUU(L)	112	0.9	CCU(P)	64	2.49	CAU(H)	62	1.68	CGU(R)	38	1.83
CUC(L)	14	0.11	CCC(P)	6	0.23	CAC(H)	12	0.32	CGC(R)	6	0.29
CUA(L)	54	0.43	CCA(P)	16	0.62	CAA(Q)	45	1.29	CGA(R)	21	1.01
CUG(L)	46	0.37	CCG(P)	17	0.66	CAG(Q)	25	0.71	CGG(R)	18	0.87
AUU(I)	250	1.75	ACU(T)	82	2.41	AAU(N)	198	1.64	AGU(S)	152	2.26
AUC(I)	35	0.25	ACC(T)	14	0.41	AAC(N)	44	0.36	AGC(S)	28	0.42
AUA(M)	167	1.2	ACA(T)	24	0.71	AAA(K)	177	1.24	AGA(S)	85	1.27
AUG(M)	111	0.8	ACG(T)	16	0.47	AAG(K)	108	0.76	AGG(S)	89	1.33
GUU(V)	233	1.95	GCU(A)	95	2.5	GAU(D)	99	1.58	GGU(G)	169	1.86
GUC(V)	33	0.28	GCC(A)	11	0.29	GAC(D)	26	0.42	GGC(G)	17	0.19
GUA(V)	115	0.96	GCA(A)	25	0.66	GAA(E)	91	1.1	GGA(G)	79	0.87
GUG(V)	97	0.81	GCG(A)	21	0.55	GAG(E)	74	0.9	GGG(G)	99	1.09

NOTE. Average codons=5678

Table S7. *Mya arenaria* mitochondrial genome codon usage

Mya arenaria											
Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU
UUU(F)	448	1.62	UCU(S)	95	1.15	UAU(Y)	177	1.47	UGU(C)	148	1.49
UUC(F)	106	0.38	UCC(S)	39	0.47	UAC(Y)	64	0.53	UGC(C)	50	0.51
UUA(L)	251	2.06	UCA(S)	74	0.9	UAA(*)	207	1.18	UGA(W)	97	0.85
UUG(L)	149	1.22	UCG(S)	33	0.4	UAG(*)	143	0.82	UGG(W)	131	1.15
CUU(L)	137	1.13	CCU(P)	41	1.34	CAU(H)	47	1.42	CGU(R)	35	1.33
CUC(L)	49	0.4	CCC(P)	26	0.85	CAC(H)	19	0.58	CGC(R)	6	0.23
CUA(L)	73	0.6	CCA(P)	33	1.08	CAA(Q)	57	0.93	CGA(R)	24	0.91
CUG(L)	71	0.58	CCG(P)	22	0.72	CAG(Q)	66	1.07	CGG(R)	40	1.52
AUU(I)	223	1.65	ACU(T)	77	1.67	AAU(N)	198	1.58	AGU(S)	137	1.66
AUC(I)	47	0.35	ACC(T)	39	0.85	AAC(N)	53	0.42	AGC(S)	46	0.56
AUA(M)	139	1.09	ACA(T)	41	0.89	AAA(K)	222	1.25	AGA(S)	112	1.36
AUG(M)	116	0.91	ACG(T)	27	0.59	AAG(K)	132	0.75	AGG(S)	124	1.5
GUU(V)	192	1.82	GCU(A)	49	1.5	GAU(D)	74	1.47	GGU(G)	146	1.43
GUC(V)	37	0.35	GCC(A)	21	0.64	GAC(D)	27	0.53	GGC(G)	46	0.45
GUA(V)	91	0.86	GCA(A)	27	0.82	GAA(E)	133	1.18	GGA(G)	94	0.92
GUG(V)	101	0.96	GCG(A)	34	1.04	GAG(E)	93	0.82	GGG(G)	122	1.2

NOTE. Average codons=5978

Table S8. *Spisula solidissima* mitochondrial genome codon usage

Spisula solidissima											
Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU
UUU(F)	503	1.61	UCU(S)	124	1.55	UAU(Y)	182	1.38	UGU(C)	168	1.35
UUC(F)	120	0.39	UCC(S)	47	0.59	UAC(Y)	81	0.62	UGC(C)	80	0.65
UUA(L)	232	1.56	UCA(S)	65	0.81	UAA(*)	182	1.17	UGA(W)	127	0.89
UUG(L)	207	1.4	UCG(S)	58	0.72	UAG(*)	130	0.83	UGG(W)	159	1.11
CUU(L)	202	1.36	CCU(P)	69	1.58	CAU(H)	42	1.33	CGU(R)	56	1.29
CUC(L)	63	0.42	CCC(P)	37	0.85	CAC(H)	21	0.67	CGC(R)	27	0.62
CUA(L)	98	0.66	CCA(P)	40	0.91	CAA(Q)	33	0.92	CGA(R)	42	0.97
CUG(L)	88	0.59	CCG(P)	29	0.66	CAG(Q)	39	1.08	CGG(R)	49	1.13
AUU(I)	170	1.51	ACU(T)	78	1.66	AAU(N)	104	1.42	AGU(S)	90	1.12
AUC(I)	55	0.49	ACC(T)	25	0.53	AAC(N)	42	0.58	AGC(S)	59	0.74
AUA(M)	118	1.06	ACA(T)	41	0.87	AAA(K)	138	1.03	AGA(S)	75	0.94
AUG(M)	105	0.94	ACG(T)	44	0.94	AAG(K)	129	0.97	AGG(S)	123	1.54
GUU(V)	196	1.74	GCU(A)	105	1.81	GAU(D)	79	1.37	GGU(G)	167	1.38
GUC(V)	50	0.44	GCC(A)	33	0.57	GAC(D)	36	0.63	GGC(G)	64	0.53
GUA(V)	91	0.81	GCA(A)	45	0.78	GAA(E)	78	0.91	GGA(G)	77	0.64
GUG(V)	114	1.01	GCG(A)	49	0.84	GAG(E)	94	1.09	GGG(G)	175	1.45

NOTE. Average codons=6249

Table S9. *Tivela mactroides* partial mitochondrial genome codon usage

Tivela mactroides (partial genome)											
Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU	Codon (aa)	N	RSCU
UUU(F)	564	1.68	UCU(S)	104	1.36	UAU(Y)	221	1.54	UGU(C)	168	1.62
UUC(F)	106	0.32	UCC(S)	40	0.52	UAC(Y)	66	0.46	UGC(C)	40	0.38
UUA(L)	269	2.19	UCA(S)	48	0.63	UAA(*)	205	1.16	UGA(W)	106	0.81
UUG(L)	207	1.68	UCG(S)	30	0.39	UAG(*)	147	0.84	UGG(W)	155	1.19
CUU(L)	121	0.98	CCU(P)	67	1.94	CAU(H)	72	1.43	CGU(R)	43	1.47
CUC(L)	38	0.31	CCC(P)	30	0.87	CAC(H)	29	0.57	CGC(R)	2	0.07
CUA(L)	55	0.45	CCA(P)	26	0.75	CAA(Q)	41	0.96	CGA(R)	35	1.2
CUG(L)	48	0.39	CCG(P)	15	0.43	CAG(Q)	44	1.04	CGG(R)	37	1.26
AUU(I)	215	1.73	ACU(T)	91	1.89	AAU(N)	183	1.63	AGU(S)	140	1.83
AUC(I)	33	0.27	ACC(T)	27	0.56	AAC(N)	42	0.37	AGC(S)	28	0.37
AUA(M)	142	1.21	ACA(T)	53	1.1	AAA(K)	217	1.22	AGA(S)	91	1.19
AUG(M)	93	0.79	ACG(T)	22	0.46	AAG(K)	138	0.78	AGG(S)	131	1.71
GUU(V)	247	1.96	GCU(A)	90	2.05	GAU(D)	82	1.59	GGU(G)	175	1.53
GUC(V)	41	0.33	GCC(A)	24	0.55	GAC(D)	21	0.41	GGC(G)	31	0.27
GUA(V)	119	0.95	GCA(A)	40	0.91	GAA(E)	120	1.1	GGA(G)	96	0.84
GUG(V)	96	0.76	GCG(A)	22	0.5	GAG(E)	99	0.9	GGG(G)	155	1.36

NOTE. Average codons=6283

ANNEXE XI.
ARCTICA ISLANDICA GENETIC DISTANCE ON NUCLEOTIDE AND AMINO-ACID DATA

Table S1. *Arctica islandica* genetic distance on nucleotide data: ATP6, COX1-3 and CYTB genes

Population 1	Population 2	ATP6	COX1	COX2	COX3	CYTB
*Arctica_islandica*_Sanger	*Arctica_islandica*_MiSeq	0.000	0.002	0.000	0.001	0.002
*Arctica_islandica*_Sanger	*Arctica_islandica*_North	0.011	0.014	0.011	0.011	0.012
*Arctica_islandica*_MiSeq	*Arctica_islandica*_North	0.011	0.014	0.011	0.012	0.012
*Arctica_islandica*_Sanger	*Arctica_islandica*_Baltic	0.000	0.001	0.000	0.001	0.002
*Arctica_islandica*_MiSeq	*Arctica_islandica*_Baltic	0.000	0.001	0.000	0.002	0.000
*Arctica_islandica*_North	*Arctica_islandica*_Baltic	0.011	0.013	0.011	0.012	0.012
*Arctica_islandica*_Sanger	*Arctica_islandica*_Iceland	0.012	0.011	0.010	0.007	0.006
*Arctica_islandica*_MiSeq	*Arctica_islandica*_Iceland	0.012	0.011	0.010	0.008	0.006
*Arctica_islandica*_North	*Arctica_islandica*_Iceland	0.004	0.003	0.005	0.006	0.005
*Arctica_islandica*_Baltic	*Arctica_islandica*_Iceland	0.012	0.010	0.010	0.008	0.006

Table S2. *Arctica islandica* genetic distance on nucleotide data: ND1-6 and ND4L genes

Population 1	Population 2	ND1	ND2	ND3	ND4	ND4L	ND5	ND6
*Arctica_islandica*_Sanger	*Arctica_islandica*_MiSeq	0.001	0.003	0.000	0.005	0.000	0.000	0.013
*Arctica_islandica*_Sanger	*Arctica_islandica*_North	0.009	0.012	0.005	0.018	0.011	0.006	0.017
*Arctica_islandica*_MiSeq	*Arctica_islandica*_North	0.010	0.011	0.005	0.015	0.011	0.006	0.004
*Arctica_islandica*_Sanger	*Arctica_islandica*_Baltic	0.000	0.000	0.000	0.004	0.000	0.001	0.013
*Arctica_islandica*_MiSeq	*Arctica_islandica*_Baltic	0.001	0.003	0.000	0.001	0.000	0.001	0.000
*Arctica_islandica*_North	*Arctica_islandica*_Baltic	0.009	0.012	0.005	0.016	0.011	0.006	0.004
*Arctica_islandica*_Sanger	*Arctica_islandica*_Iceland	0.010	0.013	0.005	0.021	0.011	0.007	0.017
*Arctica_islandica*_MiSeq	*Arctica_islandica*_Iceland	0.011	0.012	0.005	0.016	0.011	0.007	0.004
*Arctica_islandica*_North	*Arctica_islandica*_Iceland	0.003	0.005	0.000	0.005	0.000	0.001	0.000
*Arctica_islandica*_Baltic	*Arctica_islandica*_Iceland	0.010	0.013	0.005	0.017	0.011	0.008	0.004

Table S3. *Arctica islandica* genetic distance on amino-acid data: ATP6, COX1-3 and CYTB genes

Population 1	Population 2	ATP6	COX1	COX2	COX3	CYTB
*Arctica_islandica*_Sanger	*Arctica_islandica*_MiSeq	0.000	0.002	0.000	0.000	0.000
*Arctica_islandica*_Sanger	*Arctica_islandica*_North	0.000	0.009	0.012	0.000	0.000
*Arctica_islandica*_MiSeq	*Arctica_islandica*_North	0.000	0.007	0.012	0.000	0.000
*Arctica_islandica*_Sanger	*Arctica_islandica*_Baltic	0.000	0.002	0.000	0.004	0.000
*Arctica_islandica*_MiSeq	*Arctica_islandica*_Baltic	0.000	0.000	0.000	0.004	0.000
*Arctica_islandica*_North	*Arctica_islandica*_Baltic	0.000	0.007	0.012	0.004	0.000
*Arctica_islandica*_Sanger	*Arctica_islandica*_Iceland	0.000	0.004	0.009	0.000	0.000
*Arctica_islandica*_MiSeq	*Arctica_islandica*_Iceland	0.000	0.002	0.009	0.000	0.000
*Arctica_islandica*_North	*Arctica_islandica*_Iceland	0.000	0.005	0.009	0.000	0.000
*Arctica_islandica*_Baltic	*Arctica_islandica*_Iceland	0.000	0.002	0.009	0.004	0.000

Table S4. *Arctica islandica* genetic distance on amino-acid data: ND1-6 and ND4L genes

Population 1	Population 2	ND1	ND2	ND3	ND4	ND4L	ND5	ND6
*Arctica_islandica*_Sanger	*Arctica_islandica*_MiSeq	0.000	0.000	0.000	0.024	0.000	0.000	0.013
*Arctica_islandica*_Sanger	*Arctica_islandica*_North	0.000	0.009	0.000	0.033	0.000	0.000	0.013
*Arctica_islandica*_MiSeq	*Arctica_islandica*_North	0.000	0.009	0.000	0.008	0.000	0.000	0.000
*Arctica_islandica*_Sanger	*Arctica_islandica*_Baltic	0.000	0.000	0.000	0.024	0.000	0.000	0.013
*Arctica_islandica*_MiSeq	*Arctica_islandica*_Baltic	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*Arctica_islandica*_North	*Arctica_islandica*_Baltic	0.000	0.009	0.000	0.008	0.000	0.000	0.000
*Arctica_islandica*_Sanger	*Arctica_islandica*_Iceland	0.000	0.012	0.000	0.035	0.000	0.000	0.013
*Arctica_islandica*_MiSeq	*Arctica_islandica*_Iceland	0.000	0.012	0.000	0.011	0.000	0.000	0.000
*Arctica_islandica*_North	*Arctica_islandica*_Iceland	0.000	0.003	0.000	0.003	0.000	0.000	0.000
*Arctica_islandica*_Baltic	*Arctica_islandica*_Iceland	0.000	0.012	0.000	0.011	0.000	0.000	0.000

ANNEXE XII.
VALUES OF LIFE-HISTORY TRAITS AND REFERENCES ASSOCIATED FOR 76 BIVALVE SPECIES

Table S1. Life-history traits for 76 bivalve species

Species	Longevity (years)	Generation time (years)	Maximum lethal temperature (°C)	GenBank accession number/reference
Acanthocardia_tuberculata	11	-	-	NC_008452
Anodonta_anatina	28	2	-	NC_022803
Anomalocardia_brasiliana	3	0.5	-	Levivier et al, in prep
Arconaia_lanceolata	-	-	-	NC_023955
Arctica_islandica	507	32	17	NC_022709
Argopecten_irradians	2	-	35	NC_012977
Atrina_pectinata	-	-	40	NC_020028
Azumapecten_farreri	-	2	30	NC_012138
Brachidontes_exustus	-	-	-	NC_024882
Coelomactra_antiquata	-	-	-	NC_021375
Corbicula_fluminea	5	0.75	30	Levivier et al, in prep
Crassostrea_ariakensis	-	-	-	FJ841964
Crassostrea_gigas	-	-	43	EU672831
Crassostrea_iredalei	-	-	-	NC_013997
Crassostrea_virginica	-	1	36	NC_007175
Cristaria_plicata	4	-	-	NC_012716
Elliptio_sp	-	-	-	This study
Fulvia_mutica	2	1	-	NC_022194
Hiatella_arctica	126	-	-	NC_008451
Hyriopsis_cumingii	-	2	-	NC_011763
Lamprotula_gottschei	-	-	-	NC_023806
Lamprotula_leai	17	-	-	NC_023346
Lamprotula_tortuosa	-	-	-	NC_021404
Lampsilis_ornata	18	2	-	NC_005335
Lasmigona_compressa	-	-	-	NC_015481
Laternula_elliptica	36	3	9	NC_022846
Loripes_lacteus	4.9	-	-	NC_013271
Lucinella_divaricata	-	-	-	NC_013275

Table S2. Life-history traits for 76 bivalve species (suite 1)

Species	Longevity (years)	Generation time (years)	Maximum lethal temperature (°C)	GenBank accession number/reference
Lutraria_rhynchaena	-	-	-	NC_023384
Macoma_constricta	-	-	-	Levivier et al, in prep
Mactromeris_polynyma	92	12	-	Levivier et al, in prep
Margaritifera_falcata	114	9	27	NC_015476
Margaritifera_margaritifera	210	20	28	This study
Mercenaria_mercenaria	106	-	35	Levivier et al, in prep
Meretrix_larmarckii	-	-	29	NC_016174
Meretrix_lusoria	-	2	-	NC_014809
Meretrix_lyrata	11	2	-	NC_022924
Mimachlamys_nobilis	-	-	-	NC_011608
Mizuhopecten_yessoensis	16	3	26	NC_009081
Moerella_iridescens	-	-	-	NC_018371
Musculista_senhousia	2	0.67	-	GU001953
Mya_arenaria	28	1.6	32.5	NC_024738
Mytilus_californianus	-	2	37.5	NC_015993
Mytilus_edulis	24	7	28	NC_006161
Nucula_nucleus	10	-	-	EF211991
Nuttalia_olivacea	-	-	-	NC_018373
Ostrea_edulis	15	3	27.5	NC_016180
Ostrea_lurida	-	1	20	NC_022688
Panopea_generosa	-	-	-	KM580067
Paphia_amabilis	-	-	-	NC_016889
Paphia_undulata	3	0.58	-	NC_016891
Perna_viridis	3	0.42	35	NC_018362
Pinctada_margaritifera	-	2	35	NC_021638
Pinctada_maxima	-	4	-	NC_018752
Placopecten_magellanicus	25	3	21	NC_007234
Pyganodon_grandis	14	5	-	NC_013661
Quadrula_quadrula	64	6.5	-	NC_013658
Ruditapes_philippinarum	25	1	35	NC_003354
Saccostrea_mordax	-	-	-	NC_013998
Scapharca_broughtonii	20	1	-	NC_020787
Semele_scabra	-	-	-	NC_018374
Sinanodonta_woodiana	12	3	-	HQ283345
Sinonovacula_constricta	-	-	-	NC_018375
Solecurtus_divaricatus	-	-	-	NC_018376

Table S3. Life-history traits for 76 bivalve species (suite 2)

Species	Longevity (years)	Generation time (years)	Maximum lethal temperature (°C)	GenBank accession number/reference
Solemya_velum	-	-	-	NC_017612
Solen_strictus	5.5	-	-	NC_017616
Solenaia_oleivora	-	-	-	NC_022701
Soletellina_diphos	-	-	-	NC_018372
Spisula_solidissima	37	4	28	Levivier et al, in prep
Tivela_mactroides	2.50	0.42	-	Levivier et al, in prep
Toxolasma_parvus	-	-	-	NC_015483
Unio_japanensis	-	-	-	AB055625
Unio_pictorum	22	2	25	NC_015310
Utterbackia_imbecilis	-	1	-	NC_015479
Utterbackia_peninsularis	-	-	-	HM856636
Venustaconcha_ellipsiformis	15	3	-	FJ809753

Table S4. References of life-history traits for 76 bivalve species

Species	Longevity	Generation time	Maximum lethal temperature
Acanthocardia_tuberculata	(Peharda et al. 2012)	-	-
Anodonta_anatina	(Aldridge 1999)		-
Anomalocardia_brasiliana	(Silva-Cavalcanti & Costa 2011; Petracco et al. 2012)	(Gaspar et al. 2011)	-
Arconaia_lanceolata	-	-	-
Arctica_islandica	(Butler et al. 2012)	(Thorarinsdottir & Steingrimsson 2000)	(Witbaard & Bergman 2003)
Argopecten_irradians	(Guo 2009; Powell & Cummins 1985; Estabrooks 2007)	-	(Mackenzie 2008)
Atrina_pectinata	-	-	(Yamamoto et al. 1993)
Azumapecten_farreri	-	Ivin and Kalashnikov.a	(Zhang et al. 2004)
Brachidontes_exustus	-	-	-
Coelomactra_antiquata	-	-	-
Corbicula_fluminea	(Sousa et al. 2008; McMahon 2002; Mouthon 2001)	(McMahon 2002)	(NBII and ISSG; Foster et al 2015)
Crassostrea_ariakensis	-	-	-
Crassostrea_gigas	-	-	(Yamamoto et al. 1993)

Table S5. References of life-history traits for 76 bivalve species (suite 1)

Species	Longevity	Generation time	Maximum lethal temperature
Crassostrea_iredalei	-	-	-
Crassostrea_virginica	-	(Harding et al. 2013; MacKenzie Jr. 1981; Rothschild et al. 1994; Hayes & Menzel 1981)	(Kennedy 1996)
Cristaria_plicata	(Xu et al. 2011)	-	-
Elliptio_sp	-	-	-
Fulvia_mutica	(Tian & Shimizu 1998; Liu et al. 2008)		-
Hiatella_arctica	(Sejr et al. 2002)	-	-
Hyriopsis_cumingii	-	(Yoshimura et al. 2010)	-
Lamprotula_gottschei	-	-	-
Lamprotula_leai	(Ling et al. 2005)	-	-
Lamprotula_tortuosa	-	-	-
Lampsilis_ornata	(Haag & Rypel 2011; Haag & Staton 2003)	(Haag & Staton 2003)	-
Lasmigona_compressa	-	-	-
Laternula_elliptica	(Philipp, Pörtner, et al. 2005; Philipp, Brey, et al. 2005)	(Guy et al. 2014)	(Truebano et al. 2010; Rodrigues et al. 2007)
Loripes_lacteus	(Veloso et al. 2007)	-	-
Lucinella_divaricata	-	-	-
Lutraria_rhynchaena	-	-	-
Macoma_constricta	-	-	-
Mactromeris_polynyma	(MPO 2012)	(Roddick et al. 2007)	-
Margaritifera_falcata	(Vannote & Minshall 1982)	(Thomas 2008)	(Thomas 2008)
Margaritifera_margaritifera	(Ziuganov et al. 2000)	(Varandas et al. 2013; Bauer 1987; Vrignaud 2007)	(Poulleau 2009)
Mercenaria_mercenaria	(Ridgway et al. 2011b)	-	(Roegner & Mann 1991; Geog.mcgill.ca 2002)
Meretrix_larmarckii	-	-	(Higano et al. 1997)
Meretrix_lusoria	-	(Chung 2007)	-
Meretrix_lyrata	(Luu et al. n.d.)		-
Mimachlamys_nobilis	-	-	-
Mizuhopecten_yessoensis	(Silina 1996)	(Dulenina & Dulenin 2012)	(Hao et al. 2014; Ivin, VV and Kalashnikov VZ.b)

Table S6. References of life-history traits for 76 bivalve species (suite 2)

Species	Longevity	Generation time	Maximum lethal temperature
Moerella_iridescens	-	-	-
Musculista_senhousia	(Crooks 1996; Mistri 2002; Creese et al. 1997)	(Mistri 2002)	-
Mya_arenaria	(Mikkelsen 2011; MacDonald & Thomas 1980; Cardoso et al. 2009; Strasser 1999; Maximovich & Guerassimova 2003)	(Brousseau 1978; Böttger et al. 2013)	(MAPAQ n.d.)
Mytilus_californianus	-	(Paine & Trimble 2004)	(Kelley 2007)
Mytilus_edulis	(Schmidt 1999; Suchanek 1981; Powell & Cummins 1985)	(Sukhotin & Flyachinskaya 2009)	(Jones et al. 2009)
Nucula_nucleus	(Chardy et al. 1984)	-	-
Nuttalia_olivacea	-	-	-
Ostrea_edulis	(Richardson et al. 1993; Jackson and Wilding 2009;Wildscreen Arkive)	(Gendreau and Grizel 1990; Jackson and Wilding 2009)	(MarLIN)
Ostrea_lurida	-	(Bulseco 2010; COSEWIC 2011)	(Couch & Hassler 1989; Bulseco 2010)
Panopea_generosa	-	-	-
Paphia_amabilis	-	-	-
Paphia_undulata	(Agasen et al. 1998; Yan et al. 2014)	(Agasen et al. 1998)	-
Perna_viridis	(Lee 1985; Cheung 1993; Gobin et al. 2013)	(Bigatti et al. 2005; Gobin et al. 2013)	(Gobin et al. 2013)
Pinctada_margaritifera	-	(Sims 1992)2	(Yukihira et al. 2000)
Pinctada_maxima	-	(Fletcher et al. 1996)	-
Placopecten_magellanicus	(MacDonald & Bayne 1993)	(McGarvey et al. 1993)	(MAPAQ n.d.)
Pyganodon_grandis	(Anthony et al. 2001)	(Zotin & Vladimirova 2001)	-
Quadrula_quadrula	(COSEWIC 2006)		-
Ruditapes_philippinarum	(Ponurovskii 2008)	(Ekaratne & Davenport 1993; Ren et al. 2008)	(Shean 2011)
Saccostrea_mordax	-	-	-
Scapharca_broughtonii	(Sugiura et al. 2014)	(Ito et al. 1998)	-
Semele_scabra	-	-	-

Table S7. References of life-history traits for 76 bivalve species (suite 3)

Species	Longevity	Generation time	Maximum lethal temperature
Sinanodonta_woodiana	(Dudgeon & Morton 1983; Kiss & Pekli 1988; Benkö-Kiss n.d.; Spyra et al. 2012)	(Kiss & Pekli 1988; Benkö-Kiss n.d.)	-
Sinonovacula_constricta	-	-	-
Solecurtus_divaricatus	-	-	-
Solemya_velum	-	-	-
Solen_strictus	(Hong & Park 1994)	-	-
Solenaia_oleivora	-	-	-
Soletellina_diphos	-	-	-
Spisula_solidissima	(Cargnelli et al. 1999)		
Tivela_mactroides	(Turra et al. 2015)		-
Toxolasma_parvus	-	-	-
Unio_japanensis	-	-	-
Unio_pictorum	(Aldridge 1999)		(Wikipédia)
Utterbackia_imbecilis	-	(Haag 2012)	-
Utterbackia_peninsularis	-	-	-
Venustaconcha_ellipsiformis	(Badra 2007)	(USDA 2003)	-

ANNEXE XIII.
MITOCHONDRIAL GENOME ANNOTATIONS FOR TWO PALAEOHETERODONTA SPECIES

Table S1. *Elliptio* sp. mitochondrial genome annotation

	Elliptio sp.					
Gene	Location (F/R)	Size	Start codon	Stop codon	Anticodon	Intergenic nucleotides
COX1	1-1548 (R)	1548	GTG	TAG		5
COX2	1554-2246 (R)	693	ATA	TAG		11
ND3	2258-2659 (R)	402	ATT	TAG		29
tRNA-His	2689-2754 (R)	66			GTG	98
tRNA-Ala	2853-2919 (F)	67			TGC	18
tRNA-Ser2	2938-3001 (F)	64			TGA	12
tRNA-Ser1	3014-3081 (F)	68			TCT	3
tRNA-Glu	3085-3150 (F)	66			TTC	283
ND2	3434-4399 (F)	966	ATG	TAA		1
tRNA-Met	4401-4466 (F)	66			CAT	17
tRNA-Trp	4484-4549 (F)	66			TCA	8
tRNA-Arg	4558-4624 (F)	67			TCG	0
12S-Rrna	4625-5475 (F)	851				0
tRNA-Lys	5476-5542 (F)	67			TTT	2
tRNA-Thr	5545-5607 (F)	63			TGT	7
tRNA-Tyr	5615-5676 (F)	62			GTA	0
16S-Rrna	5677-6982 (F)	1306				0
tRNA-Leu	6983-7046 (F)	64			TAG	17
tRNA-Asn	7064-7129 (F)	66			GTT	14
tRNA-Pro	7144-7208 (F)	65			TGG	0
CYTB	7209-8375 (F)	1167	ATC	TAA		5
tRNA-Phe	8381-8445 (F)	65			GAA	35
ND5	8481-10217 (R)	1737	ATG	TAA		212
tRNA-Gln	10430-10495 (F)	66			TTG	9
tRNA-Cys	10505-10574 (F)	70			GCA	6
tRNA-Ile	10581-10644 (F)	64			GAT	7
tRNA-Val	10652-10715 (F)	64			TAC	5
tRNA-Leu	10721-10783 (F)	63			TAA	0
ND1	10784-11686 (F)	903	ATC	TAA		9
tRNA-Gly	11696-11758 (F)	63			TCC	26
ND6	11785-12273 (F)	489	ATC	TAG		35
ND4	12309-13646 (R)	1338	TTG	TAA		1
ND4L	13648-13956 (R)	309	ATA	TAG		4
ATP8	13953-14156 (R)	204	GTG	TAG		0
tRNA-Asp	14157-14219 (R)	63			GTC	17
ATP6	14237-14944 (R)	708	ATG	TAG		20
COX3	14965-15744 (R)	780	ATG	TAA		29

NOTE. (F) Forward. (R) Reverse

Table S2. *Margaritifera margaritifera* mitochondrial genome annotation

Margaritifera margaritifera						
Gene	Location (F/R)	Size	Start codon	Stop codon	Anticodon	Intergenic nucleotides
COX1	1-1560 (R)	1560	ATA	TAG		34
COX2	1595-2275 (R)	681	ATG	TAG		27
ND3	2303-2659 (R)	357	ATG	TAG		48
tRNA-His	2708-2771 (R)	64			GTG	115
tRNA-Ala	2887-2951 (F)	65			TGC	24
tRNA-Ser2	2976-3039 (F)	64			TGA	11
tRNA-Ser1	3051-3118 (F)	68			TCT	53
HORF	3172-3504 (F)	333	ATT	TAG		14
ND2	3519-4487 (F)	969	ATG	TAA		14
tRNA-Met	4549-4612 (F)	64			CAT	12
tRNA-Trp	4625-4687 (F)	63			TCA	1
tRNA-Arg	4689-4753 (F)	65			TCG	-1
12S-Rrna	4753-5606 (F)	854				0
tRNA-Lys	5607-5671 (F)	65			TTT	1
tRNA-Thr	5673-5738 (F)	66			TGT	2
tRNA-Tyr	5741-5805 (F)	65			GTA	0
16S-Rrna	5806-7123 (F)	1318				1
tRNA-Leu	7125-7190 (F)	66			TAG	8
tRNA-Asn	7199-7263 (F)	65			GTT	16
tRNA-Glu	7280-7344 (F)	65			TTC	70
tRNA-Pro	7415-7479 (F)	65			TGG	0
CYTB	7480-8622 (F)	1143	ATC	TAA		40
tRNA-Phe	8663-8727 (F)	65			GAA	55
ND5	8783-10549 (R)	1767	ATT	TAG		133
tRNA-Gln	10683-10751 (F)	69			TTG	17
tRNA-Cys	10769-10837 (F)	69			GCA	7
tRNA-Ile	10845-10912 (F)	68			GAT	5
tRNA-Val	10918-10982 (F)	65			TAC	0
tRNA-Leu	10983-11045 (F)	63			TAA	1
ND1	11047-11949 (F)	903	ATT	TAA		14
tRNA-Gly	11964-12024 (F)	61			TCC	13
ND6	12038-12553 (F)	516	ATT	TAA		32
ND4	12586-13899 (R)	1314	ATG	TAG		28
ND4L	13928-14224 (R)	297	ATG	TAG		5
ATP8	14230-14439 (R)	210	ATG	TAA		0
tRNA-Asp	14440-14501 (R)	62			GTC	15
ATP6	14517-15233 (R)	717	ATG	TAG		53
COX3	15287-16066 (R)	780	ATG	TAG		52

NOTE. (F) Forward. (R) Reverse

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