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1	Discordant population structure inferred from male- and female-
2	type mtDNAs from <i>Macoma balthica</i> , a bivalve species
3	characterized by doubly uniparental inheritance of mitochondria
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16	Abstract
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18	Doubly Uniparental Inheritance (DUI) of mitochondria is a remarkable exception to the
19	Strictly Maternal Inheritance (SMI) in metazoans. In species characterized by DUIalmost
20	exclusively gonochoric bivalve mollusks, females (F) transmit mitochondria to offspring of
21	both sexes, while males (M) pass on their mitochondria exclusively to their sons. Under DUI,
22	males are heteroplasmic, somatic tissues containing F-transmitted mtDNA and gametic cells
23	containing M-transmitted mtDNAs. The aforementioned transmission routes make M- and F-
24	transmitted mtDNA interesting as sex-specific markers which can differ in their effective
25	population sizes, mutation rates, and selective constraints. For these reasons, looking at both
26	markers can provide significant insights into the genetic structure of populations and

investigate its determinants. In this study, we document differences in genetic diversity,
divergence, inter-populational genetic differentiation and biogeographic structure between

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M- and F-type *cox1* mt genes in the Baltic tellin (*Macoma balthica*) to test whether *cox1m*and *cox1f* genes bear the marks of similar phylogeographic histories. Both markers were
sequenced for 302 male individuals sampled from the North Sea to the Gironde Estuary

51 sequenced for 502 male matviduals sampled from the North Sea to the Orionde Estuary

32 (Southern France). Nucleotide diversity and net divergence were over twice higher in *cox1m*

33 compared to coxlf. A strong southward decrease in nucleotide diversity was observed only at 34 cox1m. Genetic differentiation between northern and southern populations was nearly 3 times 35 higher at coxIm compared to coxIf (global $\Phi ST = 0.345$ and 0.126 respectively) and the 36 geographic localization of the strongest genetic break significantly differed between the markers (Finistère Peninsula at cox1f; Cotentin Peninsula at cox1m). A higher mutation rate, 37 38 relaxed negative selection and differences in effective population sizes (depending on 39 locations) at *cox1m* could explain differences in population genetic structure. As both F- and 40 M-type mtDNAs interact with nuclear genes for oxidative phosphorylation and ATP 41 production, geographical discordances in genetic clines could be linked to mito-nuclear genetic incompatibilities in this system. 42

43

Keywords 44

45 Doubly Uniparental Inheritance, disruption, heteroplasmy, comparative biogeography,

46 phylogeography, discordance, hybrid zone, genetic cline, mitochondria

47

48 Introduction

49 Some species show a remarkable exception to the maternal inheritance of mitochondria in 50 metazoans: the doubly uniparental mode of inheritance (DUI). In this system, both males and 51 females can transmit their mitochondria. The former transmits "female-inherited" (F-type) 52 mitochondria to all their progeny and the latter pass on "male-inherited" (M-type) 53 mitochondria to their male offspring, where the male mitogenomes (mt) are quartered in male 54 germ line and gametes (reviewed in Zouros, 2013). To date, DUI species have only been 55 discovered in the class Bivalvia, with over 100 DUI species (Gusman et al 2016) among the 56 about 11,000 contained in this taxon (Huber 2010, 2015). They are all gonochoric, except for 57 the hermaphroditic mussel Semimytilus algosus (Lubosny et al 2020). More than a simple 58 peculiarity, DUI is suspected to play a role in sex-determination and gonad differentiation 59 (Zouros 2000, Breton et al 2011, Guerra et al 2017, Capt et al 2018, 2019), and could well be 60 involved in population structure through intrinsic (e.g. genetic incompatibilities; Saavedra et 61 al 1996) and extrinsic (e.g. selection and demography, Stewart et al 1996) factors.

62

In DUI species, the divergence between F-type and M-type mitogenomes is variable 63 but generally high, ranging from 6 to over 50% (reviewed in Breton et al 2007 and Gusman et 64 al 2016), which questions the maintenance of mito-nuclear genetic coadaptation. Indeed, both F- and M-type mitochondria can be found in males and females, but in majority, females are 65

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66 homoplasmic for the F-type mtDNA whereas males are heteroplasmic, accommodating two 67 highly divergent mitogenomes (F-type in somatic tissues and M-type in sperm). The presence 68 of the M-type mtDNA in somatic tissues is considered as a paternal leak due to elimination or 69 segregation failure of sperm mitochondria in female or male embryos, respectively (Milani et 70 al 2012). Both F- and M-type mt lineages show rapid molecular evolution compared to other 71 animals, the M-type mtDNA usually evolving faster than the F-type mtDNA (Zouros et al 72 2013). Coevolution and coadaptation of mitochondrial and nuclear genes are required for 73 efficient cellular energy production (i.e. oxidative phosphorylation OXPHOS) and mito-74 nuclear genetic incompatibilities (MNIs) can lead to a desynchronization of this machinery 75 (Burton & Baretto 2012,2013). DUI could, therefore, bear on the maintenance of genetic 76 structure among populations of highly dispersive bivalve species at small spatial scales, and 77 provide key insight into the establishment and maintenance of local adaptation. 78 Indeed, barriers to gene flow can arise and be maintained by a multitude of 79 environmental and/or intrinsic factors (Barberousse et al 2010), from ecological isolation to 80 genetic incompatibilities. Hybrid zones, which correspond to E Sticky Note vainola 16.8.2022 18.09 81 spatially separated genetic stocks, are "natural laboratories" to stu intrinsic barriers and the environment, and the processes of adapta Virginia and [probably] all US populations 82 are M. petalum, Meehan is an outdated *Macoma balthica*, a species in which DUI has recently reference here. 83 84 2017), is a noteworthy model species to study hybrid zones in ma 85 al 2007, Riginos & Cunningham 2007). It has a wide distribution 86 west pacific coasts, in Japan and from Alaska to Oregon (USA, Luttikhuizen, 2003) to the 87 North Atlantic, where the species is found in the west from Arctic to Virginia (USA; Meehan, 88 1985) and in the east from the north of Russia (Hummel et al, 1997) to the Arcachon Basin 89 (Hily 2013 and this publication). The succession of glaciation and inter-glaciation periods has Inserted Text 90 resulted in colonization events of the Atlantic marked by repeated 16.8.2022 18.11 vainola colonization events (Nikula et al, 2007). These episodic colon transition from the North Sea to the Baltic Sea 91 92 multiple opportunities for secondary contacts between different genetic establishment of several hybrid zones in the Atlantic. Two subspecties of M. balihica co-occur 93 94 in North Atlantic: a Pacific lineage (*M. b. balthica*) present in the 95 Sea, and an Atlantic lineage (M. b. rubra) present in the Norwegian Sea, the North Sea and E Sticky Note 96 along the British coasts, down to the southern range limit of vainola

- 97 Luttikhuisen et al 2003, Nikula et al 2008). In Europe, genetic b
- 98 Kattegat Detroit between Sweden and Denmark (Nikula et al. 20
- 99 Finistère between the Channel and the Atlantic Ocean. Southern

For clarity, it is important to distinguish between inter-subspecies and intrasubspecies differences/zones right away.

16.8.2022 18.16

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exhibit F-type mtDNA signatures consistent with long-term isolation in the glacial refugium
of the Bay of Biscay: high genetic diversity relative to previously glaciated areas, high
prevalence of private alleles, and a sharp genetic break separating them from northern
populations (Becquet et al 2012). Multiple genes involved in the oxidative phosphorylation

104 (OXPHOS) system (including genes coding for ATP synthase Sticky Note 105 transporter were detected as significantly differentiated among value 106 populations by an F_{ST} scan (Pante et al 2012, 2019). The intra-subspecies 107 incompatibilities between mitochondrial and nuclear genes encoding OXPHOS 108 could be involved in endogenous barriers to gene flow in *M. b. rubra*.

Here, we present sharp differences in genetic diversity, divergence and population genetic structure between F- and M-type mtDNAs of *M. b. rubra* male individuals, in particular along the northeast Atlantic hybrid zone first described in Becquet et al (2012). Given the typically higher evolution rate and the potentially relaxed selection pressures acting on the M-type mitogenome, these comparative data allow us to start testing whether mitotypes are sufficiently different to cause genetic incompatibilities impeding gene flow in *M. b. rubra*.

116

117 Materials and Methods

118 Sampling

119 Individuals were collected from a total of 14 sampling sites ranging from Arcachon (southern 120 range limit of the species, France) to Le Crotoy (Somme Bay, northern France) and from 121 Kruiningen (the Netherlands) to Sylt (Germany) (Table S1). Individuals from sampling sites 122 on the French coasts were treated as follows: 70 to 100 adults from 11 mm to 23 mm were 123 randomly collected live at sexual maturity between 4th and 23rd of April 2018 at 9 locations 124 ranging from the Bay of Biscay in southern France to Somme Bay (Table S1). Individuals 125 were then held in aquaria until dissection at the LIENSs laboratory (LIttoral ENvironnement 126 et Sociétés) in La Rochelle, France, with water temperature maintained at 10°C. They were 127 fed with a multispecific microalgal mixture every other day and dead or dying individuals 128 were removed daily. For each individual, the adductor muscle was carefully severed to 129 separate the two valves, without damaging the gonad, and a sample of the mantle was taken. 130 Sex and gonadal maturation stage were then determined with a dissecting microscope (x100 131 to x400). Two types of tissue samples were collected: a gonadic sample and a somatic sample 132 (mantle). All tissue samples were flash-frozen in liquid nitrogen, and then stored at -80°C

Sticky Note ADP/ATP vainolan and northern 24.8.2022 2.23 intra-subspecies oding OXPHOS functions

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