

1 **A tale of two paths: The evolution of mitochondrial recombination in bivalves with doubly**  
2 **uniparental inheritance**

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10 Running Head: *Mitochondrial recombination in bivalves*

11 **Abstract**

12 In most animals, mitochondrial DNA is strictly maternally inherited and non-recombining. One  
13 exception to these assumptions is called doubly uniparental inheritance (DUI): a phenomenon  
14 involving the independent transmission of female and male mitochondrial genomes. DUI is  
15 known only from the molluscan class Bivalvia. The phylogenetic distribution of male  
16 mitochondrial DNA in bivalves is consistent with several evolutionary scenarios, including  
17 multiple independent gains, losses, and varying degrees of recombination with female  
18 mitochondrial DNA. In this study, we use phylogenetic methods to test male mitochondrial DNA  
19 origination hypotheses and infer the prevalence of mitochondrial recombination in bivalves with  
20 DUI. Phylogenetic modeling using site concordance factors supported a single origin of male  
21 mitochondrial DNA in bivalves coupled with recombination acting over long evolutionary  
22 timescales. Ongoing mitochondrial recombination is present in Mytilida and Venerida, which  
23 results in a pattern of concerted evolution of female and male mitochondrial DNA.  
24 Mitochondrial recombination could be favored to offset the deleterious effects of asexual  
25 inheritance and maintain mitonuclear compatibility across tissues. Cardiida and Unionida have  
26 gone without recent recombination, possibly due to an extension of the *COX2* gene in male  
27 mitochondrial DNA. The loss of recombination may be neutral but could be connected to the role  
28 of M mtDNA in sex determination or sexual development. Our results support recombination  
29 events in DUI species may occur throughout their genomes. Future investigations may reveal  
30 more complex patterns of inheritance of recombinants, which could explain the retention of  
31 signal for a single origination of male mitochondrial DNA in protein coding genes.

32

33 **Keywords:** site concordance factors, selection, concerted evolution, mitonuclear coevolution

## 34 **Introduction**

35 Mitochondria are found in almost all eukaryotic cells and possess their own independently  
36 inherited mitochondrial DNA (mtDNA). Typically, animal mtDNA is ~16 kb long and contains  
37 37 genes (13 protein-coding, two rRNAs, and 22 tRNAs) and a control region (a non-coding  
38 region that often contains the origin of replication) (Boore 1999). In most bilaterian animals,  
39 mtDNA is assumed to be strictly maternally inherited and non-recombining. However,  
40 exceptions to these generalizations have been documented across multiple phyla (Piganeau,  
41 Gardner and Eyre-Walker 2004; Barr, Neiman and Taylor 2005; Tsaousis *et al.* 2005; Ghiselli *et al.*  
42 *al.* 2021). One such exception occurs in molluscan bivalves, where several lineages show doubly  
43 uniparental inheritance (DUI). This unusual mode of mitochondrial inheritance is characterized  
44 by the transmission of two mitochondrial genomes, one passed by females to all offspring and a  
45 second passed by males to only male offspring (Hoeh, Blakley and Brown 1991; Skibinski,  
46 Gallagher and Beynon 1994). Females only possess F-mtDNA, while males are globally  
47 heteroplasmic in their somatic tissues and exclusively possess M mtDNA in their sperm (Breton  
48 *et al.* 2017, 2022; Ghiselli *et al.* 2019; Bettinazzi *et al.* 2020).

49 Doubly uniparental inheritance has been described from five bivalve orders: Cardiida,  
50 Mytilida, Nuculanida, Unionida, and Venerida (Gusman *et al.* 2016; Capt *et al.* 2020). Although  
51 the phylogenetic distribution is thought to be well characterized (Fig. 1), the origin and evolution  
52 of many aspects of DUI remains poorly understood. For example, there are conflicting  
53 hypotheses regarding whether male (M) mtDNA has originated once and has been lost multiple  
54 times (Stewart *et al.* 2009, 2021; Doucet-Beaupré *et al.* 2010), or if it has originated  
55 independently multiple times (Hoeh *et al.* 1996; Maeda *et al.* 2021). Uncertainty stems from  
56 inconsistent phylogenetic relationships between female (F) and M mtDNA, and non-monophyly  
57 of M mtDNA. Phylogenetic relationships between F and M mtDNA in DUI taxa exhibit two  
58 distinct patterns. Female and M mtDNA are reciprocally monophyletic across species in some  
59 orders, while they show sister relationships within a species in others. In other words, M mtDNA  
60 is non-monophyletic across all DUI species but shows topologies consistent with a single  
61 origination in some lineages (Unionida), independent originations in others (Mytilida,  
62 Nuculanida, Venerida), or has not been examined in more than one species (Cardiida) in yet  
63 others (Breton, Stewart and Blier 2009; Gusman *et al.* 2016). Depending on the lineage, F and M  
64 mtDNA genes can be up to 90% identical (Mytilida and Venerida) or differ by more than 50% in

65 their amino acid sequences (Unionida) (Mizi, Zouros and Rodakis 2006; Breton *et al.* 2007;  
66 Breton, Stewart and Blier 2009; Gusman *et al.* 2016).

67       Recombination events between F and M mtDNA have been documented in several DUI  
68 species (*Mytilus* spp. and *Ruditapes philippinarum*) (Ladoukakis and Zouros 2001; Burzyński *et*  
69 *al.* 2003; Passamonti, Boore and Scali 2003; Filipowicz *et al.* 2008; Ladoukakis *et al.* 2011).  
70 These events are similar to homologous recombination in bacteria, where novel fragments from  
71 the donor genome replace existing homologous genetic material in the recipient genome (Spratt  
72 *et al.* 1992). In *Mytilus*, mitochondrial recombination often precipitates a “role-reversal” in  
73 which the F mtDNA receives a M control region and is subsequently transmitted as M mtDNA  
74 (Cao *et al.* 2004; Mizi, Zouros and Rodakis 2006; Stewart *et al.* 2009; Kyriakou *et al.* 2015). In  
75 this event, recombination erases divergence between the rest of the F and M mtDNA genes (e.g.,  
76 those involved with oxidative phosphorylation (OXPHOS)). This recombination also results in a  
77 phylogenetic pattern of concerted evolution in OXPHOS genes, which could cause the observed  
78 conflict in sequence divergence and topologies of F and M mtDNA between DUI lineages  
79 (Stewart *et al.* 2009; Gusman *et al.* 2016). Recombination events have also been documented to  
80 occur in other areas of mtDNA in DUI species (Burzyński *et al.* 2003; Passamonti, Boore and  
81 Scali 2003), including within OXPHOS genes (Ladoukakis and Zouros 2001; Ladoukakis *et al.*  
82 2011). If occasional recombination in OXPHOS genes has occurred throughout the evolutionary  
83 history of bivalves, certain OXPHOS genes could retain sites informative about the origin of M  
84 mtDNA, but signal from these sites has likely been masked when using concatenation-based  
85 methods. Recent advances in site-based methodologies that estimate concordance at the level of  
86 individual sites, including the site concordance factor (Minh, Hahn and Lanfear 2020), are  
87 therefore useful for investigating the origin of M mtDNA.

88       Mitochondrial recombination is well-documented in Mytilida and Venerida, but  
89 recombination is apparently absent in Unionida. This may be due a large extension in the *COX2*  
90 gene in the M mtDNA or the presence of sex-specific open reading frames (*orfs*) in the F and M  
91 mtDNA (Stewart *et al.* 2009; Breton *et al.* 2011; Gusman *et al.* 2016). Most DUI bivalves exhibit  
92 extensions to the *COX2* gene in the M mtDNA, ranging from ~300 bp to 4.5 kb (Curole and  
93 Kocher 2002; Bettinazzi, Plazzi and Passamonti 2016; Capt *et al.* 2020), which have been  
94 hypothesized to serve as a tag for cells or organelles harboring M mtDNA (Chakrabarti *et al.*  
95 2007). Sex-specific *orfs* likely originated via duplication and have been confirmed to code for

96 proteins in Mytilida, Unionida, and Venerida (Breton *et al.* 2011; Milani *et al.* 2014; Ouimet *et*  
97 *al.* 2020). Although their function is uncertain, it is hypothesized *orfs* are involved in sex  
98 determination or sexual development (Breton *et al.* 2011, 2022; Milani *et al.* 2014; Guerra *et al.*  
99 2019; Ouimet *et al.* 2020). Although *COX2* extensions and sex-specific *orfs* are found in most  
100 DUI lineages, they have been comparably evolutionarily conserved across Unionida (Curole and  
101 Kocher 2002; Guerra *et al.* 2019), suggesting one of these two characteristics may explain why  
102 recombination is selected against.

103 In this study, we revisit the related issues of the origins of M mtDNA and recombination  
104 in mtDNA. Specifically, we use phylogenetic methods to 1) investigate the number of origins of  
105 M mtDNA, 2) infer the prevalence of mitochondrial recombination, and 3) investigate the  
106 potential drivers or inhibitors of mtDNA recombination. Our findings support a single  
107 origination of M mtDNA in bivalves with occasional recombination events causing observed  
108 non-monophyly of M mtDNA using concatenation-based methods.

109

## 110 **Materials and Methods**

### 111 *Phylogenetic distribution of doubly uniparental inheritance*

112 To provide an overview of the phylogenetic distribution of DUI in bivalves, we downloaded the  
113 phylogeny presented in Combosch *et al.* (2017). We collapsed the phylogeny to the family-level  
114 (93 families; see Table S1) and compiled DUI reports from the literature (Theologidis *et al.*  
115 2008; Gusman *et al.* 2016; Capt *et al.* 2020).

116

### 117 *Mitogenomic dataset and phylogenetic analyses*

118 We downloaded M and F mitogenomes for 37 DUI species and 10 representative orders in  
119 Bivalvia from the NCBI nucleotide collection (Table S2). *Octopus bimaculatus* (Cephalopoda)  
120 was used as an outgroup. In cases where annotations of mitogenomes were incomplete, we used  
121 MITOS2 (Bernt *et al.* 2013) to identify protein-coding genes. We excluded *ATP8* due to missing  
122 data across most species and a partial portion of *COX2* for *Limecola balthica* and *Scrobicularia*  
123 *plana* (Cardiida) M mtDNA due to a large insertion (Capt *et al.* 2020). Protein-coding genes  
124 were aligned using MACSE v 2.05 (Ranwez and Douzery 2018). We then concatenated the 12  
125 mitochondrial genes and removed all sites with missing data. The resulting concatenated  
126 alignment was used for phylogenetic analysis and consisted of 83 sequences represented by

127 2,622 amino acids (File S1). A phylogeny was estimated in IQ-TREE v 2.2.0.3 (Minh *et al.*  
128 2020) using 10 independent runs. ModelFinder (Kalyaanamoorthy *et al.* 2017) was used to select  
129 the best amino acid model of evolution (mtInv+F+I+G4) and  $10^3$  ultrafast bootstrap replicates  
130 were used to assess nodal support (Hoang *et al.* 2018).

131 We used site concordance factors (Minh, Hahn and Lanfear 2020) to test M mtDNA  
132 origination hypotheses. Briefly, site concordance factors measure the percentage of sites  
133 supporting a certain branch in a phylogeny. Hypotheses can be tested by comparing observed site  
134 concordance factors with a distribution of site concordance factors from data simulated under a  
135 given phylogenetic hypothesis (e.g., Hibbins, Gibson and Hahn 2020). We used site concordance  
136 factors from both individual genes and a concatenated alignment of all genes to test two  
137 hypotheses: 1) ten independent originations of M mtDNA (as supported by concatenation  
138 methods; Fig. 2), and 2) a single origination of M mtDNA. Specifically, our methodology  
139 evaluated these two hypotheses by directly comparing observed site concordance factors for a  
140 single origination of M mtDNA to a distribution of site concordance factors for a single  
141 origination of M mtDNA that could occur by chance under multiple origins. To generate  
142 distributions of site concordance factors for hypothesis testing from the concatenated dataset and  
143 each gene independently, we used AliSim (Ly-Trong *et al.* 2022) to simulate  $10^3$  amino acid  
144 datasets based on the resolved topology from each empirical alignment using the best model of  
145 amino acid evolution as determined by ModelFinder. We chose to use AliSim over other  
146 methods (e.g., Seq-Gen, Dawg, INDELible) to account for the non-independence of mtDNA  
147 substitutions. Next, we used Mesquite v 3.3.1 (Maddison and Maddison 2017) to create a  
148 topology from the concatenated analysis that enforced the monophyly of all M mtDNA while  
149 retaining branch length information (Fig. S1; File S2). We then calculated site concordance  
150 factors for all empirical and simulated datasets using 100 quartets. With those, we gathered site  
151 concordance factors for the branch coinciding to a single origin of M mtDNA (Fig. S1) and used  
152 one-tailed tests (with  $p = 0.05$ ) to determine if the observed site concordance factor was  
153 significantly larger than expected under 10 independent originations.

154 We investigated the hypothesis that the lack of recent recombination in Cardiida and  
155 Unionida is a result of intensified selection on M mtDNA genes that have adapted to male  
156 functions. We chose to perform this test in Cardiida given we resolved a similar phylogenetic  
157 pattern between F and M mtDNA as Unionida (Fig. 2). We used RELAX (Wertheim *et al.* 2015)

158 in HyPhy v 2.5.25 (Pond, Frost and Muse 2005) with a concatenated nucleotide alignment of 12  
159 M mtDNA genes (File S3; Table S3) to test if selection on M mtDNA in Cardiida and Unionida  
160 was significantly different than Mytilida and Venerida. Considering extensions to the *COX2*  
161 gene in the M mtDNA are shared in Cardiida and Unionida and hypothesized to be a proximate  
162 cause of the absence of recombination, we also used RELAX independently on a nucleotide  
163 alignment of M mtDNA *COX2* gene (File S4; Table S3). Codons with missing or ambiguous  
164 data in each alignment were removed. Likelihood ratio tests were used to evaluate models with a  
165 significance level of  $p = 0.05$ .

166

### 167 *Estimation of recombination frequency*

168 To estimate the frequency of recombination, we estimated divergence times between F and M  
169 mtDNA lineages. We used BEAST v 2.6.7 (Bouckaert *et al.* 2019) with a concatenated  
170 nucleotide alignment of 12 F and M mtDNA genes for all taxa sampled in Mytilida (File S5;  
171 Table S4), where recombination between M and F mtDNA has been observed and reliable fossil  
172 calibrations are available. Codons with missing or ambiguous data in each alignment were  
173 removed. The best fit model of nucleotide evolution for each codon position was selected by  
174 ModelFinder, a relaxed molecular clock was fit to each codon position, and a calibrated Yule  
175 process was used as the tree prior. We enforced priors that date the MRCA of F and M mtDNA  
176 for *Mytilus edulis*, *M. galloprovincialis*, and *M. trossolus* between 3.1 and 4.8 Mya (Rawson and  
177 Harper 2009). The analysis was run for  $10^8$  MCMC generations with an initial 10% burn-in.  
178 Tracer v1.7.1 (Rambaut *et al.* 2018) was used to determine the appropriate burn-in value and  
179 ensure convergence of all parameters ( $ESS > 200$ ), and a maximum clade credibility tree was  
180 created using TREEANNOTATOR v 2.6 (Bouckaert *et al.* 2019). To get a rough estimate of the  
181 timing of recombination events, we calculated an average divergence time between putatively  
182 recombinant F and M mtDNA lineages.

183

## 184 **Results and Discussion**

185 Phylogenetic reconstruction based on the concatenated alignment of 12 of the 13 mitochondrial  
186 protein coding OXPHOS genes showed non-monophyly of M mtDNA across bivalves (Fig. 2;  
187 File S6), as shown previously (Hoeh *et al.* 1996; Gusman *et al.* 2016; Maeda *et al.* 2021). While  
188 this topology has been previously interpreted as consistent with multiple origins or losses of M

189 mtDNA (Hoeh *et al.* 1996; Stewart *et al.* 2009; Doucet-Beaupré *et al.* 2010; Gusman *et al.* 2016;  
190 Maeda *et al.* 2021), it is also consistent with concerted evolution due to recombination between F  
191 and M mtDNA in Mytilida and Venerida, and a lack of recombination in Cardiida and Unionida  
192 (Fig. 2). As has been seen in previous studies (Gusman *et al.* 2016; Maeda *et al.* 2021), we found  
193 that F and M mtDNA within species in Mytilida and Venerida are generally sister, which is  
194 expected under the hypothesis of recombination between F and M mtDNA. One exception is  
195 *Mytilus edulis*, *M. galloprovincialis*, and *M. trossolus* (*Mytilus* spp.) have reciprocally  
196 monophyletic F and M mtDNA (Fig. 2), despite the fact that *Mytilus* spp. are known to  
197 recombine (Ladoukakis and Zouros 2001; Burzyński *et al.* 2003; Filipowicz *et al.* 2008;  
198 Ladoukakis *et al.* 2011). We estimate that recombinant M mtDNA fix less frequently (~11 My;  
199 95% CI: 7.3–14.5 My; Fig. S2) than do speciation events in *Mytilus* (~3.1–4.8 Mya). We  
200 hypothesize the reciprocal monophyly of F and M mtDNA will appear frequently across the  
201 phylogeny of certain DUI bivalve lineages at shallow taxonomic scales when data for additional  
202 taxa become available.

203 Mitochondrial recombination in Mytilida and Venerida results in a pattern of concerted  
204 evolution of F and M mtDNA, which may be favored to combat the deleterious effects of asexual  
205 inheritance and maintain mitonuclear compatibility across tissues (Muller 1964). If there are two  
206 sets of highly divergent mtDNAs within the same organism, interacting nuclear genes necessary  
207 for proper function may not cooperate efficiently with both mtDNAs, resulting in mitonuclear  
208 incompatibility for one mitogenome (Hill 2015). Mitonuclear coevolution has recently been  
209 confirmed in bivalves, with highly correlated evolution between mitochondrial and nuclear  
210 subunits involved with OXPHOS (Piccinini *et al.* 2021). However, relaxed selection on M  
211 mtDNA may be common in DUI bivalves, therefore favoring nuclear coevolution with F over M  
212 mtDNAs (Maeda *et al.* 2021). Here we suggest that mitonuclear compatibility may be restored  
213 via recombination in some DUI lineages in an analogous process to the "Fountain of Youth"  
214 (Perrin 2009). In this process, occasional recombination events are hypothesized to counteract  
215 accumulated deleterious mutations in previously non-recombining sex chromosomes (Perrin  
216 2009).

217 Analyses of energetic metabolism provide support that mitochondrial recombination may  
218 be favored to purge deleterious mutations in M mtDNA. In Mytilida and Venerida, sperm are



219 dependent on OXPHOS to sustain motility (Bettinazzi *et al.* 2019, 2020), which highlights the  
220 importance of compatibility between M mtDNA and nuclear genes. Comparative physiological  
221 studies in *M. edulis* have shown that recombination events do not have obvious deleterious  
222 effects on sperm performance (Everett *et al.* 2004). Rather, recombination may be advantageous  
223 because sperm with recently masculinized M mtDNA (i.e., those carrying F mtDNA with M  
224 control regions) swim faster than those with ancestral M mtDNA (Jha *et al.* 2007). Sperm  
225 swimming velocity has been demonstrated to be correlated with ATP levels in many taxa  
226 (Perchec *et al.* 1995; Burness, Moyes and Montgomerie 2005), and ATP production is lower in  
227 sperm with M mtDNA than eggs with F mtDNA (Bettinazzi *et al.* 2019). Mitochondrial  
228 recombination, therefore, may be favored to maximize M mtDNA ATP production in Mytilida  
229 and Venerida by replacing defective M mtDNA OXPHOS genes with more energetically robust  
230 F mtDNA OXPHOS genes (Breton, Stewart and Blier 2009). To our knowledge, physiological  
231 studies have been limited to Mytilida and Venerida (Bettinazzi *et al.* 2020), and future analogous  
232 studies in Cardiida and Unionida may further support our hypothesis.

233 We find a different pattern of phylogenetic relationships of mtDNAs in Unionida when  
234 compared to Mytilida and Venerida, consistent with previous studies (Gusman *et al.* 2016). In  
235 Unionida, F and M mtDNA are reciprocally monophyletic across species (Fig. 2). A similar  
236 relationship was recovered in Cardiida (Fig. 2), albeit based on two species. However, *L.*  
237 *balthica* (Cardiida: Tellinidae) and *S. plana* (Cardiida: Semelidae) are estimated to have diverged  
238 at or near the Cretaceous–Palogene boundary (~66 Mya) (Crouch *et al.* 2021), far greater than  
239 our estimated frequency of recombinant fixation in Mytilida (~11 My). Therefore, our data is  
240 consistent with the absence of recent recombination between F and M mtDNA in both Cardiida  
241 and Unionida. We hypothesize mitochondrial recombination was the plesiomorphic condition of  
242 DUI species and was independently lost in these lineages. This is because M mtDNA in Cardiida  
243 and Unionida would be monophyletic had recombination independently originated in Mytilida  
244 and Venerida. One possible explanation for the loss of recombination in Cardiida and Unionida  
245 involves a large extension of *COX2* in the M mtDNA (Curole and Kocher 2002), which is  
246 hypothesized to promote gender-specific mitochondrial localizations (Chakrabarti *et al.* 2007).  
247 Recombination between F and M mtDNA could disrupt proper localization and therefore be  
248 selected against.

249           Although large extensions to *COX2* may be a proximate cause for the loss of  
250 recombination in Cardiida and Unionida, its adaptive significance remains unclear. If *COX2* or  
251 additional M mtDNA genes are adapted to certain male functions, those adapted features could  
252 be lost following recombination with F mtDNA. Were this the case, we might expect to see  
253 intensified selection on *COX2* and M mtDNA genes in Cardiida and Unionida compared to  
254 Mytilida and Venerida. Our analyses reject this hypothesis, and in fact indicate significant  
255 evidence of relaxed selection in Cardiida and Unionida (*COX2*:  $K = 0.71$ ,  $p = 0.001$ ; 12 genes:  $K$   
256  $= 0.44$ ,  $p < 0.001$ ; Table S5). Another possible explanation for the loss of recombination is that  
257 mtDNA may have a role in sex determination, particularly in Unionida (Breton *et al.* 2011).  
258 Unlike other bivalve lineages with DUI, some families in Unionida (i.e., Margaritiferidae and  
259 Unionidae) have evolutionarily conserved sex-specific *orfs* (F-*orf* and M-*orf*) that have been  
260 confirmed to code for proteins (Breton *et al.* 2011). Additionally, hermaphroditism has evolved  
261 multiple times in these lineages, and each transition is often associated with the origin of a F-like  
262 mtDNA that has a hermaphrodite-specific *orf* (Breton *et al.* 2011 but see Soroka and Burzyński  
263 2017). This suggests mtDNA *orfs* are associated with sexual transitions in Unionida and may  
264 have a role in sex determination or sexual development (Breton *et al.* 2011, 2014, 2022).  
265 Recombination between F and M mtDNA would therefore be deleterious, albeit we recognize  
266 this explanation may be limited to the families Margaritiferidae and Unionidae.

267           In principle, gene trees could be used to determine the number of origins of M mtDNA.  
268 In the absence of mitochondrial recombination, a single origin of M mtDNA would result in  
269 reciprocal monophyly of F and M mtDNA across DUI species. However, it is unlikely that gene  
270 trees with the appropriate topology will be observed when there is recombination. Therefore, our  
271 phylogenetic reconstruction (Fig. 2) is consistent with either multiple origins of M mtDNA (up  
272 to 10) or a single origination of M mtDNA with recombination acting in a lineage-specific  
273 manner over long evolutionary timescales. We tested these hypotheses using site concordance  
274 factors, which supported a single origination of M mtDNA followed by lineage-specific  
275 recombination (Fig. 3; Table S6). Specifically, we found more site-level support for a single  
276 origin and can reject multiple origin hypotheses using both an individual OXPHOS gene (*ND1*:  
277  $p=0.03$ ; Fig. 3; Table S6) and a concatenated alignment of 12 genes ( $p < 0.001$ ; Fig. 3; Table  
278 S6). Our results agree with hypotheses presented in previous studies (Hoeh *et al.* 1997;  
279 Theologidis *et al.* 2008; Stewart *et al.* 2009; Doucet-Beaupré *et al.* 2010; Zouros 2013).

280           Although we can reject multiple origination hypotheses, the retention of signal in protein  
281 coding genes for a single origin of M mtDNA remains unclear. Recombination events have been  
282 documented to occur throughout mtDNA in DUI species, including within mitochondrial genes,  
283 but have been hypothesized to only occur in somatic tissue and not inherited through gametes  
284 (Ladoukakis and Zouros 2001; Ladoukakis *et al.* 2011). Given this context, our results suggest  
285 this conclusion may be unrealistic. Future investigations across DUI bivalves may reveal more  
286 complex patterns of recombination in protein coding genes and inheritance of recombinant  
287 mtDNAs, which could explain preserved signal for a single origination of M mtDNA in  
288 mitochondrial OXPHOS genes.

289

## 290 **Conclusion**

291 Our results support a single origination of M mtDNA followed by lineage-specific  
292 recombination, which has led to non-monophyly of M mtDNA using concatenation-based  
293 methods. Mitochondrial recombination events may occur to counteract the accumulation of  
294 deleterious mutations in M mtDNA to restore ATP production but are exclusive to Mytilida and  
295 Venerida (based on available data). It remains uncertain why recombination is absent in Cardiida  
296 and Unionida, but it may be selected against because of the role of mtDNAs in sex determination  
297 or sexual development in these lineages. Future studies into these topics will further contribute to  
298 the understanding of DUI and the functional significance of retaining M mtDNA in bivalves.

299

## 300 **Data Availability Statement**

301 Data used in this study can be found on GenBank with all accession numbers used as part of this  
302 research found in Supplementary Materials.

303

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309

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468 **Figure Legends**

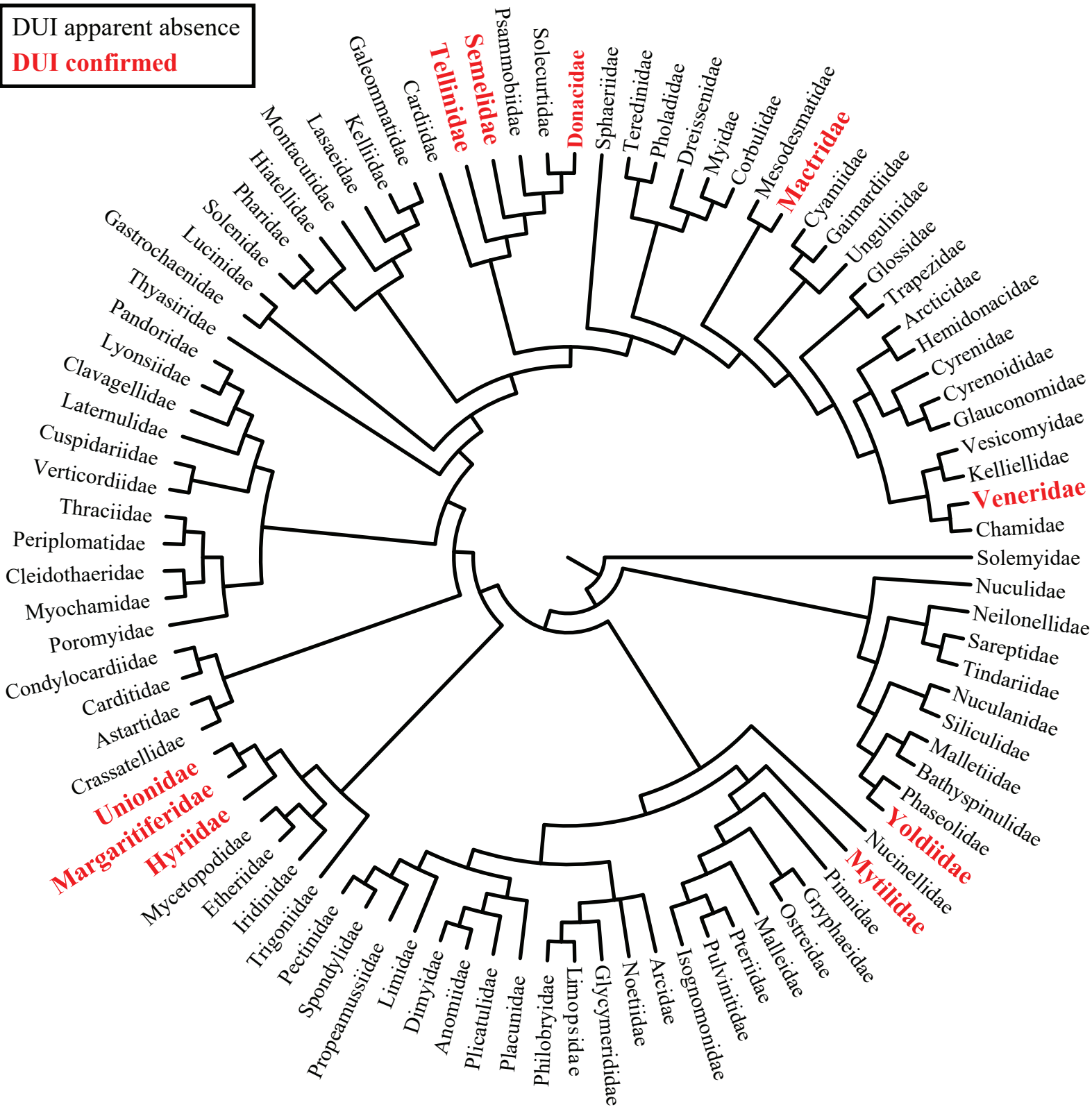
469 **Figure 1.** Phylogenetic distribution of doubly uniparental inheritance (DUI) based on a family-  
470 level tree of Bivalvia presented in Combosch *et al.* (2017). Families confirmed to exhibit DUI  
471 are bolded and colored red.

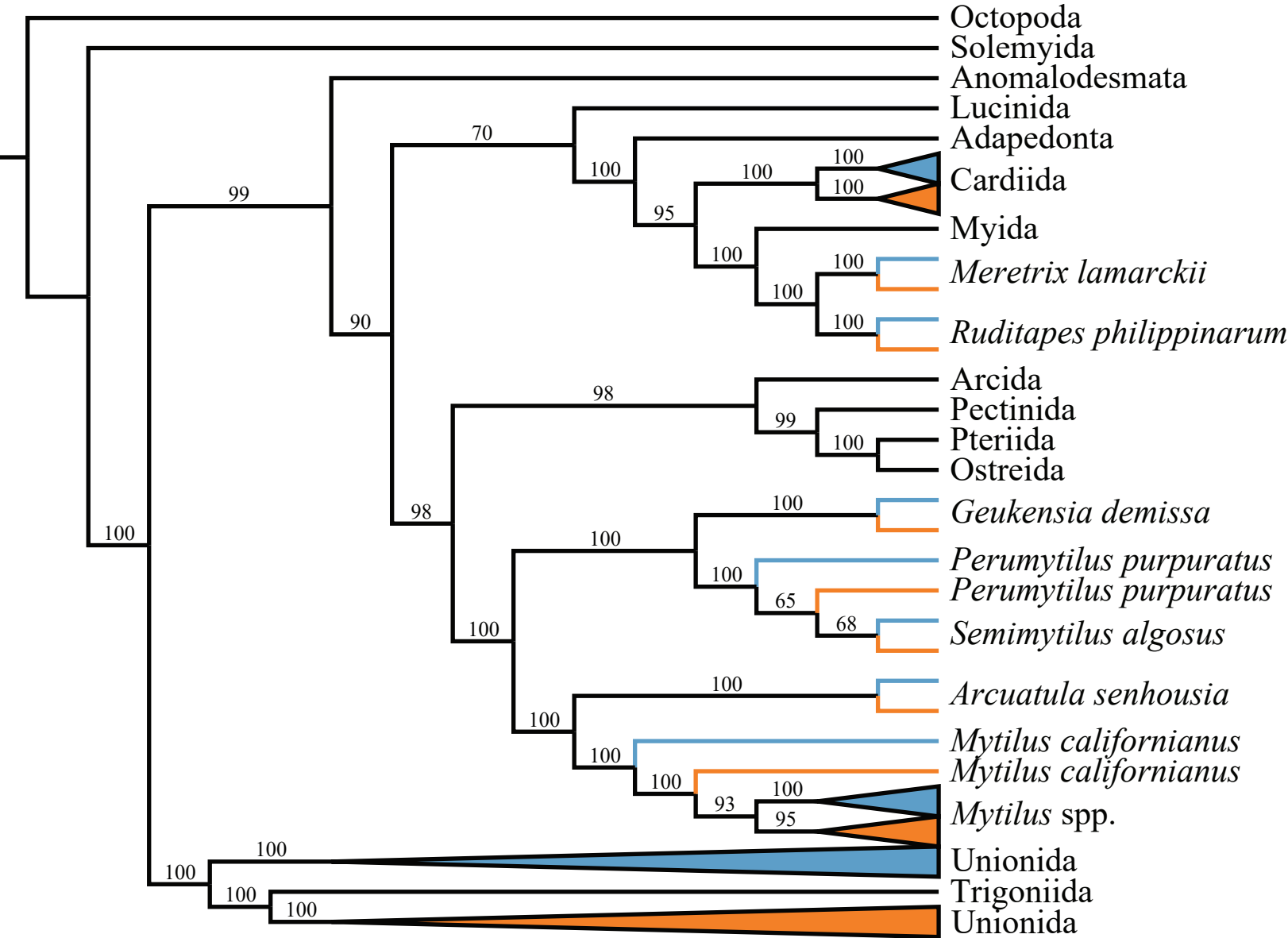
472 **Figure 2.** Phylogeny of the class Bivalvia based on amino acid sequences for 12 mitochondrial  
473 genes, showing lineages with strictly maternal inheritance (SMI), female mtDNA in DUI  
474 species, and male mtDNA in DUI species. *Mytilus* spp. refers to *M. edulis*, *M. galloprovincialis*,  
475 and *M. trossolus*. Values above branches represent ultrafast bootstrap support.

476 **Figure 3.** Null distribution and observed site concordance factors used to assess support for a  
477 single origination of male mitochondrial DNA for a concatenated alignment and *ND1*. In each  
478 plot, white bars represent the null distribution based on 1000 simulated amino acid datasets, the  
479 red arrow represents the observed value based on empirical data, and the p-value is reported.

DUI apparent absence

**DUI confirmed**

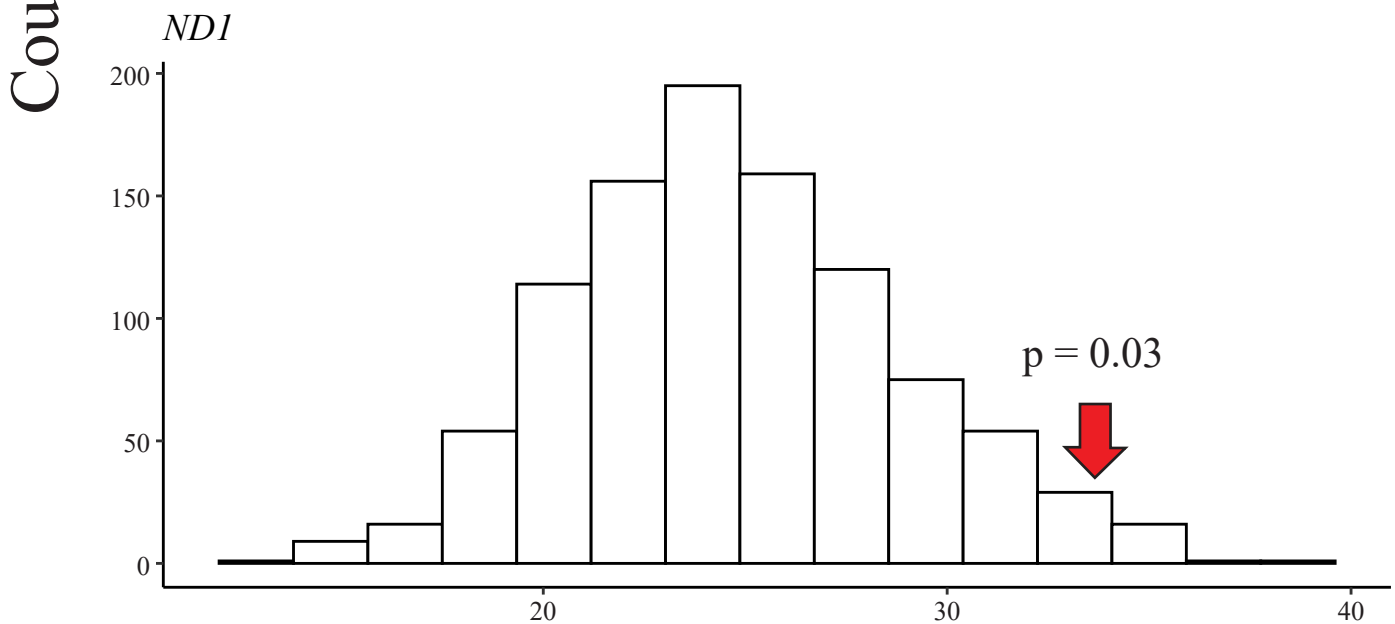
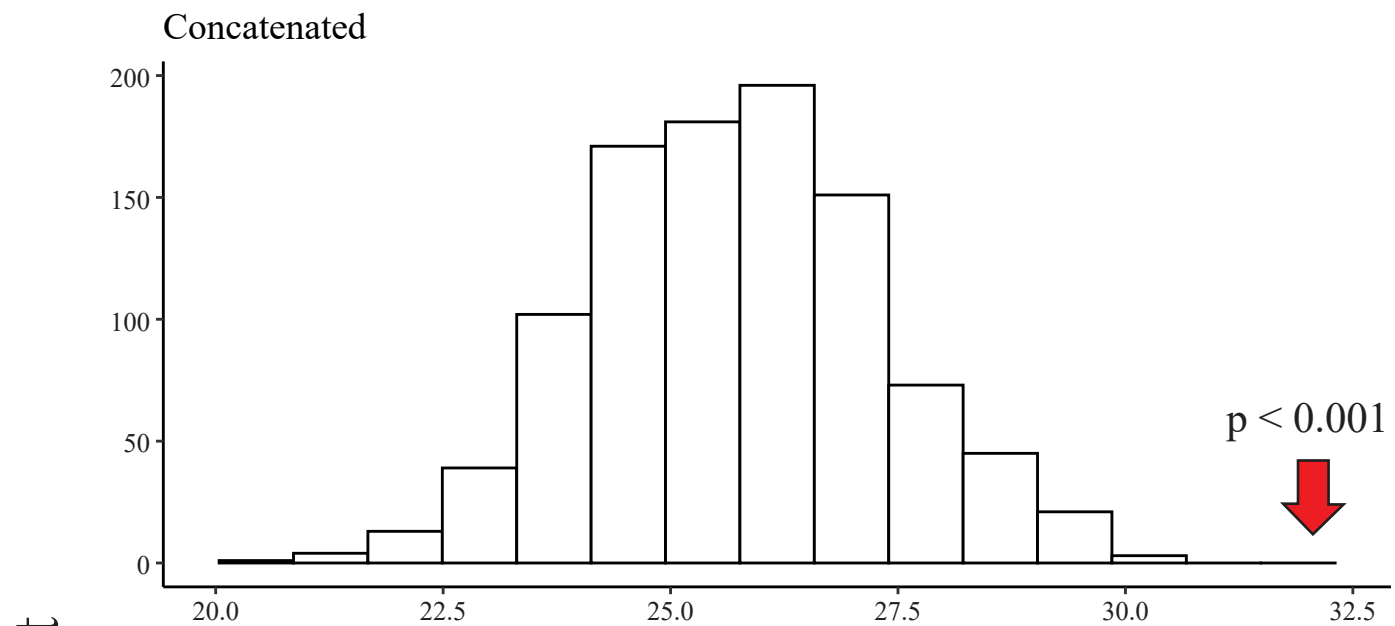




● SMI - Female mtDNA  
 ● DUI - Female mtDNA  
 ● DUI - Male mtDNA

—  
0.4

# Distribution of Site Concordance Factors



Site Concordance Factor