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Tamás Malkócs, Amélia Viricel, Vanessa Becquet, Louise Evin, Emmanuel  
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1 **Complex mitogenomic rearrangements**  
2 **within the Pectinidae (Mollusca:**  
3 **Bivalvia)**

4 Tamás Malkócs<sup>a,b,c,d</sup>, Amélia Viricel<sup>a</sup>, Vanessa Becquet<sup>a</sup>, Louise Evin<sup>a</sup>, Emmanuel  
5 Dubillot<sup>a</sup>, Eric Pante<sup>a</sup>

6 <sup>a</sup> Littoral Environnement et Sociétés (LIENSs), UMR 7266, CNRS-Université de La  
7 Rochelle, 2 rue Olympe de Gouges, F-17042 La Rochelle Cedex 01, France

8 <sup>b</sup> Pál Juhász-Nagy Doctoral School of Biology and Environmental Sciences,  
9 University of Debrecen, Egyetem tér 1, 4032 Debrecen, Hungary

10 <sup>c</sup> Institute of Biology and Ecology, University of Debrecen, Egyetem tér 1, 4032  
11 Debrecen, Hungary

12 <sup>d</sup> Institute of Aquatic Ecology, Centre for Ecological Research, 4026 Debrecen,  
13 Hungary

14 Corresponding author: Tamás Malkócs <tamas.malkocs@gmail.com>; Littoral  
15 Environnement et Sociétés (LIENSs), UMR 7266, CNRS-Université de La Rochelle,  
16 2 rue Olympe de Gouges, F-17042 La Rochelle Cedex 01, France

## 17 **Abstract**

### 18 **Background**

19 Scallops (Bivalvia: Pectinidae) present extraordinary variance in both mitochondrial  
20 genome size, structure and content, even when compared to the extreme diversity  
21 documented within Mollusca and Bivalvia. In pectinids, mitogenome rearrangements  
22 involve protein coding and rRNA genes along with tRNAs, and different genome  
23 organization patterns can be observed even at the level of Tribes. Existing pectinid  
24 phylogenies fail to resolve some relationships in the family, Chlamydinae being an  
25 especially problematic group.

### 26 **Results**

27 In our study, we sequenced, annotated and characterized the mitochondrial genome  
28 of a member of Chlamydinae, *Mimachlamys varia*—a species of commercial interest  
29 and an effective bioindicator—revealing yet another novel gene arrangement in the  
30 Pectinidae. The phylogeny based on all mitochondrial protein coding and rRNA  
31 genes suggests the paraphyly of the *Mimachlamys* genus, further commending the  
32 taxonomic revision of the classification within the Chlamydinae subfamily. At the  
33 scale of the Pectinidae, we found that 15 sequence blocks are involved in  
34 mitogenome rearrangements, which behave as separate units.

### 35 **Conclusions**

36 Our study reveals incongruities between phylogenies based on mitochondrial  
37 protein-coding versus rRNA genes within the Pectinidae, suggesting that locus  
38 sampling affects phylogenetic inference at the scale of the family. We also conclude

39 that the available taxon sampling does not allow for understanding of the  
40 mechanisms responsible for the high variability of mitogenome architecture observed  
41 in the Pectinidae, and that unraveling these processes will require denser taxon  
42 sampling.

## 43 **Keywords**

44 mitogenome, gene rearrangement, Pectinidae, de novo assembly, Variegated  
45 scallop, RNA-seq

## 46 **Background**

47 Historically, animal mitochondrial genomes were thought to have a conserved size of  
48 approximately 16 kbp, as well as a conserved structure, containing 13 protein coding  
49 genes (PCGs), two rRNA genes, and 22 tRNA genes and a single non-coding,  
50 ‘control region’ that regulates replication and transcription [1, 2]. In contrast,  
51 molluscan mitochondrial genomes provide examples of exceptional variation in size,  
52 structure and even function. Mitogenome size in Mollusca ranges from ~14 kbp up to  
53 56.2 kbp [3–7], gene rearrangements, duplications, losses and inversions are  
54 frequent e.g. [8], and there is evidence of the integration of novel genes e.g. [9].  
55 Additionally, a unique, complex mitochondrial inheritance system, called ‘doubly  
56 uniparental inheritance’ evolved in gonochoric bivalve mollusks [10–12].

57 Regarding mitogenome structure, scallops (Bivalvia: Pectinidae) constitute one of  
58 the most peculiar groups within Mollusca. They include one of the largest  
59 mitogenomes known (32–42 kbp; [7], with other species having mitogenomes of

60 different lengths ranging from 16 kbp to 21 kbp [13, 14]. They also present the most  
61 variation in mitochondrial gene order, even at relatively shallow phylogenetic depths,  
62 i.e. within subfamilies [14]. In Pectinidae, as well as among metazoans in general,  
63 gene rearrangements are most common for tRNAs [14–17]. Several pectinids  
64 contain extra tRNAs beyond the essential 22 needed for mitochondrial translation,  
65 either duplicated genes, or genes encoding for the same amino acid, but using a  
66 different anticodon [18–21]. However, even more species contain fewer than 22  
67 mitochondrial tRNAs [13, 14, 19, 22, 23], possibly coming from annotation errors,  
68 which might be the result of modified tRNA secondary structure, common in other  
69 mollusks [24–26]. Similarly, *atp8* genes were reported earlier as missing from most  
70 bivalves, including pectinids [13, 14, 18, 20], although reannotation efforts revealed  
71 its presence in several species [15, 21, 27]. The difficulty of annotating the *atp8* gene  
72 is thought to stem from the poorly conserved sequence of this gene in bivalves [28–  
73 30]. Unlike what was observed in other animal groups, mitochondrial genome  
74 rearrangements in Pectinidae often involve PCGs [13–15]. This, however, does not  
75 seem to be correlated to sequence similarities among PCGs [14]. Furthermore, like  
76 most bivalves, most pectinid mitochondrial genomes contain several inflated non-  
77 coding regions, some of which are hypothesized to function as control regions [13,  
78 14, 18, 20], although it remains controversial whether several control regions within a  
79 mitogenome could provide normal function [20].

80 Most existing pectinid phylogenies are based on one, or a handful of gene  
81 sequences, of either just mitochondrial, or of both mitochondrial and nuclear origin  
82 [31–41]. These phylogenies are often incongruent with morphological classifications  
83 of the Pectinidae [40]. Also, while they conclude that the Pectinidae is monophyletic,

84 many lower taxonomic levels are reported as being paraphyletic [31, 40].  
85 Phylogenies based on full mitogenomes have shown their utility in disentangling  
86 evolutionary relationships that were controversial when investigated using only a few  
87 genes [16, 42–44]. There are a few examples of pectinid phylogenies based on  
88 complete mitogenomes [14, 15, 18, 22, 27], however, most of these studies rely  
89 solely on genetic distance-based methods (except: [15, 22]), without considering the  
90 differences in substitution rates among different mitochondrial genes. Despite the  
91 diversity of approaches, some phylogenetic relationships remain to be resolved, for  
92 example, those within the Chlamydinae subfamily.

93 Numerous scallop species are harvested for human consumption and are used as  
94 environmental indicators. One example is the variegated scallop (*Mimachlamys varia*  
95 L., 1758), a species that inhabits the subtidal zone of the European Atlantic and the  
96 Mediterranean Sea [45]. It was demonstrated that *Mim. varia* is a potent bioindicator,  
97 as it presents significant physiological responses to chemical contaminants,  
98 including changes in biomarkers connected to oxidative stress, immune system  
99 function and mitochondrial respiration [46–48]. Most existing genetic research  
100 involving *Mim. varia* focused on the use of a few genes for phylogenetic or  
101 population genetic inference [31, 40, 49]. Viricel et al. [50] published the  
102 transcriptome of *Mim. varia*, providing a valuable resource for further  
103 ecophysiological and ecotoxicological research, including analyses of differential  
104 gene expression in response to marine pollution and comparative genomic studies  
105 within the Pectinidae.

106 In this study, our goals were to: I) assemble and characterize the mitochondrial  
107 genome of the variegated scallop (*Mimachlamys varia*); II) reconstruct the most up to  
108 date pectinid phylogeny using all available mitochondrial genomes, including *Mim.*  
109 *varia*; III) perform comparative analyses of pectinid mitochondrial gene orders, to  
110 investigate the evolution of mitogenome arrangements within this family.

## 111 **Results**

### 112 **Sequencing of the mitogenome of reference for the variegated scallop**

113 The NOVOPlasty assembly, based on a single *cox1* seed, yielded four contigs,  
114 5,289, 530, 3,458 and 13,528 bp long, recruiting about 5.9 M reads (9.67% of the  
115 61.1 M RNAseq reads submitted to the assembler), with an average depth of  
116 coverage of 84,143. NOVOPlasty never retrieved a full, circular mitogenome based  
117 on these data; the final assembly is based on the manual assembly of the four,  
118 overlapping contigs in Geneious, resulting in a complete, 20,400 bp long circular  
119 assembly. In total, 6.9 M reads were remapped onto the assembled genome. After  
120 remapping, the breadth of coverage is 99.98%, only 5 bases are not covered around  
121 the site where the circular genome was split into a linear sequence. Non-zero  
122 coverage depth values ranged from 11–4,160,626 (Fig. 1). The mean and median  
123 coverage depths were 100,437 and 14,687, respectively.

### 124 Genome composition

125 The full mitogenome contains 13 Protein Coding Genes (PCGs; including an *atp8*,  
126 reported as missing for many bivalves; [13, 14, 18, 20]), 23 tRNAs, and 2 rRNAs

127 (Fig. 1, Table 1). All genes are coded on the “+” strand. Two 2 bp overlaps were  
128 detected by MITOS2, between *nad1-trnR* and *cob-trnD*. Global A+T content is  
129 56.3%. No heteroplasmy was detected with the two MAF settings used in  
130 NOVOPlasty.

### 131 Protein-coding genes

132 All 13 PCGs commonly occurring in metazoan mitochondrial genomes were  
133 identified in *Mim. varia*. The combined length of PCGs was 10,896 bp (45.4% of the  
134 complete genome). The total amino acid (AA) length was 3619, excluding stop  
135 codons. The most frequently used AAs were Leucine (12.86%), Valine (10.39%),  
136 Phenylalanine (9.55%), Glycine (9.45%) and Serine (8.31%). Six genes (*cox1*, *atp8*,  
137 *cob*, *atp6*, *cox2*, *nad4*) had the most common start codon ATG, three had ATT  
138 (*nad6*, *nad3*, *nad5*), three TTG (*nad1*, *nad4l*, *cox3*) and *nad2* had the ATA start  
139 codon (Table 1). Eight genes had the TAG and five had the TAA stop codon.

### 140 rRNA and tRNA genes

141 The *rrnS* and *rrnL* genes were 964 bp and 1,422 bp long, respectively. Out of the  
142 total 23 tRNAs identified, most were identified by MITOS2, except *trnQ2*, which was  
143 identified by ARWEN. The length of tRNAs ranges from 63 to 73 nt. Three tRNA  
144 genes are present in two copies, *trnL* with different anticodons (*trnL1*: UAG, *trnL2*:  
145 UAA), *trnM* and *trnQ* with the same anticodon in both copies, CAU and UUG,  
146 respectively. All tRNAs were predicted to have the typical cloverleaf structure, except  
147 *trnQ2* (Additional File 1). While present in two copies in most animal mitogenomes,  
148 *trnS* was not identified in the mitogenome of *Mim. varia*.



## 149 Gene order

150 The *Mim. varia* mitogenome represents a novel gene order for the Pectinidae. The  
151 conserved pectinid gene clusters "*nad1-trnR-rnnL-cox1*" and "*nad4-trnH-trnW*" are  
152 present, however, the "*nad6-trnL2-cob*" cluster is split by the insertion of *trnF*, the  
153 MNR and *atp8*, and the "*cob-cox2*" cluster is also missing. Notable is the relocation  
154 of the "*rnnS-nad5*" pair, and the location of the MNR between the *nad6* and *cob*  
155 genes, a pattern observed in only one other species, *Pl. magellanicus*.

## 156 Structure of the MNR

157 The major non-coding region (MNR) spans 4,206 nt and has an elevated A+T  
158 content of 60.1%. There were four repeated regions, with repeat sizes between 10  
159 and 683 bp (Table 2). The largest repeat region contained 4.7 copies of a 683 bp  
160 long sequence, with a total length of 3,205 bp, making up 76.2% of the MNR. We  
161 PCR-amplified the MNR but failed to obtain its complete sequence using Sanger  
162 sequencing, possibly because of numerous stem-and loop structures and high %AT  
163 in this region (Additional file 2).

## 164 **Annotation of pectinid genomes**

165 Out of the 29 Pectinidae and 1 outgroup (*O. lurida*) mitogenomes retrieved from  
166 Genbank, only two were incomplete. All protein coding and rRNA genes identified in  
167 previous descriptions of these genomes were found and reannotated in this study.  
168 The *atp8* gene was annotated in all 25 genomes, in which it was not previously  
169 annotated, including two paralogous copies in *Mizuhopecten yessoensis* and *Pl.*  
170 *magellanicus*, and unusually long versions of the gene in two *Ar. irradians* GenBank

171 accessions—with alternative start codons—showing only 78.5% sequence similarity  
172 with conspecific *atp8* genes (Table 3, Additional file 3). Between 20 and 40 tRNA  
173 genes were annotated per mitogenome in the 29 pectinid species. Most  
174 mitogenomes contained 23 tRNA genes, compared to the 22 commonly found in  
175 animals. Gene content was mainly identical within species, but there are slight  
176 variations, some of which bears a phylogenetic pattern. For example, the *trnS1*  
177 genes in *Amusium pleuronectes*, *Pecten albicans* and *Pecten maximus* all have  
178 GCU as anticodon, and all species in the Chlamydinae subfamily contain two copies  
179 of the *trnM* gene. Some variations are present in only one species, *Pe. albicans*  
180 missing *trnL1* and *trnM*, *Mimachlamys nobilis* containing an extra *trnT* gene, *Miz.*  
181 *yessoensis* lacking five tRNA genes, while containing two supernumerary ones, and  
182 *Pl. magellanicus* containing 12 *trnM*, 4 *trnF* and 3 *trnS2* genes (Fig. 2), although  
183 most of these were identified as pseudogenes by Smith et al. [20].

#### 184 **Mito-phylogenomics**

185 All four Subfamilies and 7 out of 9 Tribes of the Pectinidae family are represented in  
186 this study (Table 4). Two different topologies can be observed among the ML trees  
187 reconstructed from the eight datasets (Fig. 3). The two rRNA datasets (G and H)  
188 show a distinct topology compared to the other 6 datasets, but the topology based  
189 on the rRNA datasets is not well-supported. All PCG ML trees had very similar  
190 topology. *Mim. varia* is placed within the same clade with the other two congeneric  
191 species only in the tree based on the E dataset (PCG amino acid sequences). In  
192 every other tree *Mim. varia* is placed in a clade with species in the Crassadomini and  
193 Chlamydini tribes. The monophyly of neither of these clades is supported. Similarly,

194 although the topology suggests that *Pl. magellanicus* is sister to all other pectinids,  
195 its placement is never supported as such, rather the topology of the Pectinidae  
196 should be considered as a trichotomy represented by the subfamilies Palliolinae,  
197 Chlamydinae and a Pectininae+Aequipectini group. The B dataset resulted in the  
198 most well-supported tree (Fig. 4A). All eight ML trees presented with proportional  
199 branch lengths are shown in Fig. 3.

200 Every Bayesian phylogenetic reconstruction run on every dataset reached  
201 convergence (ESS>200). The results of these analyses slightly differ from the results  
202 of the ML method (Fig. 4B). Support values are generally higher than in the ML  
203 approach. *Pl. magellanicus* is placed in the same clade with Chlamydinae in the  
204 trees B, E, F, G and H with moderate to high posterior probabilities (0.81-0.98). In  
205 the rRNA trees (G and H), there is a trichotomy at the root of the Pectinidae, where  
206 the three clades are formed by Palliolinae+Chlamydinae, Pectininae and  
207 Aequipectini groups, respectively. The placement of *Mim. varia* is identical to its  
208 placement in the ML trees. All eight Bayesian trees presented with proportional  
209 branch lengths are shown in Fig 5.

## 210 **Genome rearrangements**

### 211 Gene collinearity

212 Fifteen Locally Collinear Blocks were identified among 13 (1 per species) pectinid  
213 mitogenomes (Fig. 6). Most of these 15 blocks were present in all mitogenomes,  
214 except for LCB2 (missing from *Mim. nobilis*, *Mimachlamys senatoria*, *Pe. albicans*  
215 and *Pl. magellanicus*), LCB9 and LCB14 (both missing from *Pl. magellanicus*). Ten

216 blocks contained one major gene (PCG or rRNA) and none or some tRNA genes.  
217 LCB6 and LCB9 contained one or two PCGs and none or some tRNA genes, LCB7  
218 two to four PCGs and some tRNAs, while LCB2 and LCB4 both contained a set of  
219 tRNA genes only. LCBs 4, 7 and 12 were the most variable in terms of length (Fig.  
220 7), due to the fact that these three blocks contained the Major Non-coding Region in  
221 8 out of the 13 species (Fig. 6). The order of LCBs was generally more similar in  
222 more closely related species, and identical within *Argopecten*, between *Mim. nobilis*  
223 and *Mim. senatoria* and between *Chlamys farreri* and *Crassadoma gigantea*. *Mim.*  
224 *varia*, *Miz. yessoensis* and *Pl. magellanicus* presented substantially different genome  
225 organization from what could be expected by looking at their phylogenetic position  
226 alone. When we look at only a subset of the whole species list, several larger blocks  
227 can be observed. Larger blocks present in more than two species are the following:  
228 LCBs 5, 1 and 13 are present in the same order in 11 species (different in *Mim.*  
229 *yessoensis* and *Pl. magellanicus*); LCBs 5, 1, 13, 7, 8, 9 and 10 form a single block  
230 in the Pectininae and the three *Argopecten* species; LCBs 10, 14, 8, 11, 15, 12 and 4  
231 form a block in the Chlamydinae.

### 232 Common interval analysis

233 Tandem-duplication/random loss and transposition events occurred seven times  
234 along the pectinid phylogeny (Fig. 8). Out of the 7 internal nodes, only one (the  
235 common ancestor of chlamydins) was consistent, with a high level of certainty, two  
236 were k-consistent, i.e., less reliable, with an intermediate level of certainty (the  
237 common ancestor of Chlamydinae+*Placopecten* and the common ancestor of

238 Pectininae and *Argopecten*) and the rest were fallback nodes (representing the  
239 highest level of uncertainty), including the common ancestor of Pectinidae.

240 Three genes – *nad1*, *rnl* and *cox1* – were involved in the fewest rearrangement  
241 events (Additional file 4).

#### 242 Maximum likelihood analysis of gene orders

243 In the maximum likelihood phylogeny reconstructed from gene orders, *Argopecten* is  
244 sister to pectinins, as in the eight ML and eight Bayesian trees, however, within  
245 Pectininae, *Am. pleuronectes* and *Pe. maximus* form the crown (Fig. 9). The other  
246 major differences are the positions of *Mim. varia* and *Miz. yessoensis*, the latter  
247 becoming the basal species in the Chlamydiae and *Mim. varia* becoming sister to  
248 the crown of the Chlamydiae, formed by the pairs of *Mim. nobilis* – *Mim. senatoria*  
249 and *Cr. gigantea* – *Ch. farreri* (Fig. 9).

## 250 **Discussion**

251 To the best of our knowledge, our study presents the most complete mitochondrial  
252 comparative- and phylogenomic analysis to date in the Pectinidae family, including  
253 the newly-assembled mitogenome of *Mim. varia*. The composition of the latter  
254 conforms to most other pectinid mitogenomes published so far. However, it  
255 represents a completely novel gene order, previously not described in any other  
256 species of Pectinidae [13, 14, 18, 20–23, 27].

### 257 **Gene content**

258 In addition to the 12 PCGs present in every bivalve mitogenomes, the *atp8* gene was  
259 annotated in *Mim. varia*. This is not surprising, given that *atp8* is being annotated in  
260 more and more newly published bivalve mitogenomes, as well as identified in  
261 mitogenomes where it was originally thought to be missing [15]. In our study, the  
262 *atp8* gene was annotated in 25 mitogenomes in which it was not previously found, in  
263 two species (*Miz. yessoensis*, *Pl. magellanicus*) it was even detected in two  
264 paralogous copies, and in two accessions of *Ar. irradians*, only as an unusual  
265 version of the gene (alternative start codon and a total length of 222 bp),(Table 3).  
266 The *atp8* gene is very short (135 – 222 bp in the Pectinidae), and is among the most  
267 variable mitochondrial PCGs, making it very difficult to detect. The discovery of a  
268 functional *atp8* gene in most pectinids hints at it not being accessory, as previously  
269 proposed [51–53]. Duplicated genes in mitochondrial genomes are not unheard of  
270 [54–56], although whether all copies are functional remains unknown.

271 While it is common for metazoan mitochondrial genomes to contain two trnL and  
272 trnS genes, pectinid mitogenomes also commonly contain two trnM genes. In  
273 accordance, the mitogenome of *Mim. varia* contains two trnL and trnM genes,  
274 however, it contains no trnS gene, and two trnF and trnQ genes. The two trnF genes  
275 show low sequence similarity and trnF (AAA) shows low sequence identity to trnF  
276 genes in other species as well as every tRNA gene in *Mim. varia*. Although with our  
277 methodology, trnQ2 was annotated in *Mim. varia*, this might be an artifact. We base  
278 this assumption on the fact that the two trnQ genes in *Mim. varia* are found next to  
279 each other, in the gene block (trnK, trnF, trnQ2, trnQ1, trnE), but the same gene  
280 block, containing trnS2 instead of trnQ2, is identified in *Mim. senatoria* [21] and *Cr.*  
281 *gigantea* [18], and revealed during reannotation in the current study in *Mim. nobilis*,

282 *Ch. farreri* and *Miz. yessoensis* (Fig. 2, Additional file 3). Also, this trnQ2 gene shows  
283 higher sequence similarity to the trnS2 gene than to the trnQ gene in the  
284 aforementioned species. While we assume that this difference is the result of an  
285 artifact, it is not impossible that in *Mim. varia*, the trnS gene evolved into trnQ. This is  
286 supported by the fact that tRNA genes are often lost in mitochondrial genomes, and  
287 that remolding of tRNA anticodons is known to happen in mollusks [9, 21, 57].

### 288 **Structure of the MNR**

289 High A+T content and stem-loop secondary structures are the common diagnostic  
290 traits in identifying the mitochondrial control region [58–60]. Both can be observed in  
291 the MNR of *Mim. varia* (Fig. 1, Additional file 2), pointing towards it serving as the  
292 control region.

### 293 **Annotation of pectinid genomes**

294 The fact that we were able to annotate both the atp8 gene and some new tRNA  
295 genes in most pectinid accessions downloaded for this study show that the lack of  
296 these genes were most likely artifacts emerging from the difficulty of annotating  
297 these features in bivalves. With the expansion of annotation databases and the  
298 advancement of annotation tools, these difficulties are getting easier and easier to  
299 overcome, leading to progressively more precise annotations.

300 While variations in tRNA gene content presented by singular species (especially  
301 those with only one mitogenome annotated: *Pe. albicans* and *Mim. varia*) might well  
302 be resulting from sequencing errors, incomplete genomes, or simply misannotations.  
303 While the possibility of these cannot be completely excluded, even in the case of

304 species that have multiple genomes published (*Mim. nobilis*, *Miz. yessoensis*, *Pl.*  
305 *magellanicus*), those presenting a phylogenetic pattern are most probably real. A  
306 peculiar case is that of *trnM*, as it is present in more than half of all studied species  
307 (Fig. 2). This gene is present in two copies in several invertebrate mitogenomes [61,  
308 62], including some bivalves [25]. The invertebrate mitochondrial genetic code  
309 contains five start codons, most of which code for amino acids other than methionine  
310 in vertebrate mitochondrial genomes. This means that *trnM* must do ‘double duty’ as  
311 the tRNA for both methionine and formyl-methionine, matching five instead of two  
312 start codons [58], which could explain why some invertebrates, including some  
313 pectinids evolved to have more copies of it in their mitochondrial genomes.

314 Numerous pectinid mitogenomes are longer than the usual metazoan mitochondrial  
315 genome (~16 kbp), for example *Aequipecten opercularis* 21–28.2 kbp, *Chlamys*  
316 *hastata* 23.9–27.2 kbp, *Chlamys islandica* 22.5–25 kbp, *Cr. gigantea* 22.8–24.8 kbp  
317 [63], *Mim. varia* 20.4 kbp (the current study), *Miz. yessoensis* 20.5–21 kbp [14], *Pl.*  
318 *magellanicus* 30.7–40.7 kbp [20], and *Pe. maximus* [22, 63, 64], although the  
319 available mitogenome for the latter species is only 17.2 kbp long. This is mostly due  
320 to inflated non-coding regions, as demonstrated by Ghiselli et al. [58] in the whole  
321 Mollusca phylum. We identified tandem repeats of total length larger than 500 bp in  
322 the non-coding regions of *Pl. magellanicus*, *Ch. farreri* (compare with [13]), *Mim.*  
323 *varia* and *Am. pleuronectes* (Additional file 5) and repeats of around 500 bp length  
324 scattered throughout the mitogenome of *Miz. yessoensis* (Additional file 3). Gjetvaj et  
325 al. [63] also found that most of the pectinid mitogenomes they studied, contain  
326 tandem repeats of various sizes and repeat numbers in their non-coding region, and  
327 they found no significant sequence similarity among these repeats. This supports the



328 assumption that the repeats arose independently in every lineage. There is also  
329 apparent intraspecific variation in mitochondrial genome size within the Pectinidae  
330 [63–65].

331 While it is likely that correlation exist between the number and length of tandem  
332 repeats and genome size, the available number of pectinid mitogenomes does not  
333 allow to establish correlations between these traits.

334 The presence of multiple repeats make it seem very likely that tandem duplication is  
335 frequent in pectinid mitogenomes, perhaps enabled by the fact that all genes are  
336 coded on the “+” strand. However, this does not seem to influence the mitogenome  
337 architecture of other marine bivalves, most of which also code all genes on the “+”  
338 strand [13]. In plants, which usually have significantly larger mitogenomes than  
339 animals and contain numerous repeated sequences, it is suggested that these  
340 repeats promote recombination [66–68]. This might play a role in the evolution of the  
341 mitochondrial genome in the Pectinidae as well.

342 The prevalence of non-coding regions and, in some instances, extra tRNAs in  
343 Pectinidae make this group an interesting candidate to study the taxonomic  
344 distribution of small mitochondrial highly-transcribed RNAs (smithRNAs, [69]). This  
345 novel class of sncRNAs (short non-coding RNAs), initially described from the Manila  
346 clam *Ruditapes philippinarum* (class Venerida) and detected in zebrafish, fruitflies  
347 and mice [70], could influence the expression of nuclear-encoded genes and be  
348 involved in gonad formation. They were quasi-exclusively found in non-coding  
349 regions and tRNAs [69].

## 350 **Mito-phylogenomics**

351 The phylogenies presented in our study are in accordance with previous results, both  
352 with those using a few genes, and those using the complete set of mitochondrial  
353 PCGs. One novel result is that in this group, the two rRNA genes seem to have little  
354 power to resolve deep divergences, as seen in the different placement of the  
355 Pectininae in both the ML and Bayesian approaches. In accordance with Puslednik  
356 et al. [40] and Alejandrino et al. [31], we found that *Mim. varia* is not placed into one  
357 monophyletic clade with the other two *Mimachlamys* species. Generally, although  
358 Chlamydinae itself is monophyletic, its lower taxonomic levels are not well-resolved,  
359 and several genera are paraphyletic, as it was presented in previous studies [22, 27,  
360 31, 40, 71]. In contrast to Puslednik et al. [40], who concluded Aequipectini to be the  
361 basal clade in Pectinidae (similarly as shown in our rRNA datasets, G and H), we  
362 have found that Aequipectini always form a monophyletic group with Pectininae in  
363 both of our ML and Bayesian approaches, when we included PCGs in the analysis. It  
364 appears that while mitochondrial rRNA genes and the nuclear H3 gene are in  
365 accordance [40], mitochondrial PCGs paint a slightly different picture, as can also be  
366 seen in, for example, Marín et al. [22] and Yao et al. [27]. However, this could at  
367 least be partly caused by the small number of taxa involved in our study, and the  
368 different choice of outgroups, as both taxon sampling and outgroup selection are  
369 known to influence topologies [40]. Despite the Pectinidae being one of the largest  
370 families in Bivalvia, containing around 350 species [72], existing phylogenies usually  
371 contain only a handful of species, with the most making up only 31% of all species  
372 [31]. Chlamydinae is an especially problematic group within the Pectinidae, perhaps  
373 partly because of the low sampling relative to the high number of species in the

374 group. While Puslednik et al. [40] conclude that Chlamydinae—in contrast to Waller’s  
375 hypothesis (i.e., that Chlamydinae is paraphyletic, and have provided the ancestral  
376 stock for Palliolinae and Pectininae)—is in fact the crown group of the Pectinidae.  
377 However, their more recent study [31], doubled taxon sampling and used one more  
378 nuclear gene (28S), and subsequently came to the opposite conclusion, confirming  
379 Waller’s original hypothesis. Another, recent study by Smedley et al. [71] included  
380 yet another nuclear gene (18S), and—as they were studying the Pectinoidea—  
381 numerous outgroup species to Pectinidae. They revealed a similar topology to  
382 Alejandrino et al. [31], but with substantially lower support. This shows the  
383 importance of marker selection and appropriate taxon sampling on pectinid  
384 phylogenetics.

385 The phylogeny reconstructed from gene order data shows a similar, although not  
386 identical topology to the one reconstructed from sequence data. The difference  
387 between the two trees within the Pectininae can be explained with the likely  
388 incompleteness of the *Pe. albicans* mitogenome, missing a few tRNA genes. In  
389 accordance with our phylogenomic results, the gene order phylogeny fails to properly  
390 resolve Chlamydinae, which, again, can be attributed to low taxon sampling within  
391 this group.

392 The lack of sampling is even more prominent if we look at phylogenomic studies, as  
393 the number of published pectinid mitogenomes is currently low and only slowly  
394 growing. Although our study does not resolve this problem, it extends the list of  
395 published pectinid mitogenomes, shows the utility of mito-phylogenomics within the

396 Pectinidae and expands our knowledge of the evolutionary history of mitochondria  
397 within this remarkable group of bivalves.

## 398 **Genome rearrangements**

### 399 Gene collinearity

400 According to the 'punctuation model' [73], mitochondrial genomes are generally  
401 transcribed as a single polycistronic RNA from each strand, followed by the  
402 enzymatic removal of tRNAs, leading to gene-specific mRNAs [74]. In pectinids,  
403 major genes are not always separated by tRNAs (Fig. 6). It is possible that these  
404 cistrons are punctuated by secondary structures instead of tRNAs, or that they  
405 remain bicistrons. However, given that LCBs are the units of genome rearrangement,  
406 and most of them contain one major gene and some tRNA genes, it is probable that  
407 these are all separated during mRNA maturation. The one exception from this rule is  
408 LCB7, that contains the *nad6* and *cob* genes in every studied pectinid species but  
409 contains the *atp8* gene in addition to these in *Mim. varia* and  
410 Pectininae+Aequipectini, although in different position, and *atp8* together with *atp6* in  
411 *Pl. magellanicus* (Fig. 6, Additional file 3). Nevertheless, the variance in the  
412 composition of this block points towards it not being a single unit in rearrangements.  
413 The order of LCBs also shows a clear phylogenetic pattern. The divergence from this  
414 pattern in the case of the three outlier species, *Mim. varia*, *Miz. yessoensis* and *Pl.*  
415 *magellanicus* is most likely stemming from limited taxon sampling, i.e. they are  
416 relatively divergent from their closest relatives in our sampling, and their true close  
417 relatives would most likely show a similar gene order to theirs, as evidenced in other,  
418 better sampled groups in our study (e.g. *Argopecten*).

419 Common interval analysis

420 To the best of our knowledge, our study is the first attempt to reconstruct, at least  
421 partially, the ancient pectinid mitogenome. The CREx method is widely used in other  
422 animal groups, for example insects, e.g. [75, 76]. While the ancestral gene order  
423 could be inferred for Insecta, shared by most groups, and some lineages showing  
424 some rearrangement, this is not the case for Mollusca. *Katharina tunicata* [17, 77]  
425 and *Solemya velum* [30] are hypothesized to carry a mitochondrial genome similar in  
426 organization to the ancestral Molluscan and Conchiferan mitogenomes, respectively.  
427 However, pectinid mitogenomes are very divergent from these two species, and from  
428 closely related groups with available mitogenomes, which severely limits the  
429 effectiveness of using these as outgroups, given the large chance for homoplasy.  
430 Our method tries to overcome this difficulty, with first reconstructing the putative  
431 gene order of internal nodes, including the common ancestor of the Pectinidae,  
432 followed by the inference of rearrangement events. Although some internal nodes  
433 were not consistent, we recovered the putative gene order of the common ancestor  
434 of the Chlamydiae, a monophyletic group in most phylogenetic analyses, including  
435 ours. While most previous studies focusing on mitochondrial genome  
436 rearrangements in the Pectinidae involved only a few species, we compare genome  
437 rearrangements among all available pectinid mitogenomes, with methods not  
438 previously used in this group. Confirming the assumption of Marín et al. [22], we  
439 have found that TDRL (Tandem Duplication/Random Loss) events are equally  
440 important as transpositions in pectinid mitogenome rearrangements.

441

## 442 **Conclusions**

443 Scallop fisheries are commercially important and steadily growing worldwide [78, 79].  
444 Considering that mitochondrial function is implicated in growth and environmental  
445 resilience [80, 81], it is important to understand the evolution of the unusual  
446 mitochondrial genomes of pectinids, as it would aid fisheries management in our  
447 changing climate.

448 In our study, we annotate and characterize the mitochondrial genome of  
449 *Mimachlamys varia*, we demonstrate that complete mitochondrial genomes are  
450 powerful resources in reconstructing pectinid phylogenies, and we also show the  
451 utility of several tools, not previously used in this group, to investigate mitochondrial  
452 genome rearrangements within the Pectinidae. We present annotations of the *atp8*  
453 gene in several pectinid species, where it was previously thought to be missing.

454 We show incongruities between phylogenies based on mitochondrial PCGs and  
455 rRNA genes, suggesting that locus sampling affects phylogenetic inference at the  
456 scale of the Pectinidae family. Both the lack of suitable outgroups—more closely  
457 related to the Pectinidae (e.g. Limidae, Entoliidae, Spondylidae and  
458 Propeamussiidae) than the currently available species—and the lack of sufficient  
459 taxon sampling, especially within Chlamydinae and Palliolinae, are limiting factors for  
460 current research. We therefore believe that sequencing and assembly of more  
461 mitogenomes, particularly from the aforementioned groups, would greatly improve  
462 the power of further research of both mito-phylogenomics and the evolution of  
463 mitogenome rearrangements in the Pectinidae.

## 464 **Methods**

### 465 **Sequencing of the mitogenome of reference for the variegated scallop**

466 One individual of *Mim. varia* (adult male, shell length: 46 mm, shell height: 40mm)  
467 was collected in Angoulins, France, in order to sequence and annotate a reference  
468 transcriptome (nuclear and mitochondrial) for the species. RNA was purified from  
469 five tissues (digestive gland, mantle, gills, adductor muscle and gonads) using the  
470 Nucleospin RNA Set for Nucleozol kit (Macherey-Nagel). After quality control,  
471 extractions were pooled in equal amounts (4 µg of RNA per tissue type). The library  
472 was sequenced on Illumina HiSeq 2500 (2 × 300 bp). Full sample preparation and  
473 sequencing information is provided in Viricel et al. [50]; raw reads can be accessed  
474 on the NCBI Sequence Read Archive (Acc. SRP127478), and transcripts can be  
475 accessed at the Transcriptome Shotgun Assembly database (GGGO01000000).

476 Paired reads, minimally cleaned with Trimmomatic v. 0.36 ([82]; only adapters were  
477 removed as recommended in the NOVOPlasty manual), were used as input data for  
478 assembly with NOVOPlasty v. 4.2 [83] to assemble the mitochondrial genome.  
479 Quality-control before and after read filtering was done using FastQC v0.11.5 [84].  
480 The size of the mitogenome being highly variable among pectinids (from 16,079 bp  
481 in *Argopecten ventricosus*, KT161261.1, to 32,115 bp in *Placopecten magellanicus*,  
482 NC\_007234.1, [85]), we set a wide range for possible mitogenome sizes (15–35 kbp)  
483 as a parameter in NOVOPlasty. K-mer size was set to 39. We used a portion of the  
484 mitochondrial gene *cox1* as an initial seed for mitogenome assembly (we used the  
485 most common haplotype found along the coast of France, KU680872.1; [49]). When

486 NOVOPlasty runs resulted in multiple contigs (mitogenome fragments), they were  
487 assembled in Geneious v2021.0.3 (<https://www.geneious.com>). Reads were mapped  
488 onto the assembly using bowtie2 v2.3.5.1 [86] and coverage statistics were  
489 generated with samtools v1.10 [87]. Once the reference mitogenome was  
490 assembled, we used NOVOPlasty to detect intra-individual heteroplasmy [88] using  
491 a minimum Minor Allele Frequency (MAF) of 0.1 and 0.01 in two separate runs.  
492 Finally, the MITOS2 web server (<http://mitos2.bioinf.uni-leipzig.de/index.py>) [89] was  
493 used to annotate the reference with default parameters, using the RefSeq 63  
494 Metazoa database as reference and the protein-coding gene prediction method of Al  
495 Arab et al. [90]. The mitogenome was loaded into Geneious v2021.0.3. and checked  
496 manually for errors in annotations. ARWEN v1.2.3 [91], was used to look for  
497 additional tRNA genes and secondary structures of tRNAs were predicted using  
498 MITOS2 and the RNAfold web server, with default options. The Major Non-coding  
499 Region (MNR) was scanned for repeated regions using Tandem Repeats Finder v  
500 4.09 (<https://tandem.bu.edu/trf/trf.html>) [92] and secondary structures were predicted  
501 using the mfold Web Server (<http://www.unafold.org/>) [93]. The circular mitogenome  
502 was visualised using the CGView Web Server (<http://cgview.ca/>) [94], with  
503 annotations imported from Geneious.

#### 504 **Assembly and annotation of pectinid mitogenomes from GenBank.**

505 All currently available pectinid mitogenomes (as of January 2021) were downloaded  
506 from GenBank (Table 4) along with *Ostrea lurida* (Ostreidae) as an outgroup. We  
507 chose an oyster species as outgroup as there are no published mitogenomes of taxa  
508 more closely related to Pectinidae. Using only one outgroup made interpreting



509 comparisons of mitogenome rearrangements substantially less difficult. The largest  
510 mitogenome from each species was reannotated using the MITOS2 web server with  
511 the same settings as for the *Mim. varia* mitogenome. New annotations of pectinid  
512 mitogenomes were reviewed manually, in the same way as it was described for *Mim.*  
513 *varia*. If there were multiple annotations for a gene, they were filtered manually  
514 based on length and sequence similarity to other species. Additionally, tRNA genes  
515 were compared with previously published annotations. Next, this reference sequence  
516 of each species was used to annotate other sequences of the same species using  
517 the "Live Annotate & Predict" feature in Geneious.

### 518 **Mito-phylogenomics**

519 Phylogenetic analysis was conducted on the 13 protein coding genes (PCGs) and  
520 two Ribosomal RNA (rRNA) genes of all available pectinid species in GenBank, *Mim.*  
521 *varia* and *O. lurida* as an outgroup. This approach provides the most comprehensive  
522 dataset without the ambiguities arising from tRNA annotations, non-coding regions  
523 and genome rearrangements. Every gene was extracted and aligned separately in  
524 Geneious v2021.0.3. In the case of the *atp8* gene, where more copies were  
525 identified in some species, a Maximum Likelihood (ML) tree was built with the  
526 RAxML [95] plugin of Geneious using default parameters, and the least divergent  
527 copy was selected for further analysis. The *atp8* gene of two accessions of  
528 *Argopecten irradians* (EU023915; NC\_009687) were not included in the phylogenetic  
529 analyses given their unusual structure, and divergent sequences. The 13 PCG  
530 sequences were aligned separately based on translated amino-acids with MAFFT  
531 [96] with the FFT-NS-2 algorithm as implemented in TranslatorX [97]. As the initial

532 alignments contained indels and poorly aligned regions, these regions were masked  
533 with Gblocks [98] as implemented in TranslatorX, with default settings (i.e., allowing  
534 smaller final blocks, gap positions within the final blocks and less strict flanking  
535 positions). The two rRNA genes were aligned with MAFFT in Geneious using the  
536 FFT-NS-2 algorithm. Regions of uncertainty in the alignment were then masked with  
537 Gblocks using the same settings as for the PCGs. Individual gene alignments were  
538 then concatenated in Geneious resulting in eight datasets used for phylogenetic  
539 analyses: A) all PCG and rRNA genes treated with Gblocks; B) all PCG amino acid  
540 sequences and rRNA genes treated with Gblocks; C) all PCGs; D) all PCGs treated  
541 with Gblocks; E) all PCG amino acid sequences; F) all PCG amino acid sequences  
542 treated with Gblocks; G) rRNA genes; H) rRNA genes treated with Gblocks. These  
543 datasets were prepared in order to investigate the effect of rRNA and protein-coding  
544 genes as well as the presence/absence of ambiguously aligned regions on the  
545 topology and branch support of the phylogeny.

546 Maximum Likelihood phylogenetic reconstruction was achieved using IQtree v2.0.6  
547 [99] (except for the B dataset, where IQtree v1.6.12 was used), with automatic model  
548 selection and using the best partition scheme in ModelFinder (option MFP+MERGE)  
549 [100], 10,000 Ultrafast Bootstrap replicates and 10,000 replicates of the SH-like  
550 approximate likelihood ratio test (SH-aLRT) [101] on all eight datasets. Partitioning  
551 and substitution model information for every run can be found in Additional file 6.

552 Bayesian analyses were done using MrBayes v3.2.7 [102]. For each dataset, two  
553 runs were performed over 1.5 million generations, with four chains each (three  
554 heated and one cold). Trees were sampled every 500 generations, and the first 25%

555 were discarded as burn-in. Finally, a majority consensus tree was constructed to  
556 estimate posterior probabilities of branches. Convergence of each run was evaluated  
557 through Effective Sample Size (ESS) values and trace plots in the software Tracer  
558 v.1.6 [103]. Partitioning and prior model parameter settings can be found in  
559 Additional file 7.

## 560 **Genome rearrangements**

561 Three approaches were used to infer mitogenome rearrangements in the Pectinidae:  
562 1) gene collinearity analysis, which detects mosaic patterns of homology among a  
563 set of genomes, based on whole-genome sequence alignment; 2) common interval  
564 analysis, which determines pairwise rearrangement events between genomes,  
565 based on gene order data, considering several rearrangement types, but requiring  
566 identical gene content; and 3) maximum likelihood analysis of gene orders, which  
567 reconstructs phylogenies based solely on gene order data, without requiring an  
568 identical set of genes between genomes.

### 569 Gene collinearity

570 Gene collinearity among pectinid mitogenomes (one individual/species, 13  
571 individuals in total) was explored using the progressiveMauve algorithm and default  
572 parameters (automatically calculating seed weight and the minimum LCB score,  
573 computing Locally Collinear Blocks (LCBs) and using full alignments) in the MAUVE  
574 Geneious plugin [104].

### 575 Common interval analysis

576 Two joint softwares – both relying on the same algorithm – CREx [105] and TreeREx  
577 [106] were implemented for common interval analyses. Both software consider the  
578 following types of rearrangement events: transpositions, inversions, inverse  
579 transpositions and tandem-duplication/random loss (TDRL) involving the duplication  
580 of a gene block followed by the random loss of one copy of a gene in either of the  
581 two blocks. Both tools are only able to handle identical gene sets among species. To  
582 conform to this condition, only major genes (PCGs and rRNA genes) and the MNR  
583 were included in this analysis, as tRNA gene content is variable among pectinid  
584 mitogenomes.

585 The MrBayes tree based on the most supported dataset (B), with only 8 pectinids  
586 and one outgroup species representing unique gene orders, along with the gene  
587 order dataset described above, was used in the first step, using the TreeREx [106]  
588 software. TreeREx uses the phylogenetic information in the tree to ascertain  
589 rearrangement events among nodes of the tree, and to infer putative gene orders of  
590 common ancestors at internal nodes. TreeREx was used with default settings: strong  
591 consistency method applied (-s); weak consistency method applied (-w);  
592 parsimonious weak consistency method applied (-W); and the maximum number of  
593 inversions (-m) set to 2. Finally, the input dataset in the previous analysis,  
594 complemented with the putative gene orders inferred at internal nodes was put into  
595 CREx again, to visualize each rearrangement event between each pair of  
596 neighboring nodes.

597 Maximum likelihood analysis of gene orders

598 The MLGO (Maximum Likelihood analysis of Gene Order) [107] web server can be  
599 used to directly infer a phylogeny based on gene order data. The algorithm handles  
600 insertions, deletions and duplications along with transpositions. Unlike  
601 CREx/TreeREx, it is not limited to a common set of genes across taxa. Complete  
602 gene order data from 13 pectinids (one individual per species) and one outgroup (*O.*  
603 *lurida*) were used to infer a phylogeny of the Pectinidae. Branch support was  
604 computed with a bootstrap analysis of 1000 replicates.

## 605 **Declarations**

### 606 **Ethics approval and consent to participate**

607 No vertebrates or regulated invertebrates were involved in this study.

### 608 **Consent for publication**

609 Not applicable.

### 610 **Availability of data and materials**

611 Raw *Mimachlamys varia* RNAseq reads are deposited in the NCBI Sequence Read  
612 Archive (Acc. SRP127478), and transcripts can be accessed at the Transcriptome  
613 Shotgun Assembly database (GGGO01000000). The assembled mitochondrial  
614 genome is uploaded to GenBank (MZ520326), and the GenBank accession numbers  
615 of all mitogenomes used in this study can be found in Table A. The sequence  
616 alignments used this article will be uploaded upon acceptance to a public repository.

### 617 **Competing interests**

618 The authors declare no competing interests.

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## 624 **Authors' contributions**

625 ED and VB collected the samples, AV and VB performed laboratory analyses, TM,  
626 EP and LE performed data analyses, TM wrote the manuscript, AV and EP reviewed  
627 and edited the manuscript. All authors read and approved the final manuscript.

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## 948 **Figure legends**

949 **Fig. 1.** Map of the *Mimachlamys varia* mitogenome. Protein coding (CDS), rRNA and  
950 tRNA genes and the Major Non-coding Region (MNR) are marked with differently  
951 colored arrows pointing in the direction of transcription. Innermost scale represents  
952 size in kbp. GC content and GC skew are represented by black and purple-orange  
953 histograms, respectively. The outermost circle in light gray shows the natural  
954 logarithm of the depth of coverage for each base. Photo credit: Thierry Guyot.

955 **Fig. 2.** Heatmap representing mitochondrial tRNA gene sets observed in the 13  
956 pectinid species included in this study. tRNAs are represented with their one-letter  
957 amino-acid code, with the anticodon in parentheses. The deeper blue color  
958 corresponds to higher copy number of a tRNA gene.

959 **Fig. 3.** Maximum likelihood trees based on all eight datasets (A-H). Branch lengths  
960 are scaled by setting the length of the outgroup branch to 1.5 on each tree. Numbers  
961 on top of the outgroup branches represent the original length of the branches. Every  
962 genus is marked with a different color, which is identical to the color coding on  
963 Figures 4 and 5. *Mimachlamys varia* is marked with a black diamond shape, the gray  
964 line connects the corresponding internal node with the SH-like approximate  
965 likelihood ratio test (SH-aLRT) value on the left and ultrafast bootstrap support on  
966 the right. Dataset descriptions: A) all PCG and rRNA genes treated with Gblocks; B)  
967 all PCG amino acid sequences and rRNA genes treated with Gblocks; C) all PCGs;  
968 D) all PCGs treated with Gblocks; E) all PCG amino acid sequences; F) all PCG  
969 amino acid sequences treated with Gblocks; G) rRNA genes; H) rRNA genes treated  
970 with Gblocks.

971 **Fig. 4.** Phylogenetic reconstruction of 30 Pectinidae mitogenomes with *Ostrea lurida*  
972 (*Ostreidae*) as the outgroup, based on the “B” dataset (PCG amino acid and rRNA  
973 DNA sequences). Every genus is marked with a different color, identical to the color  
974 coding on Figs. 3 and 5. *Mimachlamys varia* is marked with a black diamond shape.  
975 Fully supported branches are marked with a blue circle on the corresponding node.  
976 A: Maximum likelihood tree. SH-like approximate likelihood ratio test (SH-aLRT) is  
977 presented on the left, ultrafast bootstrap support projected from the extended  
978 majority-rule consensus tree is presented on the right. B: Bayesian tree. Numbers  
979 represent posterior probabilities; the staggered and gray lines connect species  
980 names with the corresponding tips. For original branch lengths, see Fig. 3B and Fig.  
981 5B.

982 **Fig. 5.** Bayesian consensus trees based on all eight datasets (A-H). Branch lengths  
983 are scaled by setting the length of the outgroup branch to 1.5 on each tree. Numbers  
984 on top of the outgroup branches represent the original length of the branches. Every  
985 genus is marked with a different color, that is identical to the color coding of Figs. 3  
986 and 4. *Mimachlamys varia* is marked with a black diamond shape. Posterior  
987 probabilities of the corresponding internal node are presented. Dataset descriptions:  
988 A) all PCG and rRNA genes masked with Gblocks; B) all PCG amino acid  
989 sequences and rRNA genes masked with Gblocks; C) all PCGs; D) all PCGs  
990 masked with Gblocks; E) all PCG amino acid sequences; F) all PCG amino acid  
991 sequences masked with Gblocks; G) rRNA genes; H) rRNA genes masked with  
992 Gblocks.

993 **Fig. 6.** Gene collinearity analysis of 13 Pectinid species performed in Mauve v2.3.1.  
994 Color blocks represent homologous regions among different mitogenomes. The level  
995 of similarity at each position is shown in the blocks. Below these blocks, the white,  
996 red, green and pink boxes represent protein coding, rRNA, tRNA genes, and tandem  
997 repeats, respectively.

998 **Fig. 7.** Boxplot showing the length distribution of each LCB (locally colinear block)  
999 from the gene collinearity analysis.

1000 **Fig. 8.** Common interval analysis presented on the most supported Bayesian tree,  
1001 obtained from dataset "B", where each tip represents a unique gene order. Internal  
1002 nodes are named as "An", where "n" is a number between 0 and 7, and represents  
1003 ancestral gene orders. C and P indicate consistency and parsimony values,  
1004 respectively. The colors code consistent (green; highest level of certainty), k-

1005 consistent (yellow; intermediate level of certainty) and inconsistent (red; low level of  
1006 certainty) nodes. Mutation types are indicated on corresponding branches, T:  
1007 transposition, TDRL: tandem duplication and random loss.

1008 **Fig. 9.** Maximum likelihood tree based on gene orders, 42 genes in total, including  
1009 all 13 species included in this study plus the outgroup, and the complete set of genes  
1010 in each mitogenome. Numbers represent bootstrap support. *Mimachlamys varia* is  
1011 marked with a black diamond shape.



1012 **Tables**

1013 **Table 1.** Main structural features of the *Mimachlamys varia* mitogenome.

Gene	Location	Size (bp)	Start codon	Stop codon	Anticodon s	Intergenic nucleotide	A+T No. content
						AA	(%)
trnN	1–69	69			GUU	–	54
nad1	94–993	900	TTG	TAG		24 299	56.1
trnR	992–1055	64			UCG	-2	56.2
rrnL	1111–2532	1422				55	56.6
cox1	2604–4127	1524	ATG	TAG		71 507	55
nad4l	4384–4671	288	TTG	TAG		256 95	50.3
trnG	4677–4739	63			UCC	5	60.3
nad6	4774–5271	498	ATT	TAA		34 165	54.6
trnF	5414–5483	70			AAA	142	58.6
atp8	9690–9848	159	ATG	TAG		4206 52	55.3
trnL2	9859–9924	66			UAA	10	50
cob	10133–11257	1125	ATG	TAG		208 374	56
trnD	11256–11321	66			GUC	-2	47
cox3	11401–12171	771	TTG	TAA		79 256	55.5
trnK	12215–12283	69			UUU	43	46.4
trnF	12288–12352	65			GAA	4	49.2
trnQ2	12359–12425	67			UUG	6	52.2

trnQ1	12429–12497	69		UUG	3	44.1
trnE	12503–12568	66		UUC	5	54.5
atp6	12642–13313	672 ATG	TAA		73 223	56.8
cox2	13377–14078	702 ATG	TAG		63 233	54.7
nad2	14084–15040	957 ATA	TAA		41 306	56
trnV	15051–15116	66		UAC	10	50
nad3	15125–15472	348 ATT	TAG		8 115	54.9
nad4	15552–16835	1284 ATG	TAG		79 427	54.8
trnH	16837–16902	66		GUG	1	56.9
trnW	16908–16977	70		CCA	5	54.3
trnY	16981–17045	65		GUA	3	49.2
trnT	17050–17112	63		UGU	4	57.1
trnP	17116–17183	68		UGG	3	48.5
trnI	17186–17256	71		GAU	2	57.7
trnL1	17265–17333	69		UAG	8	53.6
trnM2	17336–17402	67		CAU	2	47.8
trnM1	17411–17483	73		CAU	8	41.1
trnC	17492–17559	68		GCA	8	42.6
trnA	17571–17638	68		UGC	11	59.1
rrnS	17675–18638	964			36	52.7
nad5	18690–20393	1704 ATT	TAA		51 567	55.9

1014 **Table 2.** Tandem repeats in the *Mimachlamys varia* mitogenome.

Position in	Perio	Copy	Consensus	Percent	Percent	Alignm	A	C	G	T	Entropy
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genome	d size	number	size	matches	indels	ent						(0-2)
						score						
5601–5625	13	1.9	13	100	0	50	84	0	16	0	0.63	
5894–5935	10	4.3	10	87	6	68	21	28	40	9	1.84	
5896–5933	19	2	20	94	5	69	21	26	42	10	1.85	
6440–9644	683	4.7	683	99	0	6392	28	13	22	35	1.92	

1015 **Table 3.** Newly annotated *atp8* genes.

Species	GenBank accession no.	Start	Stop	Length	AA	5'	3'
						neighbor	neighbor
<i>Placopecten magellanicus</i>	DQ088274– <i>atp8-1</i>	23484	23660	177	58	trnM	atp6
	DQ088274– <i>atp8-2</i>	64	231	168	55	trnG	trnM
	NC_007234– <i>atp8-1</i>	23484	23660	177	58	trnM	atp6
	NC_007234– <i>atp8-2</i>	64	231	168	55	trnG	trnM
<i>Chlamys farreri</i>	EF473269	10041	10187	147	48	nad5	trnD
	EU715252	10041	10187	147	48	nad5	trnD
	NC_012138	10041	10187	147	48	nad5	trnD
<i>Mizuhopecten yessoensis</i>	AB271769– <i>atp8-1</i>	9797	9943	147	48	nad4l	trnD
	AB271769– <i>atp8-2</i>	9239	9385	147	48	nad5	nad4l
	FJ595959– <i>atp8-1</i>	9797	9943	147	48	nad4l	trnD
	FJ595959– <i>atp8-2</i>	9239	9385	147	48	nad5	nad4l

	NC_009081- <i>atp8-1</i>	9797	9943	147	48	nad4l	trnD
	NC_009081- <i>atp8-2</i>	9239	9385	147	48	nad5	nad4l
<i>Crassadoma</i> <i>gigantea</i>	MH016739	11145	11303	159	52	nad5	trnD
<i>Mimachlamys</i> <i>nobilis</i>	FJ415225	7938	8090	153	50	nad4l	trnM
	FJ595958	7937	8089	153	50	nad4l	trnM
	NC_011608	7938	8090	153	50	nad4l	trnM
<i>Mimachlamys</i> <i>varia</i>	MZ520326	9690	9848	159	52	trnF	trnL2
<i>Pecten</i> <i>albicans</i>	KP900974	6000	6134	135	44	trnE	nad6
<i>Pecten</i> <i>maximus</i>	KP900975	5993	6127	135	44	trnE	nad6
<i>Argopecten</i> <i>irradians</i>	DQ665851	4272	4406	135	44	trnE	nad6
	EU023915	4185	4406	222	73	trnF	nad6
	KT161259	4272	4406	135	44	trnE	nad6
	KT161262	4272	4406	135	44	trnE	nad6
	KU589290	4272	4406	135	44	trnE	nad6
	NC_009687	4185	4406	222	73	trnF	nad6
	NC_012977	4272	4406	135	44	trnE	nad6
<i>Argopecten</i> <i>purpuratus</i>	KF601246	4276	4410	135	44	trnE	nad6

	KT161260	4276	4410	135	44	trnE	nad6
	NC_027943	4276	4410	135	44	trnE	nad6
<i>Argopecten</i>	KT161261	4238	4372	135	44	trnE	nad6

*ventricosus*

1016

1017 **Table 4.** List of species included in the present study.

1018

Subfamily	Tribe	Species	GenBank accession no.	Genome length (bp)	Source
Palliolinae	Palliolini	<i>Placopecten magellanicus</i> (Gmelin, 1791)	DQ088274	32115	La Roche et al. [77]□
			NC_007234	32115	La Roche et al. [77]□
Chlamydinae	Chlamydini	<i>Chlamys farreri</i> (Jones & Preston, 1904)	EF473269	20889	Ren et al. [11]□
			EU715252	21695	Xu et al. [18]□
			NC_012138	21695	Xu et al. [18]□

	<i>Mizuhopecten yessoensis</i> (Jay, 1857)	AB271769	20414	Sato et al. [14]□
		FJ595959	20964	Wu et al. [12]□
		NC_009081	20414	Sato et al. [14]□
Crassadomini	<i>Crassadoma gigantea</i> (J.E. Gray, 1825)	MH016739	18495	Liao et al. [13]□
Mimachlamydini	<i>Mimachlamys nobilis</i> (Reeve, 1852)	FJ415225	17963	Xu et al., unpublished
		FJ595958	17935	Wu et al. [12]□
		NC_011608	17963	Xu et al., unpublished
	<i>Mimachlamys senatoria</i> (Gmelin, 1791)	KF214684	17383	Wu et al. [16]□
		NC_022416	17383	Wu et al. [16]□
	<i>Mimachlamys varia</i> (Linnaeus,	MZ520326	20400	Malkócs et al., unpublished

		1758)			
Pectininae	Amusiini	<i>Amusium</i> <i>pleuronectes</i> (Linnaeus, 1758)	MT419374	18044	Yao et al. [23]□
	Pectinini	<i>Pecten</i> <i>albicans</i> (Schröter, 1802)	KP900974	16653	Marín et al. [17]□
		<i>Pecten</i> <i>maximus</i> (Linnaeus, 1758)	KP900975	17252	Marín et al. [17]□
Aequipectini group	Aequipectini	<i>Argopecten</i> <i>irradians</i> (Lamarck, 1819)	DQ665851	16211	Ren et al. [11]□
			EU023915	16221	Petten and Snyder, unpublished
			KT161259	16286	Li, unpublished
			KT161262	16211	Li, unpublished

			KU589290	16212	Liu, unpublished
			NC_009687	16221	Petten and Snyder, unpublished
			NC_012977	16211	Ren et al. [11]□
		<i>Argopecten purpuratus</i> (Lamarck, 1819)	KF601246	16266	Marín et al. [17]□
			KT161260	16270	Li, unpublished
			KY321561	16224	Romero et al., unpublished
			NC_027943	16270	Li, unpublished
		<i>Argopecten ventricosus</i> (G. B. Sowerby II, 1842)	KT161261	16079	Li, unpublished
Outgroup					
Family:	Ostreidae	<i>Ostrea lurida</i> Carpenter, 1864	NC_022688	16344	Xiao et al. [100]□



1019 Taxonomic authorities according to the World Register of Marine Species (WoRMS  
1020 Editorial Board, 2021). Annotation information can be found in Table 1 for *Mim. varia*,  
1021 and Additional file 3 for all other species.

## 1022 **Additional files**

1023 **File name:** Additional File 1.

1024 **File format:** PDF Document (.pdf)

1025 **Title of data:** Putative secondary structures of mitochondrial tRNAs in *Mimachlamys*  
1026 *varia*.

1027 **Description of data:** Amino acids are represented by their one-letter code. (\* :  
1028 structure predicted by RNAfold, all other structures were predicted by MITOS2)

1029 **File name:** Additional File 2.

1030 **File format:** PDF Document (.pdf)

1031 **Title of data:** Putative secondary structure of the Major Non-coding Region of  
1032 *Mimachlamys varia*.

1033 **Description of data:** There is a 100 basepair overlap kept between parts. 1: 5484–  
1034 6439; 2: 6339–7805; 3: 7705–9689.

1035 **File name:** Additional file 3.

1036 **File format:** Microsoft Word Document (.docx)

1037 **Title of data:** Annotation tables for species involved in this study.

1038 **File name:** Additional file 4.

1039 **File format:** PDF Document (.pdf)

1040 **Title of data:** Common interval analysis results presented on a Bayesian tree, where  
1041 each tip represents a unique gene order.

1042 **Description of data:** Internal nodes are named as  $A_n$ , where  $n$  is a number between  
1043 0 and 7, and represent ancestral gene orders. C and P indicate consistency and  
1044 parsimony values, respectively. The colors code consistent (green; highest level of  
1045 certainty), k-consistent (yellow; intermediate level of certainty) and inconsistent (red;  
1046 low level of certainty) nodes. Mutation types are indicated on corresponding  
1047 branches, T: transposition, TDRL: tandem duplication and random loss. Arrows point  
1048 to diagrams showing the two gene orders, and the mutation steps leading from the  
1049 more ancestral to the younger node. Frames connect gene blocks affected by a  
1050 rearrangement event, and parts of the mitogenome that is affected by a  
1051 rearrangement event.

1052 **File name:** Additional file 5.

1053 **File format:** Microsoft Word Document (.docx)

1054 **Title of data:** Tandem repeats detected in pectinid mitogenomes.