The mitochondrial genomes of *Pecten albicans* and *Pecten maximus* (Bivalvia: Pectinidae) reveal a novel gene arrangement with low genetic differentiation

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Abstract

Scallops (Bivalvia: Pectinidae) comprise more than 350 extant taxa including species of high economic importance. However, its phylogenetic classification remains unclear and so far only 7 scallop mitogenomes have been determined. In this study, the mitochondrial genomes of two congeneric scallop species *Pecten albicans* and *Pecten maximus* were determined. Both mitogenomes contain 12 protein-coding genes (*atp8* is missing), two ribosomal genes, 20-22 transfer *RNA* genes, all encoded in the same strand. Overall, both *Pecten* mitogenomes are highly similar with high nucleotide (93 %) and amino acid (98.7 %) sequence identity. Both mitogenomes also contain the same gene order arrangement, which is a novel contribution within Pectinidae. The highly similar mitochondrial organization between both *Pecten* species suggested a recent speciation event. Phylogenetic analysis based on complete protein-coding gene information as well as large synteny gene blocks confirmed the sister group relationship between the genera *Pecten* (subfamily Pectininae) and *Argopecten* (tribe Aequipectini).

Keywords • Mitochondrial genome • Pectinidae • Synteny • Phylogenetics • Amino acids • Scallops

1. Introduction

Metazoan mitochondrial genome is typically built of double-stranded circular DNA molecules of about 15-17 kb and usually encodes for 37 genes: 13 protein-coding genes (PCGs), 2 ribosomal RNA genes and 22 transfer *RNA*s that are indispensable for protein translation (Boore, 1999; Liu et al., 2013). Mitochondrial genes are considered important tools for evolutionary and phylogenetic studies because of its high mutation rates, maternal transmission (with exceptions in some animal groups) and the abundance of mitochondrial copies in cells. In most metazoan taxa, gene order is usually well conserved. However, marine bivalves are a notable exception to the common metazoan mitochondrial constitution. Marine bivalve mitogenomes are known for their high levels of gene rearrangement (Dreyer and Steiner, 2006; Yuan et al., 2012), even among species of the same family (i.e. Pectinidae; Xu et al., 2010). An extra large mitogenome size is another unusual characteristic of marine bivalves. From all Pectinidae species with available mitogenome, *Placopecten magellanicus* has the largest size (32115 bp; GenBank access number DQ088274). The presence of tandem repeats elements and transposition involving *tRNAs* or *tRNA*-like structures are responsible for the large size and length variation (30-40 kb) among individuals (La Roche et al., 1990; Smith and Snyder, 2007). Another common characteristic in most bivalve mitogenomes is the lack of *atp8* gene and certain *tRNAs* (Smith and Snyder, 2007).

The family Pectinidae comprises about 350 living species (Biscotti et al., 2007). In spite of the large fossil record, the phylogenetic relationships among scallop species are still unresolved. With 15 recognized species (World Register

of Marine Species, WORMS; http://www.marinespecies.org), scallops of the genus *Pecten* represent commercially important species distributed throughout temperate and sub-tropical seas in distant geographic areas such as Europe, Africa, Asia and Australia (Duncan and Wilson, 2012; Saavedra and Peña, 2004). *Pecten albicans* and *Pecten maximus* constitute important fisheries (Saavedra and Peña, 2004) as well as interesting evolutionary models due to their unknown divergence times. The Japanese baking scallop *P. albicans* occurs from southern Hokkaido to Kyushu in Japan (Okutani, 2000). On the other hand, *P. maximus* is distributed along the Northeast Atlantic from Norway down to North Africa (Beaumont and Gjedrem, 2007).To date, little is known about the mitochondrial genome composition of *Pecten* species and few studies have addressed the extremely low genetic differentiation among species of this genus, using only partial mitochondrial gene fragments (Saavedra and Peña, 2004). It has been shown that phylogenetic analysis based on complete mitogenome sequence data have proved to produce much robust phylogenetic reconstructions enhancing resolution and statistical confidence of inferred phylogenetic trees when compared with analyses based only on smaller portions of the mtDNA (Ingman et al., 2000 and Yuan et al., 2012).

Before this study, only seven complete mitogenomes from the family Pectinidae were available in GenBank database including 5 species from 2 subfamilies named Chlamydinae (*Mizuhopecten yessoensis*, *Chlamys farreri*, *Mimachlamys nobilis* and *Mimachlamys senatoria*) and Palliolinae: (*P. magellanicus*), and two species from the tribe Aequipectini (*Argopecten irradians* and *Argopecten purpuratus*). This is the first report on the complete

mitochondrial coding region and gene rearrangement of two Pectininae species: *P. albicans* and *P. maximus*. The results of the present study will help to get further insights into the very complex taxonomy and evolutionary relationships among Pectinidae species.

2. Materials and Methods

2.1. Sample collection and genomic DNA extraction

One individual of *P. albicans* and *P. maximus* were collected from Oki Island in Japan and Rye Bay in England, respectively. The adductor muscles were dissected and preserved in 95% ethanol and stored at -20 °C. Genomic DNA was extracted following the standard phenol-chloroform protocol and adjusted to a concentration of 50 ng/μl and used for PCR.

2.2. Long PCR amplification and sequencing

Two long PCR primer sets were designed to amplify the entire mitogenome in two fragments with overlapping segments, covering the complete circular mitochondrial genome. The primer sets 12SpectUF and PecnaXX (amplified fragments of 9922 and 9932 bp in *P. albicans* and *P. maximus*, respectively) and Pectco3F and Pect12SR (amplified fragments of approx. 13 and 15 kb in *P. albicans* and *P. maximus*, respectively) were designed based on multi-alignment comparisons of the mitochondrial 12S rRNA, *cox3*and *nad2* genes from Pectinidae species retrieved from GenBank database (primer sequences are listed in Table 1). Large PCR products were obtained using TaKaRa LA *Taq* polymerase (TaKaRa, Japan). To quicken the sequencing process, additional shorter overlapping PCR fragments (2-6 kb) were amplified (see Table 1 for

primer list) using the large PCR fragments (10-15kb) as DNA templates. Full length of both scallop mitogenomes were estimated based in the nucleotide size content of the first large PCR product amplified by primers 12SpectUF and PecnaXX (which was fully sequenced) plus the combination of overlapping large and small PCR fragments from the "second half" of the mitogenome, which were estimated based on comparison with molecular ladders of different sizes by agarose electrophoresis. All PCR amplifications were performed on an ASTEC PC816 thermal cycler in a total volume of 50 µl, containing 26.5 µl sterile H_2O, 5 µl 10 × LA PCR Taq buffer (Mg^{2+} free, TaKaRa), 5 µl $MgCl_2$ (25mM), 8 µld NTPs (2.5mM each), 2 µl each primer (10 µM), 0.5 µl LA *Taq* polymerase (5 units/µl, TaKaRa) and 1 µl of template DNA. Thermal cycling conditions were as follows: initial denaturation for 2 min at 98 °C, followed by 35 cycles of denaturation for 20 s at 94 °C, annealing for 35 s at 60 °C, and extension for 10 min at 68 °C, followed by a final extension for 10 min at 72 °C. Amplicons were electrophoresed in 1% agarose gels, stained with ethidium-bromide, and visualized under UV light. Subsequently, PCR products were extracted and purified using the QIAquick Gel Extraction kit (Quiagen, Hilden, Germany), directly sequenced in both directions by primer walking method on an ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems, Hitachi, Japan).

The largest non-coding region of the *P. albicans* and *P. maximus* mitogenomes is characterized for the presence of long tandems repeats (1.6 kb approx; Rigaa et al., 1995) and homopolymer runs, which affected the PCR reaction and DNA sequencing process by polymerase slippage. Regrettably, a fragment containing the control region was not determined (approx. 6 and 7.5 kb

in size in *P. albicans* and *P. maximus*, respectively). Thus, only the complete mitochondrial coding region is reported for both species.

Additionally, to identify the gene cluster *nad1-rrnL-cox1* in more scallop species, a highly conserved fragment including partial sequence of genes *nad1* and *cox1* flanking the complete genes *rrnL, trnR* and *trnM* were amplified and sequenced in six additional scallop species including *Amusium pleuronectes*, *Ylistrum japonicum*, *Annachlamys macassarensis*, *Bractechlamys vexillum*, *Decatopecten radula* and *Mimachlamys sanguinea*. We followed same strategy as Marín et al. (2015), using a combination of degenerated and specific primers.

2.3. Sequence assembling and gene identification

The mitogenome sequences were assembled manually using MEGA 5.2 software (Tamura et al., 2011). The mitochondrial genomes were annotated using MITOS web service (Bernt et al., 2013). PCGs were identified with ORF Finder (NCBI website) using invertebrate mitochondrial code. PCGs denoted by putative ORFs were then identified by sequence similarity with BLASTP (NCBI website). Ribosomal RNA genes were identified by multi-alignment with GenBank sequences of bivalve mitogenomes. The *tRNA* genes were identified by tRNAScan-SE 1.21 Search Server (Lowe and Eddy 1997), using the invertebrate mitochondrial codon sequence, MITOS annotation web service or using secondary structures and anticodons when necessary. The whole mitochondrial genome sequences were tested for potentially tandem repeats by TANDEM REPEAT FINDER, Version 4.0 (Benson 1999). Mitochondrial genomes were drawn using GenomeVx (Conant and Wolfe 2008). Nucleotide and amino acid sequence identities were estimated as *p*-distances using MEGA 5.2.

2.4. Phylogenetic analysis

The complete PCGs from the available mitogenomes of 7 scallop species along with the two newly developed mitogenomes were used to infer their phylogenetic relationships. The oysters *Pinctada maxima* (NC_018752) and *Crassostrea gigas* (EU672831) were used as outgroups in all the analyses. The translated amino acid sequences of 12 PCGs were aligned separately with ClustalW. Poorly aligned positions and gaps were excluded using Gblocks (Castresana, 2000) as implemented in SeaView v. 4.5.3 (Gouy et al., 2010) and concatenated to construct phylogenetic trees. The nucleotide sequence was substituted from the concatenated amino acid alignment. Substitution saturations in single codon positions from each PCGs were analyzed using DAMBE 5 (Xia, 2013). The third codon positions of the genes *atp6*, *cytb*, *cox2*, *nad1*, *nad2*, *nad4L*, *nad5*, and *nad6* showed clear signs of saturation. Thus, two dataset were used for phylogenetic analyses: nucleotide sequence of 12 PCGs including all codon positions (PCG123) and nucleotide sequence of 12 PCGs excluding the third codon positions (PCG12). Two phylogenetic methods were performed including maximum likelihood (ML) with MEGA 5.2 and Bayesian inference (BI) with MRBAYES 3 (Ronquist and Huelsenbeck, 2003). For the ML analysis, the best fit of substitution model was determined in MEGA 5.2 using Bayesian Information Criterion (BIC). The GTR+G model was selected for the PCG123 and PCG12 datasets. Node reliability was tested using 1000 bootstrap replicates. For the Bayesian approach, we used jModelTest 2 (Darriba et al., 2012) under the Akaike Information Criterion (AIC) to find the best-fit model of evolution. For both datasets (PCG123 and PCG12) BI analysis was performed

under the GTR model of nucleotide substitution with a gamma-distributed rate variation across sites and a proportion of invariable sites. Two runs were performed simultaneously, each with four Markov chains. The analyses were run for 1,000,000 generations with sampling every 100 generations. The first 25 % of the sampled trees were discarded as burn-in. All phylogenetic trees were drawn using Figtree 1.4.2 program (http://tree.bio.ed.ac.uk/software/figtree/).

3. Results

3.1. Genome composition

The mitochondrial genomes of *P. albicans* (GenBank accession KP900974) and *P. maximus* (GenBank accession KP900975) are 22.6 and 24.8 kb in length, respectively (Figure 1). As aforementioned, due to the presence of long tandem repeats within the control region of both *Pecten* species, partial mitogenomes containing the complete protein coding regions were obtained in *P. albicans* (16653 bp) and *P. maximus* (17252 bp). Excluding the main control region, the overall nucleotide sequence identity between both *Pecten* species is 93 %, with 1,089 variable sites. The structure and organization of *P. albicans and P. maximus* mitogenomes showed characteristics of a typical marine bivalve mitogenome: 12 PCGs (*atp6*, *cox1* to *cox3*, *cytb*, *nad1* to *nad6*, *nad4L*), two ribosomal RNA genes, 20 (*P. albicans*) to 22 (*P. maximus*) transfer *RNAs* and the missing *atp8* gene. In both *Pecten* species all genes are encoded in the same strand with two overlapping genes (17 nt between *nad4L* and *cox3*). In *P. albicans* the genes *nad2* and *nad3* are also overlapping by one nucleotide.

3.2. Protein-coding genes

Twelve PCGs were identified in both *Pecten* mitogenomes (Table 2). Overall, the complete length of PCGs in *P. albicans* (11403 nt) and *P. maximus* (11397 nt) accounted for 50.5% and 46.0% of their whole genome, respectively. Total A+T content from complete PCG sequences was 59.4 and 59.8 in *P. albicans* and *P. maximus*, respectively. Excluding the termination codons, a total of 3780 and 3787 amino acids are encoded by *P. albicans* and *P. maximus* mitogenomes, respectively. The amino acid frequencies are quite similar between both *Pecten* species. For both species, the upmost used amino acids are Leucine (12.9%), Valine (11.0%), Serine (10.3%), Glycine (9.9-10.0%) and Phenylalanine (9.7-9.8%). The overall amino acid sequence identity between *P. albicans* and *P. maximus* is 98.7%. The pairwise amino acid identity for homologous genes ranged from 97.2% for *cox3* to 100% for *nad4* and *nad4L* genes (Table 2). The most common eukaryotic genome start codon ATN was found in more than half of all PCGs of both species (*atp6*, *cox1*, *cox2*, *cox3, nad4, nad5, nad4L*, and *nad3* only in *P. albicans*), whereas the alternative triplets GTG and TTG are used as start codons for the genes *nad1* and *nad2* and the genes *nad6* and *cytb*, respectively. The triplet TTG was also used by *P. maximus* as start codon of the gene *nad3*. Fifty percent of the open reading frames in *P. albicans* and *P. maximus* mitogenomes end with a TAG stop codon. The other 50% ends with a TAA stop codon. No incomplete (T-, TA-) stop codons were found.

3.3. rRNA and tRNA genes

In *P. albicans* and *P. maximus*, the length of the *rrnS* and *rrnL* genes were 965 and 1423 bp and 965 and 1421 bp, respectively. In both species, the *rrnS* gene is located between *trnA* and *trnQ* genes, and the *rrnL* gene between *trnR*

241 and *cox1* genes. Sequence identity for *rrnS* and *rrnL* genes between *P. albicans*
242 and *P. maximus* is 98 % and 96.7 %, respectively. In *P. albicans* only 20 transfer
243 *RNA*s were identified. Sixteen *tRNAs* were identified with tRNAScan-SE and 3
244 *tRNAs* (*trnR*, *trnS1* and *trnS2*) using MITOS annotation web service. The *trnL1*
245 and *trnM* were not found because they might be located in the beginning of the
246 control region, which was not sequenced. The mitogenome of *P. maximus*
247 encodes a total of 22 *tRNA* genes, 19 *tRNAs* were identified with tRNAScan-SE
248 and 3 *tRNAs* (*trnM*, *trnS1* and *trnS2*) using MITOS annotation web service. The
249 *tRNAs* in both *P*ecten species ranged from 63 (*trnL2*) to 71 nucleotides (*trnQ* and
250 *trnR*). In both scallop species two *trnS*s were detected. On the other hand, a
251 second *trnL* was identified only in the beginning of the control region of *P.*
252 *maximus*. The inferred secondary structures for the two mitogenomes showed
253 that most *tRNAs* formed typical cloverleaf secondary structures (Additional file
254 S1). However, in some *tRNAs* non Watson-Crick matches, unpaired nucleotides
255 and aberrant loops were found. One non Watson-Crick base pair (C-C) was
256 detected in the D-arm of the *trnR* of *P. maximus*. Unpaired nucleotides were
257 detected in the C-arm of *trnD* from both *P*. *albicans* and *P*. *maximus*, as well as the
258 acceptor stem of *trnM* in *P*. *maximus*. Finally, the genes *trnL2* and *trnQ* from both
259 *Pecten* species formed a non-canonical oversized 9 nt anticodon loop.

260 *3.4. Gene arrangement*

261 The mitochondrial gene order of *P*. *albicans* and *P. maximus* displayed the
262 same gene order arrangement, except for last two *tRNAs* identified in *P. maximus*
263 that could not be found in *P. albicans* due to unconcluded sequencing of the
264 region downstream the gene *trnT*. The mitochondrial gene order revealed in this

study constitutes a novel gene arrangement among metazoans. Two highly conserved gene clusters (*nad6-trnL-cytb* and *nad1-rrnL-cox1*) were detected in most scallop species with available mitogenomes (see Fig. 2), except for *P. magellanicus* (subfamily Palliolinae). The sequences spanning the 3' end of the *nad1* through 5' end of the *cox1* gene were determined in 6 different scallop species (GenBank access numbers from KP900976 to KP900981). Depending on species, the insertion of *trnR* between the genes *nad1* and *rrnL*, and *trnM* between the genes *rrnL* and *cox1* were detected (Fig. 2). Overall, gene cluster *nad1-rrnL-cox1* is present in the mitogenomes of 13 scallop species, whereas the gene cluster *nad6-trnL-cytb* was detected in a total of 8 scallop species. The mitogenomes of the genera *Argopecten* (tribe Aequipectini) and *Pecten* (subfamily Pectininae) shared two large similar gene clusters *rrnS-trnQ-trnV-nad1-trnR-rrnL-cox1-trnaF-trnE-nad6-trnL-cytb-cox2-nad4L-cox3-nad3-nad4* and *trnH-trnW-trnP-nad5-atp6-trnC-trnY-trnT-trnL-trnM*, with just few gene insertion and deletion differences (Fig. 3). At least 9 rearrangement events were identified between the mitogenomes of *A. purpuratus* and *P. maximus* including two *trna* gene insertions, one *trna* gene deletion, and the translocation of four *trna* genes, one PCG and the control region (Fig. 3).

3.5. Phylogenetic analysis

Results based on nucleotide sequences deduced from amino acid information from all 12 PCGs are shown in Fig. 4. Both phylogeny estimation approaches (ML and BI) inferred with two datasets (PCG123 and PCG12) showed very similar topologies, similar branch lengths and high bootstrap support and posterior probabilities. The 9 scallop species were recovered in 3 clear

289 monophyletic groups. The sister taxa *Pecten* (subfamily Pectininae) and
290 *Argopecten* (tribe Aequipectini) scattered in two subclades within a larger basal
291 clade. Within the subfamily Chlamydinae, two discrete subclades (*M. nobilis* and
292 *M. senatoria*) + (*C. farreri* and *M. yessoensis*) were recovered. Finally, *P.*
293 *magellanicus* (subfamily Palliolinae) split in a single branch after the
294 Chlamydinae clade.

295 **4. Discussion**

296 In this study, two new mitogenomes belonging to the subfamily
297 Pectininae were revealed, including a novel gene arrangement. The complete
298 mitochondrial genome lengths of both *Pecten* species were estimated based on
299 their PCR amplicon sizes. Previously, Rigaa et al. (1995) described the presence
300 of a tandem repeat unit of 1.6 kb occurring from two to five times within the
301 control region of *P. maximus*. Accordingly, a mitogenome of 20 and 24.8 kb
302 should contain two and five repeat units, respectively. Taking into consideration
303 the results by Rigaa et al. (1995), the complete mitogenome of the *P. maximus*
304 analyzed in this study (24.8 kb in length), should contain five repeat units, which
305 explains the large mitochondrial molecule size. The increased mitochondrial size
306 caused by the presence of large tandem repeats within the control region is a
307 common feature among scallops (Gjetvaj et al., 1992; Rigaa et al., 1995). Large
308 mitochondrial genomes have been found in *M. yessoensis* (20964 bp; Wu et al.,
309 2009), *P. magellanicus* (32115 bp; Smith and Snyder, 2007), *Crassodoma*
310 *gigantea* 22.8-24.8 kb, *Aequipecten opercularis* 21-28.2 kb, *Chlamys hastata* 23.9-
311 27.2 kb, *C. islandica* 22.2-25 kb (Gjetvaj et al., 1992). To date, all described
312 scallop mitogenomes encode all PCGs in one strand. Placement of all coding

genes on the same strand and lacking of *atp8* gene are one of the most distinctive characteristics of marine bivalve mitogenomes (He et al., 2011). Besides, it is believed that coding on both strands may inhibit mitogenome rearrangement, and marine bivalve mitogenomes are known for showing high levels of gene rearrangement (Ren et al., 2010a). Lack of the *atp8* gene is another common signature in marine bivalve mitogenomes. Indeed, the *atp8* gene is absent or highly modified in different animal groups including Chaetognatha, Rotifera, most Mollusca Bivalvia, Nematoda and Platyhelminthes (Gissi et al., 2008). Its high nucleotide variability together with its short and variable length might have been hampered the annotation of the of the *atp8* gene in many studies (Gissi et al., 2008; Smietanka et al., 2010). Thus, "putative" *atp8* gene has been reported in some bivalve species such as *Mytilus* spp (Breton et al., 2010; Smietanka et al., 2010)

Overall, both *Pecten* mitogenomes showed similar start codons. About 40 % of the PCGs from *Pecten* mitogenomes used alternative start codons GTG or TTG. The non-conventional start codons GTG and TTG are not usual in molluscan mitogenomes, however their use have been reported in other scallop species (Marín et al., 2014; Smith and Snyder, 2007; Wu et al., 2009) and in many gastropods (Yuan et al., 2012). Duplication of transfer *RNA*s is common in mollusks (Ren et al., 2010b) and total *tRNAs* number identified in Pectinidae varies depending on species, ranging from 16 in *M. yessoensis* (Wu et al., 2009) to 32 in *P. magellanicus* (Smith and Snyder 2007). In *P. maximus* and *P. albicans* two *trnS*s were detected. On the contrary, lack of *trnS* has been reported in some scallop species including *C. farreri*, *M. nobilis* and *M. yessoensis* (Wu et al., 2009),

A. irradians irradians (Ren et al., 2010b), and *A. purpuratus* (Marín et al., 2014). Previous mollusk mitogenome studies have revealed that among the *tRNA* genes, *trnS* is the least conserved (Ren et al., 2010b).

The small conserved gene block *nad4-trnH-trnW* was observed in *C. farreri*, *Mimachlamys* spp. (Chlamydinae), *P. magellanicus* (Palliolinae) and *Pecten* spp. (Pectininae), and the gene block *trnL-cytb-cox2* in *P. magellanicus* (Palliolinae), *Pecten* spp. and *Argopecten* spp. (Pectininae) (Fig. 2). Additionally, as described by Ren et al. (2010b), the gene order rearrangement of *M. yessoensis* closely resembles those of *Chlamys farreri* and *Mimachlamys* spp., all belonging to the subfamily Chlamydinae (Fig. 2). Three conserved gene clusters (*nad4L-nad6-trnL-cytb*, *trnD-cox3-trnK-trnF-trnQ-trnE-atp6-cox2-nad2*, and *nad1-trnR-rrnL-trnM*) are shared among these three genera. Similarly, the synapomorphy of two large gene clusters identified in our results (*rrnS-trnQ-trnV-nad1-trnR-rrnL-cox1-trnaF-trnE-nad6-trnL-cytb-cox2-nad4L-cox3-nad3-nad4* and *trnH-trnW-trnP-nad5-atp6-trnC-trnY-trnT-trnL-trnM*) between genera *Argopecten* and *Pecten*, supports the sister relationship between tribe Aequipectini (genus *Argopecten*) and subfamily Pectininae (genus *Pecten*) (Fig. 3). The usefulness of gene rearrangements for phylogenetic reconstructions of distant Pectinidae groups might be limited due to the drastic variability of mitochondrial gene order in this family. Nevertheless, our results confirm that Pectinidae gene rearrangement can be used to determine and support phylogenetic relationships among closer groups. In this study, additional scallop species mitogenomes were found to contain the highly conserved gene clusters reported by Ren et al. (2010b): *nad1-rrnL-cox1* (now detected in 13 species; Fig.

2) and *nad6-trnL-cytb* (now detected in 8 species, Fig. 2). These conserved blocks may have been inherited from a common ancient scallop ancestor (Ren et al., 2010b) and therefore could be used as molecular markers for a deeper understanding of mitochondrial genome evolution in members of Pectinidae.

At least 9 rearrangement events occurred between the mitogenomes of *A. purpuratus* and *P. maximus*. Because of the relatively complex rearrangement events, their evolutionary pathways of gene rearrangements cannot be explained by one rearrangement mechanism alone, instead it might be a result of a combination of more mechanisms such as tandem duplication and random loss (TDRL) model (Boore, 1999), transposition (Macey et al., 1997) and intramolecular recombination (Lunt and Hyman 1997). Comparisons among mitogenomes at lower taxonomic levels (e.g., within genera) are highly useful for further interpretation and understanding of mitochondrial gene rearrangement mechanisms (Gissi et al., 2008). Thus, the analysis of additional mitogenomes from species with intermediate stages can be useful to trace missing mitochondrial evolutionary pathways in Pectinidae. Interestingly, most of the rearrangement events occurred in genes flanking the control region of *Argopecten* and *Pecten* species (Fig. 3). This result suggests that the control region in the family Pectinidae is a potential hotspot for gene rearrangement. In metazoan mitogenomes, the control region neighborhood has been recognized as one of the gene rearrangement hotspots (Kurabayashi et al., 2008).

Phylogenetic relationships in Pectinidae are still a matter of debate, due to the incongruent results between genetic and morphological studies. In this study, the concatenation of 12 PCGs allowed the recovery of strong supported

385 phylogeny based on two different phylogenetic approaches (ML and BI). Overall,
386 our results strongly supported the basal position and sister group relationship
387 between *Argopecten* and *Pecten*, and the apical position of Chlamydinae and
388 Palliolinae subfamilies. The groups formed by Aequipectini + Pectinidae and
389 Chlamys + Palliolinae have been inferred as the most ancient and recently
390 derived groups from the family Pectinidae, respectively (Puslednik et al., 2008).
391 Our results strongly supported the existence of the subfamily Palliolinae, which
392 is in agreement with previous studies (Puslednik et al., 2008; Saavedra and Peña,
393 2006; Waller, 2006). However, our phylogenetic results recovered the subfamily
394 Palliolinae in the tip of the tree, just after the subfamily Chlamydinae. In this
395 regard, our results slightly differed from previous studies. For example,
396 Puslednik et al. (2008) used partial mitochondrial and nuclear genes to infer the
397 phylogenetic relationships of 46 scallop species. In the same study, members of
398 the subfamily Palliolinae (covered by 3 species) were recovered in a
399 monophyletic group between Chlamydinae and Pectininae clades. In this study,
400 we used the only available mitochondrial genome from Palliolinae (*P.*
401 *magellanicus*). It has been shown that the inclusion of dense taxa dataset results
402 in more accurate estimation of evolutionary relationships (Heath et al., 2008).
403 Thus, the inclusion of complete mitogenomes from more representatives of the
404 subfamily Palliolinae will provide an accurate estimation of the phylogenetic
405 position of this subfamily within Pectinidae.

406 *Pecten* species are characterized for a broad geographic range
407 distribution, inhabiting the coasts of four continents. However, in spite of the
408 clear allopatric populations, very low mitochondrial genetic distances (ranging

409 from 0.006 to 0.042) have been reported for members of this genus (Peña and
410 Saavedra, 2012). The estimated genetic distance between *P. albicans* and *P.*
411 *maximus* mitogenomes are in agreement with previous results by Peña and
412 Saavedra (2012). Relatively low genetic distance (7 %) and quite similar amino
413 acid sequence identity (98.7 %) found between the mitogenomes of *P. albicans*
414 and *P. maximus* suggest a recent divergence event. Further studies using
415 complete mitochondrial genome information from other *Pecten* species are
416 needed to corroborate the recent diversification within this genus. Recently
417 diverged species are challenging for identification (van Velzen et al., 2012).
418 Previous studies have demonstrated the great utility of the mitochondrial 16S
419 gene for scallop species identification (Marín et al., 2013a, 2015). Interestingly,
420 our results showed that the genes with the highest pairwise sequence diversities
421 were *nad1* (p=0.079) and *nad6* (p=0.081). Thus, the *nad1* and *nad6* genes can be
422 also considered as alternative potential markers in further identification and
423 population studies of *Pecten* species, which are characterized for showing
424 extremely low genetic variation. Finally, our results will also be of great utility to
425 clarify the possible conspecificity between *P. maximus* and *P. jacobaeus* (Canapa
426 et al., 2000).

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Figures

Fig. 1. Circular and linearized mitochondrial genome organization of *Pecten albicans* and *Pecten maximus*. All genes are encoded in the same strand. Protein-coding genes are marked in blue (gene name abbreviations: *atp6,* ATP synthase subunit 6; cytb, apocytochrome *b*; *cox1-3*, cytochrome *c* oxidase subunits; *nad1–4*, *4L*, *5–6*, NADH dehydrogenase subunits), ribosomal genes in red (gene name abbreviations: *rrnL*, *rrnS*, large and small subunit rRNAs), *tRNAs* (abbreviated using universal amino acid code) in yellow, and control region in grey.

Fig. 2. Synteny among the linear-mapping mitogenomes of 12 scallop species, including the novel *Pecten maximus* mitogenome developed in this study. All mitogenomes are opened downstream of control region for easier comparison. Species inside pink box belong to the subfamily Chlamydinae (*Mimachlamys senatoria* is omitted because of its high similarity with *Mimachlamys nobilis*), white box represents subfamily Palliolinae, purple box represents subfamily

Pectininae (*Pecten albicans* is not included because of its high similarity with *Pecten maximus*), and green box the tribe Aequipectini (*Argopecten irradians* is not included because of its high similarity with *Argopecten purpuratus*). Conserved gene cluster *nad1-rrnL-cox1* is represented in 6 additional species *Amusium pleuronectes*, *Ylistrum japonicum*, *Annachlamys macassarensis*, *Bractechlamys vexillum*, *Decatopecten radula* and *Mimachlamys sanguinea*. Black horizontal bars indicate the conserved gene cluster "*nad1-rrnL-cox1*". Blue bars indicate the conserved gene cluster "*nad6-L-cytb*". Orange and red bars represent the conserved gene clusters "*L2-cytb-cox2*" and "*nad4-H-W*", respectively.

Fig. 3. Linear-mapping mitogenomes rearrangement comparison between *Pecten maximus* and *Argopecten purpuratus.* Protein-coding genes are marked in blue, ribosomal genes in red, *tRNAs* (abbreviated using universal amino acid code) in yellow, and control region in grey. Large conserved gene blocks are located inside black line rectangles. The blue stars represent gene insertions, red stars represent gene deletions and black lines indicate six gene translocations.

Fig. 4. Maximum likelihood (A) and Bayesian Inference (B) phylogenetic consensus trees constructed based on the concatenation of 12 mitochondrial protein-coding genes in 9 scallop species. The two species with newly determined mitogenomes developed in this study (*Pecten albicans* and *Pecten maximus*, subfamily Pectininae) are highlighted in bold. The conserved gene cluster *nad1-trnR-rrnL-cox1* is represented by a black circle, *nad1-trnR-rrnL-trnM-cox1* is represented by a black triangle, *nad1-trnR-rrnL-trnM-rrnS-cox1* is represented by a black square (indicated in all species except for *Placopecten magellanicus*, where the gene cluster is absent). Bootstrap values (maximum

likelihood) and posterior probabilities (Bayesian inference) at correspondent nodes are shown in percentages. The oysters *Pinctada maxima* (NC_018752) and *Crassostrea gigas* (EU672831) were used as outgroups.

Table 1 Primer list

Table 2 Mitochondrial gene annotation

Supplemental Material Inferred secondary structure of *tRNAs* in *Pecten albicans* and *Pecten maximus*.

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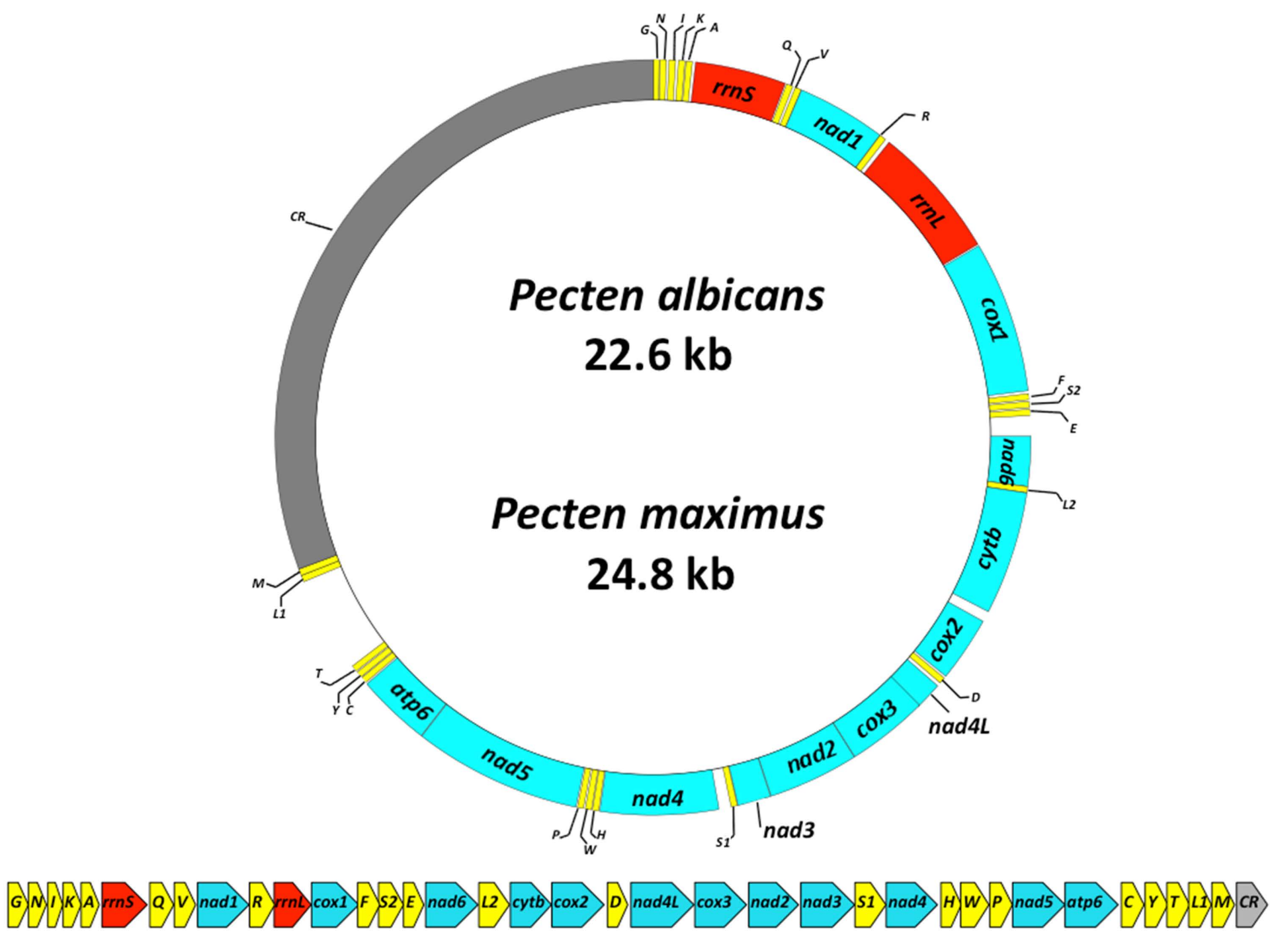
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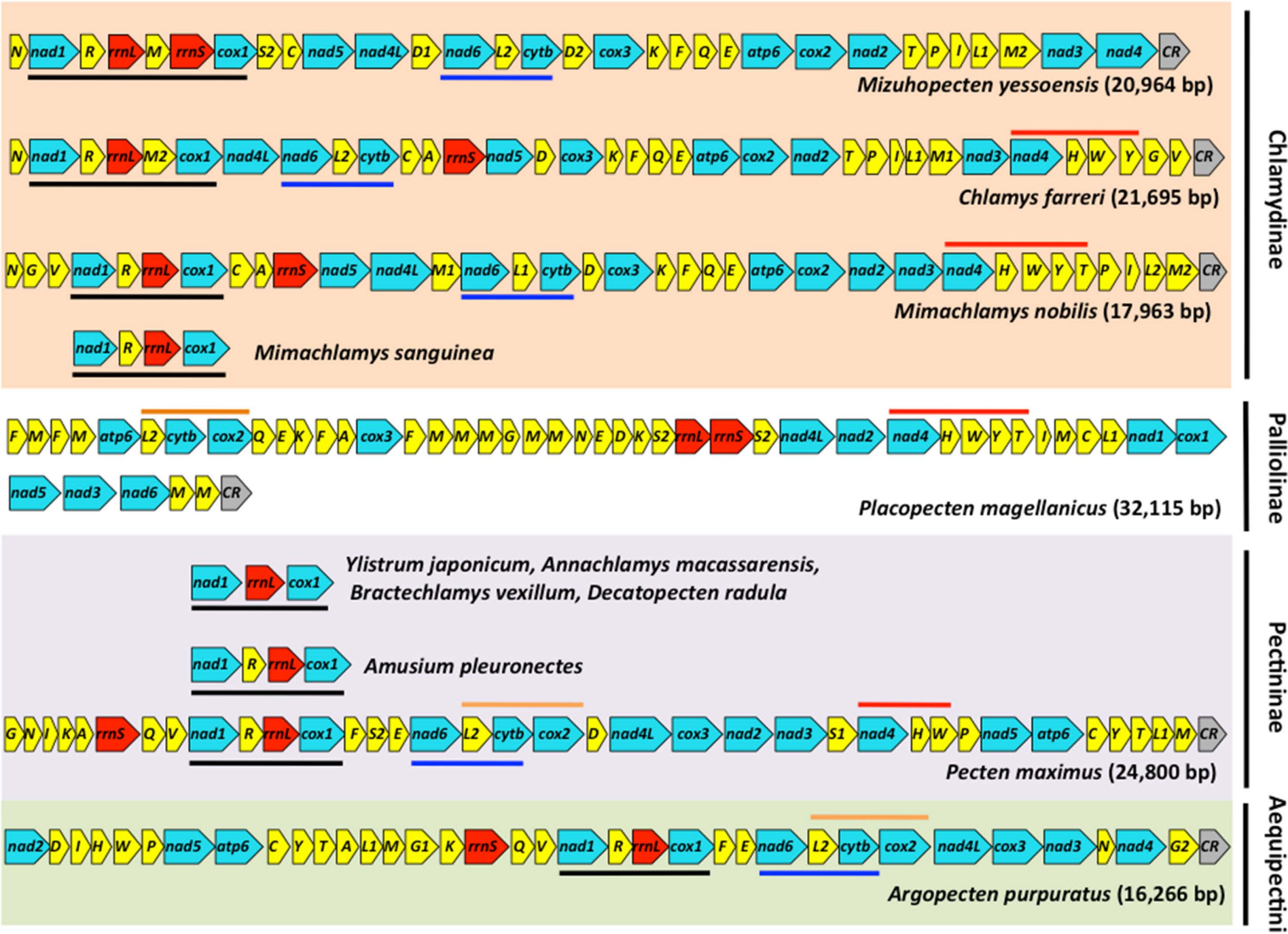
22.6 kb
Pecten maximus
24.8 kb
G N I K A rrnS Q V nad1 R rrnL cox1 F S2 E nad6 L2 cytb cox2 D nad4L cox3 nad2 nad3 S1 nad4 H W P nad5 atp6 C Y T L1 M CR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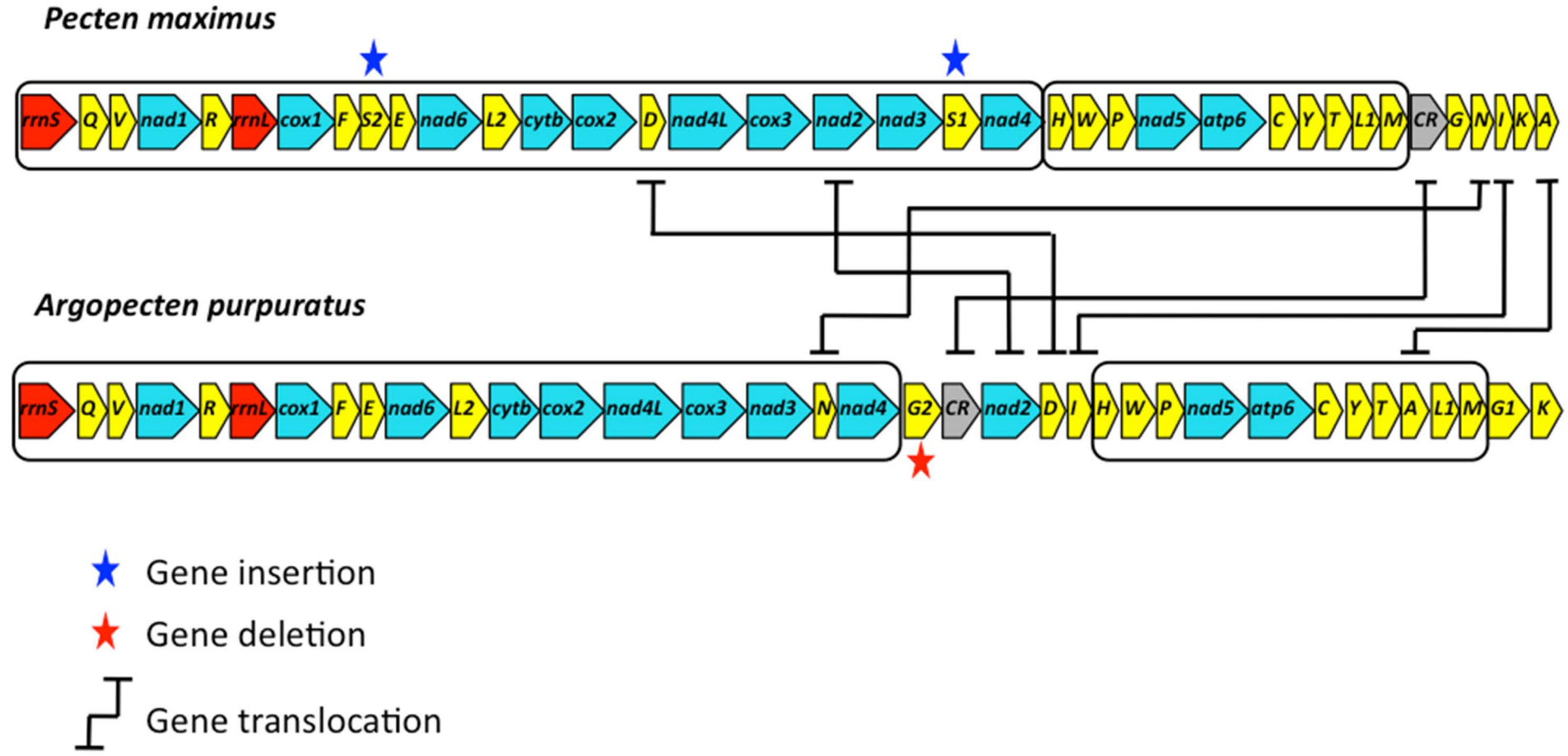
Mizuhopecten yessoensis (20,964 bp)
N nad1 R rrnL M2 cox1 nad4L nad6 L2 cytb C A rrnS nad5 D cox3 K F Q E atp6 cox2 nad2 T P I L1 M1 nad3 nad4 H W Y G V CR
Chlamys farreri (21,695 bp)
N G V nad1 R rrnL cox1 C A rrnS nad5 nad4L M1 nad6 L1 cytb D cox3 K F Q E atp6 cox2 nad2 nad3 nad4 H W Y T P I L2 M2 CR
Mimachlamys nobilis (17,963 bp)
nad1 R rrnL cox1
Mimachlamys sanguinea
Chlamydinae
F M F M atp6 L2 cytb cox2 Q E K F A cox3 F M M M G M M N E D K S2 rrnL rrnS S2 nad4L nad2 nad4 H W Y T I M C L1 nad1 cox1
nad5 nad3 nad6 M M CR
Placopecten magellanicus (32,115 bp)
Palliolinae
nad1 rrnL cox1
Ylistrum japonicum, Annachlamys macassarensis,
Bractechlamys vexillum, Decatopecten radula
nad1 R rrnL cox1
Amusium pleuronectes
G N I K A rrnS Q V nad1 R rrnL cox1 F S2 E nad6 L2 cytb cox2 D nad4L cox3 nad2 nad3 S1 nad4 H W P nad5 atp6 C Y T L1 M CR
Pecten maximus (24,800 bp)
Pectininae
nad2 D I H W P nad5 atp6 C Y T A L1 M G1 K rrnS Q V nad1 R rrnL cox1 F E nad6 L2 cytb cox2 nad4L cox3 nad3 N nad4 G2 CR
Argopecten purpuratus (16,266 bp)
Aequipectini


Pecten maximus
rrnS Q V nad1 R rrnL cox1 F S2 E nad6 L2 cytb cox2 D nad4L cox3 nad2 nad3 S1 nad4 H W P nad5 atp6 C Y T L1 M CR G N I K A
Argopecten purpuratus
rrnS Q V nad1 R rrnL cox1 F E nad6 L2 cytb cox2 nad4L cox3 nad3 N nad4 G2 CR nad2 D I H W P nad5 atp6 C Y T A L1 M G1 K
Gene insertion
Gene deletion
Gene translocation

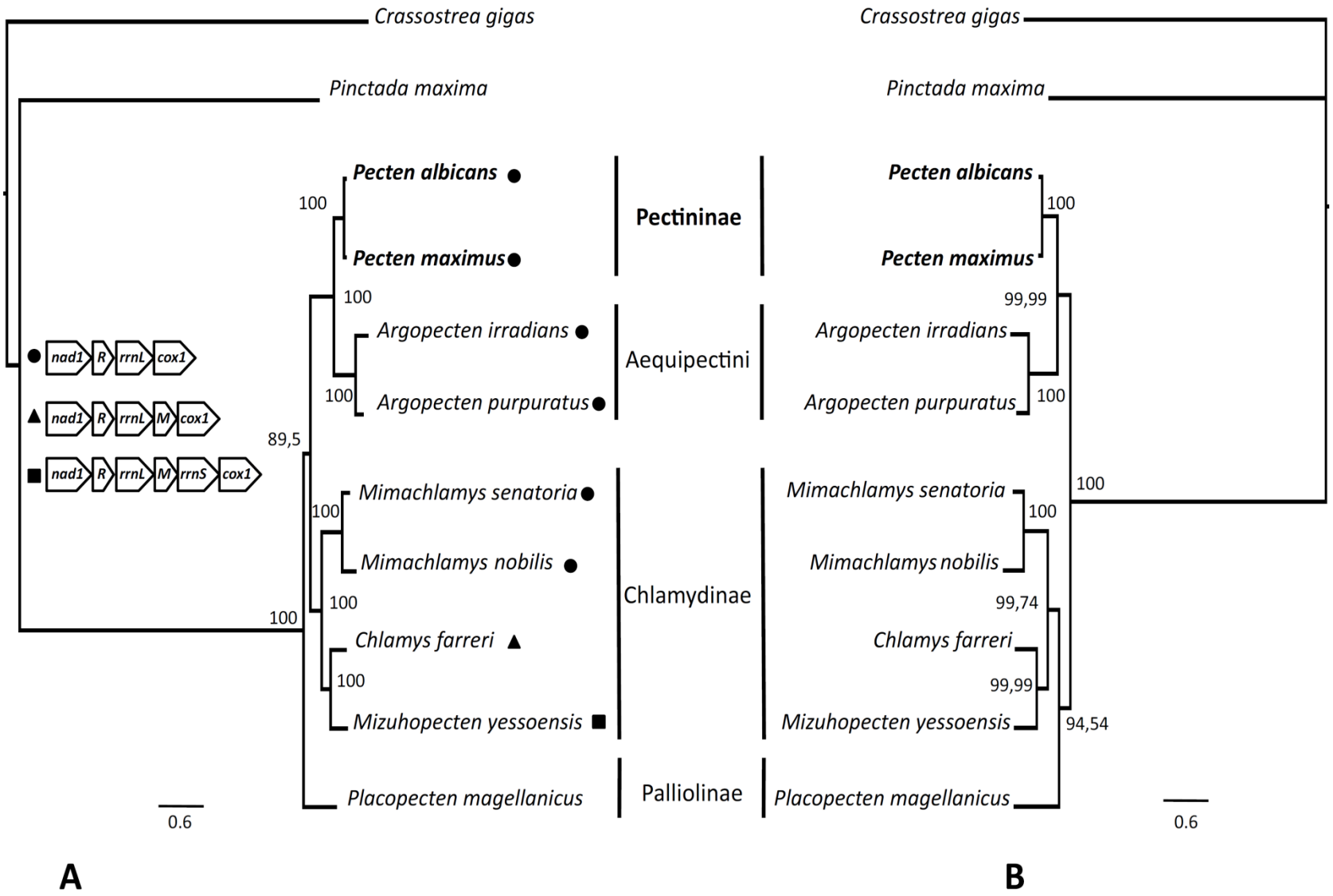

Crassostrea gigas
Pinctada maxima
Pecten albicans
Pecten maximus
Argopecten irradians
Argopecten purpuratus
Mimachlamys senatoria
Mimachlamys nobilis
Chlamys farreri
Mizuhopecten yessoensis
Placopecten magellanicus
Pectininae
Aequipectini
Chlamydinae
Palliolinae
nad1
R
rrnL
cox1
M
rrnS
100
89,5
99,99
99,74
99,99
94,54
0.6
A
B

Table 1

List of universal and *Pecten*-specific primers used to amplify the complete mitochondrial genomes of *Pecten albicans* (PA) and *Pecten maximus* (PM). Long PCR products were used as templates in all subsequent amplifications of short PCR products.

Primer name	Sequence 5'-3'	Direction	Species	Gene	Source
LONG PCR					
12SpectUF	AGTCCAACCAGGTGCCAGCA	Forward	PA/PM	*rrnS*	Marín et al. (2013a)
PecnaXX	CCCCTGCAAACATAAAGAATTCAAC	Reverse	PA/PM	*nad2*	This study
Pectco3F	TGGCCTCAGGAGATTTATACAATACC	Forward	PA/PM	*cox3*	This study
Pect12SR	CAACAAGCTGACAACGGCAATACACC	Reverse	PA/PM	*rrnS*	This study
SHORT PCR					
12SpectUF	AGTCCAACCAGGTGCCAGCA	Forward	PA/PM	*rrnS*	Marín et al. (2013a)
CO1AB	GGTGCTGGGCAGCcayatnccngg	Reverse	PA/PM	*cox1*	Marín et al. (2013b)
16SCC	GCGTAATCCGTCTTGACAGT	Forward	PA/PM	*rrnL*	Marín et al. (2013b)
PecnaXX	CCCCTGCAAACATAAAGAATTCAAC	Reverse	PA/PM	*nad2*	This study
Cox3F1	TGGCCTCAGGAGATTTATACAATACC	Forward	PA/PM	*cox3*	This study
Nad4R1	CCAGTATGGGCAATAGAAGAGTA	Reverse	PA	*nad4*	This study
Nad5R1	AAGAACTGTGGCAAGGATAGAACAA	Reverse	PM	*nad5*	This study
Nad3F2	GAGGGTTTTTAGTGGTTCTTG	Forward	PA	*nad3*	This study
TrnpR2	CCCAATGTACTAACTTATACTACCC	Reverse	PA	*trnP*	This study
Nad5F2	GCGGTGGCTTTGGCTGGTGATACTT	Forward	PM	*nad5*	This study
Atp6R2	CCCCTCATTGTTCAATCAAACTG	Reverse	PM	*atp6*	This study
TrnHF3	TGGTGTAGAAAGGCACGAAAGGTTG	Forward	PA	*trnH*	This study
CRpa3	ATACTACCCTGCTCAAACAAGTGG	Reverse	PA	*CR*	This study
Nad5F3	TTCCGTGCCATGTTAGGAAGGCATT	Forward	PM	*nad5*	This study
CRpm3	AGGCTCCACTGACCTACCTATAAAC	Reverse	PM	*CR*	This study
CRpm4	GGTCAGTGGAGCCTCTTTAGAGCTA	Forward	PA/PM	*CR*	This study
Pect12SR	CAACAAGCTGACAACGGCAATACACC	Reverse	PA/PM	*rrnS*	This study

Table 2

Complete mitochondrial annotation, number of amino acids in each gene, pairwise amino acid and nucleotide identity based on the mitochondrial genomes of *Pecten albicans* (PA) *and Pecten maximus* (PM).

Gene	Location		Size (bp)		Start codon		Stop codon		Anticodon		Intergenic nucleotides		No. Amino acid		% Amino acid identity	% Nucleotide identity
	PA	PM	PA	PM	PA	PM	PA	PM	PA	PM	PA	PM	PA	PM	PA/PM	PA/PM
trnG	1-66	1-66	66	66					TCC	TCC	-	-				
trnN	75-141	75-141	67	67					GTT	GTT	8	8				
trnI	170-239	168-237	70	70					GAU	GAU	28	26				
trnK	269-337	267-335	69	69					TTT	TTT	29	29				
trnA	349-414	348-413	66	66					TGC	TGC	11	12				
rrnS	450-1414	447-1411	965	965							35	33				98
trnQ	1430-1500	1427-1497	71	71					TTG	TTG	16	15				
trnV	1527-1593	1524-1590	67	67					TAC	TAC	26	26				
nad1	1597-2544	1594-2541	948	948	GTG	GTG	TAA	TAA			3	3	315	315	99	92.1
trnR	2551-2621	2544-2615	71	72					TCG	TCG	6	2				
rrnL	2665-4087	2662-4082	1423	1421							43	46				96.7
cox1	4106-5716	4101-5711	1611	1611	ATG	ATG	TAA	TAA			18	18	536	536	99.1	93.2
trnF	5749-5812	5743-5806	64	64					GAA	GAA	32	31				
trnS2	5830-5895	5823-5888	66	66					TGA	TGA	17	16				
trnE	5913-5978	5906-5971	66	66					TTC	TTC	17	17				
nad6	6204-6731	6188-6715	528	528	TTG	TTG	TAG	TAG			225	216	175	175	97.7	91.9
trnL2	6734-6796	6718-6780	63	63					TAA	TAA	2	2				
cob	6800-8110	6784-8094	1311	1311	TTG	TTG	TAA	TAA			3	3	436	436	99.1	93.3
cox2	8224-8919	8208-8903	696	696	ATG	ATG	TAG	TAG			113	113	231	231	98.3	93.7
trnD	8944-9011	8927-8994	68	68					GTC	GTC	24	23				
nad4L	9051-9344	9034-9327	294	294	ATA	ATG	TAA	TAA			39	39	97	97	100	92.2
cox3	9328-10194	9311-10177	867	867	ATG	ATG	TAG	TAG			-17	-17	288	288	97.2	93.3
nad2	10200-11162	10183-11145	963	963	GTG	GTG	TAA	TAA			5	5	320	320	98.4	93.3
nad3	11162-11521	11151-11504	360	354	ATA	TTG	TAG	TAG			-1	5	119	117	98.3	95.8
trnS1	11615-11681	11511-11577	67	67					TCT	GCT	93	6				
nad4	11722-12972	11707-12957	1251	1251	ATG	ATG	TAG	TAG			40	129	416	416	100	93.4
trnH	12985-13050	12970-13035	66	66					GTG	GTG	12	12				
trnW	13061-13127	13046-13112	67	67					CCA	CCA	10	10				
trnP	13146-13210	13131-13195	65	65					TGG	TGG	18	18				
nad5	13226-15013	13212-14999	1788	1788	ATG	ATG	TAG	TAG			15	16	595	595	98.7	94.2
atp6	15020-15805	15006-15791	786	786	ATA	ATA	TAA	TAA			6	6	261	261	98.1	95.3
trnC	15824-15889	15810-15875	66	66					GCA	GCA	18	18				
trnY	15900-15965	15886-15951	66	66					GTA	GTA	10	10				
trnT	15979-16046	15965-16032	68	68					TGT	TGT	13	13				
trnL1	-	17051-17120	-	70					-	TAG		1018				
trnM	-	17122-17193	-	72						CAU		1				

bp: base pairs.

The mitochondrial genomes of *Pecten albicans* and *P. maximus* (Bivalvia: Pectinidae) reveal a novel gene arrangement with low genetic differentiation

Biochemical Systematics and Ecology

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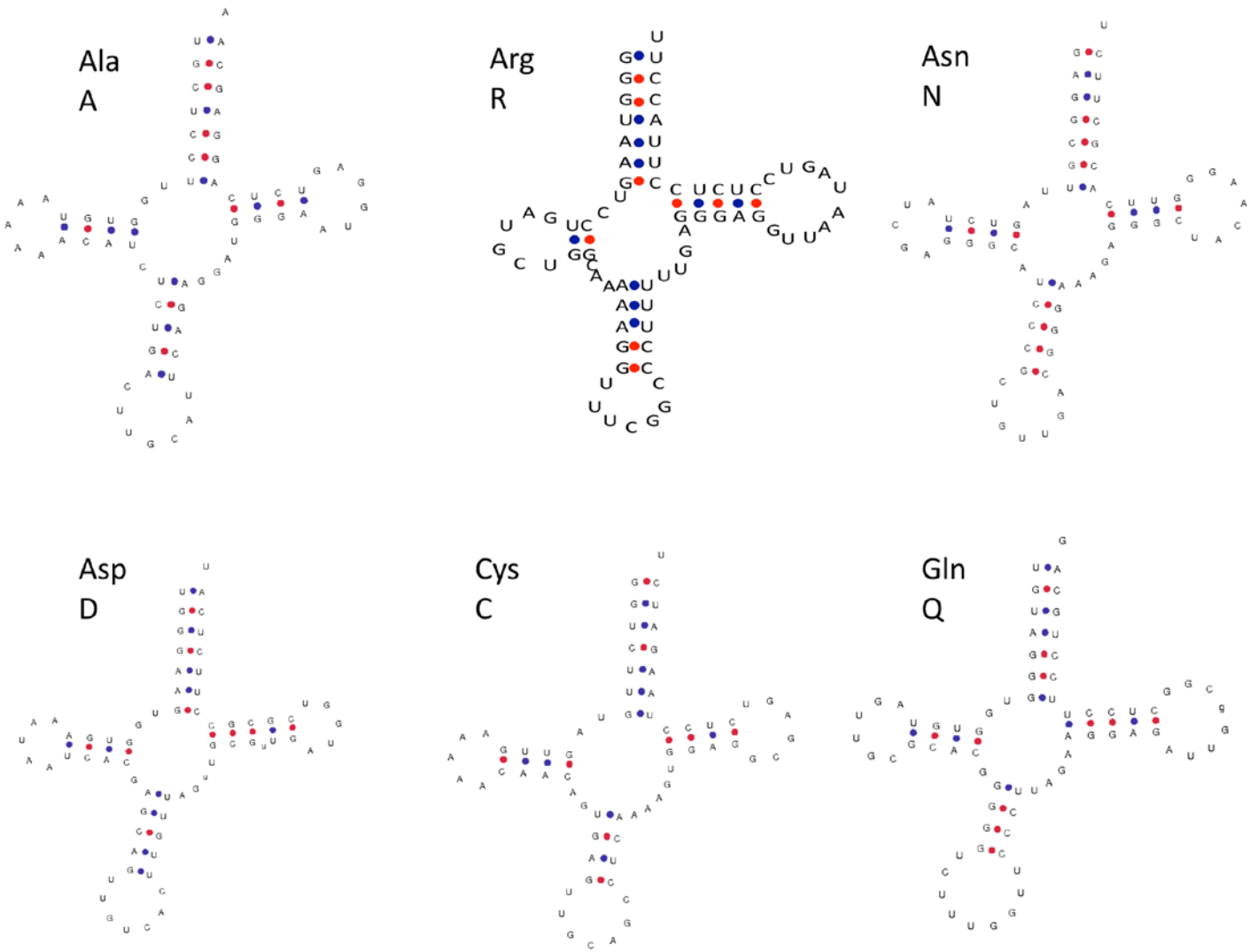
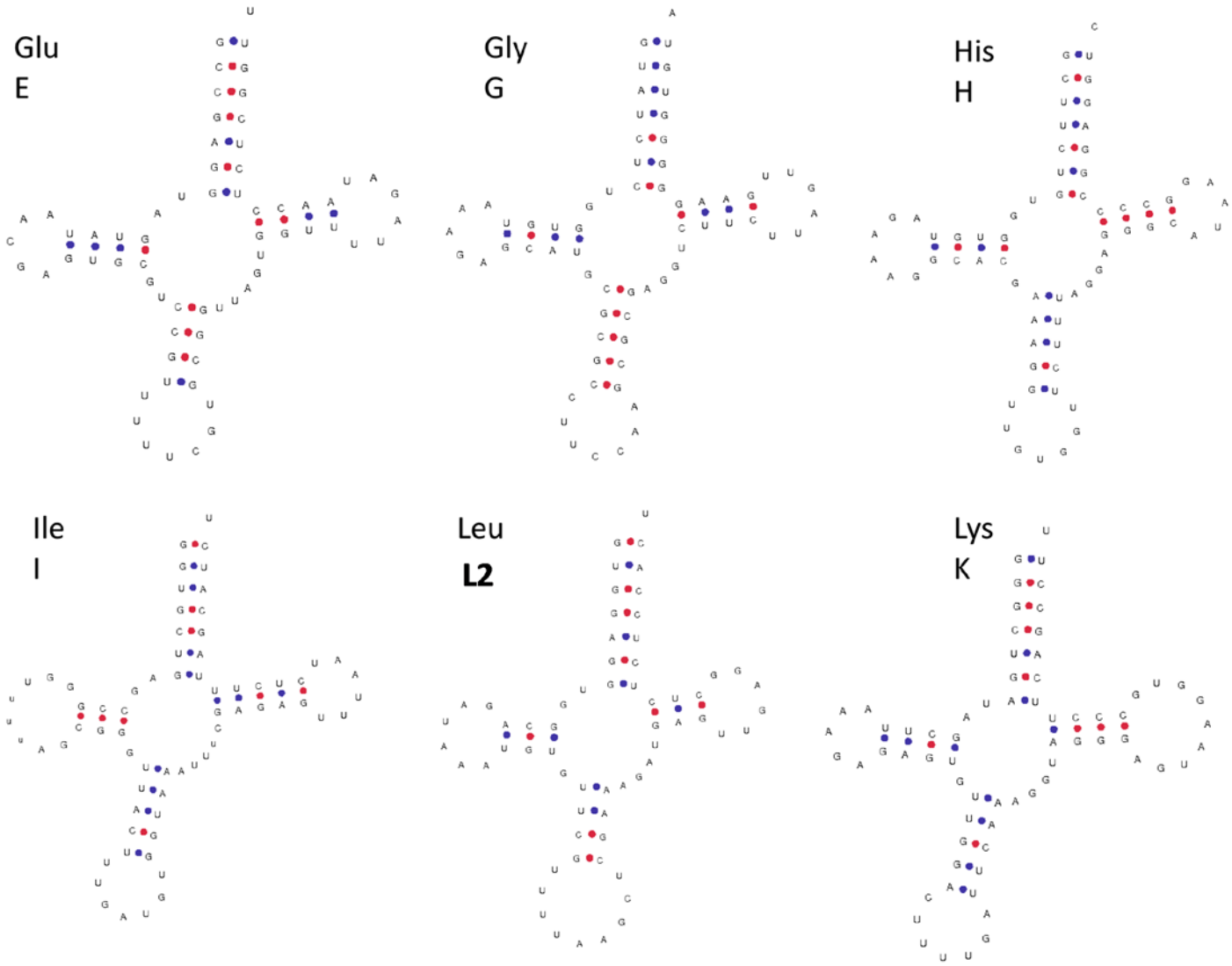
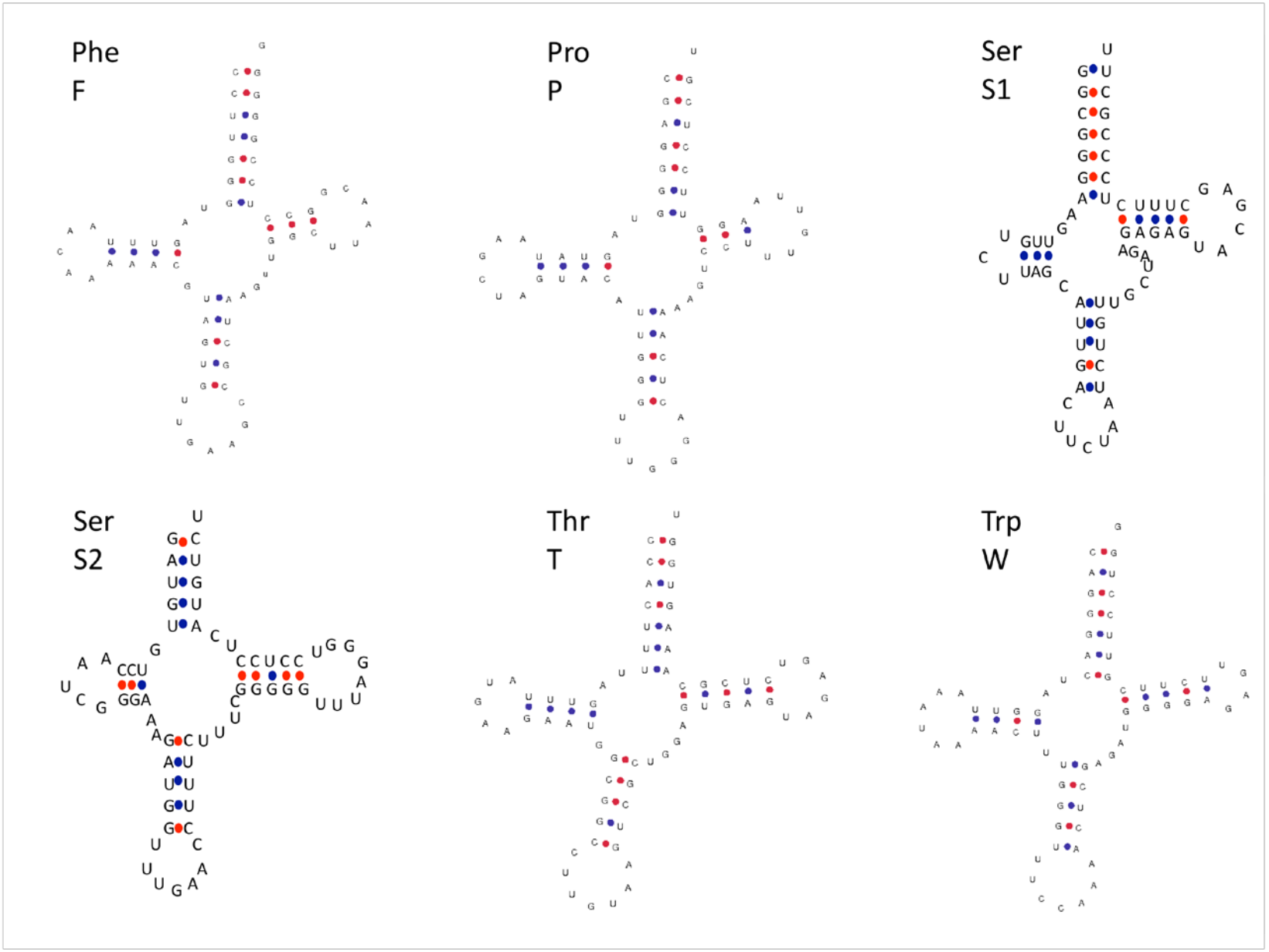


Supplementary 1a. Inferred secondary structure of the identified transfer RNAs in *Pecten albicans*. Blue and red circles represent U-A or U-G and C-G pairings, respectively



Supplementary 1a. (Continued)



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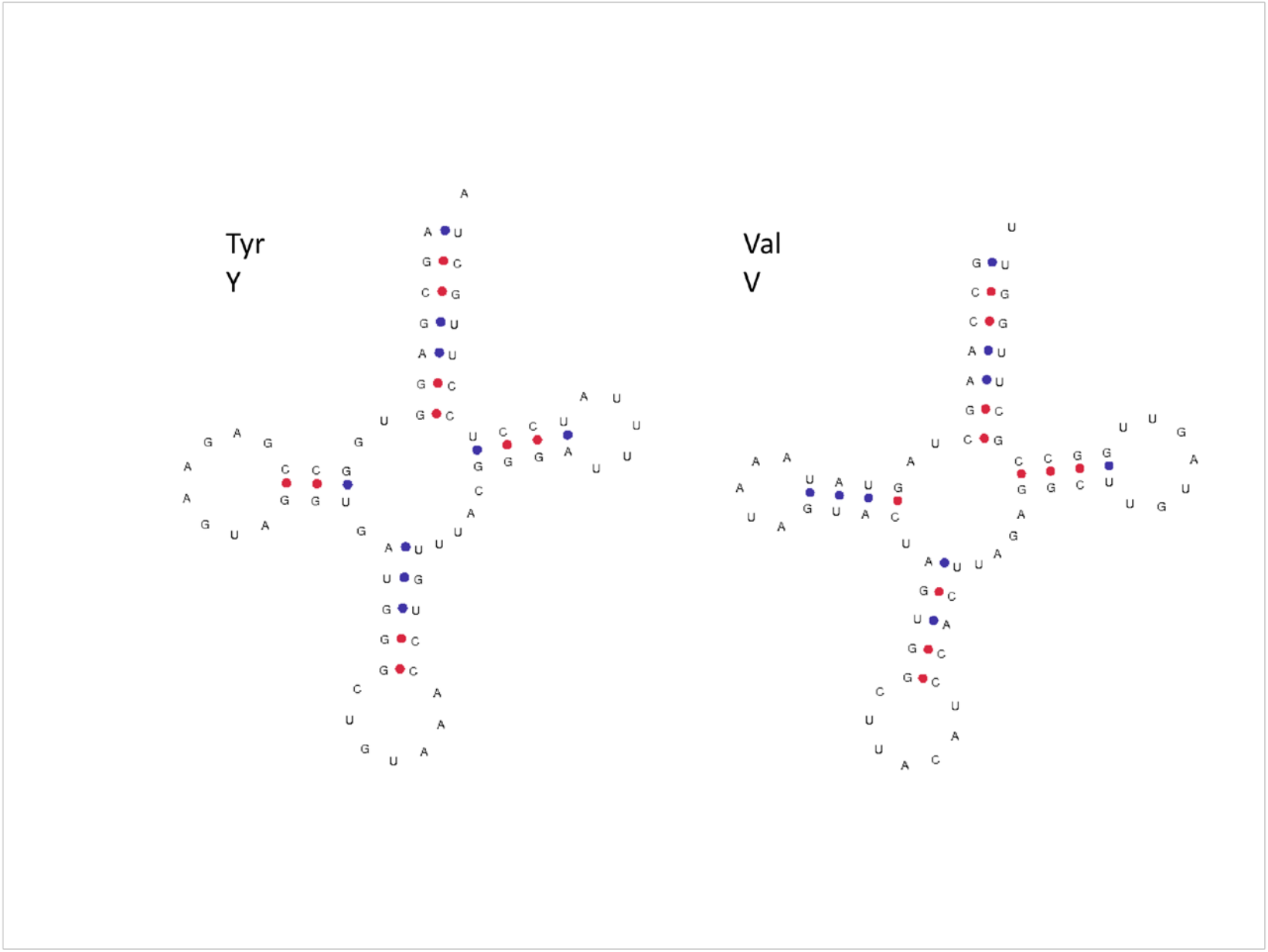
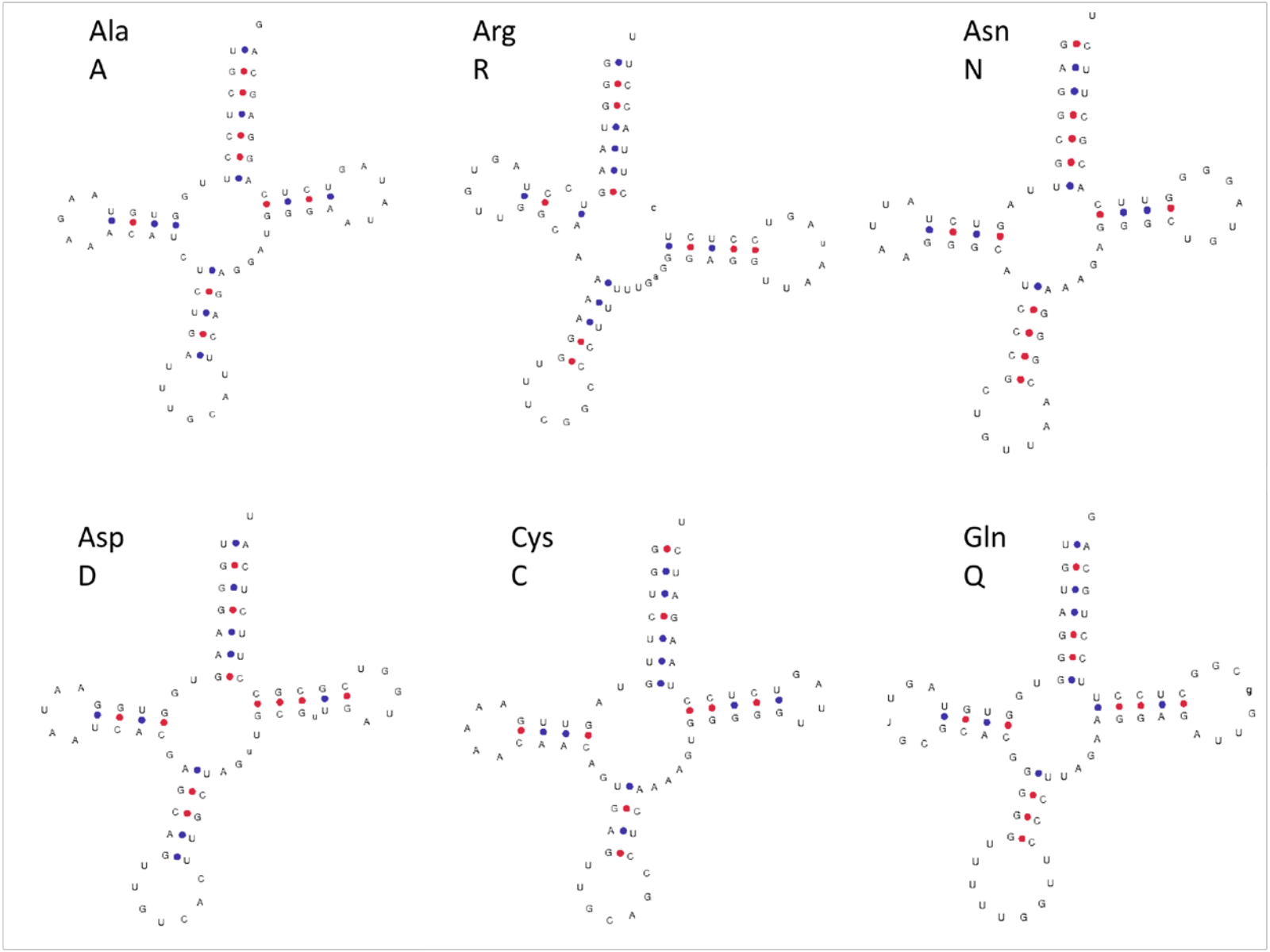
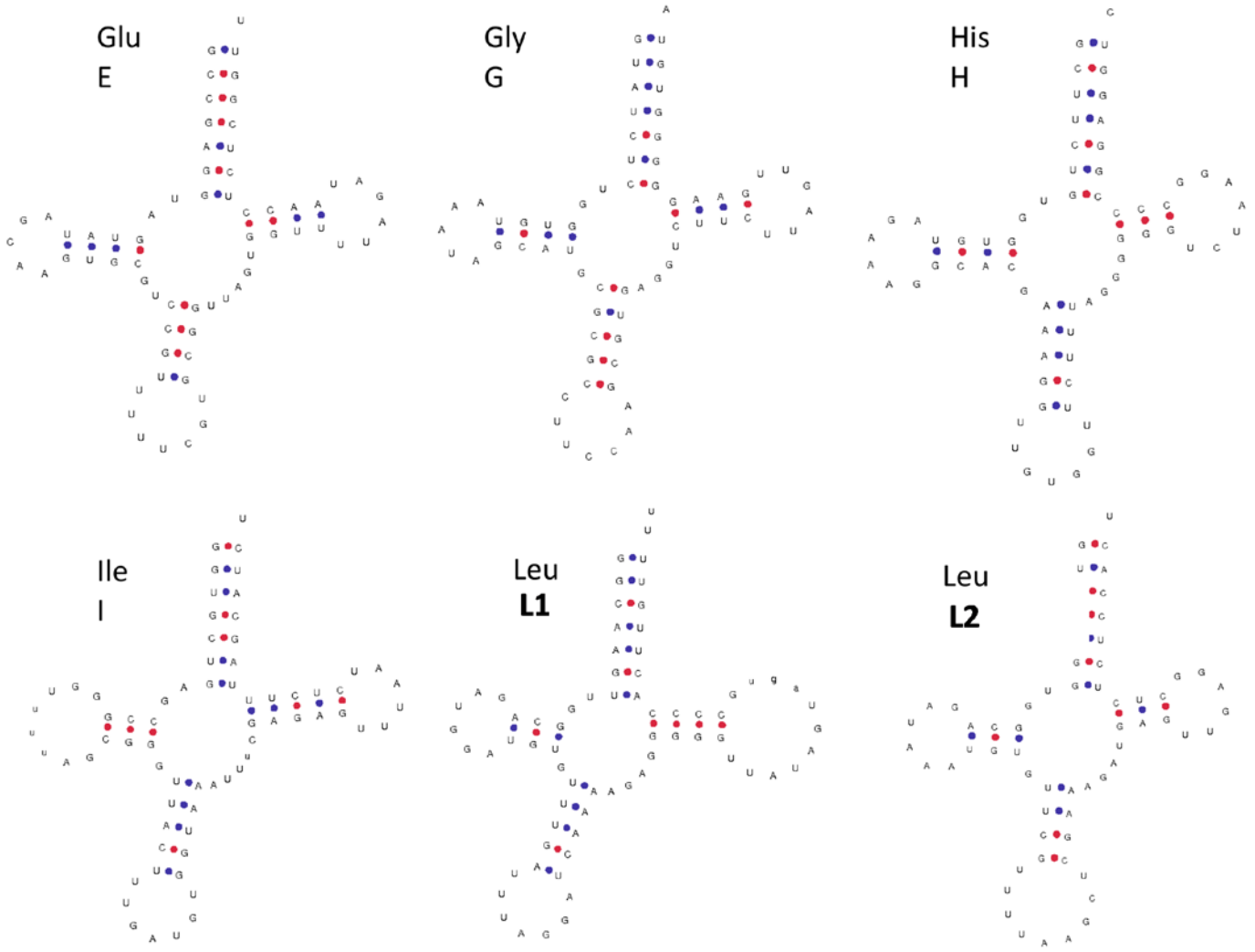


Supplementary 1a. (Continued)



Supplementary 1b. Inferred secondary structure of the identified transfer RNAs in *Pecten maximus*. Blue and red circles represent U-A or U-G and C-G pairings, respectively



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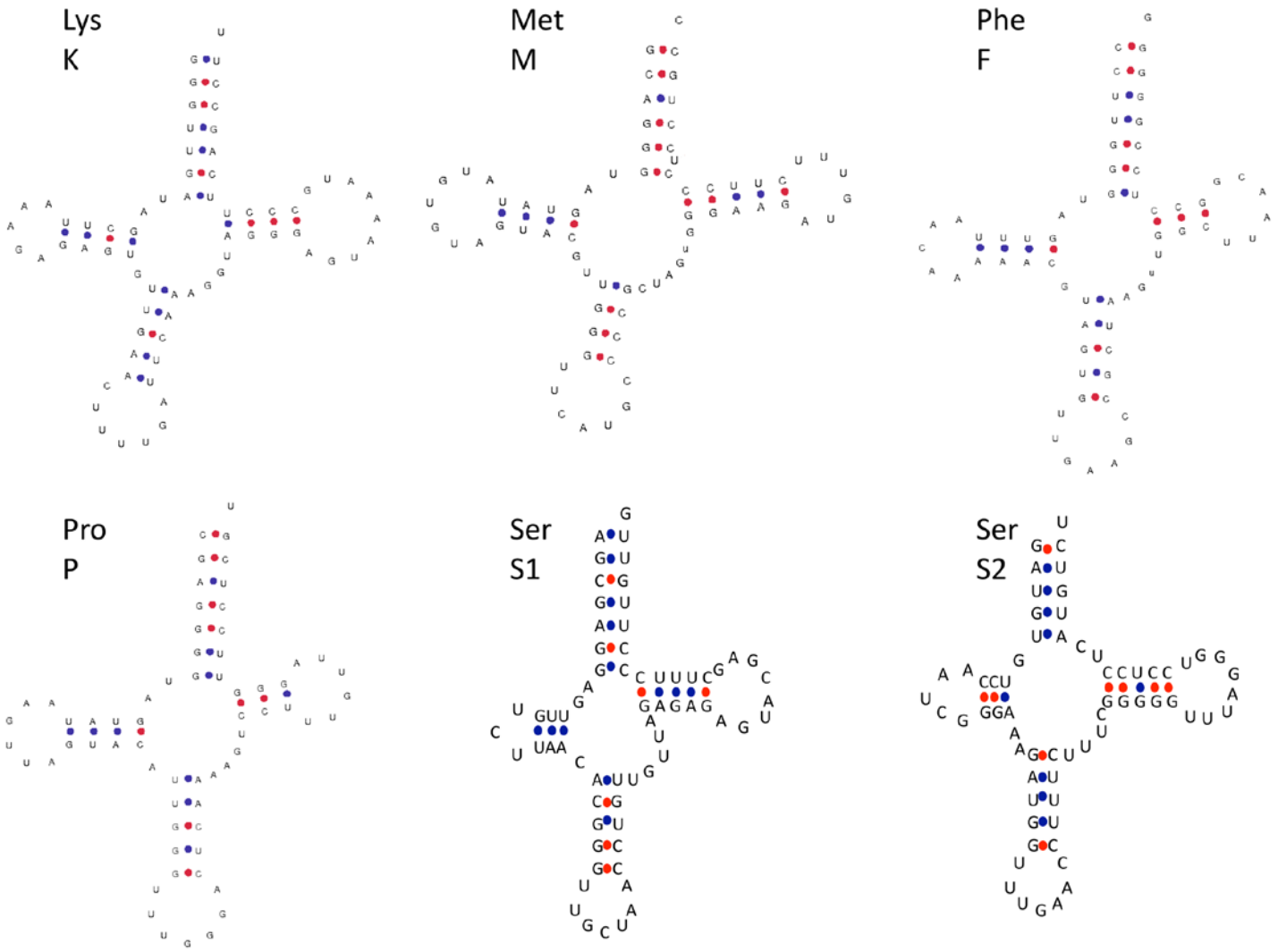
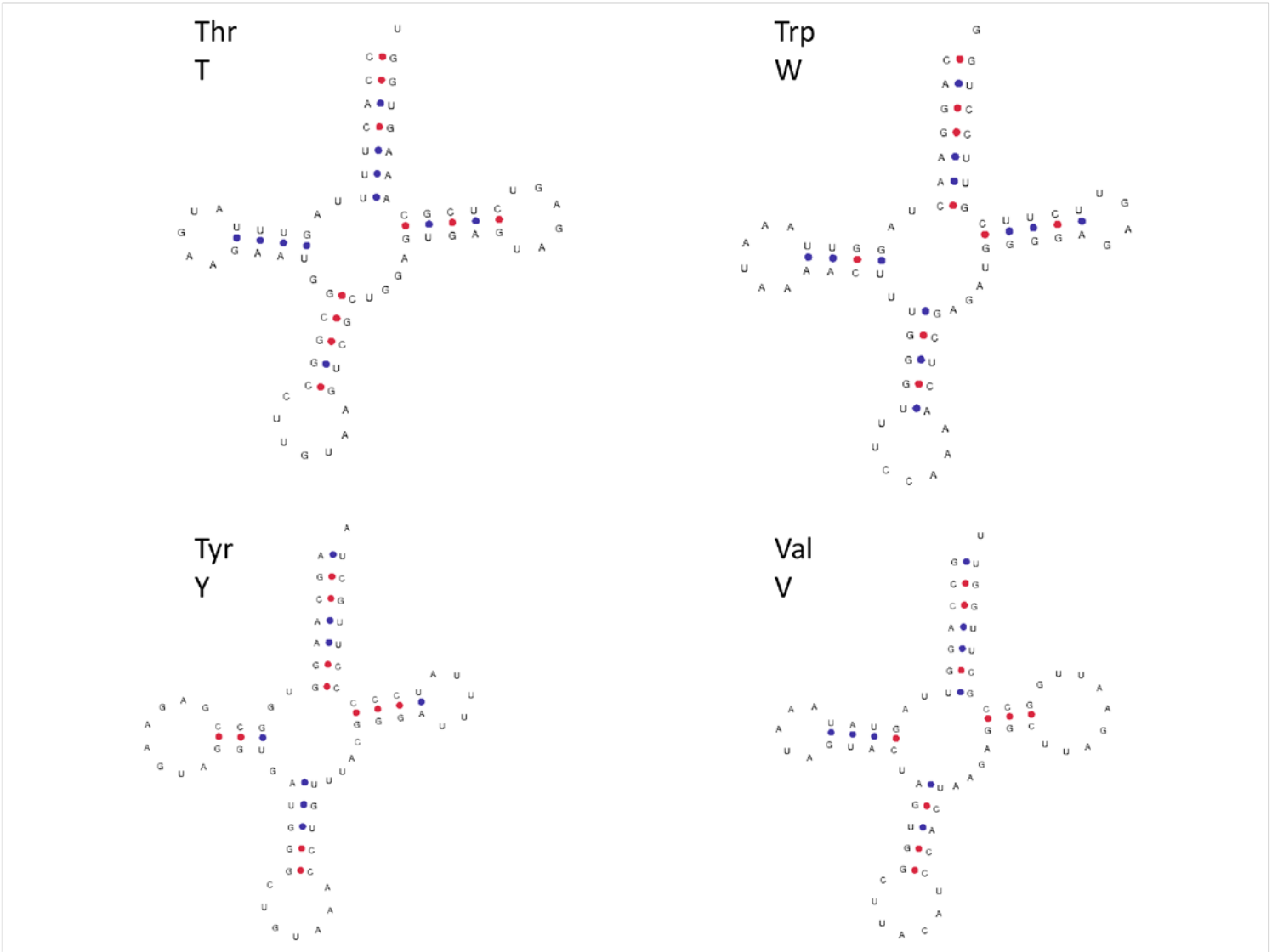


Supplementary 1b. (Continued)



Supplementary 1b. (Continued)