First complete female mitochondrial genome in four bivalve species genus Donax and their phylogenetic relationships within the Veneroida order

Jenyfer Fernández-Pérez¹ *, Ana Nantón¹, Francisco J. Ruiz-Ruano², Juan Pedro M. Camacho², Josefina Méndez¹

¹ Grupo Xenomar, Departamento de Biología, Facultade de Ciencias and CICA (Centro de Investigaciones Científicas Avanzadas), Universidade da Coruña, Campus de A Zapateira, A Coruña, Spain,
² Departamento de Genética, Facultad de Ciencias, Universidad de Granada, Granada, Spain

* jenyfer.fernandez.perez@udc.es

Abstract

Background

Four species of the genus Donax (D. semistriatus, D. trunculus, D. variegatus and D. vittatus) are common on Iberian Peninsula coasts. Nevertheless, despite their economic importance and overexploitation, scarce genetic resources are available. In this work, we newly determined the complete mitochondrial genomes of these four representatives of the family Donacidae, with the aim of contributing to unveil phylogenetic relationships within the Veneroida order, and of developing genetic markers being useful in wedge clam identification and authentication, and aquaculture stock management.

Principal findings

The complete female mitochondrial genomes of the four species vary in size from 17,044 to 17,365 bp, and encode 13 protein-coding genes (including the atp8 gene), 2 rRNAs and 22 tRNAs, all located on the same strand. A long non-coding region was identified in each of the four Donax species between cob and cox2 genes, presumably corresponding to the Control Region. The Bayesian and Maximum Likelihood phylogenetic analysis of the Veneroida order indicate that all four species of Donax form a single clade as a sister group of other bivalves within the Tellinoidea superfamily. However, although Tellinoidea is actually monophyletic, none of its families are monophyletic.

Conclusions

Sequencing of complete mitochondrial genomes provides highly valuable information to establish the phylogenetic relationships within the Veneroida order. Furthermore, we provide here significant genetic resources for further research and conservation of this commercially important fishing resource.
Introduction

Bivalve molluscs of the genus Donax (Donacidae family) are an important constituent of the macrofauna of sandy beaches in temperate, tropical and subtropical zones, being the dominant organisms in this type of environment [1]. In the littoral of Iberian Peninsula, the five European species of Donax live sympatrically in the same beaches [2, 3]: D. trunculus (Linnaeus, 1758) (Atlantic and Mediterranean), D. vittatus (Da Costa, 1778) (Atlantic), D. variegatus (Gmelin, 1791) (Atlantic and Mediterranean), D. semistriatus (Poli, 1775) (Atlantic and Mediterranean) and D. venustus (Poli, 1775) (Atlantic and Mediterranean) [4, 5, 6, 7]. Nevertheless, D. venustus is practically non-existent in the Iberian Peninsula as a single individual has been found between the years 2000 and 2006 along the south coast of Portugal [3].

Few species of the genus Donax are commercially exploited, but some are consumed locally or used as fishing bait. D. trunculus is exploited in many countries bordering the Mediterranean Sea and Atlantic Ocean, including Portugal [8, 9], Italy [10], France [11], and Spain [12, 13]. Only in Iberian Peninsula, the recorded captures since 1999 to 2014 equal 10,156 tons, with a maximum production of 1,042 tons in 2005 followed by an incessant decline reaching only 250 tons in 2014 [14]. Although this data only reflects production since fishermen were obliged to declare their captures [8], the species has been subjected to intense exploitation over the last decades and, currently, some D. trunculus populations seem to be at high long-term risk of extinction [15]. Furthermore, this species constitutes an important shellfish resource due to its high economical value. For instance, in Galicia (northwest of Spain), D. trunculus is a species with a high contribution rate, being the bivalve with greater commercial value (38.52 €/kg in the year 2016) [16] in markets during last years. Due to the similarity in size, shape and colour of the Donax clams in different species, captures of D. trunculus in natural beds may contain other species of the genus with lesser economical value and may be marketed together. However, despite their overexploitation and economic importance, relatively few genetic resources are available for this species [15, 17] and the whole genus [18, 19].

In order to preserve this important fishing resource, genetic tools should be employed. Molecular genetics has proven highly informative to determine the level of genetic variability, which is an essential feature to consider when defining conservation priorities, as well as to better understand the (recent) evolutionary history of species groups. Within the molecular resources, mitochondrial (mt) genome stands out to be considered a useful tool for population genetic and phylogenetic studies, not only because complete mt genomes are often more informative than single genes, but also because they reveal some genome-level details, such as the rearrangement of genes, which are valuable information for studies of evolutionary relationships among species [20, 21, 22, 23]. Moreover, mitochondrial DNA (mtDNA) is particularly important in helping to differentiate species that are morphologically similar, contributing to the identification and authentication of commercial food species to detect and avoid fraud, to protect consumer rights and to achieve other quality objectives, such as certificate of origin.

Most metazoan mitochondrial genomes are typically closed circular molecules of ~16 kb, encoding 37 genes: 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes and two ribosomal RNA (rRNA) genes [24]. In addition, at least one extensive non-coding sequence is present which contain elements that control the initiation of replication and transcription [25]. Mitochondrial genome has several valuable features that make it exclusive, including its small size, high evolutionary rates, limited recombination, relatively conserved gene content and organization, and maternal inheritance [22, 26]. Though, an extreme exception to the paradigm of strict maternal inheritance of animal mtDNA (SMI) is found in some bivalve lineages, which possess an unusual system known as doubly uniparental inheritance (DUI) ([27, 28, 29] for reviews). Species showing DUI display two different kinds of mitochondrial genomes,
i.e. male (M) and female (F) mitogenomes. While females have only the F genome, males are heteroplasmic and possess F and M genomes, which the F type predominating in somatic tissues and the M one in gonads [30, 31]. To date, the vast majority of species with DUI which have been reported belong to the orders Mytiloida, Nuculanoida, Unionoida and Veneroida [32], including the wedge clam *D. trunculus* [33].

In this study, we determine, for the first time, the complete female mitochondrial (mt) genome sequences in four species of *Donax* from the Iberian Peninsula, and compare them with those of other marine bivalves. In addition, the four newly sequenced mitogenomes, together with the veneroids mt genomes available in GenBank, were used to construct the phylogenetic relationships in the Veneroida order. This work should be of importance not only for better understanding the phylogenetic relationships within the Veneroida order, but also for the development of genetic markers useful in wedge clams aquaculture and restoration effects, as well as for the identification and authentication of commercial species.

**Materials and methods**

**Ethics statement**

All clams handling was conducted in accordance with the guidelines and regulations established by the University of A Coruña and the local governments. Field sampling did not require specific permissions but was in accordance with general governmental regulations. No endangered or protected species were involved.

**Samples collection and DNA extraction**

Given that DUI has been described in *D. trunculus* [33] and we have found evidence for it in *D. vittatus* and *D. semistriatus* [34], and since the goal of our work was on female mtDNA, we used somatic cells of female specimens as the only source for mtDNA sequencing. Therefore, each of the four *Donax* complete mt genomes sequenced here was obtained from a single female specimen in each species, sampled at natural beds. The *D. trunculus* sample was collected at Corrubedo (A Coruña, northwestern Spain) while the *D. semistriatus*, *D. variegatus* and *D. vittatus* samples came from the Portuguese coast (Table 1). Gender determination was performed on each individual by microscopic examination of gametogenic tissue from the visceral mass, and was based on the presence of eggs or sperm. Specimens were taxonomically identified using Pereira *et al.* 2012 [18] and Nantón *et al.* 2015 [19] molecular protocols developed in our laboratory. Voucher specimens and their shells were deposited at the malacology collections of the Museo Nacional de Ciencias Naturales (MNCN), Madrid (Spain) (Table 1).

Total genomic DNA was extracted from about 40 mg of ethanol-preserved foot muscle tissue of female specimens using DNAeasy Blood and Tissue Kit (Qiagen, Germany) following manufacturer’s instructions with only a minor modification, namely EB (10mM Tris-Cl, pH 8.5) rather than AE (10mM Tris-Cl, 0.5 mM EDTA, pH 9.0) buffer was used to avoid possible interference of EDTA with Nextera enzyme.

**Molecular procedures and sequencing**

The purified genomic DNA was assessed by spectrophotometry (NanoDrop ND-1000, Technologies, Inc.), fluorometry (Qubit HS, Invitrogen, USA) and 1% agarose gel electrophoresis. After quality controls, four libraries (one per species) were prepared using the NEBNext<sup>®</sup> Ultra™ DNA Library Prep Kit for Illumina<sup>®</sup> and sequenced in the Illumina HiSeq 4000 platform yielding about 20 Gb data for *D. vittatus* and 10 Gb for each of the three other species, subdivided into 2x150 nt paired-end reads.
Mitogenome assembly and annotation

The mt genomes were reconstructed using 2x1,000,000 reads per species with the MITObim assembler [35]. We performed a first assembly with the -quick option, which resulted in a partial mt genome sequence of about 10,000 bp. In order to get the complete sequence, we extracted the sequence of the COI gene from the previous assembly to be used as starting sequence in MITObim with the -seed option. This yielded sequence of about 17,000 bp whose quality and completeness were assessed on the basis of their average coverage along their whole length, by mapping, in each species, the same 2x1,000,000 reads used in the assembly against the inferred mitogenome sequence. For this purpose, we used the SSAHA2 software [36] with a minimum score of 100. Then we extracted coverage information from these mapping using pysamstats (available at: http://github.com/alimanfoo/pysamstats).

The mt genomes were annotated using the MITOS Web Server [37] applying the invertebrate mitochondrial genetic code and followed by manual validation of the coding regions using the NCBI ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/). Based on ORF Finder result, the sqn files generated from MITOS were edited and submitted to NCBI. The annotations of PCGs were refined, while the annotations of tRNA genes were kept unchanged. tRNA genes were detected using MITOS, tRNAscan-SE v.2.0 [38] and ARWEN v.1.2 [39]; and secondary structures of tRNAs were inferred using MITOS in default search mode. Mitogenome maps were drawn using GenomeVx online tool [40] followed by manual modification. Repeat sequence patterns in the longest non-coding region (NCR) were checked using the web-based software server Tandem Repeats Finder (http://tandem.bu.edu/trf/trf.html) [41].

Phylogenetic analyses

To investigate the phylogenetic relationships between species of the Veneroida order, we used the 33 mitogenomes currently available in GenBank (last accessed 17 January 2017), in addition to the four newly determined in this work. Lucinella divaricata and Loripes lacteus, belonging to the order Lucinoida, were used as outgroups (Table 2). Owing to the fact that a lack of the Atpase subunit 8 (atp8) gene has been reported in some bivalve species, we investigated the possibility that its presence might have gone unnoticed in these species by actively searching for atp8 sequence in an annotation with MITOS and aligning with other mitogenomes using Geneious Pro v.4.8.5 [42]. We found the atp8 gene in eight species where previous analyses had concluded the absence of this gene. The alignment of the amino acid sequences for each of the 13 mitochondrial PCGs was performed with the MUSCLE plug-in in Geneious Pro v.4.8.5 [42] with default parameters. We removed poorly aligned regions with Gblocks v.0.91b [43], with options allowing gaps for all positions and 85% of the number of sequences for flanking positions. The 13 separate amino acid sequence alignments were then concatenated into a single large dataset consisting of 2617 sites (S1 File).

Phylogenetic analyses were performed under Maximum Likelihood (ML) using RaxML [44] in a web server (http://embnet.vital-it.ch/raxml-bb/) and Bayesian inference (BI) using MrBayes v3.2.6 [45] and PhyloBayes [46]. The best fit models of amino acid evolution were
chosen by ProtTest v.3.4.2 [47], with default settings, based on Akaike Information Criterion (AIC). The optimal chosen methods were: LG + I + G + F for cox1, cox3 and nad5 genes; LG + G + F for cox2, nad6 and atp8; MtArt + I + G + F for cob, atp6, nad2 and nad4; MtArt + I + G + F for nad1, nad3 and nad4l. However, as the MtArt evolutionary model is not available in MrBayes, the LG model (the second best-fit model according to ProtTest) was used in Bayesian analysis, being therefore: LG + I + G + F for cox1, cox3, cob, nad1, nad2, nad3, nad4 and nad4l genes; LG + G + F for cox2, atp6, nad6 and atp8; and LG + G for nad4l. The ML analyses consisted of 1000 bootstrap iterations using the CAT model for each partition. BI analysis consisted of two independent Markov chain Monte Carlo (MCMC) runs, each comprising four

Table 2. List of the species whose mitogenome sequences were used in the phylogenetic analysis.

| Species                | Classification                  | GB Accession no. | Reference       |
|------------------------|---------------------------------|------------------|----------------|
| Donax semistriatus     | Veneroida; Tellinoidea; Donacidae | KY780363         | This study      |
| Donax trunculus        | Veneroida; Tellinoidea; Donacidae | KY780364         | This study      |
| Donax variegatus       | Veneroida; Tellinoidea; Donacidae | KY780365         | This study      |
| Donax vittatus         | Veneroida; Tellinoidea; Donacidae | KY780366         | This study      |
| Macoma balthica       | Veneroida; Tellinoidea; Tellinidae | KM373200         | [50]           |
| Moerella iridescens   | Veneroida; Tellinoidea; Tellinidae | JN398362         | [51]           |
| Nuttallia olivacea     | Veneroida; Tellinoidea; Psammobiidae | JN398364        | [51]           |
| Semele scabra        | Veneroida; Tellinoidea; Semelidae | JN398365         | [51]           |
| Solecurtus divaricatus | Veneroida; Tellinoidea; Solecurtidae | JN398367        | [51]           |
| Solettellina diphus    | Veneroida; Tellinoidea; Psammobiidae | JN398363        | [51]           |
| Sinonovacula constricta | Veneroida; Solenoidae; Pharidiae | JN398366        | [51]           |
| Solen grandis         | Veneroida; Solenoidae; Solenidae | HQ703012         | [56]           |
| Solen strictus        | Veneroida; Solenoidae; Solenidae | JN786377         | [57]           |
| Cyclina sinensis      | Veneroida; Veneroidae; Veneridae | KU097333         | [75]           |
| Meretrix lamarkii       | Veneroida; Veneroidae; Veneridae | GUJ071281        | [76]           |
| Meretrix lusoria       | Veneroida; Veneroidae; Veneridae | GQ903339         | [62]           |
| Meretrix lyra         | Veneroida; Veneroidae; Veneridae | KC832317         | [77]           |
| Meretrix meretrix      | Veneroida; Veneroidae; Veneridae | GQ463598         | [78]           |
| Meretrix pelachialis   | Veneroida; Veneroidae; Veneridae | EU145977         | [79]           |
| Paphia amabilis       | Veneroida; Veneroidae; Veneridae | JF969276         | [49]           |
| Paphia euglypta       | Veneroida; Veneroidae; Veneridae | GU269271         | [80]           |
| Paphia textile        | Veneroida; Veneroidae; Veneridae | JF969277         | [49]           |
| Paphia undulata       | Veneroida; Veneroidae; Veneridae | JF969278         | [49]           |
| Ruditapes philippinarum | Veneroida; Veneroidae; Veneridae | KT001084         | [81]           |
| Saxidomus purpuratus  | Veneroida; Veneroidae; Veneridae | KP419933         | [82]           |
| Acanthocardia tuberculata | Veneroida; Cardioidea; Cardiidae | DO632743        | [59]           |
| Fulvia mutica        | Veneroida; Cardioidea; Cardiidae | NC_022194        | [83]           |
| Tridacna squamosa     | Veneroida; Cardioidea; Cardiidae | KP205428         | [84]           |
| Corbicula fluminea   | Veneroida; Corbiculoidae; Corbiculidae | KX254564 | Tao et al., unpublished |
| Geloina coxans       | Veneroida; Corbiculoidae; Corbiculidae | KP999913 | Zhou, unpublished |
| Calypgotheca magnifica | Veneroida; Glossoidea; Vesicomoridae | KR862368 | [85] |
| Arctica islandica   | Veneroida; Arcticoidea; Arcticidae | KF363951        | [86]           |
| Coelomacra antiquata | Veneroida; Mactroidea; Mactridiae | KC503290         | [87]           |
| Lutraria rynchena    | Veneroida; Mactroidea; Mactridiae | NC_023384        | [88]           |
| Mactra chimensis     | Veneroida; Mactroidea; Mactridiae | KJ754823         | [89]           |
| Lucinella divaricata | Lucinoida; Lucinoidea; Lucinidae | EF043342         | Dreyer et al., unpublished |
| Loripes lacteus      | Lucinoida; Lucinoidea; Lucinidae | EF043341         | Dreyer et al., unpublished |

https://doi.org/10.1371/journal.pone.0184464.t002
linked chains (one cold and three heated; as default settings). They were performed for 1,000,000 generations, sampling every 100 generations to allow adequate time for convergence. The convergence of the two runs was assessed by stopping the analysis when the average standard deviation was below 0.01 (stoprule = yes and stopval = 0.01 in the mcmc command). 1,000,000 generations were enough to reach adequate average standard deviation (<0.01). By default, the first 25% trees were discarded as burn-in. BI analyses were also conducted at the amino-acid level using the CAT + GTR model in PhyloBayes [46]. Two independent MCMC analyses were run in parallel for 4,000 generations. The first 1,000 samples were discarded as burn-in. From the remaining samples, we sampled a tree every 10 cycles to compute a consensus tree. The convergence between the two chains were considered acceptable when the max-diff parameter was below 0.3 (maxdiff = 0.218586) and the minimum effective size (MES) was >50 (MES = 64).

**Results and discussion**

**Sequencing and mitogenome assembly**

A total of about 92,000,000 paired reads (2x150 nt) were obtained for *D. semistriatus*, about 85,000,000 for *D. trunculus*, about 82,000,000 for *D. variegatus* and about 185,000,000 for *D. vittatus*. We selected 2x1,000,000 reads that were used to assemble the mitogenome in each species, yielding average coverages of 45x in *D. semistriatus*, 31x in *D. trunculus*, 37x in *D. variegatus*, and 58x in *D. vittatus*. Coverage profiles were uniform along the mt genomes (see S1 Fig).

**Genome composition**

The mitogenomes of the four *Donax* species sequenced in this study were circular molecules, as revealed by the MITObim assembly. They are composed of 37 genes: 13 PCGs (including the *atp8* gene), two ribosomal RNA genes and 22 transfer RNA genes (Fig 1). Their main structural features are summarized in Table 3. The complete mt genomes of *D. semistriatus*, *D. trunculus*, *D. variegatus* and *D. vittatus* vary in size from 17,044 bp (*D. semistriatus*) to 17,365 bp (*D. trunculus*). Length differences are mostly due to the size variation of the non-coding region. The A+T content of the four mitogenomes ranges from 58.9% (*D. trunculus*) to 63.5% (*D. vittatus*). Although gene organization is known to vary extensively, even among species from the same genus [22, 48, 49], all four complete *Donax* mt genomes showed the same gene order and they are located on the “+” strand, likewise in *Macoma balthica*, other member of the Tellinoidea superfamily for which the whole mt genome is available [50]. The only difference was noted in the location of the longest NCR which, in *M. balthica*, is situated between *rrnS* and *tRNA-Met*, whereas in *Donax* clams it is located between *cob* and *cox2* genes (Fig 1). Therefore, in consistency with the highly rearranged gene order in bivalves, the longest NCR is not conserved at the same position among bivalve mt genomes [51, 52].

**Protein coding genes**

The typical 13 PCGs were identified in the four new mitogenomes analyzed here, including the *atp8* gene, which had been reported as missing in several bivalve species [51, 53, 54, 55, 56, 57, 58], but subsequent analysis found its presence in several of them [48, 50, 52, 59, 60, 61, 62]. It was suggested that the short and variable length of this protein, along with its high variation in amino acid composition, might hinder the finding of this gene due to annotation difficulties [22]. However, using the same bioinformatic approach employed in *Donax* species, we found the *atp8* gene in publicly available mitogenome sequences of most Veneroida order
species available in the databases (Table 4). Moreover, we found other discrepancies with GenBank annotations. The tRNA-Lys annotation for *Mactra chinensis* (KJ754823) was modified (from 9945–10028 to 13611–13677) and in the following cases, the previous *rrnS* annotations were also edited: *rrnS* for *M. meretrix* (GQ463598) and *M. petechialis* (EU145977) were edited from 7093–8673 to 7089–8569; for *C. antiquata* (KC503290) from 7898–9197 to 7898–9096; and for *L. rhynchaena* (NC_023384) from 6870–8244 to 6870–8161.

Fig 1. Maps of the mitochondrial genomes of *Donax* species. Genome lengths are shown in the middle of each map, genes are all on “+” strand and NCR indicates the longest non-coding region.

https://doi.org/10.1371/journal.pone.0184464.g001
The location of the \textit{atp8} gene within the mitogenome is the same in the eight species of the Tellininae superfamily (all four \textit{Donax} species, \textit{M. balthica}, \textit{M. iridescens}, \textit{S. divaricatus} and \textit{S. diphos}), i.e. between \textit{tRNA-Met} and \textit{tRNA-Ser1}. In \textit{Donax} species, this short gene encoded a 42 amino acids protein starting with methionine (ATG, in the four species) and ending with a

| Table 3. Main structural features of the four sequenced mt genomes in this study. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Total length                    | Donax semistriatus | Donax trunculus | Donax variega tus | Donax vittatus |
| Total length                    | 17044            | 17365           | 17195           | 17070          |
| A+T%                            | 61.9             | 58.9            | 60.4            | 63.5           |
| \textit{cox2}                   | 846 (ATG/TAA)    | 846 (ATG/TAA)   | 831 (ATG/TAG)   | 846 (ATG/TAA)  |
| \textit{tRNA-Val}               | 62               | 64              | 64              | 64             |
| \textit{tRNA-Trp}               | 69               | 68              | 69              | 69             |
| \textit{tRNA-Gly}               | 64               | 65              | 66              | 66             |
| \textit{rrnS}                   | 863 (ATG/TAG)    | 860             | 859             | 865            |
| \textit{tRNA-Met}               | 65               | 65              | 65              | 65             |
| \textit{atp8}                   | 126 (ATG/TAG)    | 126 (ATG/TAG)   | 126 (ATG/TAG)   | 126 (ATG/TAG)  |
| \textit{tRNA-Ser1}              | 68               | 69              | 69              | 68             |
| \textit{nad6}                   | 576 (ATG/TAG)    | 573 (ATG/TAA)   | 540 (ATG/TAA)   | 576 (ATG/TAG)  |
| \textit{rml}                    | 1373             | 1367            | 1383            | 1386           |
| \textit{atp6}                   | 714 (ATG/TAA)    | 714 (ATG/TAA)   | 711 (ATG/TAG)   | 714 (ATG/TAG)  |
| \textit{cox3}                   | 891 (ATG/TAG)    | 915 (ATA/TA A)  | 891 (ATG/TAG)   | 891 (ATG/TAG)  |
| \textit{nad2}                   | 1062 (ATG/TAA)   | 1062 (TTG/TAG)  | 1062 (ATG/TAA)  | 1062 (ATG/TAA) |
| \textit{tRNA-Pro}               | 67               | 68              | 67              | 67             |
| \textit{tRNA-Gln}               | 65               | 66              | 66              | 65             |
| \textit{tRNA-Cys}               | 65               | 66              | 67              | 65             |
| \textit{tRNA-Ala}               | 64               | 65              | 65              | 65             |
| \textit{tRNA-Phe}               | 63               | 64              | 63              | 63             |
| \textit{cox1}                   | 1710 (ATG/TAA)   | 1710 (ATG/TAA)  | 1710 (ATG/TAA)  | 1710 (ATG/TAA) |
| \textit{nad4}                   | 1347 (TTG/TAA)   | 1356 (TTG/TAA)  | 1332 (TTG/TAA)  | 1347 (TTG/TAA) |
| \textit{tRNA-His}               | 66               | 66              | 66              | 64             |
| \textit{tRNA-Ser2}              | 66               | 65              | 66              | 65             |
| \textit{tRNA-Glu}               | 63               | 64              | 63              | 63             |
| \textit{nad3}                   | 363 (ATG/TAA)    | 363 (ATG/TAA)   | 363 (ATG/TAA)   | 363 (ATG/TAA)  |
| \textit{tRNA-Ile}               | 69               | 69              | 69              | 69             |
| \textit{tRNA-Lys}               | 65               | 63              | 64              | 64             |
| \textit{nad41}                  | 288 (TTG/TAG)    | 288 (TTG/TAG)   | 288 (ATG/TAA)   | 288 (TTG/TAG)  |
| \textit{tRNA-Tyr}               | 64               | 64              | 66              | 65             |
| \textit{tRNA-Thr}               | 65               | 66              | 66              | 65             |
| \textit{tRNA-Leu1}              | 65               | 66              | 65              | 65             |
| \textit{tRNA-Asp}               | 63               | 62              | 64              | 63             |
| \textit{tRNA-Leu2}              | 65               | 66              | 65              | 66             |
| \textit{nad1}                   | 924 (ATG/TAG)    | 924 (ATG/TAG)   | 924 (ATG/TAG)   | 924 (ATG/TAG)  |
| \textit{tRNA-Asn}               | 65               | 64              | 66              | 65             |
| \textit{nad5}                   | 1734 (ATG/TAA)   | 1734 (ATG/TAG)  | 1734 (ATG/TAA)  | 1734 (ATG/TAA) |
| \textit{tRNA-Arg}               | 63               | 63              | 63              | 63             |
| \textit{cob}                    | 1215 (ATG/TAA)   | 1218 (ATA/TAA)  | 1206 (ATG/TAA)  | 1215 (ATG/TAA) |

For each mt genome, total length (in bp), the percent of overall A+T content, and size (bp) of the protein coding genes (start and stop codons in brackets), tRNAs, \textit{rml} and \textit{rrnS} are given.

https://doi.org/10.1371/journal.pone.0184464.t003
stop codon (TAG in *D. semistriatus*, *D. trunculus* and *D. vittatus*; or TAA, in *D. variegatus*) (Table 4), so that ATP8 proteins show 83.7% amino acid identity among species. Finally, it has been suggested that the *atp6* and *atp8* genes are adjacent in most animal mitochondrial genomes, often with overlapping reading frames [63]. However, in *Donax* species *atp6* and *atp8* genes are physically separated by 1,917 (D. *trunculus*)– 1,928 bp (D. *vittatus*). Likewise, these two genes also fail to be adjacent in the mitogenome of other heterodont bivalves, such as *Hiatella arctica* [59], *M. balthica* [50] and *Meretrix lamarckii* [64]. On the contrary, they are

| Species                  | *atp8* | Size  | Position   | Start/Stop codons | Reference                   |
|-------------------------|--------|-------|------------|-------------------|-----------------------------|
| *Donax semistriatus*    | Yes    | 126   | 2396–2521  | ATG/TAG           | This study                  |
| *Donax trunculus*       | Yes    | 126   | 2419–2544  | ATG/TAG           | This study                  |
| *Donax variegatus*      | Yes    | 126   | 2352–2477  | ATG/TAA           | This study                  |
| *Donax vittatus*        | Yes    | 126   | 2310–2435  | ATG/TAG           | This study                  |
| *Macoma balthica*       | Yes    | 129   | 75–203     | ATT/TAA           | [50]                        |
| *Moerella iridescens*   | Yes    | 132   | 11625–11756| ATA/TAG           | This study                  |
| *Nuttallia olivacea*    | Yes    | 132   | 12930–13061| ATA/TAG           | This study                  |
| *Semele scabra*         | Yes    | 129   | 11969–12100| ATT/TAA           | This study                  |
| *Solecurtus divaricatus*| Yes    | 135   | 11321–11455| GTG/TAG           | [52]                        |
| *Solepellina diphus*    | Yes    | 135   | 11214–11342| GTG/TAG           | This study                  |
| *Sinovacuola constricta*| Yes    | 114   | 14288–14401| ATG/TAA           | This study                  |
| *Solen grandis*         | Yes    | 114   | 13703–13816| GTG/TAG           | This study                  |
| *Solen strictus*        | Yes    | 114   | 13473–13586| ATG/TAG           | This study                  |
| *Cyclina sinensis*      | Yes    | 117   | 8568–8684  | ATG/TAG           | This study                  |
| *Meretrix lamarckii*    | Yes    | 120   | 8835–8954  | ATG/TAA           | [76]                        |
| *Meretrix lusoria*      | Yes    | 120   | 8642–8761  | ATG/TAG           | [62]                        |
| *Meretrix lyra*         | Yes    | 120   | 8753–8872  | ATG/TAG           | [77]                        |
| *Meretrix merex*        | Yes    | 141   | 8532–8672  | ATA/TAG           | [52]                        |
| *Meretrix pelichi*      | Yes    | 141   | 8532–8672  | ATA/TAG           | [52]                        |
| *Paphia amabilis*       | Yes    | 114   | 14035–14148| ATG/TAG           | [49]                        |
| *Paphia euglypta*       | Yes    | 117   | 12994–13110| ATA/TAA           | This study                  |
| *Paphia textile*        | Yes    | 114   | 13019–13132| ATA/TAG           | [49]                        |
| *Paphia undulata*       | Yes    | 114   | 12642–12755| ATG/TAA           | [49]                        |
| *Ruditapes philippinarum*| Yes    | 120   | 5968–6087  | ATT/TAG           | [52]                        |
| *Saxidomus purpuratus*  | Yes    | 117   | 9557–9673  | ATG/TAA           | This study                  |
| *Acanthocardia tuberculata* | Yes | 103   | 12546–12648| GTG/CCT           | [52]                        |
| *Fulvia mutica*         | Yes    | 114   | 11341–11454| TTG/TAA           | [83]                        |
| *Tridacna squamosa*     | Yes    | 117   | 8525–8641  | ATG/TAG           | This study                  |
| *Corbicula fluminea*    | Yes    | 114   | 5480–5593  | ATG/TAA           | Tao et al., unpublished     |
| *Geloina coxans*        | Yes    | 114   | 12249–12362| TTG/TAG           | Zhou, unpublished           |
| *Calypgothena magnifica*| Yes    | 114   | 5440–5553  | ATG/TAA           | [85]                        |
| *Arctica islandica*     | Yes    | 151   | 10343–10493| TTG/AGT           | [52]                        |
| *Coelomactra antiquata* | Yes    | 114   | 9097–9210  | ATG/TAA           | This study                  |
| *Lutraria rhychnaena*   | Yes    | 118   | 8162–8275  | ATG/TAG           | This study                  |
| *Mactra chinesis*       | Yes    | 114   | 10000–10113| ATG/TAG           | This study                  |
| *Lucinella divaricata*  | Yes    | 114   | 15861–15974| ATT/TAA           | Dreyer et al., unpublished  |
| *Loripes lacteus*       | Yes    | 118   | 14442–14589| ATT/ACT           | Dreyer et al., unpublished  |

For each *atp8* sequence, size (bp), position (from-to), and start and stop codons.

https://doi.org/10.1371/journal.pone.0184464.t004
adjacent in the Unionidae [65] and Solemyidae [66], as well as in basal molluscs like Chaetoderma nitidulum (EF211990) and Katharina tunicata [67]. This suggests that the association of these genes might be an example of an ancestral state that has later been lost in derived bivalves.

Total length of the 13 PCGs ranged from 11,718 bp (D. variegatus) to 11,829 bp (D. trunculus), accounting for 68.1–69.2% of its total mt genome length. The longest PCG is nad5, with a size of 1,734 bp (577 aa), whereas nad2, cox1, nad4 and cob exceed 1,000 bp. However, nad3 and nad4l genes are shorter than 400 bp and atp8 gene is the shortest PCG with 126 bp (41 aa). These features are similar to those previously reported in M. balthica [50] and five other species of the Tellinoidea superfamily (Moerella iridescens, Sanguinolaria diphos, Sanguinolaria olivacea, Semele scabra and Solecurtus divaricatus) [51].

The ATN conventional start codon is used in most PCGs (ATG, N = 41; ATA, N = 2; the last codon being classically found in the invertebrate mitochondrial genetic code, particularly in bivalves [50]). However, like most invertebrate mt genomes, Donax mtDNA shows alternative start codons, and some PCGs start with NTG codons (TTG, N = 8; GTG, N = 1). In contrast, the observed stop codons are TAA (N = 32) and TAG (N = 20), and all 13 PCGs of the four mt genomes end in a full termination codon.

Transfer and ribosomal RNA genes
Standard rRNAs were found in the four mt genomes of Donax species analyzed here. The small-subunit ribosomal RNA (rrnS) was flanked by tRNA-Gly and tRNA-Met in all four mt genomes, and its size ranged from 859 bp (D. variegatus) to 865 bp (D. vittatus), with A+T content between 63.8 (D. semistriatus) and 68.5% (D. vittatus). On the other hand, the large-subunit ribosomal RNA (rrnL) was located between nad6 and atp6, just like in M. balthica [50], M. iridescens, S. diphos, S. olivacea, S. scabra, S. constricta and S. divaricatus [51]. Its size varied from 1,367 bp (D. semistriatus) to 1,386 bp (D. vittatus), and its A+T content ranged between 63.5 (D. variegatus) and 67.2% (D. semistriatus).

Twenty-two discrete nucleotide sequences (ranging from 62 to 69 bp) were predicted to fold into the typical secondary structures of tRNAs (see S2–S5 Figs). The predicted structures of tRNA genes showed cloverleaf shape with four arms in the four species, although some of them exhibited folding differences. Sixteen tRNAs showed a small supplemental stem loop (four in D. semistriatus: tRNA-Pro, tRNA-Phe, tRNA-Ile and tRNA-Leu2; two in D. trunculus: tRNA-Ile and tRNA-Thr; six in D. variegatus: tRNA-Val, tRNA-Pro, tRNA-Gln, tRNA-His, tRNA-Ile and tRNA-Arg; and four in D. vittatus: tRNA-Pro, tRNA-Phe, tRNA-Ile and tRNA-Leu2). Seven tRNAs showed no terminal TΨC loop (three in D. semistriatus: tRNA-His, tRNA-Thr and tRNA-Arg; one in D. trunculus: tRNA-Asn; and three in D. vittatus: tRNA-His, tRNA-Thr and tRNA-Asp). In addition, tRNA-Ser2 in D. trunculus showed the dihydrouracil (DHU) stem replaced by a big DHU loop. Finally, the single unpaired nucleotide, which is usually present at the 5’ end in other tRNAs, appeared at the 3’ end in tRNA-Tyr, with the only exception of D. variegatus where this tRNA lacks this unpaired nucleotide. These features have previously been found in mtDNAs of other bivalve species, such as M. balthica [50] and M. lamarckii [64].

Non-coding regions
As in most bivalves, the four species of the genus Donax analyzed here contained a large number of NCRs. The number of intergenic sequences varied from 17 (D. trunculus and D. vittatus) to 22 (D. variegatus), with 1,679 bp (representing 9.9% of the whole mitogenome) in D. semistriatus to 1,985 bp (11.4% of the mt genome) in D. trunculus (Table 5). The longest NCR was
located between *cob* and *cox2* genes in the four species, with length ranging from 1,549 bp (*D. semistriatus*) to 1,863 bp (*D. trunculus*). The other NCRs ranged from 1 to 21 bp. The longest NCR is thought to contain the Control Region (CR) because it presents some peculiar patterns, such as AT-rich or tandem repeats, believed to play a role in initiating and/or regulating mitochondrial transcription and replication [24, 68, 69]. The A+T content of the longest NCR in each mt genome was higher (*D. semistriatus*, *D. variegatus* and *D. vittatus*) or slightly lower (*D. trunculus*) than that of the whole mt genome (Table 5).

Six tandem repeats were also found in the longest NCRs of the four mt genomes, four of which were distinct tandem repeat units. The first motif consisted of 2.7 nearly identical copies of a 122 bp unit located at positions 48–386 from the 5´-end of the longest NCR in *D. semistriatus*. The second was 2.1 copies of 126 bp located at positions 17042–17309 in *D. trunculus*. In addition, microsatellite-like repeats, (TA)_{12} in *D. semistriatus* and (TA)_{12}ACACTTGTGA (TA)_{10} in *D. trunculus*, were detected near the 5´-end of the longest NCR. The third tandem repeat consisted in 2.1 copies of 137 bp located between positions 57 and 344 in *D. variegatus*, and the last one included 2 copies of 122 bp located at positions 47–304 in *D. vittatus*. Such long tandem repeats have also been reported in other bivalves of the Veneroida order [51, 55, 59, 62]. The study of tandem repeats in the CR is important for the light it sheds on a variety of processes, including the molecular mechanisms arising them and their possible functional implications [70].

### Phylogenetic analysis in Veneroida

To further study the relationships among *Donax* species and its position within the Veneroida order, ML and BI trees based on amino acid sequences of 13 concatenated PCGs belonging to 37 species were performed (Fig 2). Tree topologies were congruent and received high support in most nodes, with the exception of *S. scabra*, which showed a less basal position in the PhyloBayes phylogeny ((*M. balthica* + *M. iridescens*) + *S. scabra*) with 0.57 posterior probability as branch support.

We perform here the first phylogeny including the species of the genus *Donax* from the Iberian Peninsula (*D. trunculus*, *D. semistriatus*, *D. variegatus* and *D. vittatus*). Our analysis has shown that the four species form a single clade as a sister group to other bivalves of the superfamily Tellinoidea. All ten species of this superfamily belong to five different families and form a strongly supported clade, thus corroborating the monophyly of this superfamily [71, 72]. Nevertheless, our phylogenetic tree indicated, with high support by BI and ML, that *S. diphos* (Psammobiidae) shows closer relationship with *S. divaricatus* (Solecurtidae), *M. balthica* and *M. iridescens* (Tellinidae), *S. scabra* (Semelidae) and *Donax* species (Donacidae) rather than with *N. olivacea* (Psammobiidae), which implies that these two species (*S. diphos* and *N. olivacea*) do not form monophyletic groups. This result is also reported by Yuan et al. 2012 [51] and Ozawa et al. 2017 [73], and it is in agreement with the conclusion put forward by Taylor et al. 2007 [71] when analysed familial relationships within Tellinoidea, as Semelidae,

**Table 5. Comparison of non-coding regions (NCRs) within the four mt genomes.**

| Species         | No. of NCR | Total length (bp) | Proportion of the mt genome (%) | Longest NCR | A+T % |
|-----------------|------------|-------------------|--------------------------------|-------------|-------|
| *Donax semistriatus* | 18         | 1679              | 9.9                            | 1549        | 66.6  |
| *Donax trunculus*  | 17         | 1985              | 11.4                           | 1863        | 51.8  |
| *Donax variegatus* | 22         | 1869              | 10.9                           | 1718        | 62.6  |
| *Donax vittatus*   | 17         | 1697              | 9.9                            | 1580        | 67.5  |

[https://doi.org/10.1371/journal.pone.0184464.t005](https://doi.org/10.1371/journal.pone.0184464.t005)
Donacidae and Tellinidae do not form monophyletic groups. Tellinoidea is actually monophyletic, but none of its families are monophyletic [72], suggesting the need for a more exhaustive study within this commercially important marine bivalve clade.

Gene arrangement within mitogenomes is highly conserved in many taxonomic groups. For instance, most vertebrates share the same gene order [74]. However, in other animal groups, like the class Bivalvia, the mitochondrial genome arrangement is more variable [51, 52]. We compare here the gene arrangements of four newly sequenced mitogenomes to other closed related species belonging to Tellinoidea superfamily. This comparison was previously done by Yuan et al. (2012), without taking into account the *atp8* gene and without including Donax species and *M. balthica*, and their results supported the conclusion that comparisons of mitochondrial gene order rearrangements are, to some extent, a useful tool for phylogenetic studies. Seven out of the ten Tellinoidea mitogenomes hitherto analyzed (including the four Donax species analyzed by us, *M. balthica*, *M. iridescens* and *S. divaricatus*) show completely identical gene order, and *S. diphos* only differs in lacking a tRNA-Phe. Remarkably, the *atp8* gene shows the same location within the mitogenome of these eight species of the Tellinoidea superfamily, specifically between *tRNA-Met* and *tRNA-Ser1*. This result is consistent with the main phylogenetic conclusions from the 37 mitogenomes analyzed here (see above), and remarks the interest of performing additional full mitogenome sequencing, especially including more veneroid families and subfamilies, with gene order being a useful hallmark helping to clarify phylogenetic relationships within the order.

**Future implications**

This is a basic research work where we describe and characterize, for the first time, the female mitochondrial genome in four bivalve molluscs belonging to the genus Donax. This has provided new interesting information for the scientific community which can be feasible for
application in aquaculture. In fact, the mtDNA sequences contributed here add significantly useful genetic markers for i) helping to differentiate these commercial food species being morphologically similar, ii) detecting and avoiding fraud, iii) protecting consumer rights and achieving other quality objectives, such as certificate of origin, and iv) for using in population genetics studies and aquaculture stock management in Donax species. However, this possible applicability requires a broader work, where the different markers will be tested in a higher number of individuals, not only fresh individuals but also processed, packaged or frozen ones, as well as in a high number of females and males given that male genomes are still not available.

Conclusions

In this study, we determined the complete mt genomes of four bivalve species of the genus Donax, which are the first representatives from the family Donacidae being analyzed at this respect. Not only we have increased the number of complete mt genomes sequenced within Veneroida order, but also, we have illustrated the phylogenetic relationships among Donax species and their position within this order. Our results demonstrate that the sequencing of complete mitogenomes provides highly valuable information for phylogenetic analysis in bivalves. Furthermore, the mtDNA sequences contributed here add significantly useful genetic markers for use in species identification and authentication, phylogeny, population genetics, and aquaculture stock management in species of Donax.

Supporting information

S1 File. The alignment of 37 mitogenomes sequences used for phylogenetic analyses. Sequences include concatenated thirteen mitochondrial protein-coding genes.

S1 Fig. Coverage profiles for the four newly sequenced mitochondrial genomes. Blue line represents coverage along the mitochondrial sequences for the four Donax species. Red dashed lines represent the average coverage values: 45.46x in D. semistriatus, 30.94x in D. trunculus, 37.12x in D. variegatus, and 58.10x in D. vittatus.

S2 Fig. Predicted tRNA structures in D. semistriatus. 22 tRNAs are identified in the mitogenome of D. semistriatus and their cloverleaf secondary structures are inferred with MITOS annotation pipeline.

S3 Fig. Predicted tRNA structures in D. trunculus. 22 tRNAs are identified in the mitogenome of D. trunculus and their cloverleaf secondary structures are inferred with MITOS annotation pipeline.

S4 Fig. Predicted tRNA structures in D. variegatus. 22 tRNAs are identified in the mitogenome of D. variegatus and their cloverleaf secondary structures are inferred with MITOS annotation pipeline.

S5 Fig. Predicted tRNA structures in D. vittatus. 22 tRNAs are identified in the mitogenome of D. vittatus and their cloverleaf secondary structures are inferred with MITOS annotation pipeline.
Acknowledgments

We would like to thank Dra D. Martínez Patiño and S. Nóvoa from Centro de Cultivos Marí- nos de Ribadeo–CIMA (Xunta de Galicia) and Dr. M.B. Gaspar from Instituto Portugués do Mar e da Atmosfera–IPMA (Portugal) for providing specimens. The authors wish to thank the three anonymous reviewers for helpful remarks and suggestions that improved the quality of the manuscript. We also thank Dr. D. Huchon for useful comments during manuscript review. This work was supported by the Ministerio de Economía y Competitividad (Spain) through project AGL2016-75288-R AEI/FEDER, UE.

Author Contributions

Conceptualization: Jenyfer Fernández-Pérez, Juan Pedro M. Camacho, Josefina Méndez.

Data curation: Jenyfer Fernández-Pérez, Ana Nantón, Francisco J. Ruiz-Ruano, Juan Pedro M. Camacho, Josefina Méndez.

Formal analysis: Jenyfer Fernández-Pérez, Francisco J. Ruiz-Ruano, Juan Pedro M. Camacho.

Funding acquisition: Josefina Méndez.

Investigation: Jenyfer Fernández-Pérez, Josefina Méndez.

Methodology: Jenyfer Fernández-Pérez, Ana Nantón, Francisco J. Ruiz-Ruano, Juan Pedro M. Camacho, Josefina Méndez.

Project administration: Josefina Méndez.

Supervision: Josefina Méndez.

Writing – original draft: Jenyfer Fernández-Pérez.

Writing – review & editing: Jenyfer Fernández-Pérez, Ana Nantón, Francisco J. Ruiz-Ruano, Juan Pedro M. Camacho, Josefina Méndez.

References

1. Ansell AD. The Biology of the Genus Donax. In: McLachlan A, Erasmus T, editors. Sandy Beaches as Ecosystems. W Junk, The Hague; 1983. pp. 607–636.
2. Salas C, Tirado C, Manjón–Cabeza ME. Sublethal foot–predation on Donacidae (Mollusca: Bivalvia). J Sea Res. 2001; 46:43–56.
3. Rufino MM, Gaspar MB, Pereira AM, Maynou F, Monteiro CC. Ecology of megabenthic bivalve communities from Sandy beaches on the south coast of Portugal. Sci Mar. 2010; 74:163–178.
4. Ansell AD, Lagardère F. Observations on the biology of Donax trunculus and D. vittatus at Ile d’Oléron (French Atlantic coast). Mar Biol. 1980; 57:287–300.
5. Salas–Casanova C. The Donacidae of the Bay of Malaga (Spain). Taxonomy. Basteria. 1987; 51:33–50.
6. Salas–Casanova C, Hergueta E. The functional morphology of the alimentary canal in Donax venustus Poli and Donax semistriatus Poli. In: Morton B., editors. The bivalvia. Hong Kong University Press, Hong Kong; 1990. pp. 213–222.
7. Gaspar MB, Santos MN, Vasconcelos P, Monteiro CC. Shell morphometric relationships of the most common bivalve species (Mollusca: Bivalvia) of the Algarve coast (southern Portugal). Hydrobiologia. 2002; 477:73–80.
8. Gaspar MB, Ferreira R, Monteiro CC. Growth and reproductive cycle of Donax trunculus L., (Mollusca: Bivalvia) off Faro, southern Portugal. Fish Res. 1999; 41(3):309–316.
9. Chicharo L, Chicharo A, Gaspar M, Alves F, Regala J. Ecological characterization of dredged and non–dredged bivalve fishing areas off south Portugal. J Mar Biol Assoc UK. 2002; 82(1):41–50.
10. Zeichen MM, Agnesi S, Mariani A, Maccaroni A, Ardizzone GD. Biology and population dynamics of *Donax trunculus* L. (Bivalvia: Donacidae) in the South Adriatic Coast (Italy). Est Coast Shelf Sci. 2002; 54(6):971–982.

11. Thébaud O, Verón G, Fitas S. Incidences des épisodes d’efflorescences de micro algues toxiques sur les écosystèmes et sur les pêcheries de coquillages en baie de Douarnenez. In: Rapport Ifremer R.INT. DCE/DE–DCE/STH/UDPP 05–010– Brest, France; 2005. pp. 88.

12. Ramón M, Cano J, Peña JB, Campos MJ. Current status and perspectives of mollusc (bivalves and gastropods) culture in the Spanish Mediterranean. Bol Inst Oceanogr. 2005; 21:361–373.

13. Molares J, Parada JM, Navarro–Pérez E, Fernández A. Variabilidad interanual de las ventas de los principales recursos marisqueros de Galicia y su relación con las condiciones ambientales. Rev Gal Rec Mar (Art Inf Tecn). 2008; 2(1):1–42.

14. FAO–FIGIS: In Fisheries Global Information System http://www.fao.org/fishery/statistics/global-capture-production/query/en (2017). Accessed 10 Mar 2017.

15. Marie AD, Lejeusne C, Karapatsiou E, Cuesta JA, Drake P, Macpherson E, et al. Implications for management and conservation of the population genetic structure of the wedge clam *Donax trunculus* across two biogeographic boundaries. Sci Rep. 2016; 6:39152. https://doi.org/10.1038/srep39152 PMID: 27991535

16. Consellería do medio rural e do mar, Xunta De Galicia. In Pesca de Galicia—Plataforma tecnolóxica da pesca http://www.pescadegalicia.gal/estadisticas/ (2017). Accessed 17 Mar 2017.

17. Nantón A, Arias–Pérez A, Méndez J, Freire R. Characterization of nineteen microsatellite markers and development of multiplex PCRs for the wedge clam *Donax trunculus* (Mollusca: Bivalvia). Mol Biol Rep. 2014; 41:5351–5357. https://doi.org/10.1007/s11033-014-3406-0 PMID: 24852303

18. Pereira AM, Fernández-Tajes J, Gaspar MB, Méndez J. Identification of the wedge clam *Donax trunculus* by a simple PCR technique. Food Control. 2012; 23(1):268–270.

19. Nantón A, Freire R, Arias–Pérez A, Gaspar MB, Méndez J. Identification of four *Donax* species by PCR–RFLP analysis of cytochrome c oxidase subunit I (COI). Eur Food Res Technol. 2015; 240:1129–1133.

20. Boore JL, Brown WM. Big trees from little genomes: mitochondrial gene order as phylogenetic tool. Curr Opin Genet Dev. 1998; 8(6):668–674. PMID: 9914213

21. Rokas A, Holland PWH. Rare genomic changes as a tool for phylogenetics. Trends Ecol Evol. 2000; 15(11):454–459. PMID: 11050348

22. Gissi C, Iannelli F, Pesole G. Evolution of the mitochondrial genome of Metazoa as exemplified by comparison of congeneric species. Heredity (Edinb). 2008; 101(4):301–320.

23. Shen X, Ma X, Ren J, Zhao F. A close phylogenetic relationship Sipuncula and Annelida evidenced from the complete mitochondrial genome sequence of *Phascolosoma esculenta*. BMC Genomics. 2009; 10:136. https://doi.org/10.1186/1471-2164-10-136 PMID: 19327168

24. Wolstenholme DR. Animal Mitochondrial DNA: Structure and Evolution. Int Rev Cytol. 1992; 141:173–216. PMID: 1452431

25. Hoffman RJ, Boore JL, Brown WM. A novel mitochondrial genome organization for the blue mussel, *Mytilus edulis*. Genetics. 1992; 131:397–412. PMID: 1386586

26. Zhou Y, Zhang JY, Zheng RQ, Yu BG, Yang G. Complete nucleotide sequence and gene organization of the mitochondrial genome of *Paa spinosa* (Anura: Ranoidae). Gene. 2009; 447(2):86–96. https://doi.org/10.1016/j.gene.2009.07.009 PMID: 19631263

27. Breton S, Doucet-Beaupré H, Stewart DT, Hoeh WR, Blier PU. The unusual system of doubly uniparental inheritance of mtDNA: isn’t one enough? Trends Genet. 2007; 23(9):465–74. https://doi.org/10.1016/j.tig.2007.05.011 PMID: 17681397

28. Passamonti M, Ghiselli F. Doubly uniparental inheritance: two mitochondrial genomes, one precious model for organelle DNA inheritance and evolution. DNA Cell Biol. 2009; 28:79–89. https://doi.org/10.1089/dna.2008.0807 PMID: 19196051

29. Zouros E. Biparental Inheritance Through Uniparental Transmission: The Doubly Uniparental Inheritance (DUI) of Mitochondrial DNA. Evol Biol. 2013; 40:1–31.

30. Stewart DT, Saavedra C, Stanwood RR, Ball AO, Zouros E. Male and female mitochondrial DNA lineages in the blue mussel (*Mytilus edulis*) species group. Mol Biol Evol. 1995; 12:735–747. PMID: 7476121

31. Sutherland BW, Stewart DT, Kenchington E, Zouros E. The fate of paternal mitochondrial DNA in developing female mussels, *Mytilus edulis*: implications for the mechanism of doubly uniparental inheritance of mitochondrial DNA. Genetics. 1998; 148:341–347. PMID: 9475744
32. Gusman A, Lecomte S, Stewart D.T., Passamonti M, Breton S. Pursuing the quest for better understanding the taxonomic distribution of the system of doubly uniparental inheritance of mtDNA. PeerJ. 2016; 4:e2760. https://doi.org/10.7717/peerj.2760 PMID: 27994972
33. Theologidis I, Fodelianakis S, Gaspar MB, Zouros E. Doubly uniparental inheritance (DUI) of mitochondrial DNA in Donax trunculus (Bivalvia: Donacidae) and the problem of its sporadic detection in Bivalvia. Evolution. 2008; 62(4):959–970. https://doi.org/10.1111/j.1558-5646.2008.00329.x PMID: 18208565
34. Fernández-Pérez J, Froufe E, Nantón A, Gaspar MB, Méndez J. Genetic diversity and population genetic analysis of Donax vittatus (Mollusca: Bivalvia) and phylogeny of the genus with mitochondrial and nuclear markers. Est Coast Shelf Sci. 2017; In press.
35. Hahn C, Bachmann L, Chevreux B. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads—a baiting and iterative mapping approach. Nucleic Acids Res. 2013; 41(13):e129. https://doi.org/10.1093/nar/gkt371 PMID: 23661685
36. Ning Z, Cox AJ, Mullikin JC. SSAHA: A Fast Method for Large DNA Databases. Genome Res. 2001; 11:1725–1729. https://doi.org/10.1101/gr.194201 PMID: 11591649
37. Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, et al. MITOS: Improved de novo Metazoan Mitochondrial Genome Annotation. Mol Phylogenet Evol. 2013; 69(2):313–319. https://doi.org/10.1016/j.ympev.2012.08.023 PMID: 22982435
38. Lowe TM, Chan PP. tRNAscan–SE On–line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res. 2016; 44(Web Server issue):W54–W57. https://doi.org/10.1093/nar/gkw413 PMID: 27174935
39. Laslett D, Canbäck B. ARWEN, a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. Bioinformatics. 2008; 24:172–175. https://doi.org/10.1093/bioinformatics/btm573 PMID: 18033792
40. Conant GC, Wolfe KH. GenomeVx: Simple web–based creation of editable circular chromosome maps. Bioinformatics. 2008; 24:861–862. https://doi.org/10.1093/bioinformatics/btm598 PMID: 18227121
41. Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, et al. Geneious v4.8.5. 2009. Available at: http://www.geneious.com/
42. Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol. 2000; 17:540–552. PMID: 10742046
43. Stamatakis A, Hoover P, Rougemont JA. Rapid Bootstrap Algorithm for the RAxML Web–Servers, Syst Biol. 2008; 57(6):758–771. https://doi.org/10.1080/10635150802429642 PMID: 18853362
44. Ronquist F, Huelsenbeck JP, Larget B, Huelsenbeck J, Huelsenbeck J, Huelsenbeck J, et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 2012 61:539–542. https://doi.org/10.1093/sysbio/syr028 PMID: 22357727
45. Lartillot N, Philippe H. A Bayesian Mixture Model for Across-Site Heterogeneities in the Amino-Acid Replacement Process. Mol Phylogenet Evol. 2004; 21(6):1095–1109. https://doi.org/10.1016/j.ympev.2012.07.032 PMID: 22846367
46. Yuan Y, Li Q, Yu H, Kong L. The Complete Mitochondrial Genomes of Six Heterodont Bivalves (Tellinidae and Solenoida): Variable Gene Arrangements and Phylogenetic Implications. PLoS ONE. 2012; 7(2):e32353. https://doi.org/10.1371/journal.pone.0032353 PMID: 22842247
47. Plazzi F, Puccio G, Passamonti M. Comparative Large-Scale Mitogenomics Evidence Clade-Specific Evolutionary Trends in Mitochondrial DNAs of Bivalvia. Genome Biol Evol. 2016; 8(8):2544–2564. https://doi.org/10.1093/gbe/evw187 PMID: 27503296
53. Ren J, Shen X, Jiang F, Liu B. The mitochondrial genomes of two scallops, Argopecten irradians and Chlamys farreni. (Mollusca: Bivalvia): The most highly rearranged gene order in the family Pectinidae. J Mol Evol. 2010; 70(1):57–68. https://doi.org/10.1007/s00239-009-9308-4 PMID: 20013337

54. Smith DR, Snyder M. Complete Mitochondrial DNA Sequence of the Scallop Placopecten magellanicus: Evidence of Transposition Leading to an Uncharacteristically Large Mitochondrial Genome. J Mol Evol. 2007; 65(4):380–391. https://doi.org/10.1007/s00239-007-9016-x PMID: 17922075

55. Meng X, Zhao N, Shen X, Hao J, Liang M, Zhu X, et al. Complete mitochondrial genome of Coelomactra antiquata (Mollusca: Bivalvia); The first representative from the family Mactridae with novel gene order and unusual tandem repeats. Comp Biochem Physiol Part D Genomics Proteomics. 2012; 7(2):175–179. https://doi.org/10.1016/j.cbd.2012.02.001 PMID: 22381378

56. Yuan Y, Li Q, Kong L, Yu H. The complete mitochondrial genome of the grandjackknife clam, Solen grandis (Bivalvia: Solenidae): a novel gene order and unusual non-coding region. Mol Biol Rep. 2012; 39(2):1287–1292. https://doi.org/10.1007/s11033-011-0861-8 PMID: 21598108

57. Yuan Y, Li Q, Kong L, Yu H. The complete mitochondrial genome of Solen strictus (Bivalvia: Solenidae). Mitochondrial DNA A DNA Mapp Seq Anal. 2012; 23(2):112–114.

58. Shen X, Song J, Meng X, Tian M, Yan B, Cheng H, et al. The first representative of Coelomactra antiquata mitochondrial genome from Liaoning (China) and phylogenetic consideration. Mitochondrial DNA Part B. 2016; 1(1):525–527.

59. Dreyer H, Steiner G. The complete sequences and gene organization of the mitochondrial genomes of Hiatella arctica and the first record for a putative AtPase subunit 8 gene in marine bivalves. Front Zool. 2006; 3:13. https://doi.org/10.1186/1742-9994-3-13 PMID: 16948842

60. Breton S, Stewart DT, Hoeh WR. Characterization of a mitochondrial ORF from the gender–associated mtDNAs of Mytilus spp. (Bivalvia: Mytilidae): identification of the “missing” ATPase gene. Mar Genomics. 2010; 3(1):11–18. https://doi.org/10.1016/j.margen.2010.01.001 PMID: 21798192

61. Śmietanka B, Burzyński A, Wenne R. Comparative genomics of marine mussels (Mytilus spp.) gender associated mtDNA: rapidly evolving atp8. J Mol Evol. 2010; 71(5–6):385–400. https://doi.org/10.1007/s00239-010-9393-4 PMID: 20931184

62. Wang H, Zhang S, Li Y, Liu B. Complete mtDNA of Meretrix lusoria (Bivalvia: Veneridae) reveals the presence of an atp8 gene, length variation and heteroplasmoy in the control region. Comp Biochem Physiol Part D: Genomics and Proteomics. 2010; 5(4):256–264.

63. Boore JL. Animal mitochondrial genomes. Nucleic Acids Res. 1999; 27(8):1767–1780. PMID: 10101183

64. Bettinazzi S, Pizzai F, Passamonti M. The Complete Female–and Male–Transmitted Mitochondrial Genome of Meretrix lamarkki. PLoS One. 2016; 11(4):e0153631. https://doi.org/10.1371/journal.pone.0153631 PMID: 27083010

65. Breton S, Doucet-Beaupré H, Stewart DT, Piontkivska H, Karmakar M, Bogan AE, et al. Comparative mitochondrial genomics of freshwater mussels (Bivalvia:Unionoida) with doubly uniparental inheritance of mtDNA: gender–specific open reading frames and putative origins of replication. Genetics. 2009; 183(4):1575–1589. https://doi.org/10.1534/genetics.109.110700 PMID: 19822725

66. Pizzai F, Ribani A, Passamonti M. The complete mitochondrial genome of Solemya velum (Mollusca: Bivalvia) and its relationships with conchifera. BMC Genomics. 2013; 14:409. https://doi.org/10.1186/1471-2164-14-409 PMID: 2377315

67. Boore JL, Brown WM. Complete DNA sequence of the mitochondrial genome of the black chiton, Katharina tunicata. Genetics. 1994; 138(2):423–443. PMID: 8728825

68. Faber JE, Stepien CA. Tandemly repeated sequences in the mitochondrial DNA control region and phylogeography of the Pike-perches Sizostedion. Mol Phylogenet Evol. 1998; 10(3):310–322. https://doi.org/10.1006/mpev.1998.0530 PMID: 10051384

69. Saito S, Tamura K, Aotsuka T. Replication origin of mitochondrial DNA in insects. Genetics. 2005; 171(4):1695–16705. https://doi.org/10.1534/genetics.105.046243 PMID: 16118189

70. Mundy NL, Winchell CS, Woodruff DS. Tandem Repeats and Heteroplasmy in the Mitochondrial DNA Control Region of the Loggerhead Shrike (Lanius ludovicianus). J Hered. 1996; 87(1):21–26. PMID: 8742819

71. Taylor JD, Williams ST, Glover EA, Dyal P. A molecular phylogeny of heterodonta bivalves (Mollusca: Bivalvia: Heterodonta): new analyses of 18S and 28S rRNA genes. Zool. Scr. 2007; 36:587–606.

72. Combsch DJ, Collins TM, Glover EA, Graf DL, Harper EM, Healy JM, et al. A family–level Tree of Life for bivalves based on a Sanger–sequencing approach. Mol Phylogenet Evol. 2017; 107:191–208. https://doi.org/10.1016/j.ympev.2016.11.003 PMID: 27840226
73. Ozawa G, Shimamura S, Takaki Y, Yokobori S, Ohara Y, Takishita K, et al. Updated mitochondrial phylogeny of Pteriomorph and Heterodont Bivalvia, including deep–sea chemosymbiotic Bathymodiolus mussels, vesicomyid clams and the thyasirid clam Concholela cf. bisecta. Mar Genomics. 2017; 31:43–52. https://doi.org/10.1016/j.margen.2016.09.003 PMID: 27720682

74. Pereira SL. Mitochondrial genome organization and vertebrate phylogenetics. Genet Mol Biol. 2000; 23:754–752.

75. Dong P, Ma G, Chang L, Zhu Y, Tian X. The complete mitochondrial genome of Cyclina sinensis (Veneroida:Veneridae). Mitochondrial DNA Part B. 2016; 1(1):173–174.

76. Wang H, Zhang S, Xiao G, Liu B. Complete mtDNA of the Meretrix lamarcki (Bivalvia: Veneridae) and molecular identification of suspected M. lamarcki based on the whole mitochondrial genome. Mar Genomics. 2011; 4(4):263–71. https://doi.org/10.1016/j.margen.2011.06.006 PMID: 22118638

77. Wu X, Xiao S, Li X, Li L, Shi W, Yu Z. Evolution of the rRNA gene family in mitochondrial genomes of five Meretrix clams (Bivalvia, Veneridae). Gene. 2014; 533(1):439–46. https://doi.org/10.1016/j.gene.2013.09.077 PMID: 24084366

78. He CB, Wang J, Gao XG, Song WT, Li HJ, Li YF, et al. The complete mitochondrial genome of the hard clam Meretrix meretrix. Mol Biol Rep. 2011; 38(5):340–9.

79. Ren J, Shen X, Sun M, Jiang F, Yu Y, Chi Z, et al. The complete mitochondrial genome of the clam Meretrix petechialis (Mollusca: Bivalvia). Mitochondrial DNA. 2009; 20(4):78–87. https://doi.org/10.1080/1940173090266425 PMID: 19479624

80. Xu X, Wu X, Yu Z. The mitogenome of Paphia euglypta (Bivalvia: Veneridae) and comparative mitogenomic analyses of three venerids. Genome. 2010; 53(12):1041–52. https://doi.org/10.1139/G10-096 PMID: 21164537

81. Hwang JY, Han GG, Park JY, Kim EM, An CM, Kang JH, et al. Complete sequence and polymorphisms of female Ruditapes philippinarum (Mollusca: Bivalvia) mitochondria genome. Mitochondrial DNA A DNA Mapp Seq Anal. 2016; 27(5):3462–3. https://doi.org/10.3109/19401736.2015.1066348 PMID: 26248000

82. Bao X, He C, Gao X, Li Y, Gao L, Jiang B, et al. The complete mitochondrial genome of Saxidomus purpuratus (Veneroida: Veneridae). Mitochondrial DNA A DNA Mapp Seq Anal. 2016; 27(5):3648–9. https://doi.org/10.3109/19401736.2015.1079595

83. Imanishi Y, Tanaka M, Fujiiwara M. Complete mitochondrial genome sequence of Japanese cockle Fulvia mutica (Cardiidae). Fish Sci. 2013; 79(6):949–57.

84. Gan HM, Gan HY, Tan MH, Penny SS, Willan RC, Austin CM. The complete mitogenome of the giant clam Tridacna squamosa (Heterodonta: Bivalvia: Tridacnidae). Mitochondrial DNA A DNA Mapp Seq Anal. 2016; 27(5):3220–1. https://doi.org/10.3109/19401736.2015.1007355 PMID: 25648928

85. Liu H, Cai S, Zhang H, Vrijenhoek RC. Complete mitochondrial genome of hydrothermal vent clam Calyptogena magnifica. Mitochondrial DNA A DNA Mapp Seq Anal. 2016; 27(6):3433–3435. https://doi.org/10.3109/19401736.2016.1089488 PMID: 26462964

86. Glöckner G, Heinze I, Platzer M, Held C, Abele D. The mitochondrial genome of Artica islandica; Phylogeny and variation. PLoS One. 2013; 8(12):e82857. https://doi.org/10.1371/journal.pone.0082857 PMID: 24312674

87. Yuan Y, Kong L, Li Q. Mitogenome evidence for the existence of cryptic species in Coelomactra anti-quata. Genes Genomics. 2013; 35(6):693–701.

88. Gan HM, Tan MH, Thai BT, Austin CM. The complete mitogenome of the marine bivalve Lutraria rhynchaena Jonas 1844 (Heterodonta: Bivalvia: Mactridae). Mitochondrial DNA A DNA Mapp Seq Anal. 2016; 27(1):335–6. https://doi.org/10.3109/19401736.2014.892104 PMID: 24617474

89. Shen X, Meng XP, Chu KH, Zhao NN, Tian M, Liang M, et al. Comparative mitogenomic analysis reveals cryptic species: A case study in Mactridae (Mollusca: Bivalvia). Comp Biochem Physiol Part D Genomics Proteomics. 2014; 12:1–9. https://doi.org/10.1016/j.cbd.2014.08.002 PMID: 25247670
|  | Val | Trp | Gly | Met | Ser1 |
|---|-----|-----|-----|-----|------|
|  | Pro | Gln | Cys | Ala | Phe  |
|  | His | Ser2| Glu | Ile | Lys  |
|  | Tyr | Thr | Leu1| Asp | Leu2 |
|  | Asn | Arg |     |     |      |
| Val | Trp | Gly | Met | Ser1 |
|-----|-----|-----|-----|------|
| Pro | Gln | Cys | Ala | Phe  |
| His | Ser2| Glu | Ile | Lys  |
| Tyr | Thr | Leu1| Asp | Leu2 |
| Asn | Arg |     |     |      |