Additional taxonomic coverage of the DUI (Doubly Uniparental Inheritance) in bivalves: evidence of sex-linked heteroplasmy in the razor clam *Solen marginatus* Pulteney, 1799, but not in the lagoon cockle *Cerastoderma glaucum* (Bruguière, 1789)

Running Title: Evidence of DUI in the razor clam *Solen marginatus* Pulteney, 1799

Livia Lucentini [1*], Federico Plazzi[2*], Andrea Augusto Sfriso[3], Claudia Pizzirani[1], Adriano Sfriso[4] and Stefania Chiesa[5,6#]

[1]University of Perugia, Department of Chemistry, Biology and Biotechnologies, Via Pascoli, 1, 06123 Perugia, Italy

[2]University of Bologna, Department of Biological, Geological and Environmental Sciences, via Selmi 3, 40126 Bologna, Italy

[3]University of Ferrara, Department of Chemical and Pharmaceuticals Sciences, Via Fossato di Mortara 17, 44121 Ferrara, Italy

[4]Ca' Foscari University of Venice, Department of Environmental Sciences, Informatics and Statistics, Via Torino 155, 30170 Mestre Venice, Italy

[5]Ca' Foscari University of Venice, Department of Molecular Sciences and Nanosystems, Via Torino 155, 30170 Mestre Venice, Italy

[6]ISPRA, Institute for Environmental Protection and Research, Via Vitaliano Brancati 48, 00144, Rome, Italy

*** Livia Lucentini and Federico Plazzi should be considered joint first author**

#Corresponding Author: **Stefania Chiesa. E-mail:** ***stefania.chiesa@unive.it; stefania.chiesa@isprambiente.it***

Keywords: DUI (Doubly Uniparental Inheritance), bivalves, sex-linked heteroplasmy, *Solen marginatus*, *Cerastoderma glaucum*

Abstract

In animals, Doubly Uniparental Inheritance (DUI) is a major exception to the common Strict Maternal Inheritance of mitochondria. To date, DUI has only been found in many bivalve species, but its distribution is still unclear. Given the great species richness of the class, much effort is needed to further investigate the occurrence of DUI in unsampled species. A compelling evidence of DUI is generally the presence of a sex-linked heteroplasmy, where two divergent mitochondrial lineages are found: one is isolated from the male germline, the other one is isolated from the female germline and, normally, from the soma of both sexes. In the present study, we investigated the sex-linked heteroplasmy in the razor clam *Solen marginatus* Pulteney, 1799 and in the lagoon cockle *Cerastoderma glaucum* (Bruguière, 1789) using two mitochondrial markers (*cox1* and *rrnL*). We found evidence of DUI in the species *S. marginatus*, with a divergence up to 21% for the *rrnL* gene, but not in *C. glaucum*. Moreover, our phylogenetic reconstruction includes all the available data for heterodont species with sex-linked heteroplasmy and suggests multiple origins of DUI in this subclass, as well as the presence of DUI in other species of the genus *Solen*.

1. Introduction

Mitochondria, beside being the well-known cell compartments where the TCA cycle and oxidative phosphorylation take place, play different, yet pivotal roles in many eukaryotic cellular processes, spanning from apoptosis to aging, from cell differentiation to fertilization, from signaling to nuclear gene regulation through ncRNAs (see as examples Spikings, Alderson, & St. John, 2007; Scheffler, 2008; Van Blerkom, 2011; López-Otín, Blasco, Partridge, Serrano, & Kroemer, 2013; Chandel, 2014; Babayev et al., 2016; Bottje et al., 2017; Pozzi, Plazzi, Milani, Ghiselli, & Passamonti, 2017; Prieto & Torres, 2017; Riggs et al., 2018; Hill, 2019). As a keynote feature of these multifaceted organelles, mitochondrial inheritance was also shown to involve different mechanisms. While the Strict Maternal Inheritance (SMI) of mitochondria probably represents the rule in animals (Birky, 2001), a mechanism alternative to SMI is the Doubly Uniparental Inheritance (DUI), which has been reported in many species of bivalve molluscs (Breton, Doucet-Beaupré, Stewart, Hoeh, & Blier, 2007; Passamonti & Ghiselli, 2009; Zouros, 2013; Gusman, Lecomte, Stewart, Passamonti, & Breton, 2016; Zouros & Rodakis, 2019).

In DUI species, both parental mitochondrial lineages pass to the zygote: a paternal, male type (M), which occurs in sperm, and a maternal, female type (F), occurring in oocytes. Thus, zygotes are heteroplasmic: in embryos developing to females, M-type mitochondria are dispersed and disrupted; in embryos developing to males, they are clustered together towards the primordial germline (Cao, Kenchington, & Zouros, 2004; Milani, Ghiselli, & Passamonti, 2012). Among adult specimens, females are typically homoplasmic for the F lineage, whereas males maintain heteroplasmy: the germline is dominated by M-type mitochondria and somatic cells show different proportions of M-type and (often dominating) F-type mitochondria (Garrido-Ramos, Stewart, Sutherland, & Zouros, 1998; Chakrabarti et al., 2007; Kyriakou, Zouros, & Rodakis, 2010; Batista, Lallias, Taris, Guerdes-Pinto, & Beaumont, 2011; Ghiselli, Milani, & Passamonti, 2011; Obata, Sano, & Komaru, 2011, Brannock, Roberts, & Hilbish, 2013).

DUI is generally detected using sex-linked heteroplasmy as a proxy (for example, Passamonti & Scali, 2001; Theologidis, Fodelianakis, Gaspar, & Zouros, 2008; Boyle & Etter, 2013; Plazzi, Cassano, & Passamonti, 2015; Plazzi, 2015; Vargas, Pérez, Toro, & Astorga, 2015; Dégletagne, Abele, & Held, 2016; Gusman et al., 2016). Over one hundred species have been currently reported to show this peculiar phenomenon; however, it is possible that for many other bivalves, if not molluscans, DUI species are still to be discovered (Gusman et al., 2016). Furthermore, evidence is growing towards a multiple-origin scenario: the scattered distribution of DUI within bivalve species (Gusman et al., 2016; Plazzi & Passamonti, 2019), as well as significant molecular differences

among different DUI systems (Zouros, 2013; Plazzi, 2015; Plazzi & Passamonti, 2019; and reference therein), are consistent with the idea that DUI evolved multiple times in different groups of bivalves (Milani, Ghiselli, Guerra, Breton, & Passamonti, 2013; Milani, Ghiselli, & Passamonti, 2016; Zouros, 2013; Plazzi & Passamonti, 2019). However, with more than 3,500 extant and extinct genera (Millard, 2001), the diversity of bivalves overwhelms the availability of empirical data on sex-linked heteroplasmy, and the current knowledge of DUI distribution within the class is still insufficient to draw conclusions. The DUI phenomenon is apparently restricted to bivalves, although only a limited research has been carried out among gastropods (Parakatselaki, Saavedra, & Ladoukakis, 2016; Gusman, Azuelos, & Breton, 2017); however, DUI is probably widespread within bivalves (Gusman et al., 2016).

The razor clam *Solen marginatus* Pulteney, 1799, order Solenoidea, family Solenidae, has a broad geographic distribution extending from Norway to the Mediterranean Sea, North Africa, the Southeast and Western coasts of England (Darriba Couñago & Fernandez Tajes, 2011; Ayache et al., 2016). The so-called "razor clam" is an infaunal bivalve (Semeraro et al., 2016) living in soft sea beds and present in the deepest sediments, generally up to 20–50 cm below the surface (Macedo, Macedo & Borges, 1999). Despite its economic interest, especially in the Southern Mediterranean area (Ayache et al., 2016), limited data are available on its biology, ecology and bioaccumulation profiles (see Sfriso et al., 2018 and references within). Moreover, few data are available on molecular markers (Fernandez Tajes & Mendez, 2007; Francisco Candeira, Gonzalez Tizon, Varela, & Martinez Lage, 2007), genetic diversity of its populations (Semeraro et al., 2016; Hmida, Fassatoui, Ayed, Ayache, & Romdhane, 2012), gene structures and arrangements (Gonzalez Romero, Ausio, Mendez, & Eirin-Lopez, 2009; Mesías Gansbiller, et al., 2012) and cytogenetics (Fernandez Tajes, Gonzalez-Tizon, Martinez-Lage, & Mendez, 2003). To date, sex-linked heteroplasmy has been suggested for the congeneric, Indo-Pacific species *Solen grandis* Dunker, 1862; however, only three sequences have been released in GenBank (Accession Numbers AB064983, AB064984 and AB064985) and they are still unpublished.

The lagoon cockle *Cerastoderma glaucum* (Bruguière, 1789), order Veneroida, family Cardiidae, is also a benthic bivalve occurring in surface soft bottom sediments (Karray et al., 2015) or inside the algal biomass. The species is distributed from the Atlantic coast of Norway to the Caspian Sea (Brock, 1979) and in Mediterranean coastal lagoons. Compared to the closely related common cockle *Cerastoderma edule* (Linnaeus, 1758), *C. glaucum* prefers semi-enclosed, shallow and nontidal lagoons (Brock, 1979) or choked areas. *C. glaucum* has been used in different environments as a bioindicator species of environmental contamination (see Karray et al., 2015; Sfriso et al., 2018 and citations within). Moreover, since it represents an interesting model of a

benthic organism with a fragmented distribution, genetic diversity of its populations have been extensively investigated, by traditional Sanger sequencing of ITS and mtDNA sequences (Nikula & Vainola, 2003; Freire, Arias, Mendez, & Insua, 2010; Ladhar Chaabouni, Hamza Chaffai, Hardivillier, Chenais, & Denis, 2010; Tarnowska, Chenuil, Nikula, Feral, & Wolowicz, 2010; Tarnowska et al., 2012; Vergara Chen, Gonzalez Wanguemert, Marcos, Perez Ruzafa, 2013; Sromek et al., 2016) by allozymic (Mariani, Ketmaier, & de Matthaeis, 2002; Sromek et al., 2016 and references within), and by microsatellite markers (Sromek et al., 2016). More recently, population genomics has been investigated by NGS-based RAD markers (Sromek, Forcioli, Lasota, Furla, & Wolowicz, 2019). Data have also been collected on its karyotype (Thiriot Quievreux & Wolowicz, 1996).

Despite the occurrence of genetic and genomic data available for lagoon cockle, to our knowledge there are no published papers regarding the possible occurrence of DUI phenomenon; up to now, there is no evidence of DUI from cardiids (Gusman et al., 2016).

Therefore, in the present study *S. marginatus* and *C. glaucum* have been selected as target species for a new study on the DUI phenomenon in bivalves.

2. Materials and methods

2.1 Sample collection and tissue preparation

Mature specimens of *S. marginatus* (3 females and 6 males) and *C. glaucum* (5 females and 7 males) were collected in Summer 2017 in the Venice Lagoon (Northern Adriatic Sea) in two stations facing the west side of the Malamocco-Marghera Canal. Razor clams were collected at Verto Sud (sexagesimal coordinates: VS - 45.382987°/12.254941°); lagoon cockles were collected at Torretta Bianca (TB - 45.393239°/12.264009°). The individuals were sampled by hand and transported to the laboratory in an aerated basin with seawater.

Sample dissections were carried out within 24 hours following protocols already tested for previous DUI analyses (Gusman et al., 2016). In detail, each individual was dissected, and the gonadal content was analyzed under a light microscope (100×) to identify the occurrence of eggs or sperm. Unambiguously sexed individuals were then selected for genetic analyses.

Somatic tissues (mantle and foot) and gonadal content were carefully separated for each specimen and preserved in absolute ethanol at −20°C for DNA extractions (see next section).

2.2 DNA extraction and purification

Total DNA was isolated individually from both the mantle/foot and the gonadal content, using the DNeasy Blood & Tissue kit (Qiagen, Germantown, MD, USA), following the manufacturer's instructions.

The quality and quantity of DNA were assessed by electrophoresis on 1% agarose gels and spectrophotometric analysis.

2.3 Mitochondrial marker amplification and sequencing

Amplification of two different mitochondrial gene regions was carried out to investigate the occurrence of intraspecific F and M haplotypes (Gusman et al., 2016): cytochrome *c* oxidase subunit 1 (*cox1*) and 16S (*rrnL*).

Amplifications were performed by using universal primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') for the *cox1* fragment (Folmer, Black, Hoeh, Lutz, & Vrijenhoek 1994) and by more specific primers 16 Sar-ALT (5'-CGCCTGTTTATCAAAAACATSG-3') and 16 Sbr-ALT (5'-CCGGTCTGAACTCAGATCACGT-3') designed for bivalves for *rrnL* fragment (Mikkelsen, Bieler, Kappner, & Rawlings, 2006).

The amplification reactions were performed in a total volume of 25 µl, including 15.2 µl of sterilized distilled water, 5 µl of 5× colorless GoTaq reaction buffer (7.5 mM $MgCl_2$), 1 µl of each 10 µM primer, 0.5 µl of dNTP mixture, 0.3 µl Go Taq G2 (Promega, Madison, WI, USA), and 2 µl of DNA.

For the *cox1* gene fragment, PCR was carried out for 10 min at denaturation temperature of 95°C, followed by thirty-five cycles of 30 sec at 95 °C, 40 sec at 47 °C and 60 sec at 72 °C, followed by a final extension of 10 min at 72 °C.

For the *rrnL* gene fragment, PCR amplifications were performed by denaturing DNA for 2 min at 95 °C, followed by thirty-five cycles of 30 sec at 94 °C, 40 sec at 52 °C and 1 min at 72 °C, and a final extension of 10 min at 72 °C.

The amplification products were checked by electrophoresis in TBE buffer and in 2% agarose gel containing SafeView Nucleic Acid Stain (NBS Biologicals, Huntingdon, Cambridgeshire, UK) and visualized under UV light: products were approximately 750 and 510 bp long for *cox1* and *rrnL* amplicons, respectively.

Amplicons were then purified with EXOSAP-IT (Thermo Fisher Scientific, Affymetrix Inc., Santa Clara, CA 95051, USA) following the standard protocol and Sanger sequencing was conducted by Eurofins Genomics Germany GmbH.

178 ***2.4 Phylogenetic analysis***

179 Electropherograms were handled and edited using MEGA X (Kumar, Stecher, Li, Knyaz, &
180 Tamura 2018). The taxonomic identity of the obtained sequences was evaluated using BLAST+
181 (Camacho et al., 2009). Uncorrected p-distances within and between female and male samples were
182 computed using MEGA X.

183 The complete sequences of *cox1* and *rrnL* genes were downloaded from complete mitochondrial
184 genomes available in GenBank from the bivalvian clade Imparidentia *sensu* Combosch et al.
185 (2017), using the anomalodesmatan *Lyonsia norwegica* (Gmelin, 1791) (GenBank Accession
186 Number NC_034302) as an outgroup. *Ruditapes philippinarum* (Adams & Reeve, 1850) and
187 *Meretrix lamarckii* Deshayes, 1853 are DUI species whose complete mtDNA is available in
188 GenBank and were therefore included in the analysis.

189 Moreover, all the (currently) known *cox1* and *rrnL* sequences related to a sex-linked heteroplasmy
190 among Imparidentia were added to the dataset, following the list compiled by Gusman et al. (2016):
191 *Cyclina sinensis* (Gmelin, 1791), *Donax trunculus* Linnaeus, 1758, *Donax cuneatus* Linnaeus,
192 1758, *Donax faba* Gmelin, 1791, *Pseudocardium sachalinensis* (Schrenck, 1862), and
193 *Scrobicularia plana* (da Costa, 1778). The putative M-type sequence of *Solen grandis* Dunker,
194 1862 was released in GenBank under the Accession Number AB064985 (Gusman et al., 2016);
195 however, it has never been published and it is consistently placed outside the family Solenidae in all
196 preliminary analyses. As a possible contamination, we decided to exclude this sequence from our
197 dataset, along with the putative, unpublished F-type sequence extracted from the female gonad
198 (GenBank Accession Number AB064983), retaining only somatic sequences of *S. grandis*. All
199 sequences obtained for this study and downloaded from GenBank are listed in Supporting
200 Information Table S1.

201 Sequences were aligned with the T-Coffee algorithm (Notredame, Higgins & Heringa, 2000), using
202 the packages PSI-BLAST (Altschul et al., 1997), Muscle (Edgar, 2004), ProbconsRNA (Do,
203 Mahabhashyam, Brudno & Batzoglou, 2005), RNAplfold (Lorenz et al., 2011), and MAFFT (Katoh
204 & Standley 2013); the option Psicoffee was set for *cox1* amino acids and the MR-Coffee mode was
205 set for *rrnL* nucleotides. Aligned amino acids were retro translated into nucleotides using a custom-
206 tailored R script; sites with low or noisy phylogenetic signal were masked using
207 masking_package_v1.1 (Plazzi, Puccio, & Passamonti, 2016; available at
208 https://github.com/mozoo/masking_package), retaining sites selected as phylogenetically useful by
209 at least four of the five tool Aliscore 2.0 (Misof & Misof, 2009), BMGE 1.1 (Criscuolo & Gribaldo,
210 2010), Gblocks 0.91b (Castresana, 2000), Noisy (Dress et al., 2008), and Zorro (Wu, Chatterji, &

Eisen, 2012). The *cox1* alignment was further subdivided into the three codon positions using a custom-tailored Python script, obtaining four datasets: cox1_1, cox1_2, cox1_3, and *rrnL*.

We estimated the degree of saturation in our datasets using the substitution saturation test developed by Xia and colleagues (Xia & Lemey, 2009; Xia, Xie, Salemi, Chen, & Wang, 2003); moreover, we used the distmat application of EMBOSS 6.6.0 (Rice, Longden, & Bleasby, 2000) to compute pairwise (uncorrected) p-distances and plotted them over pairwise ML distances computed with RAxML 8.2.12 (Stamatakis, 2014). Since the cox1_3 partition was detected to be highly saturated (Supporting Information Figure S1, Supporting Information Table S2), it was excluded from subsequent analyses.

The three remaining datasets were concatenated into the final dataset; the phylogenetic inference was carried out using IQ-TREE 1.7-beta7 (Nguyen, Schmidt, von Haeseler, & Minh, 2015) with 1000 ultrafast bootstrap replicates (Hoang, Chernomor, von Haeseler, Minh, & Vinh, 2018). Substitution models were selected using ModelFinder (Kalyaanamoorthy, Minh, Wong, von Haeseler, & Jermiin, 2017) and the best partitioning scheme was selected with the greedy strategy implemented in ModelFinder (Chernomor, von Haeseler, & Minh, 2016; Lanfear, Calcott, Ho, & Guindon, 2012). Nodes with an ultrafast bootstrap support value lower than 85 were collapsed with PhyloWidget (Jordan & Piel, 2008) and the phylogenetic tree was graphically edited with Dendroscope 3.6.3 (Huson & Scornavacca, 2012).

3. Results and Discussion

We obtained 13 sequences of *S. marginatus cox1* gene (2 from female germline, 1 from female soma, 5 from male germline, 5 from male soma), and 10 sequences of the *C. glaucum cox1* gene (4 from female soma, 3 from male germline, 3 from male soma). Most *cox1* sequences ranged from 592 to 644 bp in length; due to a poor electropherogram quality, the *S. marginatus* F8 and *C. glaucum* M7 somatic sequences were trimmed to 366 and 406 bp, respectively, and the *S. marginatus* sequences obtained from the male gonad were trimmed to 140-231 bp, with the exception of M7 (594 bp). All sequences were deposited in GenBank under the Accession Numbers MN630857-MN630869 for *S. marginatus* and MN613229-MN613238 for *C. glaucum* (see Supporting Information Table S1).

Conversely, 18 sequences of *S. marginatus rrnL* gene (3 from female germline, 3 from female soma, 6 from male germline, 6 from male soma) and 21 sequences of *C. glaucum rrnL* gene (2 from female germline, 5 from female soma, 7 from male germline, 7 from male soma) were produced, globally ranging from 419 to 469 bp. All sequences were deposited in GenBank under the

Accession Numbers MN603377-MN603394 for *S. marginatus* and MN602566-MN602586 for *C. glaucum* (see Supporting Information Table S1).

Variable positions of *cox1* and *rrnL* alignments are shown in Figure 1. Within-group (i.e., within-mitotype) uncorrected p-distances are generally low, ranging for nucleotides from 0.0004 for *S. marginatus* F *rrnL* to 0.0871 for *S. marginatus* M *cox1* and for amino acids from 0 for *C. glaucum* *cox1* to 0.0886 for *S. marginatus* M *cox1* (Table 1). However, while average uncorrected p-distance between mitotypes is comparably low for *C. glaucum* (up to 0.0071 for *cox1* nucleotides), it is two or three orders of magnitude higher for *S. marginatus* (up to 0.2122 for *rrnL*), which entails that average sequence similarity between F-type and M-type lineages is not higher than ~85% for *cox1* and ~80% for *rrnL* (Table 1). Therefore, there is no evidence for sex-linked heteroplasmy in *C. glaucum* *cox1* and *rrnL* genes, while we provide strong evidence of sex-linked heteroplasmy for *S. marginatus*. The only exception to this is the *cox1* sequence of the male specimen number 7 (see Figure 1): it has been extracted from the gonad, but it turned out to be a F-type sequence, most likely because of somatic tissue contaminating the germline.

The final dataset was comprised by 95 sequences and 614 sites: the phylogenetic tree is shown in Figure 2 and supports the same conclusion about sex-linked heteroplasmy. The family Cardiidae was retrieved as monophyletic with an ultrafast bootstrap (UF-Boot) support value of 100. The cluster *Fulvia mutica* (Reeve, 1844) + *Vasticardium flavum* (Linnaeus, 1758) is the sister group of remaining cardiids, which split into Tridacninae on one side, and *Acanthocardia* + *Cerastoderma* (UF-Boot support value = 100) on the other side. However, *C. glaucum* sequences were uniformly distributed and there were no strongly supported clusters with respect to sex or tissue.

Conversely, the family Solenidae was also recovered as monophyletic (UF-Boot support value = 100), but the cluster of *S. marginatus* M-type sequences (i.e., sequences extracted from male germline) is strongly supported to be monophyletic (UF-Boot support value = 100) and the sister group of the remaining F-type sequences (i.e., sequences extracted either from male soma or from female tissues), which are also strongly supported (UF-Boot support value = 99). Notably, however, the cluster of F-type sequences is comprised by all included F-type sequences from the genus *Solen*: F-type sequences from *S. marginatus*, which were newly obtained for this study, are the sister group of a cluster with the topology *S. strictus* + *S. grandis* (UF-Boot support value = 100).

All this considered, we suggest the presence of the DUI phenomenon in the species *S. marginatus*. Contrastingly, there is no evidence supporting the same for *C. glaucum*. Actually, we did not find sex-linked heteroplasmy in the latter species, which would have strongly suggested the presence of DUI (as is the case for *S. marginatus*), but this cannot be taken as a definitive proof of the absence of this phenomenon.

As repeatedly observed (e.g., Theologidis et al., 2008; Passamonti & Plazzi, submitted) a sex-linked heteroplasmy might be present, but standard PCR-based methods may fail to detect it. If the two mitochondrial genomes are significantly divergent, the selected primer pair might amplify only either, typically the female one: therefore, recall that a minimal amount of contaminating somatic cells are always present in gonadal extracts, this would result in the amplification of the female genome in all the considered tissues. Notably, most *cox1* sequences obtained from sperm in *S. marginatus* were the shortest in the alignment because of the low quality of the electropherograms, which in turn is most probably due to a lower efficiency of the universal primers on the male allele. Conversely, if the divergence between the two genomes is very low (e.g., due to a young origin of DUI in this species, or due to a recent masculinization event; Stewart, Breton, Blier, & Hoeh, 2009; Zouros, 2013; and reference therein), two markers might be not enough to detect diagnostic substitutions. Thus, additional types of data (e.g., massive sequencing of amplicons) are required in order to completely dismiss the hypothesis of *C. glaucum* to be a DUI species.

Conversely, the detection of sex-linked heteroplasmy in *S. marginatus* is a strong clue for the existence of DUI in this species; moreover, the phylogenetic reconstruction suggests that DUI arose before the separation of the three species included in our dataset. However, as aforementioned the only available putative M-type sequence from *S. grandis* is possibly contaminated, thus additional samples of the male germline from other species of the genus *Solen* are mandatory to confirm the present finding.

Moreover, this sex-specific pattern is neither the rule nor an exception in our phylogenetic tree. Given our relatively restricted dataset, the present phylogenetic reconstruction of Imparidentia mitochondrial lineages has definitely to be taken as preliminary: there is sure enough evidence of little saturation in our datasets (Supporting Information Figure S1, Supporting Information Table S2) and many UF-Boot support values ranged from 60 to 95. Nonetheless, the pattern of sex-linked heteroplasmy in the family Veneridae is completely different: for each species (*R. philippinarum*, *M. lamarckii*, and *C. sinensis*) the F- and the M- type cluster together. In this family, three independent origins of the DUI phenomenon can be claimed, recalling that masculinization, which is common among mytilids and would reset the divergence between the two lineages (Zouros, 2013), has never been directly observed for venerids (Plazzi & Passamonti, 2019; and reference therein).

Within the family Mactridae, the DUI species *P. sachalinensis* shows a species-specific pattern similar to that shown by Veneridae, but in this case a single species with sex-linked heteroplasmy is currently known. The finding of more mactrid species with a sex-linked heteroplasmy will allow to test for the consistency of this pattern. The situation is more difficult to disentangle for the

superfamily Tellinoidea. The relationships between the different species are scarcely supported and the present reconstruction would be compatible with both a sex-specific and a species-specific pattern. Very long branches, like those leading to *D. faba* or *S. plana* male sequences, may hamper the phylogenetic inference.

As a second conclusion, the first Imparidentia phylogenetic tree spanning over all the available sex-linked sequences, as well as over many complete mitochondrial sequences, is presented in this study. It supports the hypothesis of multiple DUI origins (Figure 2), which has become more than a speculation in recent years (Milani et al., 2013, 2016; Plazzi & Passamonti, 2019; Zouros, 2013). More information is needed to further clarify the distribution and the patterns of DUI evolution among Imparidentia, and the complete mitochondrial genomes of DUI species are mandatory in order to obtain robust phylogenetic results. Finally, we report strong evidence for the existence of a DUI system in the genus *Solen* (corroborating a previous claim by Gusman et al., 2016), and specifically for the European species *S. marginatus*, which deserves further characterization *per se*.

4. Conclusions

The present study focused on the taxonomic coverage of the DUI (Doubly Uniparental Inheritance) in bivalves. In particular, the occurrence of DUI has been investigated in two species, namely razor clam *S. marginatus* and lagoon cockle *C. glaucum*.

Cytochrome *c* oxidase subunit 1 (*cox1*) and 16S (*rrnL*) mitochondrial regions were selected to test the presence of intraspecific F and M haplotypes in these two species.

Results herein collected suggested the occurrence of DUI phenomenon in the razor clam *S. marginatus*, with a divergence up to the 21% for the *rrnL* gene, but not in the lagoon cockle *C. glaucum*. Moreover, our phylogenetic reconstruction suggests multiple origins of DUI in the heterodont subclass, as well as the presence of DUI in other species of the genus *Solen*, which should be furtherly investigated.

Acknowledgements

The Authors would like to thank the two referees who revised the manuscript for their useful suggestions to improve the paper.

5. References

Altschul, S., Madden, T., Schaffer, A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, 25, 3389–3402.

Ayache, N., Hmida, L., Cardoso, J. F. M. F., Haouas, Z., Da Costa, F., & Romdhane, M. S. (2016). Reproductive cycle of the razor clam *Solen marginatus* (Pulteney, 1799) in the Southern Mediterranean Sea (Gulf of Gabes, South Tunisia). *Journal of Shellfish Research*, 35, 289–397.

Babayev, E., Wang, T., Szigeti-Buck, K., Lowther, K., Taylor, H. S., Horvath, T., & Seli, E. (2016). Reproductive aging is associated with changes in oocyte: mitochondrial dynamics, function, and mtDNA quantity. *Maturitas*, 93, 121–130.

Batista, F. M., Lallias, D., Taris, N., Guerdes-Pinto, H., & Beaumont, A. R. (2011). Relative quantification of the M and F mitochondrial DNA types in the blue mussel *Mytilus edulis* by real-time PCR. *Journal of Molluscan Studies*, 77, 24–29.

Bottje, W. G., Khatri, B., Shouse, S. A., Seo, D., Mallmann, B., Orlowski, S. K., Pan, J., Kong, S., Owens, C. M., Anthony, N. B., Kim, J. K., & Kong, B. C. (2017). Identification and Differential Abundance of Mitochondrial Genome Encoding Small RNAs (mitosRNA) in Breast Muscles of Modern Broilers and Unselected Chicken Breed. *Frontiers in Physiology*, 8, 816.

Boyle, E. E., & Etter, R. J. (2013). Heteroplasmy in a deep-sea protobranch bivalve suggests an ancient origin of doubly uniparental inheritance of mitochondria in Bivalvia. *Marine Biology*, 160, 413–422.

Brannock, P. M., Roberts, M. A., & Hilbish, T. J. (2013). Ubiquitous heteroplasmy in *Mytilus* spp. resulting from disruption in doubly uniparental inheritance regulation. *Marine Ecology Progress Series*, 480, 131–143.

Breton, S., Doucet-Beaupré, H., Stewart, D. T., Hoeh, W. R., & Blier, P. U. (2007). The unusual system of doubly uniparental inheritance of mtDNA: isn't one enough? *Trends in Genetics*, 23, 465–474.

Brock, V. (1979). Habitat selection of two congeneric bivalves, *Cardium edule* and *C. glaucum* in sympatric and allopatric populations. *Marine Biology*, 54, 149–156.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: architecture and applications. *BMC Bioinformatics*, 10, 421.

Cao, L., Kenchington, E., & Zouros, E. (2004). Differential Segregation Patterns of Sperm Mitochondria in Embryos of the Blue Mussel (*Mytilus edulis*). *Genetics*, 166, 883–894.

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, 17, 540–552.

376 Chakrabarti, R., Walker, J. M., Chapman, E. G., Shepardson, S. P., Trdan, R. J., Curole, J. P.,
377 Watters, G. T., Stewart, D. T., Vijayaraghavan, S., & Hoeh, W. R. (2007). Reproductive Function
378 for a C-terminus Extended, Male-Transmitted Cytochrome *c* Oxidase Subunit II Protein Expressed
379 in Both Spermatozoa and Eggs. *FEBS Letters*, 581, 5213–5219.
380 Chandel, N. S. (2014). Mitochondria as signaling organelles. *BMC Biology*, 12, 34.
381 Chernomor, O., von Haeseler, A., & Minh, B. Q. (2016). Terrace aware data structure for
382 phylogenomic inference from supermatrices. *Systematic Biology*, 65, 997–1008.
383 Combosch, D. J., Collins, T. M., Glover, E. A., Graf, D. L., Harper, E. M., Healy, J. M., Kawauchi,
384 G. Y., Lemer, S., McIntyre, E., Strong, E. E., Taylor, J. D., Zardus, J. D., Mikkelsen, P. M., Giribet,
385 G., & Bieler, R. (2017). A family-level Tree of Life for bivalves based on a Sanger-sequencing
386 approach. *Molecular Phylogenetics and Evolution*, 107, 191–208.
387 Criscuolo, A., & Gribaldo, S. (2010). BMGE (Block Mapping and Gathering with Entropy):
388 selection of phylogenetic informative regions from multiple sequence alignments. *BMC*
389 *Evolutionary Biology*, 10, 210.
390 Darriba Couñago, S., & Fernandez Tajes, J. (2011). Systematics and distribution. In: A. Guerra, C.
391 L. Seijo, M. Gaspar & F. Da Costa (Eds.), *Razor clams: biology, aquaculture and fisheries* (pp. 65–
392 87). A Coruna, Spain: Xunta de Galicia: Conselleria do Mar.
393 Dégletagne, C., Abele, D., & Held, C. (2016). A distinct mitochondrial genome with DUI-like
394 inheritance in the ocean quahog *Arctica islandica*. *Molecular Biology and Evolution*, 33, 375–383.
395 Do, C. B., Mahabhashyam, M. S. P., Brudno, M., & Batzoglou, S. (2005). PROBCONS:
396 Probabilistic Consistency-based Multiple Sequence Alignment. *Genome Research*, 15, 330–340.
397 Dress, A. W. M., Flamm, C., Fritzsch, G., Grünewald, S., Kruspe, M., Prohaska, S. J., & Stadler, P.
398 F. (2008). Noisy: Identification of problematic columns in multiple sequence alignments.
399 *Algorithms for Molecular Biology*, 3, 7.
400 Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high
401 throughput. *Nucleic Acids Research*, 32, 1792–1797.
402 Fernandez Tajes, J, Gonzalez-Tizon, A, Martinez-Lage, A, & Mendez, J. (2003). Cytogenetics of
403 the razor clam *Solen marginatus* (Mollusca: Bivalvia: Solenidae). *Cytogenetic and Genome*
404 *Research*, 101, 43-46.
405 Fernandez Tajes, J, & Mendez, J. (2007). Identification of the razor clam species *Ensis arcuatus*, *E.*
406 *siliqua*, *E. directus*, *E. macha*, and *Solen marginatus* using PCR-RFLP analysis of the 5S rDNA
407 region. *Journal of Agricultural and Food Chemistry*, 55, 7278-7282.

Folmer O., Black M., Hoeh W., Lutz R., & Vrijenhoek R. (1994). DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I form diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.

Francisco Candeira, M., Gonzalez Tizon, A., Varela, M. A., & Martinez Lage, A. (2007). Development of microsatellite markers in the razor clam *Solen marginatus* (Bivalvia: Solenidae). *Journal of the Marine Biological Association of the United Kingdom*, 87, 977-978.

Freire, R., Arias, A., Mendez, J., & Insua, A. (2010). Sequence variation of the internal transcribed spacer *(ITS*) region of ribosomal DNA in *Cerastoderma* species (Bivalvia: Cardiidae). *Journal of Molluscan Studies*, 76, 77-86.

Garrido-Ramos, M. A., Stewart, D. T., Sutherland, B. W., & Zouros, E. (1998). The distribution of male-transmitted and female-transmitted mitochondrial DNA types in somatic tissues of blue mussels: Implications for the operation of doubly uniparental inheritance of mitochondrial DNA. *Genome*, 41, 818–824.

Ghiselli, F., Milani. L., & Passamonti, M. (2011). Strict sex-specific mtDNA segregation in the germ line of the DUI species *Venerupis philippinarum* (Bivalvia: Veneridae). *Molecular Biology and Evolution*, 28, 949–961.

Gonzalez Romero, R., Ausio, J., Mendez, J., & Eirin-Lopez, J. M. (2009). Histone genes of the razor clam *Solen marginatus* unveil new aspects of linker histone evolution in protostomes. *Genome*, 52, 597-607.

Gusman, A., Azuelos, C., & Breton, S. (2017). No evidence of sex-linked heteroplasmy or doubly-uniparental inheritance of mtDNA in five gastropod species. *Journal of Molluscan Studies*, 83, 119–122.

Gusman, A., Lecomte, S., Stewart, D. T., Passamonti, M., & Breton, S. (2016). Pursuing the quest for better understanding the taxonomic distribution of the system of doubly uniparental inheritance of mtDNA. *PeerJ*, 4, e2760.

Hill, G. E. (2019). *Mitonuclear Ecology*. Oxford: Oxford University Press.

Hmida, L., Fassatoui, C., Ayed, D., Ayache, N., & Romdhane, M. S., 2012. Genetic characterization of the razor clam *Solen marginatus* (Mollusca: Bivalvia: Solenidae) in Tunisian coasts based on isozyme markers. *Biochemical Systematics and Ecology*, 40, 146-155.

Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, 35, 518–522.

Huson, D. H., & Scornavacca, C. (2012). Dendroscope 3: An interactive tool for rooted phylogenetic trees and networks. *Systematic Biology*, 61, 1061–1067.

441 Jordan, G. E., & Piel, W. H. (2008). PhyloWidget: web-based visualizations for the tree of life
442 *Bioinformatics*, 24, 1641–1642.
443 Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., & Jermiin, L. S. (2017).
444 ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates, *Nature Methods*, 14,587–
445 589.
446 Karray, S., Tastard, E., Moreau, B., Delahaut, L., Geffard, A., Guillon, E., Denis, F., Hamza
447 Chaffai, A., Chénais, B., & Marchand, J. (2015). Transcriptional response of stress regulated genes
448 to industrial effluent exposure in the cockle *Cerastoderma glaucum*. *Environmental Science and*
449 *Pollution Research*, 22, 17303–17316.
450 Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7:
451 improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772–780.
452 Kumar, S., Stecher G., Tamura K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis
453 Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33: 1870-1874
454 Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular
455 Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35,
456 1547–1549.
457 Kyriakou, E., Zouros, E., & Rodakis, G. C. (2010). The atypical presence of the paternal
458 mitochondrial DNA in somatic tissues of male and female individuals of the blue mussel species
459 *Mytilus galloprovincialis*. *BMC Research Notes*, 3, 222.
460 Ladhar Chaabouni, R., Hamza Chaffai, A., Hardivillier, Y., Chenais, B., & Denis, F. (2010). A pilot
461 study of genetic differentiation between two phenotypes of a Mediterranean population of the
462 bivalve *Cerastoderma glaucum* and genetic discrimination with other *Cerastoderma glaucum* and
463 *Cerastoderma edule* populations outside the Mediterranean. *Marine Ecology-An Evolutionary*
464 *Perspective*, 31, 355-363.
465 Lanfear, R., Calcott, B., Ho S. Y., & Guindon, S. (2012). Partitionfinder: combined selection of
466 partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and*
467 *Evolution*, 29, 1695–1701.
468 López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The hallmarks of
469 aging. *Cell*, 153, 19994–21217.
470 Lorenz, R., Bernhart, S. H., Hoener zu Siederdissen, C., Tafer, H., Flamm, C., Stadler, P. F., &
471 Hofacker, I. L. (2011). ViennaRNA Package 2.0. *Algorithms for Molecular Biology*, 6, 26.
472 Macedo, M.C., Macedo, M.I., & Borges, J.P. (1999). *Conchas Marinhas de Portugal*. Lisbon, PT:
473 Verbo (497 pp).

Mariani, S., Ketmaier, V., & de Matthaeis, E. (2002). Genetic structuring and gene flow in *Cerastoderma glaucum* (Bivalvia: Cardiidae): evidence from allozyme variation at different geographic scales. *Marine Biology*, 140, 687-697.

Mesías Gansbiller, C., Sánchez, J. L., Pazos, A. J., Lozano, V., Martínez Escauriaza, R., & Pérez-Parallé, M. L. (2012). Conservation of Gbx genes from EHG homeobox in bivalve molluscs. *Molecular Phylogenetics and Evolution*, 63, 213-217.

Mikkelsen, P.M., Bieler, R., Kappner, I., & Rawlings, T. (2006). Phylogeny of Veneroidea (Mollusca: bivalvia) based on morphology and molecules. *Zoological Journal of the Linnean Society*, 148, 439–521.

Milani, L., Ghiselli, F., & Passamonti, M. (2012). Sex-Linked Mitochondrial Behavior During Early Embryo Development in *Ruditapes philippinarum* (Bivalvia Veneridae) a Species with the Doubly Uniparental Inheritance (DUI) of Mitochondria. J*ournal of Experimental Zoology - Part B Molecular and Developmental Evolution*, 318, 182–189.

Milani, L., Ghiselli, F., & Passamonti, M. (2016). Mitochondrial selfish elements and the evolution of biological novelties. *Current Zoology*, 62, 687–697.

Milani, L., Ghiselli, F., Guerra, D., Breton, S., & Passamonti, M. (2013). A Comparative Analysis of Mitochondrial ORFans: New Clues on Their Origin and Role in Species with Doubly Uniparental Inheritance of Mitochondria. *Genome Biology and Evolution*, 5, 1408–1434.

Millard, V. (2001). *Classification of Mollusca: A classification of worldwide Mollusca. 2nd edition*. South Africa.

Misof, B., & Misof, K. (2009). A Monte Carlo approach successfully identifies randomness in multiple sequence alignments: a more objective means of data exclusion. *Systematic Biology*, 58, 21–34.

Nguyen, L.-T., Schmidt, H. A., von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution*, 32, 268–274.

Nikula, R., & Vainola, R. (2003). Phylogeography of *Cerastoderma glaucum* (Bivalvia: Cardiidae) across Europe: a major break in the Eastern Mediterranean. *Marine Biology*, 143, 339-350.

Notredame, C., Higgins, D. G., & Heringa, J. (2000). T-Coffee: A novel method for fast and accurate multiple sequence alignment. *Journal of Molecular Biology*, 302, 205–217.

Obata, M., Sano, N., & Komaru, A. (2011). Different transcriptional ratios of male and female transmitted mitochondrial DNA and tissue-specific expression patterns in the blue mussel, *Mytilus galloprovincialis*. *Development Growth & Differentiation*, 53, 878–886.

507 Parakatselaki, M. E., Saavedra, C., & Ladoukakis, E. D. (2016). Searching for doubly uniparental
508 inheritance of mtDNA in the apple snail *Pomacea diffusa*. *Mitochondrial DNA Part A*, 27, 4000–
509 4002.
510 Passamonti, M., & Ghiselli, F. (2009). Doubly Uniparental Inheritance: two mitochondrial
511 genomes, one precious model for organelle DNA inheritance and evolution. *DNA and Cell Biology*,
512 28, 79–89.
513 Passamonti, M., & Plazzi, F. (submitted). DUI and beyond: the contribution of the Manila clam
514 *Ruditapes philippinarum*. *Journal of Zoological Systematics and Evolutionary Research*.
515 Passamonti, M., & Scali, V. (2001). Gender-associated mitochondrial DNA heteroplasmy in the
516 venerid clam *Tapes philippinarum* (Mollusca Bivalvia). *Current Genetics*, 39, 117–124.
517 Plazzi, F. (2015). The detection of sex-linked heteroplasmy in *Pseudocardium sachalinensis*
518 (Bivalvia: Mactridae) and its implications for the distribution of doubly uniparental inheritance of
519 mitochondrial DNA. *Journal of Zoological Systematics and Evolutionary Research*, 53, 205–210.
520 Plazzi, F., & Passamonti, M. (2019). Footprints of unconventional mitochondrial inheritance in
521 bivalve phylogeny: Signatures of positive selection on clades with doubly uniparental inheritance.
522 *Journal of Zoological Systematics and Evolutionary Research*, 57, 258–271.
523 Plazzi, F., Cassano, A., & Passamonti, M. (2015). The quest for doubly uniparental inheritance in
524 heterodont bivalves and its detection in *Meretrix lamarckii* (Veneridae: meretricinae). *Journal of*
525 *Zoological Systematics and Evolutionary Research*, 53, 87–94.
526 Plazzi, F., Puccio, G., & Passamonti, M. (2016). Comparative large-scale mitogenomics evidences
527 clade-specific evolutionary trends in mitochondrial DNAs of Bivalvia. *Genome Biology and*
528 *Evolution*, 8, 2544–2564.
529 Pozzi, A., Plazzi, F., Milani, L., Ghiselli, F., & Passamonti, M. (2017). SmithRNAs: could
530 mitochondria "bend" nuclear regulation? *Molecular Biology and Evolution*, 34, 1960–1973.
531 Prieto, J., & Torres, J. (2017). Mitochondrial Dynamics: In Cell Reprogramming as It Is in Cancer.
532 *Stem Cells International*, 2017, 8073721.
533 Rice, P., Longden, I., & Bleasby, A. (2000). EMBOSS: The European Molecular Biology Open
534 Software Suite. *Trends in Genetics*, 16, 276–277.
535 Riggs, C. L., Summers, A., Warren, D. E., Nilsson, G. E., Lefevre, S., Dowd, W. W., Milton, S.,
536 Podrabsky, J. E. (2018). Small Non-coding RNA Expression and Vertebrate Anoxia Tolerance.
537 *Frontiers in Genetics*, 9, 230.
538 Scheffler, I.E. (2008). *Mitochondria*. Hoboken: John Wiley & Sons.
539 Semeraro, A., Geba, K.M., Arias, A., Anadon, N., Garcıa-Vazquez, E., & Borrell, Y.J. (2016).
540 Genetic diversity and connectivity patterns of harvested and aquacultured molluscs in estuaries

from Asturias (northern Spain). Implications for management strategies. *Aquaculture Research*, 47, 2937–2950.

Sfriso, A.A., Chiesa, S., Sfriso, A., Buosi, A., Gobbo, L., Boscolo Gnolo, A. & Argese, E. (2018). Spatial distribution, bioaccumulation profiles and risk for consumption of edible bivalves: a comparison among razor clam, Manila clam and cockles in the Venice Lagoon. *Science of the Total Environment*, 643: 579-591.

Spikings, E. C., Alderson, J., & St. John, J. C. (2007). Regulated Mitochondrial DNA Replication During Oocyte Maturation Is Essential for Successful Porcine Embryonic Development. *Biology of Reproduction*, 76, 327–335.

Sromek, L., Forcioli, D., Lasota, R., Furla, P., Tarnowska-Marini, K., Wolowicz, M., & Chenuil, A. (2016). Strong genetic structuring of the cockle *Cerastoderma glaucum* across Europe: new insights from an intronic marker and multivariate analysis. *Journal of Molluscan Studies*, 82, 515-524.

Sromek, L., Forcioli, D., Lasota, R., Furla, P., & Wolowicz, M. (2019). Next-generation phylogeography of the cockle *Cerastoderma glaucum*: Highly heterogeneous genetic differentiation in a lagoon species. *Ecology and Evolution*, 9, 4667-4682.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30, 1312–1313.

Stewart, D. T., Breton, S., Blier, P. U., & Hoeh, W. R. (2009). Masculinization events and doubly uniparental inheritance of mitochondrial DNA: a model for understanding the evolutionary dynamics of gender-asssociated mtDNA in mussels. In P. Pontarotti (Ed.), *Evolutionary biology from concept to application II* (pp. 163–173). Berlin: Springer-Verlag.

Tarnowska, K., Chenuil, A., Nikula, R., Feral, J. P., & Wolowicz, M. (2010). Complex genetic population structure of the bivalve *Cerastoderma glaucum* in a highly fragmented lagoon habitat. *Marine Ecology Progress Series*, 406, 173-184.

Tarnowska, K., Krakau, M., Jacobsen, S., Wolowicz, M., Feral, J. P., & Chenuil, A. (2012). Comparative phylogeography of two sister (congeneric) species of cardiid bivalve: Strong influence of habitat, life history and post-glacial history. *Estuarine Coastal and Shelf Science*, 107, 150-158.

Theologidis, I., Fodelianakis, S., Gaspar, M. B., & Zouros, E. (2008). Doubly uniparental inheritance (DUI) of mitochondria DNA in *Donax trunculus* (Bivalvia: Donacidae) and the problem of its sporadic detection in Bivalvia. *Evolution*, 62, 959–970.

Thiriot Quievreux, C., & Wolowicz, M. (1996). Karyotypes of *Cerastoderma glaucum* (Bivalvia) from Baltic and Mediterranean populations. *Hydrobiologia*, 324, 149-155.

Van Blerkom, J. (2011). Mitochondrial function in the human oocyte and embryo and their role in developmental competence. *Mitochondrion*, 11, 797–813.

575 Vargas, J., Pérez, M., Toro, J., & Astorga, M. P. (2015). Presence of two mitochondrial genomes in
576 the mytilid *Perumytilus purpuratus*: phylogenetic evidence for doubly uniparental inheritance.
577 *Genetics and Molecular Biology*, 38, 173–181.
578 Vergara Chen, C., Gonzalez Wanguemert, M., Marcos, C., & Perez Ruzafa, A. (2013). Small-scale
579 genetic structure of *Cerastoderma glaucum* in a lagoonal environment: potential significance of
580 habitat discontinuity and unstable population dynamics. *Journal of Molluscan Studies*, 79, 230-240.
581 Wu, M., Chatterji, S., & Eisen, J. A. (2012). Accounting for alignment uncertainty in
582 phylogenomics. *PLoS One*, 7, e30288.
583 Xia, X., & Lemey, P. (2009). Assessing substitution saturation with DAMBE. In P. Lemey, M.
584 Salemi & A.-M. Vandamme (Eds.), *The Phylogenetic Handbook: A Practical Approach to DNA*
585 *and Protein Phylogeny. 2nd edition.* (pp. 615-630). Cambridge: Cambridge University Press.
586 Xia, X., Xie, Z., Salemi, M., Chen, L., & Wang, Y. (2003). An index of substitution saturation and
587 its application. *Molecular Phylogenetics and Evolution*, 26, 1–7.
588 Zouros, E. (2013). Biparental inheritance through uniparental transmission: the doubly uniparental
589 inheritance (DUI) of mitochondrial DNA. *Evolutionary Biology*, 40, 1–31.
590 Zouros, E., & Rodakis, G. C. (2019). Doubly Uniparental Inheritance of mtDNA: An Unappreciated
591 Defiance of a General Rule. *Advances in Anatomy Embryology and Cell Biology*, 231, 25–49.
592

Figure legends

Figure 1. Variable sites of *cox1* and *rrnL* alignments of newly obtained sequences. "SoMa" ("*Solen marginatus*") is followed by the sex of the specimen (either "F" or "M"), a specimen ID and the source tissue ("G" for "gonad" and "S" for "soma"; but see text for specimen SoMaM7G). Site numbers referring to the complete matrix are printed above each alignment. Pink color indicates F-type sequences, blue color indicates M-type sequences.

Figure 2. Maximum Likelihood phylogenetic reconstruction of Imparidentia *sensu* Combosch et al. (2017) using partial sequences of the mitochondrial markers *cox1* and *rrnL*. Node support is shown as ultrafast boostrap support value as computed by IQ-TREE. Newly obtained sequences are indicated with the picture of relative species, and the species names are followed by either "F" for "F-type" or "M" for "M-type" and a specimen ID. The entangled blue and pink rings pinpoint systems with sex-linked heteroplasmy: again, species names are followed by either "F" or "M" in that case. For newly obtained sequences and whenever available, the source tissue is also shown ("G" for "gonad" and "S" for "soma"; but see text for specimen SoMaM7G).

List of Supporting Information

Supporting Information Figure S1. Pairwise uncorrected p-distances plotted over pairwise Maximum Likelihood distances for the four available datasets.

Supporting Information Table S1. Sequences downloaded from GenBank for the present study. If the complete mitochondrial genome was available and used to extract *cox1* and *rrnL* sequences, the corresponding GenBank Accession Number is given; otherwise, separated Accession Numbers for *cox1* and/or *rrnL* are shown. When applicable, sex ("F" for "female, "M" for "male"), sequence progressive number and source tissue ("G" for "gonad", "S" for "soma") are also provided after the species name. The first entry is the outgroup. Taxonomy follows the World Register of Marine Species available at http://www.marinespecies.org.

Supporting Information Table S2. Test of substitution saturation for the three *cox1* codon positions and for *rrnL*. The analysis was performed on fully resolved sites only, assuming an asymmetrical topology and removing duplicate sequences. For the sake of clarity, given the sample size only results for 32 OTUs are shown.

Table 1. Uncorrected p-distances[†] within and between F-type and M-type sequences.

		cox1 (nt[‡])			*cox1* (aa[§])			*rrnL*		
Solen marginatus	F	0.0010	±	0.0007	0.0031	±	0.0023	0.0004	±	0.0004
	M	0.0871	±	0.0112	0.0886	±	0.0217	0.0006	±	0.0006
	F vs M	0.1597	±	0.0205	0.1477	±	0.0347	0.2122	±	0.0178
Cerastoderma glaucum	F	0.0080	±	0.0020	0.0000	±	0.0000	0.0040	±	0.0019
	M	0.0094	±	0.0029	0.0000	±	0.0000	0.0040	±	0.0021
	F vs M	0.0071	±	0.0018	0.0000	±	0.0000	0.0037	±	0.0017

[†] Mean within- and between-groups uncorrected p-distance with pairwise deletion of gaps ± standard deviation (1,000 bootstrap replicates).

[‡] nt, nucleotides.

[§] aa, amino acids.