No evidence of DUI in the Mediterranean alien species *Brachidontes pharaonis* (P. Fisher, 1870) despite mitochondrial heteroplasmy

Marek Lubośny, Beata Śmietanka, Marco Arculeo & Artur Burzyński

Two genetically different mitochondrial haplogroups of *Brachidontes pharaonis* (p-distance 6.8%) have been identified in the Mediterranean Sea. This hinted at a possible presence of doubly uniparental inheritance in this species. To ascertain this possibility, we sequenced two complete mitogenomes of *Brachidontes pharaonis* mussels and performed a qPCR analysis to measure the relative mitogenome copy numbers of both mtDNAs. Despite the presence of two very similar regions composed entirely of repetitive sequences in the two haplogroups, no recombination between mitogenomes was detected. In heteroplasmic individuals, both mitogenomes were present in the generative tissues of both sexes, which argues against the presence of doubly uniparental inheritance in this species.

*Brachidontes pharaonis* (P. Fisher, 1870) is a Lessepsian mussel species that invaded the Mediterranean Sea after the opening of the Suez Canal in 1869 connecting the Indian Ocean through the Red Sea. Due to intrinsic plasticity and overlapping morphological traits, this species is often mistaken for *B. variabilis*, which inhabits regions of the Indian Ocean and the West Pacific Ocean. It is a gonochoristic species, with a white gonad-bearing mantle in males and brown mantle in females. There is only one reported case of a clearly hermaphroditic individual in this species, and the sex determination system is otherwise quite stable.

Many bivalve species possess a unique system of mitochondrial inheritance called doubly uniparental inheritance (DUI). Under DUI male individuals are heteroplasmic with an additional, divergent mitogenome located mostly in their gonads. Furthermore, this divergent mitogenome is passed through the sperm to the progeny, unlike the male mitogenome of non-DUI animals, which is lost upon fertilization. In normal circumstances, germlines are homoplasmic towards one of the mitogenomes, M-type in males and F-type in females. However, in rare cases this second male-type mitogenome can also be detected in female individuals (for such exceptions, see). After fertilization, initially heteroplasmic embryos behave in one of two ways. If the embryo is a male, the M-type mitochondria group together during the first cell divisions, becoming the dominant mitochondrial fraction in gonads of adult male (somatic tissues are mostly dominated by F-type mitochondria). On the other hand, if the embryo develops into a female individual, the M-type mitochondria get dispersed during the first few division cycles and the signal from the M-type mitochondrial DNA disappears. The mechanism of this elimination is unknown.

Genetic studies based on *cox1* and 16S rRNA gene markers revealed the presence of two (p-distance 6.8%) different haplogroups (M and L; referred to respectively A and B later on due to possible misinterpretation of the M haplogroup as male-type mtDNA) in *B. pharaonis*, suggesting the existence of cryptic species. No heteroplasmic individuals were identified within the Mediterranean population, and the presence of a particular mitochondrial haplogroup did not correlate with the sex of the individuals. Nevertheless, many studies suggested the presence of DUI in this genus. However, there are well-known difficulties with detecting DUI by end-point PCR with universal primers. The variability of the sequence divergence between mitogenome pairs ranges from around 5% to over 50%. This makes the whole approach problematic for the following reasons. In the cases of low divergence, the amplification of the most prevalent mitogenome, or the one with sequence slightly better...
It is also greater than the distance between several interspecies pairs of mitogenomes: *M. solisianus* paraphyletic, due to the presence of *M. solisianus*. As a side note, phylogenetic analysis revealed the following inconsistency: genus *Brachidontes* the mitogenomes, including possible mitochondrial introgression events within this genus can not be concluded. However, due to the small number of available complete mitogenomes, the analysis of taxonomic affinities of *Brachidontes* pharaonis* samples (p-distance 0.071 for coding genes and 0.086 for the whole mtDNA). The preliminary results of the end-point PCR (Table 1) suggested not only the existence of heteroplasmy, but also showed that it was not linked with sex of individuals in this species. The results between PCR and qPCR differ because not every homoplasmic individual in PCR was checked with qPCR and some of the heteroplasmic individuals were resolved as homoplasmic after the qPCR. This was due to the very low estimated copy number of the second mitogenome, much lower than the copy number of a nuclear gene. Very high Cq values, close to the NTC control, were observed in these samples. Since such results could be either caused by non-specific signal (primer-dimer formation) or the truly very low gene copy number (much lower than that of atpα nuclear gene), these were classified as likely caused by sample contamination and not by true heteroplasmy. In the absence of any sex-bias, instead of naming those mitogenomes as M-type for male and F-type for female, we will refer to them neutrally as type A and type B. The mitogenomes (Fig. 1) are similar in length (type A: 20,066 bp, type B: 20,072 bp), code the typical set of 13 proteins, 2 rRNAs and 23 tRNAs, all on the same strand and in the same order. The striking feature of both mitogenomes is the presence of two long noncoding regions, rich in repetitive sequences, that divide the mitogenomes almost perfectly in half. Fragments of open reading frames in noncoding regions were identified as repeats of the 5′ end of *atp6*, suggesting the occurrence of multiple tandem duplication random loss events that encompass fragments of the coding sequences, throughout the evolution of these mitogenomes.

Given the overall relatively small divergence in mitochondrial sequences, the long repetitive sequences, and heteroplasmy, the possibility of widespread mitochondrial recombination at one of the long noncoding regions was considered. Such recombination would impair our ability to detect the type of mitogenome with just one small qPCR fragment. To address this issue, four pairs of specific qPCR primers were used to quantify the two parts of mitogenomes A and B separately (Supplementary data Table S1 and S2). Under the no recombination hypothesis the four quantities, one per part of the mitogenome, should match according to the mitogenome type, whereas if recombination is involved, the four estimates would be mismatched. Analysis of 30 *B. pharaonis* individuals revealed no noticeable difference between the halves of mitogenome copy number (Fig. 2). We have observed seven cases of heteroplasmy divided more or less evenly (no statistically significant differences for Fisher’s exact test) between sexes. The heteroplasmic individuals were: 4 females, 3 males in qPCR (23%) and 6 females, 7 males in end-point PCR analysis (33%). Both A and B mitogenomes were detectable in males and females (Supplementary data Table S5–S10 and Fig. S1).

| PCR   | All | Female | Male | qPCR   | All | Female | Male |
|-------|-----|--------|------|--------|-----|--------|------|
| Only A| 11  | 7      | 4    | Only A | 8  | 3      | 5    |
| Only B| 15  | 8      | 7    | Only B | 15 | 6      | 9    |
| A and B| 13 | 6      | 7    | A and B| 7  | 4      | 3    |
| N     | 39  | 21     | 18   | N      | 30 | 13     | 17   |
|       |     |        |      | A > B  | 3  | 1      | 2    |
|       |     |        |      | B > A  | 4  | 3      | 1    |

Table 1. Detection of the two mitogenomes (A and B) by PCR and qPCR. Number of tested individuals (N) in which A or B mitogenome was detected. For qPCR, each heteroplasmic individual showed an excess of one mitogenome over the other, which is also indicated here for mantle tissues.

Matching with PCR primers, may mask the presence of the second mitogenome. In the cases of high divergence, universal primers may not be universal enough to pick up the second genome at all. Moreover, regardless of the divergence, there is always a possibility that a mitogenome of another, contaminating, biological entity, would be co-amplified. This is facilitated by high sensitivity of end-point PCR protocols[12,21,27]. All this prompted us to use a quantitative mitogenomic approach to further corroborate the possibility of DUI in this species.

**Results**

We were able to detect and sequence two slightly divergent mitogenomes in *Brachidontes pharaonis* samples (p-distance 8.6%) in which A or B mitogenome was detected. For qPCR, each heteroplasmic individual showed an excess of one mitogenome over the other, which is also indicated here for mantle tissues.

**Discussion**

We reported two mitogenomes (p-distance 8.6%) in *B. pharaonis*. These two mitogenomes are quite frequently present in the same individuals (Table 1). Is it possible that the observed heteroplasmy was not true but the result of a recent *numt* which is segregated in the population? We dismiss this possibility for the following reasons.
Both mitogenomes were highly expressed at RNA level (31% of all NGS RNAseq sequencing reads mapped onto the A-type mtDNA and 4% onto the B-type mtDNA sequence), which is not a typical feature of a numt.

Figure 1. Genetic map of *Brachidontes pharaonis* mitochondrial genomes. The white arrows with orange bands represent protein-coding genes with predicted transmembrane domains. The dark arrows represent the rRNA and tRNA genes; the white boxes indicate the location of repetitive sequences. The figure and compositional indices were generated with MITOCONSTRICTOR as in33,34.
Moreover, numts are usually much smaller than the complete mitogenomes (but look here35), and their sequence is degenerated36. Here both very large mitogenomes have all their genes intact and seemingly functional. Finally, the ratios of copy numbers of mitogenomes to the nuclear genome are not constant. A tight correlation between a numt and nuclear copy number is expected, which is not the case here. What can be the reason for the existence of two such haplotypes within this species?

Since DUI was postulated for some Brachidontes species based solely on sequence divergence, it is reasonable to consider it here3,24. One may ask what sequence divergence threshold (if there is one) can be unequivocally associated with DUI? The situation present in the Baltic Sea Mytilus mussels37,38, where the divergent paternal mitogenome was replaced by the masculinized F-type mitogenome, is exceptional. Consequently, besides the differences in the noncoding control region, the M and F mitogenomes are identical in that case. If such extreme cases are not considered, the lowest divergence of the complete mitochondrial genomes observed and counted as DUI belongs to Arctica islandica28,29, with the M-F divergence (nucleotide p-distance) at 5.1% for coding genes and 6.9% for the whole mtDNA (Fig. S2). Even if a wider species range is considered, for which only cox1 gene fragments are known, the least divergent is also Arctica islandica: 2.5% at the protein level and 5.2% at the nucleotide level. So, can we count the divergence observed in B. variabilis3 3.6% at the nucleotide level as a suggestion of DUI existence, with the lowest known divergence in the cox1 gene? Or is this just a high haplotype diversity within a species, not linked to sex-related heteroplasmy? This rhetorical question also applies to B. pharaonis.

In the context of DUI, divergence alone is just a secondary factor, depending only on the time of separation of the two lineages and their evolutionary rates. Nevertheless, for the emerging mitogenomes to play different physiological roles, there must be functional differences between their proteins, which is problematic for low genetic distances. However, even with a small number of nonsynonymous substitutions in the sequence, the encoded protein may become different enough (like in quickly evolving viruses or targeted enzyme engineering39,40) to differ in activity. Unfortunately, tracing such substitutions in the context of DUI is difficult, but the possibility that a low-divergence mitogenome may still play distinct physiological role can’t be discounted. Are there any other features that could help classify a mitogenome as involved in DUI? Perhaps we should consider additional open reading frames (ORFs) or gene extensions with no homology to the second mitogenome as a DUI marker? Is this a universal feature of DUI mitogenomes? Indeed, this seems to be true for all known pairs of DUI mitogenomes.

**Figure 2.** The ratio of mtDNA to nDNA in seven heteroplasmic individuals of B. pharaonis. F represents female individuals; M represents male individuals. The yellow and green colored bars represent sequence copy number per nuclear DNA for type A mtDNA; The pink and blue bars represent sequence copy number per nuclear DNA for type B mtDNA. The target genes atp6 and nd4 are located on the opposite sites of the respective mitogenomes separated by repetitive sequences. Samples described as “Mantle” contained mainly gonadal tissues, not just the somatic mantle.
(Table 2): each of them has some gender-specific structural features, but this is not the case for *B. pharaonis*. Here, both mitogenomes are structurally identical.

Another feature of mtDNA, which has been reported in the context of DUI, is mitochondrial recombination\(^6^5,^6^6\). The existence of very large repetitive elements suggests that at least intramolecular recombination would be present in *B. pharaonis*. Yet, we have not found any evidence for the exchange of large parts between A and B mitogenomes. They apparently maintain their integrity, despite the conditions, which should favor the exchange\(^6^7\). The qPCR method would not detect low frequency, short span recombination so this has to be considered cautiously. However, no detectable signature of recombination is present within the A and B mitogenomic sequences, therefore even if such recombination is present, it is limited to somatic cells.

Several studies postulated the existence of cryptic species within the *Brachidontes* genus (*B. pharaonis/variabilis*\(^3\) and *B. puniceus/exustus*\(^2^4,^6^8\)). These were usually argued by the distinct divergence (p-distance) at the nucleotide level (~7–20%) between mitochondrial sequences. However, at the protein level, these distances were much lower (p-distance ~ 0–1.5%). These must be interpreted cautiously because only short fragments of one mitochondrial gene are available (*cox1*: *AY621835-AY621860; AY621862-AY621865; AY621909; AY621911; AY825105-AY825108; DQ836012; DQ836013; DQ836019-DQ836021) and the mitogenomic distances may be quite different (Fig. 4). However, all nonsynonymous substitutions in the mentioned *cox1* fragments stay within the group of nonpolar, mostly aliphatic amino acids (Ile, Val, Leu, and in two cases also Met and Phe). This suggests no alternation in the overall protein structure/reactivity as is indicated by amino acid substitution matrices\(^6^9,^7^0\) derived from empirical data. The issue of cryptic species identification becomes even more complicated when specimens are erroneously assigned\(^6^9\) to their respective species, as in the case of *Brachidontes/...
which in fact is a nuclear hybrid of mitochondrial markers comes from the case of Baltic Mytilus trossulus, edulis here for M/F recombination66,76). If only the mitogenomic protein level p-distances are considered, the cryptic and are prone to introgression72,73. A good example of potential pitfalls associated with such a simplistic use of not be based solely on mitochondrial markers, which are known to evolve under strong selective constraints

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genera. This is the example of Mytilaster solisianus (d’Orbigny, 1842), known earlier as Brachidontes solisianus (d’Orbigny, 1842). The current taxonomy classifies this species as Mytilaster, but the mitogenomic phylogenetic tree (Fig. 3) does not support this. Furthermore, the M. solisianus mitogenome deposited in GenBank has been wrongly annotated as Perna perna43. A taxonomic revision of Brachidontes seems warranted but should not be based solely on mitochondrial markers, which are known to evolve under strong selective constraints and are prone to introgression72,73. A good example of potential pitfalls associated with such a simplistic use of mitochondrial markers comes from the case of Baltic Mytilus trossulus, which in fact is a nuclear hybrid of M. edulis and M. trossulus that lost their native mitochondrial genome towards one from M. edulis72,74,75 (also see here for M/F recombination66,76). If only the mitogenomic protein level p-distances are considered, the cryptic species hypothesis is becoming less likely. However, the fact that heteroplasmic individuals were consistently observed suggests that anomalies in the mitochondrial inheritance may be involved.

In conclusion, B. pharaonis represents a species possessing two slightly different mitogenomes (p-distance 8.6%) in every possible combination between the sexes. Total homoplasy for mitogenomes A or B, as well as heteroplasy of both mitogenomes within a single individual are possible. This heteroplasy is not correlated

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| Family        | Species                  | Feature                                      |
|---------------|--------------------------|----------------------------------------------|
| Mytilidae     | Arcuatula senhousia      | duplicated m-cox2 with 3’ extension          |
|               | Grunenstia demissa       | m-cox2 extension                             |
|               | Mytilus californianus    | f-ORF; a bit longer m- atp8 gene             |
|               | Mytilus galloprovincialis| f-ORF; a bit longer m- atp8 gene             |
|               | Mytilus edulis           | f-ORF; a bit longer m- atp8 gene             |
|               | Mytilus trossulus        | f-ORF; a bit longer m- atp8 gene             |
|               | Perumytilus purpuratus   | nd2 like f-ORF                               |
|               | Semimytilus patagonicus  | m-atp8 extension; ORFs                       |
| Veneridae     | Raditapes philippinarum  | m-ORF (rphm21)                               |
|               | Meretrix lamarcii         | Insertion in m-cox2; additional ORFs         |
| Semelidae     | Scrobicularia plana      | Insertion in m-cox2; additional m-ORFs       |
| Tellinidae    | Limecola balthica        | Insertion in m-cox2; additional m-ORFs       |
| Arctidae      | Arctica islandica        | f-ORFs: nd6 × nd2 hybrid gene duplication    |
| Hyridae       | Echiuridaella menzienii  | m-cox2 3’ extension; ORFs                   |
| Margaritiferida | Margaritifera margaritifera| m-cox2 3’ extension; ORFs                 |
|               | Pseudonio maroacanu      | m-cox2 3’ extension; ORFs                   |
|               | Cumberlandia monodonta   | m-cox2 3’ extension; ORFs                   |
|               | Venuscaencha ellipsiformis| m-cox2 3’ extension; ORFs               |
|               | Utterbackia peninsularis | m-cox2 3’ extension; ORFs                   |
|               | Uniio tumidus            | m-cox2 3’ extension; ORFs                   |
|               | Uniio pictorum           | m-cox2 3’ extension; ORFs                   |
|               | Uniio delphinus          | m-cox2 3’ extension; ORFs                   |
|               | Uniio crassus            | m-cox2 3’ extension; ORFs                   |
|               | Solenaia carinata        | m-cox2 3’ extension; ORFs                   |
|               | Quadrula quadrala        | m-cox2 3’ extension; ORFs                   |
|               | Peganodon grandis        | m-cox2 3’ extension; ORFs                   |
|               | Potamilius diatus        | m-cox2 3’ extension; ORFs                   |
|               | Lampilis silquoida       | m-cox2 3’ extension; ORFs                   |
|               | Lampilis powelli         | m-cox2 3’ extension; ORFs                   |
|               | Aculampretula tortuosa   | m-cox2 3’ extension; ORFs                   |
|               | Lamprotula leai          | m-cox2 3’ extension                         |
|               | Pronodularia japonensis  | m-cox2 3’ extension; ORFs                   |
|               | Sinohyriopsis cumingii   | m-cox2 3’ extension; ORFs                   |
|               | Anodonta anatina         | m-cox2 3’ extension; ORFs                   |
|               | Sinanodonta woodiata     | m-cox2 3’ extension; ORFs                   |
|               | Lanceolaria lanceolati   | m-cox2 3’ extension; ORFs                   |
|               | Potoniida littoralis     | m-cox2 3’ extension; ORFs                   |
|               | Microconchylaea bonellii | m-cox2 3’ extension; ORFs                   |
|               | Chamberlainia hainesiana | m-cox2 3’ extension; ORFs                   |
|               | Pilshyriocha exilis      | m-cox2 3’ extension; ORFs                   |
|               | Monodontina vondembuschia| m-cox2 3’ extension; ORFs                   |

Table 2. Features correlating with doubly uniparental inheritance observed in complete pairs of mitogenomes (when both M and F are available).
with sex, which excludes DUI. Did we observe the first stages of emerging DUI in this species or is heteroplasmic *B. pharaonis* a hybrid of two very similar cryptic species and the observed heteroplasmasy represents paternal leakage? Future studies concerning *Brachidontes* populations are needed to conclusively answer this question. On a more practical level, we advise that the use of somatic tissues77, in phylogenetic studies on bivalves, usually practiced as a precaution to avoid amplification of the potential M mitogenome, may not always work as planned78. In the case of *B. pharaonis* such an approach would lead to seemingly random amplification of one of the two mitogenomes present in an individual.

Methods

Samples of *Brachidontes pharaonis* mussels were gathered in June 2014–2015 at the salt pan “infersa” of the Marsala lagoon (northwest of Sicily—Italy). Individuals were sectioned with a sterile scalpel blade, checked for male or female gametes under a light microscope, and stored frozen in − 80 °C until further use. DNA was extracted using the modified CTAB extraction method79,80. Small tissue samples (~ 50 mg) were incubated overnight in the 700 µl of extraction buffer (2% CTAB, 0.1 M Tris–HCl, 1.4 M NaCl, 20 mM EDTA, 1 mg/ml proteinase K and 35 mM 2-mercaptoethanol). The digested samples were then extracted with chloroform (1:1 vol/vol ratio) and centrifuged three times at 20,000 × g for 10 min. Then the DNA remaining in the aqueous phase was precipitated by mixing with cold isopropanol (1:1 vol/vol), incubated for 20 min at − 20 °C, and centrifuged (20,000 × g for 30 min at 4 °C). The recovered DNA pellets were washed twice with 75% ethyl alcohol and dried in a vacuum concentrator. At the final stage, DNA pellets were resuspended in Tris–EDTA buffer, checked for DNA concentration and integrity by gel electrophoresis and Epoch microplate Spectrophotometer. RNA was extracted with the GenElute Mammalian Total RNA miniprep kit (Sigma).

Total RNA from three mantles of male individuals was pooled and sent to Macrogen Inc (Korea) for high throughput NGS sequencing (MiSeq Illumina, TruSeq NGS library 2 × 150 bp). Raw sequencing reads have been submitted to the SRA GenBank database under accession number SRR19141670. The acquired data were processed according to the Oyster river protocol34,81 and assembled into the raw transcriptome. Long-range PCR primers for amplification of overlapping mitogenome fragments have been designed based on assembled contigs containing mitochondrial genes identified with Wise2 software82. PCRs were carried out in a volume of 20 µl, containing 25 ng of DNA, primers at 0.4 µM each, dNTP at 200 µM, 1.5 mM MgCl₂ and 0.4 U Phusion High-Fidelity polymerase (Thermo Scientific) suspended in GC buffer for difficult GC-rich templates. The PCR conditions were as follows: initial denaturation at 98 °C for 30 s; 35 cycles of denaturation at 98 °C for 10 s, annealing (Table S3 and S4) for 30 s and extension at 72 °C for 8 min. The final extension at 72 °C lasted 10 min. The amplified products were then assigned to their respective mitogenome (A and B) and sent for another NGS sequencing (MiSeq Illumina, TrueSeq NGS library, 2 × 300 bp). Complete mitochondrial DNA sequences have been recovered with NOVOplasty83 software and validated by mapping NGS reads onto the assembled mitogenomes in CLC Genomics Workbench 9.5 (QIAGEN). The two acquired mitogenomes were then annotated with CRITICA84, Wise282, GLIMMER85, ARWEN86, and nhmmer87. Mitogenome circular diagrams and compositional indices were drawn with MITOCONSTRICTOR33,34 (https://github.com/aburzynski/mitoconstrictor). Annotated mitochondrial genomes for *Brachidontes pharaonis* were deposited in the GenBank under accession numbers ON464163 and ON464164.

Based on assembled transcriptome and two mitogenome sequences, a set of five qPCR primer pairs spanning: nuclear *atpα* gene and mitochondrial *nad4*, *atp6* (from both mitogenomes; type A and type B) were designed in Primer388. The specificity of each primer pair was verified, and there was no cross-amplification between A and B mitogenomic fragments. Quantitative PCR efficiency has been verified by running standard curves in nine repetitions with seven dilution points of samples. Reactions quantifying A and B mtDNA, as well as nuclear DNA, were performed on an ECO48 (Illumina/now PCRmax) Real-Time PCR System according to the qPCR kit (EurX) manufacturer instructions, in 10 µl reaction volume containing 1× SG-qPCR Master Mix, 2 µl of DNA (at ~ 15 ng/µl) and 0.5 µM of each primer. The thermal profile was as follows: initial denaturation at 95 °C for

![Figure 4.](https://example.com/image.png)

The divergence between A and B type mitogenome of *B. pharaonis*. The blue color represents the p-distance for nucleotide sequences, and dark red color represents p-distance for translated protein genes.
was checked with Tracer92, the effective sample size for estimated parameters was greater than 200. ML was
GTR + F + I + 4G was the best fitting for all protein genes except
10,000 replicates. Substitution models for every partition were chosen with build-in ModelFinder tool. Model
performed in IQ-TREE93, under the default parameters with ultrafast bootstrap approximation parameter set to
1. Sirna Terranova, M, Lo Brutto, S., Arculeo, M. & Mitton, J. B. Population structure of Brachidontes pharaonis (P. Fisher, 1870)
(Bivalvia, Mytilidae) in the Mediterranean Sea, and evolution of a novel mtDNA polymorphism. Mar. Biol. 150, 89–101 (2006).

10 min, followed by 35 cycles of 10 s denaturation at 94 °C, annealing at 60 °C for 30 s, elongation at 72 °C for
30 s, and a melting curve 55–95 °C. Primer sequences, reaction efficiency, and tabulated results have been placed
in Supplementary data S1 and S2. Statistical Fisher's exact test (Statistica 7, StatSoft) was used to calculate the
significance of the association between sex and heteroplasmy.

Table 3. List of mitogenomes used in phylogenetic analysis, with species, accession numbers, and references
(wherever available). All species names have been updated according to the WoRMS (World Register of Marine
Species) database (access date July 2021).

| Species and references | Acc. no | Species and references | Acc. no |
|------------------------|---------|------------------------|---------|
| Arcuatula senhousia F89 | GU001953 | Modiolus nipponicus89 | MK721547 |
| Arcuatula senhousia M89 | GU001954 | Modiolus philippinarum89 | KY705073 |
| Bathymodiolus adyoide89 | MT916741 | Mytilopsis virgata89 | MK721548 |
| Bathymodiolus azoricus89 | MT916742 | Mytilaster solisianus89 | KM655841 |
| Bathymodiolus brooksi89 | MT916743 | Mytilopsis keenae89 | MK721542 |
| Bathymodiolus japonicus89 | AP014560 | Mytilopsis virgata89 | MK721548 |
| Bathymodiolus maritondicus89 | MT916745 | Mytilus californianus F89 | GQ227172 |
| Bathymodiolus manumensis89 | KY270856 | Mytilus californianus M89 | GQ227173 |
| Bathymodiolus secretorferma89 | NC_099552 | Mytilius chilenus89 | KT966847 |
| Bathymodiolus septemarcis93 | AP014562 | Mytilius unguiculatus89 | KJ577549 |
| Bathymodiolus thermophiles89 | MK721544 | Mytilius edulis F89 | MF407676 |
| Brachidontes exsustus89 | KM233636 | Mytilius edulis M89 | HM488974 |
| Brachidontes mutabilis89 | MK721541 | Mytilius galloprovincialis M89 | FJ890850 |
| Brachidontes pharaonis A | ON464163 | Mytilius galloprovincialis F89 | FJ890849 |
| Brachidontes pharaonis B | ON464164 | Mytilius trossulus F89 | HM462080 |
| Crenomytilus grayanus89 | MK721543 | Mytilius trossulus M89 | HM462081 |
| Geukenia demissa F94 | MN449487 | Perna canaliculus93 | MG766134 |
| Geukenia demissa M94 | MN449488 | Perna perna | MT388202 |
| Gigantidas childress99 | MT916744 | Perna viridis93 | JQ970425 |
| Gigantidas haimaenasa99 | MT916746 | Perumytilus purpuratus NF90 | MH330332 |
| Gigantidas platifrons97 | AP014561 | Perumytilus purpuratus NM90 | MH330330 |
| Gregeria coraiophaga94 | MK721545 | Perumytilus purpuratus SF90 | MH330333 |
| Limnoperna fortunei95 | K976905 | Perumytilus purpuratus SM90 | MH330331 |
| Leioseolenis lischi89 | MK721546 | Semimytilus patagonicus F94 | MT026712 |
| Modiolus calminus99 | MN602036 | Semimytilus patagonicus M90 | MT026713 |
| Modiolus kuriensia | KY242717 | Septifer bilocularis99 | MK721549 |
| Modiolus modiolus98 | KX821782 | | |

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Institutional affiliations.

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Correspondence and requests for materials should be addressed to M.L.

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Additional information

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The authors declare no competing interests.

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Author contributions

M.L., M.A., B.S. and A.B. designed and conceived the experiments. M.L. and B.S. performed the experiments. M.A. gathered and sampled tissues. M.L. and A.B. performed data analysis. M.L., M.A., B.S. and A.B. wrote the manuscript. All authors reviewed drafts of the paper and approved the final manuscript.

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