Molecular characterization of the bivalves *Mya arenaria*, *Mya truncata* and *Hiatella arctica*

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**RESEARCH NOTES**

Bivalves are commonly studied for food chain involvement,1,2 predator/prey relationship,3 immunity,4 genetic analysis,5–7 population structure8,9 and morphology.10 Bivalve populations have been extensively studied as indicators of pollution11–14 and for indicating gene damage resulting from pollution.15,16 However, species identification using molecular techniques is poorly2–5,17,18 measurable, but planktonic or damaged samples (crushed organisms in sediments or digested bivalves in predator guts) are far more difficult to identify.3,18 Molecular approaches may overcome some of these problems. In this paper, we report new PCR-based tests for the identification of three bivalve species within the Myoidae order: *Mya arenaria*, *Mya truncata*, and *Hiatella arctica* based on the internal transcribed spacer (ITS-1) ribosomal DNA region located between the 18S and 5.8S ribosomal RNA genes.

Marine bivalves were collected between 1998 and 2000 on the East and West coasts of Scotland, and identified by morphology. Individuals from the following species, which are taxonomically close and commonly found on the coasts of UK, were used in this study: *Cerastoderma edule* (Linnaeus, 1758), *Dosinia exoleta* (Linnaeus, 1758), *Hiatella arctica* (Linnaeus, 1767), *Mya arenaria* (Linnaeus, 1758), *Mya truncata* (Linnaeus, 1758), *Mytilus edulis* (Linnaeus, 1758), and *Venerupis senegalensis* (Gmelin, 1791).

DNA was extracted from whole *Hiatella arctica* specimens, whereas for bigger bivalves, such as the *Mya* species, *Cerastoderma edule*, *Dosinia exoleta*, and *Venerupis senegalensis*, we have used only some parts of the animal (foot or gills). Tissues were digested and DNA was extracted by using a DNeasy Tissue kit (QIAGEN Ltd, Crawley, UK) using the protocol recommended by the manufacturer. Purified DNA samples were stored at –20°C until needed.

A PCR reaction (using previously published ITS2 and ITS5 primers20) was performed on different bivalve species. Unfortunately, bands of 550 bp were amplified from DNA extracted for all the species tested in this study even when the Tm was raised to 56°C instead of 48°C, as recommended20 (data not shown) and, therefore, could not be specifically identified. *Mya* species according to the size of the amplified DNA. Since only a few sequences have been published for these organisms and none are so far in the GenBank for *Mya* species, we sequenced amplified products from two different *Mya* species and representatives of *Hiatella arctica*.

DNA fragments were excised from ethidium bromide-stained agarose gels using the QIAquick gel extraction kit (QIAGEN Ltd, Crawley, UK) and were sequenced on an ABI 310 DNA sequencer from Applied Biosystems (Warrington, UK) using the Big Dye Terminator kit. Sequences were aligned using the MultiAlin software.19 Sequences can be retrieved on the GenBank database under the following accession numbers: AF348985, AF348986, and AF348987 for *Mya arenaria*; AF348988, AF348989, and AF348990 for *M. truncata*; AF348991, AF348992, and AF348993 for *Hiatella arctica*.

We were able to design three new forward primers (5’-ATGCGTGGGCCGCCGGC-3’; 5’-GGGATGCGTGGGCGG-3’; 5’-CGGATGATCTATTAAAGCACGT-3’) designed from the aligned ITS sequences allowing a species-specific reaction for the identification of *Mya arenaria*, *M. truncata*, and *Hiatella arctica*, respectively (Figure 1). The reverse primer was in all cases the previously published ITS2 primer.

For the three new species-specific PCR, the amplification reaction (50 μl) performed on a TouchGene apparatus (Techne Cambridge, UK) comprised: 1.5 mM MgCl2, 20 mM dNTPs (dATP, dGTP, dCTP); 0.5 unit of SuperTaq polymerase (HT Biotechnology Ltd, Cambridge, UK), 0.1 units of Uracil DNA glycosylase (Life Technologies, Paisley, UK), 0.275 μg of TaqStart Antibody (Clontech Laboratories UK Ltd, Basingstoke, UK), and 0.5 μl of DNA template.

![Figure 1. PCR results with species-specific primers](Link to figure)

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25 pmol of each primer. A first denaturation step of 5 min at 95°C was followed by 32 amplification cycles (45 s denaturation at 95°C, 30 s annealing at a variable melting temperature (from 56°C to 61°C), 45 s extension at 72°C). An extension step of 5 min at 72°C and a hold at 4°C concluded the PCR programme. Amplified products were observed under UV after electrophoresis in a 2.0% agarose gel using a 0.5× TBE buffer.

Specific PCR reactions for *Mya arenaria* and *Mya truncata* produced fragments of 500 bp and 530 bp, respectively, for each individual within the species (Figure 1). The third PCR, targeting *Hiatella arctica*, showed DNA amplification with product sizes ranging between 520 bp and 680 bp (Figure 1). The taxonomy of *Hiatella arctica* is controversial and morphological features suggest that this species may, in fact, be a mixture of two species.21

None of these three species-specific PCR reactions cross-reacted with DNA from any of the other bivalve species used in this study.

DNA sequences for *Mya arenaria* obtained by Caporale et al.20 belonged to two groups. Alignment of the sequences obtained in this study revealed that Scottish samples belonged to group 1.20 No *Mya arenaria* group 2 DNA sequence was observed. ITS sequences are generally subject to rapid evolution changes and can be used for sub-specific typing.22 The recovery of animals with identical sequences from North fields of the Atlantic indicates wide geographical dispersion of the same population.

Our results will be of interest for scientists working with damaged animals without a recognizable shell (digested animals in predator guts for instance), or interested to follow the larvae of these species within the plankton as direct identification of larvae forms requires specialist knowledge and is time demanding. Molecular techniques are in these cases very useful, do not need a large amount of tissue, can be non-destructive, and are very sensitive.

REFERENCES


