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PCR amplification of a middle repetitive element detects larval stone crabs (Crustacea: Decapoda: Menippidae) in estuarine plankton samples

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ABSTRACT: Planktonic larval dispersal and recruitment can be major determinants of the structure and dynamics of marine communities. However, these processes have been difficult to study because of their natural variability and the limitations of methods used to collect and analyze plankton samples. In particular, the use of microscopy to determine the composition of plankton samples is time-consuming and often limited by a lack of reliable morphological characters for species identification. The need for methods of greater accuracy and efficiency has led to the development of molecular approaches to plankton analysis, including detection by DNA hybridization, amplification of DNA from plankton samples by the polymerase chain reaction (PCR) and taxonomic characterization by ribosomal DNA sequence analysis. Here we describe a PCR-based method that detects larval crabs in estuarine plankton samples. This technique is unusually expedient and relatively cost-effective. It is based on the detection of a middle repetitive sequence characteristic of the stone crab *Menippe mercenaria*, as well as the closely related species *M. adina*. Amplification by PCR of a 585 base pair region of this sequence from plankton samples accurately indicates the presence of either species. Because of the high abundance of this sequence in the genome of *Menippe*, single larvae can be detected in typical plankton samples. Unlike methods based on 'universal' sequences (rRNA or regions of the mitochondrial genome), the amplification of a PCR product of the expected size is a reliable indication of the presence of the target species, and no further characterization is necessary. This technique is intended to facilitate the large-scale processing of plankton samples that is necessary for accurate determination of the temporal and spatial distributions of individual species in plankton communities.

KEY WORDS: Plankton · Larval ecology · Middle repetitive DNA · Estuarine · PCR · Crustacea · *Menippe*

INTRODUCTION

It is now widely recognized that the supply of planktonic larvae available for recruitment can influence the distribution and abundance of benthic adult populations (Gaines & Roughgarden 1985, Gaines et al. 1985, Lewin 1986, Young 1987). This has led to renewed interest in the ecology of planktonic larvae and their transport by near-shore oceanographic processes (Grosberg & Levitan 1992, Gaines & Bertness 1993, Todd 1998). However, studies of the distributions of planktonic organisms are often limited by the labor required to determine the presence and abundance of particular taxa in large numbers of field-collected samples (Silberman & Walsh 1992, Fuhrman et al. 1994, Medeiros-Bergen et al. 1995). Identification of larvae can be complicated by either a lack of morphological differentiation between taxa or phenotypic plasticity within taxa (Smith 1977, Medeiros-Bergen et al. 1995). Phenotypic plasticity of larvae may be a source of bias in the analysis of plankton samples because it may reflect responses to environmental factors such as food (Biodron-Metairon 1988), source of water (Wilson & Armstrong 1961) or temperature (Shirley et al. 1987). Consequently, the use of light microscopy and traditional

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morphological identification to analyze plankton samples can be tedious and subjective.

Molecular techniques have been used to distinguish among bacterioplankton and the planktonic larvae of metazoan invertebrates (Coffroth & Mulawka 1995, Medeiros-Bergen et al. 1995, Lim 1996). One of the earliest such studies, by Giovannoni et al. (1990), used PCR amplification and sequencing of large subunit rDNA (LSRDNA) sequences, often referred to as '16S sequences', to identify 3 distinct lineages of bacterioplankton present in the Sargasso Sea. Comparisons of LSRDNA sequences have also been used to distinguish the larvae of closely related marine invertebrate taxa (Ward et al. 1990, Olsen et al. 1991, Fuhrman et al. 1992, 1994).

An alternative to complete analysis of LSRDNA DNA sequences for species identification is the use of LSRDNA hybridization probes (Rehnstam et al. 1993, Fuhrman et al. 1994, Medeiros-Bergen et al. 1995). These probes specifically hybridize with the DNA of a target genus or species. Medeiros-Bergen et al. (1995) used this approach to identify 3 morphologically indistinguishable species of sea cucumber larvae. Although this method offers the advantages of increased sensitivity, it is labor-intensive and costly.

Randomly amplified polymorphic DNA (RAPD) analysis (Williams et al. 1990) requires fewer manipulations and is less expensive than hybridization analysis or direct sequencing of PCR products. RAPD polymorphisms have been used as species-specific markers for 5 species of gorgonian corals (Coffroth & Mulawka 1995). This technique is expedient because only the sizes of PCR products need to be determined, which can be accomplished easily by agarose gel electrophoresis. However, the reproducibility of RAPD analysis may be compromised by variable sample conditions, and the genetic basis for the observed polymorphisms is generally unknown (reviewed by Fritsch & Reiseberg 1996). These limitations make this method unsuitable for the analysis of marine plankton samples. Plankton samples are subjected to variable collection and preservation conditions that would reduce the reproducibility of RAPD analysis. More importantly, plankton samples typically include a diverse array of species, each of which could potentially produce confounding PCR products in a RAPD analysis.

Middle repetitive sequence elements have seldom been used for species identification, although their general characteristics suggest they may be suitable for this purpose. Middle repetitive sequences are defined as sequences present in 10^3 to 10^5 copies genome^{-1} (Hardman 1986), and are typically dispersed throughout a genome (Felger & Sperlich 1989). Divergence in the middle repetitive sequences of different species is often greater than divergence in other sequences used for species identification, including mitochondrial DNA and nuclear gene sequences. As a result, homologous repetitive DNA sequences can generally be detected in closely related species by DNA hybridization, but not in species that have diverged for more than about 5 million yr (Felger & Hunt 1993). Therefore, we expect most middle repetitive sequences to be characteristic of either species or recently evolved genera. Among Crustacea, families of repetitive elements have been found in multiple species, but the similarity of these sequences decreases rapidly with taxonomic divergence (Graham & Skinner 1973). Recent analysis of LSRDNA sequences from the 2 *Menippe* species that occur and hybridize in the Gulf of Mexico (*M. adina* and *M. mercenaria*) indicates that they probably separated less than 2 million yr before present (Schneider-Broussard et al. 1998). Thus, we expect a middle repetitive sequence from *M. adina* to be characteristic of this species pair.

In the present study, PCR amplification of a middle repetitive element was used for specific detection of DNA from *Menippe adina* and *M. mercenaria* under a variety of conditions, including DNA in mixtures obtained from plankton samples. Our methods are intended as a less costly and less laborious alternative to either microscopic examination or methods that require DNA sequence characterization.

MATERIALS AND METHODS

Isolation and characterization of a middle repetitive sequence from *Menippe*. Stone crabs (*M. adina* and *M. mercenaria*) were trapped in wire crab pots from 3 locations by local fisherman and by D.L.F. *M. adina* specimens were collected from upper Terrebonne Bay near Cocodrie, Louisiana; *M. mercenaria* were collected from Rookery Bay, near Naples, Florida, from Harbor Branch Marine Institute harbor, north of Fort Pierce, Florida, and from a locale near Brunswick, Georgia. Total genomic DNA was isolated from fresh tissues of *M. adina* and *M. mercenaria*. DNA from 1 specimen of *M. adina* was used to construct a genomic library in the plasmid pUC19 (Maniatis et al. 1982, Neigel et al. 1991). One recombinant plasmid, pMACC36, contained the middle repetitive sequence used in this study (henceforth designated MACC36). The sequence of MACC36 was determined by the dideoxy method (Sanger et al. 1977) and the primers shown in Table 1. The complete sequence of MACC36 is available from GenBank (Accession no. AF058687).

The sequence of MACC36 was used to design PCR primers (36F and 36R in Table 1) for the amplification of related sequences from both *Menippe adina* and *M. mercenaria*. PCR amplifications of the expected 1.7 kb

Table 1. PCR primer sequences used for sequencing MACC36 and for detection of homologous sequences in *Menippe mercenaria* and *M. adina*

Primer	Sequence
MACC36 sequencing	
36F	AATGAGCGTCTTCTGAGCATATAG
36R	GGATCTTGAGGAATGCACATTCTT
36F3	GCTCAAGTCTGCACAAAT
35R3	GAGAGTCTTCTAAGGAGGAGGCGTTGA
36F4	CATAATTGTCGATAGTAAGT
Larval detection	
36F5	CACACACTTCTTGAGAAAAGG
36R5	GTTCTTTTTTGTTTCCTCCAC

sequences homologous to MACC36 were routinely obtained from genomic DNA after reaction conditions were optimized. These products were cloned by TA ligation into the pCRII cloning vector (Invitrogen Corp.). Fifteen recombinant plasmids with the expected insert size were mapped using 3 restriction endonucleases (*Eco*RI, *Hin*dIII and *Pst*I) and compared to the pMACC36 insert. Seven of these inserts were also partially sequenced using the dideoxy method (Sanger et al. 1977) and are referred to as TACC36 sequences.

The copy number of sequences homologous to MACC36 was estimated by hybridization of an MACC36 probe to genomic DNA from *Menippe adina* and *M. mercenaria*. A Bio-Rad slot blot manifold (Bio-Rad 2000®) was used to apply genomic DNA samples and MACC36 standards to a nitrocellulose membrane. Across each row of slots, 100 ng aliquots of genomic DNA were alternated with standard amounts of purified MACC36 that ranged from 6.3 pg to 2 ng in 2-fold increments, with 100 ng of salmon sperm DNA added to each standard to provide a uniform background of non-target DNA. Three replicates of each sample were included to control for random variation in hybridization intensity across the membrane. Non-radioactive labeling of the probe and immunological detection were performed using the 'Genius DNA Labeling and Detection Kit' (Boehringer Mannheim) according to the manufacturer's directions. The probe contained 3 µg of digoxigenin-labeled pMACC36 in 10 ml of prehybridization solution (5 × SSC, 0.1% N-lauroylsarcosine, 0.02% SDS, 1% blocking reagent), and hybridization was performed overnight at 69°C.

Larval detection. Stone crab first zoeal stage larvae (Z1 larvae) were hatched from eggs of ovigerous female specimens of *Menippe adina* and *M. mercenaria* collected at Cocodrie and Fort Pierce respectively. To provide a standard non-*Menippe* control, naupliar larvae of *Artemia* sp. were cultured from cysts (Red Jungle Brand O.S.I®) at 23°C in 23‰ salinity artificial seawater. Mixtures of estuarine zooplankton from Lake Pelto, LA, were collected with a 0.5 mm mesh plankton net of mouth dimensions 0.45 × 0.2 m towed at ~1 m s^{-1} along a fixed 240 m path. All larvae and plankton were gently strained, rinsed with ethanol and placed immediately in 95 to 100% ethanol. Prior to DNA isolation, larvae and plankton were placed on Miracloth (Calbiochem®) and rinsed with deionized water. DNA was isolated following the phenol:chloroform DNA extraction procedure of Palumbi et al. (1991).

For detection of larval DNA, a portion of the MACC36 sequence was amplified using the primers 36F5 and 36R5 (Table 1). Amplifications were performed in a Perkin-Elmer® N801-0150 DNA Thermal Cycler: 1 cycle at 94°C for 1 min; 35 cycles of 94°C for 1 min, 53°C for 1 min, ramp for 2 min, 72°C for 90 s; 1 cycle at 72°C for 5 min; 15°C soak. The 25 µl reactions contained final concentrations of 2 mM dNTP, 2 µM of each primer, 1 × Perkin Elmer's PCR Buffer with 1.5 mM $MgCl_2$, 1 U of Amplitaq Gold™ polymerase, and 1 µl of template DNA. Sizes of amplification products were determined by electrophoresis of 10 µl from each reaction on 2% agarose gels.

To test for the specificity of detection, reactions were run with 20 to 100 ng of DNA isolated from other brachyuran crabs, from *Artemia*, or from mixed plankton samples that did not contain larval *Menippe* (Table 2). All PCRs with DNA from non-target species were performed using an annealing temperature of 45°C (rather than 53°C) to lower the stringency of primer annealing and thus provide a more conservative test of non-specific amplification. The suitability of non-target DNA for PCR was verified by amplification of a LSRDNA sequence from each sample using the procedure of Palumbi et al. (1991) for the primers 16sar-L and 16sbr-H.

The sensitivity of the PCR assay was tested with template DNA from *Menippe* in amounts ranging from 10 pg to 100 ng per 25 µl reaction. This range was also used in reactions to which either 10 ng of naupliar *Artemia* DNA or 10 ng of mixed estuarine plankton

Table 2. Non-target DNA samples used as negative controls for PCRs to test for the specificity of the 36F5 and 36R5 primers as diagnostics for *Menippe*. Between 20 to 100 ng of DNA was used in each reaction

Panopeus americanus	*Sesarma reticulatum*
P. simpsoni	*Callinectes sapidus*
P. lacustris	*Ocypode quadrata*
Eurypanopeus dissimilis	*Artemia* sp.
E. depressus	Estuarine mixed plankton
Cataleptodius floridanus	

Table 3. Sources and dilutions of *Menippe* DNA samples used to test the sensitivity of *Menippe*-specific PCR amplification across the geographic range of *Menippe*. DNA from non-target sources were added to test for interactions or interference between DNA from *Menippe* and DNA from other taxa. Successful amplifications of the sequence (585 bp) from *Menippe*: +. EP: estuarine mixed plankton

Sample	100 ng	10 ng	1 ng	100 pg	10 pg	1 pg
M. mercenaria (Brunswick, GA)	+	+	+	+	+	–
M. mercenaria (Fort Pierce, FL)	+	+	+	+	+	–
M. adina (Cocodrie, LA)	+	+	+	+	+	–
M. mercenaria (FL) and 10 ng *Artemia* DNA	+	+	+	+	+	–
M. mercenaria (FL) and 10 ng EP DNA	+	+	+	+	+	–

DNA were added, to test for interactions or interference between DNA from *Menippe* and non-target species (Table 3). An amplification was considered successful if a band could be easily visualized on a UV transilluminator, which required a product DNA concentration of at least 5 ng μl^{-1}.

Nauplii of *Artemia* were used to provide a standard background of larvae from a non-target species against which larval *Menippe* could be detected. Concentrated suspensions of nauplii were removed from culture, rinsed with deionized water, drained of excess liquid on filter paper, and divided into 100 µl volume aliquots. Each aliquot contained approximately 1500 to 2000 nauplii. A similar procedure was used for the preparation of 100 µl aliquots of estuarine plankton that were collected during times of the year when larval *Menippe* are not present. Z1 larval *Menippe* were then added to these aliquots prior to DNA isolation. Negative control PCRs (without template DNA) were prepared in parallel with each set of PCRs to test for contamination of reagents and buffers.

RESULTS

Isolation and characterization of a middle repetitive sequence from *Menippe*

The nucleotide sequence of MACC36 did not resemble any published sequences and did not appear to encode a protein. A BLAST search (Altschul et al. 1990) of the GenBank database did not locate any sequences with statistically significant matches. The longest open reading frame found in conceptual translations of MACC36 was only 372 bp (base pairs). To examine the possibility that the sequence could be a retroviral element, conceptual translations of all reading frames were compared with known reverse transcriptase peptide sequences, which contain several highly conserved amino acid residues at specific positions (Finnegan 1989). These conserved residues were not found in any reading frame for MACC36.

Sequences from *Menippe adina* and *M. mercenaria* that are homologous to MACC36 were variable and did not exhibit species-specific characteristics. Fifteen individual TACC36 sequences from *M. adina* and *M. mercenaria* were amplified by PCR, cloned and compared by restriction mapping (Roberts 1995). All except 3 appeared to share at least 1 restriction site with others, but they varied in the number and position of individual sites as well as overall length. From one individual 8 distinct restriction maps were observed among 9 sequences, and from another individual all 4 sequences had distinct restriction maps. The isolation of more than 2 sequences from single individuals indicates that sequences homologous to MACC36 are present in multiple copies per genome. Seven of the TACC36 clones were partially sequenced from each end. Between 111 and 186 bp were obtained for each sequence. All were clearly homologous to MACC36. Divergence between sequences homologous to MACC36 and a consensus sequence ranged from 0.9 to 6.3%, and averaged 2.7%. In addition, up to 8 insertions/deletions (indels) were observed in comparisons with a consensus sequence, including up to 7 that would cause frameshifts within a coding region (Roberts 1995). There were no characteristics that could be used to distinguish TACC36 sequences isolated from *M. adina* from those isolated from *M. mercenaria*.

Estimates of the copy number of sequences homologous to MACC36 were obtained by quantification of the hybridization of a MACC36 probe to genomic DNA samples from *Menippe adina* and *M. mercenaria*. There was some variation in hybridization intensity among genomic DNA samples from different individuals, but replicate extractions were not performed to determine if this reflected actual variation in copy number. From a comparison of hybridization intensities of the genomic DNA samples with the MACC36 standards, we estimated the mass of DNA in each sample that consisted of sequences homologous to MACC36. These estimates were converted to estimates of copy number (Table 4) for a sequence 3.0 kb in length (Roberts 1995) and a haploid genome size of

Table 4. Copy number estimates of the MACC36 sequences for several genomic DNAs. Copy number of sequences homologous to MACC36 was estimated by hybridization of a non-radioactively labeled probe to *Eco*RI digested genomic DNA from *Menippe adina* and *M. mercenaria*. A Bio-Rad slot blot manifold (Bio-Rad 2000®) was used to apply genomic DNA samples and MACC36 standards to a nitrocellulose membrane. Three replicates of each sample were included to control for variation in hybridization intensity

Species	Ind.	Avg. copy no.	Range
Menippe adina	55	8300	6800–9600
M. mercenaria	43	12400	9800–14200
M. mercenaria	95	18500	18000–19000
M. mercenaria	96	15200	11400–18500
M. mercenaria	101	15400	14000–16400

2.7 pg (Rheinsmith et al. 1974). The average of these estimates was 9.3 × 10^4 copies.

Larval detection

The specificity of the primers 36F5 and 36R5 for *Menippe* was tested in PCRs with DNA from other crustacean species, including other representatives of the superfamily Xanthoidea and in plankton samples that did not include larval *Menippe*. None of these DNA samples produced amplification products (Table 2). This cannot be attributed to general unsuitability of the samples for PCR because LSRDNA sequences were successfully amplified from each DNA sample. While we cannot extrapolate these results to species that have not been tested, our results show that amplification products are not obtained from crustaceans in general, from several xanthoid species that are likely to occur with *Menippe*, or from a diverse array of organisms found in estuarine plankton samples.

The sensitivity of our PCR assay was first determined for genomic DNA isolated from adult specimens of *Menippe adina* and *M. mercenaria*, and purified by ultracentrifugation. Amplifications were consistently successful over a 10^4-fold range of DNA template concentrations, down to 10 pg of DNA per reaction (Table 3), which represents less DNA than would be present in 2 diploid cells. Furthermore, amplifications were successful over the same range when 10 ng of DNA from either *Artemia* or from mixed plankton samples was added to each reaction. Thus, even when present at 1000-fold greater concentration, the presence of non-target species DNA does not compromise the sensitivity of this assay.

DNA isolated from mixtures of plankton could differ substantially in quality from the purified DNA used in the experiments described above, and DNA quality could limit the sensitivity of a PCR-based assay. To investigate this possibility, we isolated total DNA from mixtures that included between 1 and 200 larval *Menippe* and a substantially greater biomass of either

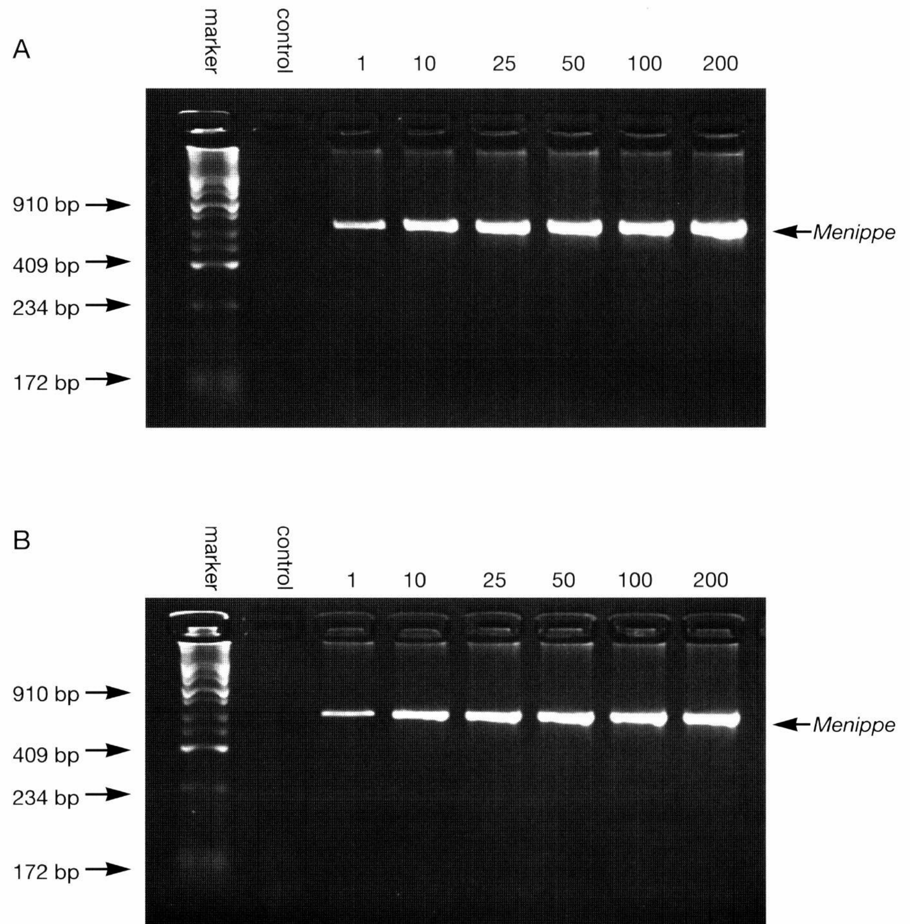

Fig. 1. Agarose gel electrophoresis of *Menippe*-specific PCR products. 100 µl aliquots of naupliar *Artemia* and estuarine plankton were prepared, and 1, 10, 25, 50, 100 or 200 first stage zoeal larvae from *Menippe* were added to these aliquots prior to DNA isolation. PCR products of target DNA from Menippe were identified for increasing numbers of zoeal larvae detected in the presence of (A) naupliar *Artemia* and (B) estuarine plankton. Lengths in base pairs are indicated for size standard fragments from pUC 18 digested with *Dde*III (Lane 1)

naupliar *Artemia* (between 1500 and 2000 nauplii), or field collected samples of estuarine plankton that contained assorted non-target species of crustacean larvae and other zooplankton. In every case, amplifications were successful (Fig. 1). These results demonstrate that the PCR assay is sensitive enough to detect even a single larval *Menippe* that is greatly diluted in a sample of mixed zooplankton.

DISCUSSION

Although middle repetitive DNA sequences have been characterized from many species, they are seldom used as phylogenetic or population genetic markers. Middle repetitive sequences have been of interest to evolutionary biologists primarily because many of them are transposable elements (Spradling & Rubin 1981, Georgiev et al. 1982). For example, about half of the middle repetitive DNA in the genome of *Drosophila melanogaster* is comprised of about 30 families of transposable elements (Spradling & Rubin 1981). Functional transposable elements may have distinctive characteristics, such as terminal repeats, and at least some elements must encode a transposase. We cannot determine if the family of sequences represented by MACC36 has terminal repeats because our sequence represents only a 2 kb portion of a canonical sequence that is at least 3 kb in length. However, the degree of variation and the presence of numerous indels found in comparisons of homologous TACC36 sequences suggest that these sequences do not encode functional proteins.

Middle repetitive sequences are of limited usefulness for phylogenetic inference because they are usually restricted to one or a few species and often exhibit unusual modes of evolution. However, the characteristics of the MACC36 family of sequences made it a useful target for the larval detection assay described here. The length and complexity of MACC36 allowed us to design PCR primers that appear to be specific for *Menippe* and result in the amplification of a product of convenient size. The sequence appears to be unconstrained by a protein coding function, which may explain the variation we observed between members of the sequence family and its absence from closely related taxa. The high copy number of this sequence allows it to be detected easily in amounts of genomic DNA that correspond to only a few cells. Under practical conditions, a single Z1 larva can be detected in a plankton sample.

It should be possible to isolate repetitive sequences that have properties similar to MACC36 for the detection of other planktonic species. In length and copy number, MACC36 is similar to a middle repetitive sequence isolated from the land crab *Gecarcinus lateralis* by Fowler et al. (1985). This sequence was found to have a length of 2.1 kb (LaMarca et al. 1981) and a copy number of approximately 1.6×10^4 per haploid genome (Skinner et al. 1982). Restriction analysis of independent clones showed similar restriction patterns in 70% of the sequences, but 30% of the sequences had multiple arrangements (Stringfellow et al. 1985). Like MACC36, this sequence exhibits length polymorphism, insertions and deletions, and point mutations. Highly repetitive sequences (those present at greater than 10^5 copies per genome) may generally be too short to be useful for taxon-specific detection. However, Bilodeau and co-workers (Bilodeau et al. 1999) identified a sequence present at 5×10^5 copies per genome that is approximately 600 bases in length, and appears to be specific for the grapsid crab genus *Sesarma*.

Because of their high abundance, repetitive sequences can be isolated from very small genomic libraries. Our experience suggests that 10 to 20 plasmids with insert sizes of 3 to 5 kb should be sufficient. Southern blot analysis (Southern 1975) can be used to both estimate copy number and roughly determine the size of the repeat. Some experimentation with the design of PCR primers may be useful; we found that the sensitivity of our assay differed considerably for different primer pairs (data not shown). In our experience, amplification products of about 500 bp are ideal for detection of a target sequence. They can usually be distinguished from non-specific amplification products, which are generally smaller, but are not so large as to be difficult to amplify from partially degraded DNA.

Detection of a specific genus or species by amplification of a diagnostic middle repetitive sequence is relatively inexpensive and efficient. DNA isolation, PCR amplification, and gel electrophoresis can be performed simultaneously on a large number of samples. Most significantly, with suitable controls for the specificity and reproducibility of the assay, additional characterization of the diagnostic PCR products should be unnecessary. In contrast, if DNA sequencing is used to determine whether a specific sequence is present in a mixture generated by PCR, it is necessary to first isolate single sequences by cloning and then sequence each clone individually (e.g. Fuhrman et al. 1994). Even techniques that employ DNA hybridization rather than sequencing to characterize LSRDNA, rDNA, and other sequences that are amplified with 'universal' primers require additional steps; these include the transfer of PCR products to a membrane, hybridization with a diagnostic probe, and visualization of the hybridization signal (Medeiros-Bergen et al. 1995).

The sensitivity of detection afforded by the use of repetitive sequences is unlikely to be matched by methods based on low copy number nuclear sequences or mitochondrial sequences. For example, successful PCR amplification of the mitochondrial small subunit ribosomal RNA gene from a variety of organisms typically requires at least 10 to 20 ng of genomic DNA (Palumbi et al. 1991), which is 1000-fold more DNA than was required for our assay. We have found that about 50 ng of genomic DNA can be isolated from a single Z1 larva of *Menippe* by routine methods. However, in a typical application only a small proportion of the total DNA isolated from a plankton sample is used as a template for each PCR. Thus, it is unlikely that a mitochondrial sequence would be detected from a single Z1 larva of *Menippe* present in a plankton sample.

We have demonstrated that larval *Menippe* can be detected with extreme sensitivity by PCR amplification of a repetitive sequence. It is our hope that this demonstration will stimulate further development of this approach to address significant problems in larval ecology. While an ideal assay would provide quantification as well as detection of planktonic species, detection alone would be sufficient for some applications. For example, the spread of an invading species could be monitored by detection of its arrival in new habitats or its occurrence in samples of ballast water. Detection could also be used to provide an efficient initial screen of plankton samples in which the species of interest occurs infrequently. After the species was detected, replicate samples could then be subjected to thorough analysis by microscopy or other methods.

Although detection of a planktonic species would be sufficient for some applications, it should also be possible to extend our approach to quantify the abundance or biomass of a target species. Rough estimates of larval density could be obtained by use of our detection assay to implement the 'most probable number' method, a method which is often used to estimate bacterial densities (Roser et al. 1987). Samples are progressively diluted to the point where no organisms are detected, and the dilution factor is then used to estimate the density of organisms in the initial sample. We are also investigating the use of competitive PCR (Wang et al. 1989) to estimate the biomass of *Menippe* in plankton samples. Competitive PCR includes an internal standard to control for variation in amplification efficiency, and thus addresses a common problem with field-collected samples.

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