qualities that made the product amenable for working with blood samples would be useful for whole organisms as well. We describe this modified technique and demonstrate its utility for PCR amplification of multiple loci from single harpacticoid copepods.

## Results and Discussion

Initially, PCR amplifications of template DNA isolated from pooled samples of 1 to 10 copepods following the manufacturer's protocol did not yield a satisfactory success rate (less than 10%). Template DNA yield was much more consistent when the protocol was modified as follows. A single copepod, (frozen at −80°C or preserved in 95% ethanol) was picked with an Irwin loop while viewed under a dissecting microscope directly into 10 μl of ice-cold PCR Buffer II (500 mM KCl, 100 mM Tris-HCl, pH 8.3; Perkin-Elmer Cetus) in a 500 μl microcentrifuge tube. The tube was centrifuged briefly, and 1 μl of a10 mg/ml proteinase K (PK) solution was added and mixed by pipetting. The tube was transferred to a water bath and incubated at 55°C for 3 hours (tubes were gently mixed every hour and tap-spun to collect the condensate). Following incubation, each tube was placed in a 100°C heating block for five minutes to ensure complete inactivation of the PK (we have found that this heating step is crucial for successful template isolation and subsequent PCR amplification). Subsequently, 10 μl of GeneReleaser was added to each extraction, and the GeneReleaser cycling protocol was run as per the manufacturer's instructions. Subsequently, each tube was centrifuged for one minute at 13,000 *g*, and the clear supernatant (approximately 20 μl) was transferred to a fresh 500 μl tube. Typically, for a 50-μl reaction, 2 μl of this supernatant was sufficient template to produce an easily visible product on a 1% agarose gel after 40 cycles of the PCR (50 mM KCl, 10 mM Tris-HCl, 2.5 mM $MgCl_2$, pH 8.3, 200 μM each dNTP, 5 pmol forward and reverse primer, and 1 unit *Taq* DNA polymerase, Perkin-Elmer Cetus).

Using this extraction method, we have been able to amplify successfully portions of the mitochondrial cytochrome oxidase subunit I gene (COI; forward and reverse primers were modified versions of those described in Folmer et al., 1994) from single specimens of the harpacticoid copepod *Microarthridion littorale* (Figure 1). Direct sequencing of the COI gene product from several individuals

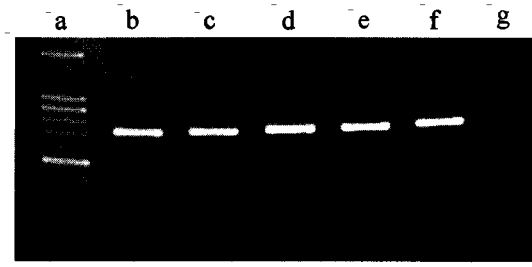


**Figure 1.** Cytochrome oxidase subunit I PCR products from individual *M. littorale* (using 2 μl template DNA from an approximately 20 μl PK-GeneReleaser extraction). Lane a, Commercial 100 bp ladder (Promega); lanes b–f, PCR products from individual copepods; lane g, Negative control.

and comparison with other sequences in GenBank (Release 100.0) indicated high homology between *M. littorale* COI and COI from the only other harpacticoid copepod in record, *Tigriopus californicus* (Burton and Lee, 1994). This protocol extends to other marine taxa and has been used to amplify the mitochondrial COI locus from other benthic taxa including copepod taxa other than *M. littorale*, polychaete juveniles, barnacles, and amphipods (N.V.S., unpublished data). These results can be generalized beyond the assay of mitochondrial genes to include nuclear gene loci. We have been able to amplify consistently the nuclear gene tubulin (primers were from Palumbi, 1996) from single specimens of *M. littorale* with success rates comparable to that observed with the mitochondrial COI locus (data not shown).

The advantages of this technique include the high rate of successful extraction and amplification from small animals and the ability to assay individual animals for multiple loci. We have performed over 500 PCR reactions using this method with a success rate of greater than 95%. We believe that this success rate can be partly attributed to the fact that precipitation (and associated losses) is unnecessary with our method. Indeed, only a single transfer of an aqueous phase is required, reducing the possibility of template loss and the chance of contamination among samples. Overall, we estimate that the PK-GeneReleaser method provides sufficient DNA template for 8 to 10 PCR amplifications from small metazoans such as *M. littorale*, facilitating the assay of multiple loci per individual. We believe this approach is well suited for isolating and amplifying DNA from a diverse assemblage of small, benthic organisms and might prove amenable to other larger, "problematic" taxa as well.

## Acknowledgments

We thank Lirong Shi for technical assistance and Joe Jones, Thomas Merritt, and Regina Wetzer and for valuable comments on earlier versions of the manuscript. This research was supported by National Science Foundation grant OCE-9402855 (J.M.Q.) and EPA grant G6J10429 (G.T.C., B.C.C., and J.M.Q.).

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