JOURNAL OF PLANKTON RESEARCH | VOLUME 33 | NUMBER 6 | PAGES 917–925 | 2011

DNA extraction, amplification and analysis of the 28S rRNA portion in sediment-buried copepod DNA in the Great Wall Bay and Xihu Lake, Antarctica

ZHI-HUAN XU[1], XIAO-DONG JIANG[2], GUI-ZHONG WANG[1]*, JIAN-FENG HE[2], MING-HONG CAI[2], LI-SHENG WU[1], JIE-LAN JIANG[1] AND XUE-LEI CHEN[1]

[1]DEPARTMENT OF OCEANOGRAPHY, STATE KEY LABORATORY OF MARINE ENVIRONMENTAL SCIENCE, XIAMEN UNIVERSITY, XIAMEN 361005, CHINA AND [2]KEY LABORATORY FOR POLAR SCIENCE OF STATE OCEANIC ADMINISTRATION, POLAR RESEARCH INSTITUTE OF CHINA, SHANGHAI 200136, CHINA

*CORRESPONDING AUTHOR: gzwang@xmu.edu.cn

Received July 18, 2010; accepted in principle December 9, 2010; accepted for publication December 11, 2010

Corresponding editor: Roger Harris

The Antarctic region, characterized by a constant low temperature, is viewed as an ideal place for protecting biomolecules. In this study, five different DNA extraction methods were used to analyze copepod DNA buried in Antarctic marine and lake sediments for potential studies on copepod distribution and composition in the past. After the comprehensive comparison of DNA extraction efficiency, purity of DNA extracts, time spent and cost per extraction, the E.Z.N.A.™ Soil DNA Kit was viewed as the most suitable DNA extraction method for studying sediment-buried copepod DNA in the polar area. Furthermore, the DNA extracts using this method were subjected to DNA cloning and sequencing. A homology tree based on a ~300-bp fragment of partial 28S rRNA was established, and two distinct groups were observed: the species *Boeckella poppei* dominated the lake group, but the marine group was more diverse with a similarity rate as low as 75% among some copepod species. The present study provided a suitable DNA extraction method for analyzing sediment-buried copepod DNA in Antarctica and also offered reliable results on the distribution of sediment-buried copepod DNA. The inferred information could be applied to reconstruct copepod communities in the past and assess the evolutionary processes involved.

KEYWORDS: DNA extraction; copepod DNA; sediment; Antarctica

INTRODUCTION

Copepods always dominate zooplankton communities and perhaps are the most numerous metazoans on earth, playing important roles in aquatic ecosystems and biogeochemical cycles. Studies on copepods have focused on the taxonomy, distribution, production, metabolism and roles of the larger copepod species in the pelagic food web (e.g. Hirche, 1983; Marcus and Boero, 1998; Zervoudaki *et al.*, 2007) in the last few decades. However, past community structure and species composition could not be well documented until

doi:10.1093/plankt/fbq165, available online at www.plankt.oxfordjournals.org. Advance Access publication January 11, 2011

the advent of PCR technology and the development of other molecular techniques (Pääbo *et al.*, 1989). With the help of molecular techniques, even a small amount of copepod DNA can be amplified and used for identifying species composition and distribution in sediments (Bissett *et al.*, 2005), DNA barcoding (Bucklin *et al.*, 1995, 2003; Caudill and Bucklin, 2004) and molecular systematic and phylogenetic assessments (Bucklin *et al.*, 2003; Machida *et al.*, 2006). So far, a larger number of publications have resulted from the studies of fossil DNA in sediments (e.g. Coolen and Overmann, 1998; Hofreiter *et al.*, 2001; Willerslev *et al.*, 2003, 2004, 2007; Coolen *et al.*, 2004), especially the study of Bissett *et al.* (Bissett *et al.*, 2005) who successfully amplified a small portion of copepod specific fragment from five Antarctic lakes and identified species composition in different sediment layers.

There is a lack of identifiable copepod morphological remains in sedimentary records, and so the analysis of fossil DNA seems to be the only way to include these environmental indicator species in the reconstruction of past marine and lake environments. With the development of molecular techniques, many biomarkers such as mitochondrial 16S rRNA (Braga *et al.*, 1999; Caudill and Bucklin, 2004; Zeng *et al.*, 2009b), nuclear small subunit 18S rRNA (Bucklin *et al.*, 2003), 28S rRNA (Braga *et al.*, 1999) and cytochrome c oxidase subunit I mitochondrial gene (Bucklin *et al.*, 2003; Machida *et al.*, 2006; Adamowicz *et al.*, 2007) are now widely applied to systematic assessments, and some of them are used for copepod phylogenetic studies and DNA barcoding. Bissett *et al.* (Bissett *et al.*, 2005) used a ~300-bp fragment of 28S rRNA to successfully identify species composition and distribution in Antarctic lake sediments and proved it to be efficient in identifying sediment-buried copepod DNA.

Antarctica is an ideal place for preserving ancient genes due to the constant low temperature (Willerslev *et al.*, 2004), and even small amounts of permafrost contain ancient plant DNA as old as 300–400 kyr (Willerslev *et al.*, 2003). Examining the past biotic diversity in benthic sediments is greatly facilitated using powerful molecular techniques (e.g. Limburg and Weider, 2002; Caudill and Bucklin, 2004; Coolen *et al.*, 2004; Yebra *et al.*, 2006; Coolen and Overmann, 2007; Rogers, 2007; Coolen *et al.*, 2009). Existing DNA extraction protocols facilitate genetic analysis of sediment microorganisms (Qu *et al.*, 2008; Zhang *et al.*, 2008, 2009a), although few reports directly mention DNA extraction methods which can be used to analyze sediment-buried copepod genes.

The aim of this study was to determine a valid and reliable sediment DNA extraction method. We compared DNA extraction efficiency, extracted DNA quality, extraction time spent and costs using five different sediment DNA extraction methods (Tsai and Olson, 1991; Coolen and Overmann, 1998; FastDNA™ Spin Kit for Soil, E.Z.N.A.™ Soil DNA Kit and PowerMax® Soil DNA Isolation Kit) for the surface and the 10-cm layers of sediment cores from Antarctic marine (Great Wall Bay) and lake (Xihu Lake) sediments. DNA extracted using the E.Z.N.A.™ Soil DNA Kit was chosen for further PCR amplification and DNA cloning. The distribution and species composition of the two habitats at different sediment depths were observed from the homology tree constructed based on the partial sequences of 28S rRNA, and the results were used to choose a suitable method for analyzing sediment-buried copepod DNA and recognizing the species composition and distribution in Antarctic sediments.

METHOD

Sediment collection

Sediments were collected from Great Wall Bay (S7) and Xihu Lake (S8) in the Antarctica Peninsula during the 22nd Chinese Antarctic Research Expedition in 2006. Xihu Lake is near the Antarctic Great Wall Station of China which is located in the Fildes Peninsula (Fig. 1). All sediment cores were sectioned into 1 cm segments, placed into sterile plastic bags and stored at 4°C during the 1-week transportation, then transferred to −20°C refrigerator for longer storage until analysis. A 12-cm

Fig. 1. Map showing sampling sites.

sediment core (marked S7-2) from Great Wall Bay and a 10-cm sediment core (marked S8-1) from Xihu Lake were chosen for this study. The top 2-cm layers of each sediment core were mixed well to represent the surface layers of S7-2 and S8-1 and were marked S7-2-1 and S8-1-1. Similarly, the 9- and 10-cm depth layers from S7 and S8 were mixed well to represent the 10-cm depth samples (and marked S7-2-10 and S8-1-10).

DNA extraction from the sediments

Five DNA extraction methods, including three commercially available sediment DNA extraction kits and two manual sediment DNA extraction protocols, were used in the experiment. Blank controls, which used sterile water instead of sediment samples, were included in each method during the DNA extraction procedures to confirm the absence of foreign DNA contamination.

DNA extraction protocols for the three commercial soil DNA extraction kits: i.e. the FastDNA™ Spin Kit for Soil (MP Biomedicals, USA); the E.Z.N.A.™ Soil DNA Kit (Omega Biotek, USA) and the PowerMax® Soil DNA Isolation Kit (MoBio Laboratories, USA), followed the instructions for each kit. For the E.Z.N.A.™ Soil DNA Kit and the FastDNA™ Spin Kit for soil methods, 0.5 g of sediment was used, and for the PowerMax® Soil DNA Isolation Kit 5 g; and the final DNA extracts were dissolved in 50 μL, 80 μL and 5 mL for these respective methods. In addition, DNA extracts from the PowerMax® Soil DNA Isolation Kit were concentrated in a final volume of 500 μL according to the kit's instructions. All DNA extracts from the three kits were stored at −20°C for further analysis.

Methods for the two manual sediment DNA extractions followed the extraction procedures of Tsai and Olson (Tsai and Olson, 1991) and Coolen and Overmann (Coolen and Overmann, 1998), except that the first step of the latter method used 2 g of sediment instead of 5. The DNA extracted was dissolved in 1 × TE (pH = 8.0) to a final volume of 80 μL and stored at −20°C until analysis.

The DNA extracted and blank controls were subjected to 1% agarose gel electrophoresis (6 V/cm). The gel was stained with ethidium bromide and visualized under UV in a transilluminator (Bio-Rad, USA).

DNA assessment

The DNA extracted was subjected to DNA concentration assessment using a Quant-iT™ Picogreen® dsDNA kit (Invitrogen, USA), and a high-range DNA standard curve was set according to the kit's instruction. Each DNA sample was diluted by 200-fold in 10 mM Tris−HCl, 1 mM EDTA, pH 7.5 (TE), to a final volume of 1 mL and then added to a 1 mL Quant-iT™ PicoGreen® dsDNA working solution. The mixture was cultivated at room temperature for 5 min followed by further fluorescence assessment. The samples were excited at 480 nm and the fluorescence emission intensity was measured at 520 nm using a spectrofluorometer of Genios-DNA (Tecan, Sweden). DNA concentration assessment was performed twice for each DNA extract. The DNA concentration was expressed as the mean ± standard deviation. The differences among methods, sediment types and depths were tested by the three-way ANOVA followed by the *post hoc* Duncan's test using SPSS version 16.0. The data from the E.Z.N.A.™ Soil DNA Kit were tested by the two-way ANOVA on the effects of different sediment types and depths.

The 260/280 ratio of the extracted DNA was assessed using the NanoDrop® ND-1000 (NanoDrop Technologies, USA) and 1 μL of extracted DNA was loaded for each DNA extract.

PCR amplification

The DNA extracted and the blank controls were subjected to nested PCR which amplified a portion of the 28S rRNA gene using primers F63 (GCATATC AATAAGCGGAGGAAAAG) and R635 (GGTCCGT GTTTCAAGACGG), followed by CopF2 (TGTGTGG TGGTAAACGGAG) and CopR1 (CCGCCGACCTAC TCG). These primers amplified a portion of the D domain of the nuclear large subunit rDNA (Wuyts *et al.*, 2001) from three orders of calanoid, cyclopoid and harpacticoid copepods. Thermocycle conditions for the F63-R635 PCR were as follows: at 94°C for 4 min; 18 cycles at 94°C for 30 s, 62°C for 45 s (decreasing by 0.5°C each cycle) and 72°C for 60 s; 10 cycles at 94°C for 30 s, 52°C for 30 s and 72°C for 60 s; and a final 72°C extension for 4 min. For CopF2-CopR1 PCR, the thermocycler conditions were as follows: at 94°C for 60 s; 29 cycles at 94°C for 5 s, 61°C for 20 s and 72°C for 30 s; and a final 72°C extension step for 10 min (Bissett *et al.*, 2005). PCR products were running in 1.5% agarose gel (6 V/cm) with ethidium bromide staining. The gel was visualized under UV in a transilluminator (Bio-Rad, USA).

DNA cloning and sequencing

Nested PCR products from the E.Z.N.A.™ Soil DNA Kit method were used for further DNA cloning and sequencing. The PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega,

USA), and purified PCR products were ligated into the pMD18-T vector (Takara, Japan) at 16°C for half an hour and then transformed into DH5α competent cells, followed by incubating on ice for half an hour, 2 min at 42°C and 2 min on ice. Next, 890 μL of LB liquid was added to each tube and they were incubated at 37°C and 2 g for an hour. Transformants were screened using LB plates containing 100 μg/mL of ampicillin. PCR was introduced to analyze positive transformants directly. Five positive clones were picked from each plate for further sequencing. Positive clones were sequenced with M13 forward and reverse primers using a 3730 × l 96-capillary DNA analyzer (ABI, USA).

RESULTS

Most of the DNA extracts from the five extraction methods formed highlight DNA strips mainly around 23.1 kb except for the S7-2-10 sample (Fig. 2). Owing to cautious manipulation during the extraction procedures, no DNA fragments were detected in the five blank controls (Fig. 2). Among the three commercial DNA extraction kits, DNA strips from S7-2 were much brighter than S8-1, especially for the PowerMax® Soil DNA Isolation Kit, which obtained the brightest DNA strip in sample S7-2-1 (Fig. 2). Small DNA fragments were observed in nearly all DNA extracts, which covered a range from 23.1 kb to 564 bp, and some of them were even smaller than 564 bp (Fig. 2).

The DNA concentration varied significantly among the five methods ($F_{4,33} = 7.029$; $P < 0.001$) and two sediment types ($F_{1,33} = 25.191$; $P < 0.001$), but there was no significant difference between the two depths ($F_{1,33} = 3.201$; $P = 0.083$) tested by the three-way ANOVA. Moreover, the mean DNA concentration of the two depths and two sediment types using the E.Z.N.A.™ Soil DNA Kit was significantly higher than the method of FastDNA™ Spin Kit for Soil and two manual DNA extraction methods (*post hoc* Duncan's test, $P < 0.05$) but was not significantly higher than the PowerMax® Soil DNA Isolation Kit (*post hoc* Duncan's test, $P = 0.341$). Since the three-way ANOVA followed by *post hoc* Duncan's test indicated that E.Z.N.A.™ Soil DNA Kit had the best extraction efficiency, the effects of different sediment depths and types have been further tested in this treatment (the two-way ANOVA): the DNA concentration from the marine sediment (S7-2) was significantly higher ($F_{1,5} = 44.286$; $P = 0.001$) than from lake sediment (S8-1) and the top sediment samples (S7-2-1 and S8-1-1) contained relatively higher DNA concentrations than those from deeper sediment samples (S7-2-10 and S8-1-10) but the difference was not significant ($F_{1,5} = 0.214$; $P = 0.663$). The 260/280 ratios shown by the three commercial soil DNA extraction kits were much higher than those of the two manual DNA extraction methods (Table I).

Nested PCR amplification was tested on each DNA extract and blank control. A ~300-bp DNA fragment of partial 28S rRNA was detected from all samples except the blank controls. Purified PCR products from the E.Z.N.A.™ Soil DNA Kit method were used for DNA cloning. In all, 17 sequences were obtained from 20 clones; others (two clones from S8-1-10 and one clone from S7-2-1) had no sequencing signal. These 17 sequences were used to construct a homology tree with five reported copepod portion 28S rRNA sequences from GenBank. Two distinct groups were observed: one major clade was a marine group (constituting of sequences from S7-2) and another lake group was composed of sequences from S8-1 (Fig. 3). In the lake group, species composition was simple and mainly dominated by *Boeckella poppei*, and the inner group similarity rate was as high as 98% (Fig. 3). Most sequences

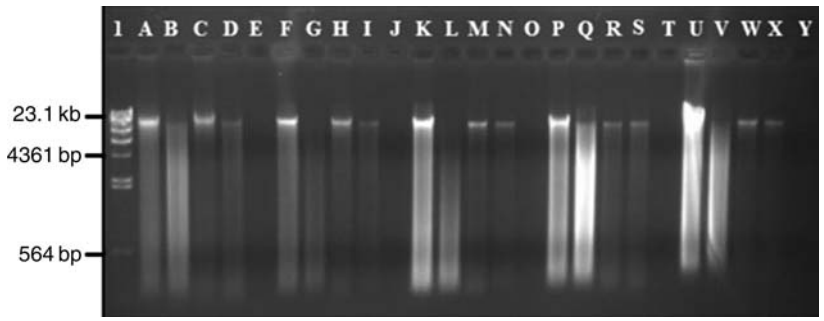

Fig. 2. Agarose gel electrophoresis of the total DNA from five extraction methods. Lane 1: lambda DNA/HindIII (MBI Fermentas). Lanes A–E: S7-2-1, S7-2-10, S8-1-1, S8-1-10, blank control (Coolen and Overmann, 1998). Lanes F–J: S7-2-1, S7-2-10, S8-1-1, S8-1-10, blank control (Tsai and Olson, 1991). Lanes K–O: S7-2-1, S7-2-10, S8-1-1, S8-1-10, blank control (FastDNA™ Spin Kit for Soil). Lanes P–T: S7-2-1, S7-2-10, S8-1-1, S8-1-10, blank control (E.Z.N.A.™ Soil DNA Kit). Lanes U–Y: S7-2-1, S7-2-10, S8-1-1, S8-1-10, blank control (PowerMax® Soil DNA Isolation Kit).

Table I: Characterization of DNA extracted from S7-2 and S8-1 sediment cores

Sediment samples	DNA extraction methods	DNA concentration [μg/g(dry wt); mean ± SD]	260/280 ratios
S7-2-1	Coolen and Overmann (1998)	0.222 ± 0.014	1.25
	Tsai and Olson (1991)	0.282 ± 0.008	1.22
	FastDNA™ Spin Kit for Soil	8.185 ± 1.316	1.83
	E.Z.N.A.™ Soil DNA Kit	11.955 ± 4.060	1.87
	PowerMax® Soil DNA Isolation Kit	12.962 ± 1.274	1.85
S7-2-10	Coolen and Overmann (1998)	0.103 ± 0.030	1.23
	Tsai and Olson (1991)	0.084 ± 0.016	1.23
	FastDNA™ Spin Kit for Soil	1.902 ± 0.201	1.74
	E.Z.N.A.™ Soil DNA Kit	10.754 ± 2.646	1.86
	PowerMax® Soil DNA Isolation Kit	4.900 ± 1.159	1.82
S8-1-1	Coolen and Overmann (1998)	0.049 ± 0.008	1.44
	Tsai and Olson (1991)	0.136 ± 0.069	1.47
	FastDNA™ Spin Kit for Soil	0.804 ± 0.144	1.83
	E.Z.N.A.™ Soil DNA Kit	1.170 ± 2.646	1.79
	PowerMax® Soil DNA Isolation Kit	0.697 ± 0.203	1.66
S8-1-10	Coolen and Overmann (1998)	0.044 ± 0.008	1.36
	Tsai and Olson (1991)	0.089 ± 0.051	1.45
	FastDNA™ Spin Kit for Soil	0.526 ± 0.172	2.11
	E.Z.N.A.™ Soil DNA Kit	0.938 ± 0.041	1.88
	PowerMax® Soil DNA Isolation Kit	0.625 ± 0.029	1.76

[S7-2-1(1), S7-2-1(2), S7-2-1(3), S7-2-10(1), S7-2-10(3) and S7-2-10(5)] in the marine group had a similarity rate as high as 98%. However, the sequences from the marine group had low similarity rates (75–82%) with the three reported copepod species (GenBank accession numbers: EF460771, FJ967119 and AY997801; Fig. 3). The sequences from the surface layers and 10 cm depth layers shared a high similarity rate both in the marine and lake group.

DISCUSSION

We used five different DNA extraction methods to make a comparison of their extraction efficiency for copepod DNA contained in Antarctic marine and lake sediments since there was no ready method for extracting copepod genes directly from sediments, and since the complex composition of organisms in the sediments varied among different habitats (Gray and Elliott, 2009). On the basis of the total DNA amount extracted from sediment samples, the E.Z.N.A.™ Soil DNA Kit method exhibited the best extraction efficiency and obtained the highest mean DNA concentration among the five DNA extraction methods. Moreover, except for the PowerMax® Soil DNA Isolation Kit method, the mean DNA concentration obtained by the E.Z.N.A.™ Soil DNA Kit was significantly higher ($P < 0.001$) than the other methods. The great extraction efficiency of the three commercial DNA extraction kit was mainly attributed to the filter column in each kit. Although the filter columns in each kit were different, they all could "catch" the DNA tightly and efficiently, so that later the DNA could be washed from the filter columns with the elution buffers contained in each kit. Especially for the E.Z.N.A.™ Soil DNA Kit method, we balanced the filter column before we started the experiment based on the kit's instructions, and this improved the ability of the filter column to absorb DNA. This might result in the highest mean DNA concentration when using the E.Z.N.A.™ Soil DNA Kit.

The purity of DNA extracts is viewed as an important index of DNA quality. The 260/280 ratio is a widely used index for assessing the quality of extracted DNA, with the acceptance range being between 1.8 and 2.0, and 1.8 taken as "a rule of thumb" for DNA (Pinto *et al.*, 2009). However, because of the existence of inhibitors such as humic acids in the sediments (which have negative impacts on downstream PCR and DNA cloning protocols; Hales *et al.*, 1996) and also affect the ratio of 260/280) in our study, relatively the lower 260/280 ratios were observed using the two manual DNA extraction methods than when using the three commercial DNA extraction kits. In particular, the DNA extraction method of Coolen and Overmann (Coolen and Overmann, 1998) introduced a reagent called PVPP during its DNA extraction procedures. PVPP used to be viewed as efficient in removing inhibitors from sediments (Miller *et al.*, 1999), but Zhao *et al.* (Zhao *et al.*, 2003) found that when the amount of sediment is >0.5 g, the PVPP cannot perform well enough to remove most inhibitors. Bearing this latter finding in mind, the relatively low value of the 260/280 ratio, which was observed when using the Coolen and Overmann (Coolen and Overmann, 1998) method, was not so surprising. We believed that PVPP could remove inhibitors from sediments and help to improve DNA quality after further purification. The relatively low DNA concentration obtained using the Coolen and Overmann (Coolen and Overmann, 1998) method also affected the efficiency of the CsCl density centrifuge, and a narrow and dim strip was observed after centrifuging. In this case, DNA could not have been efficiently

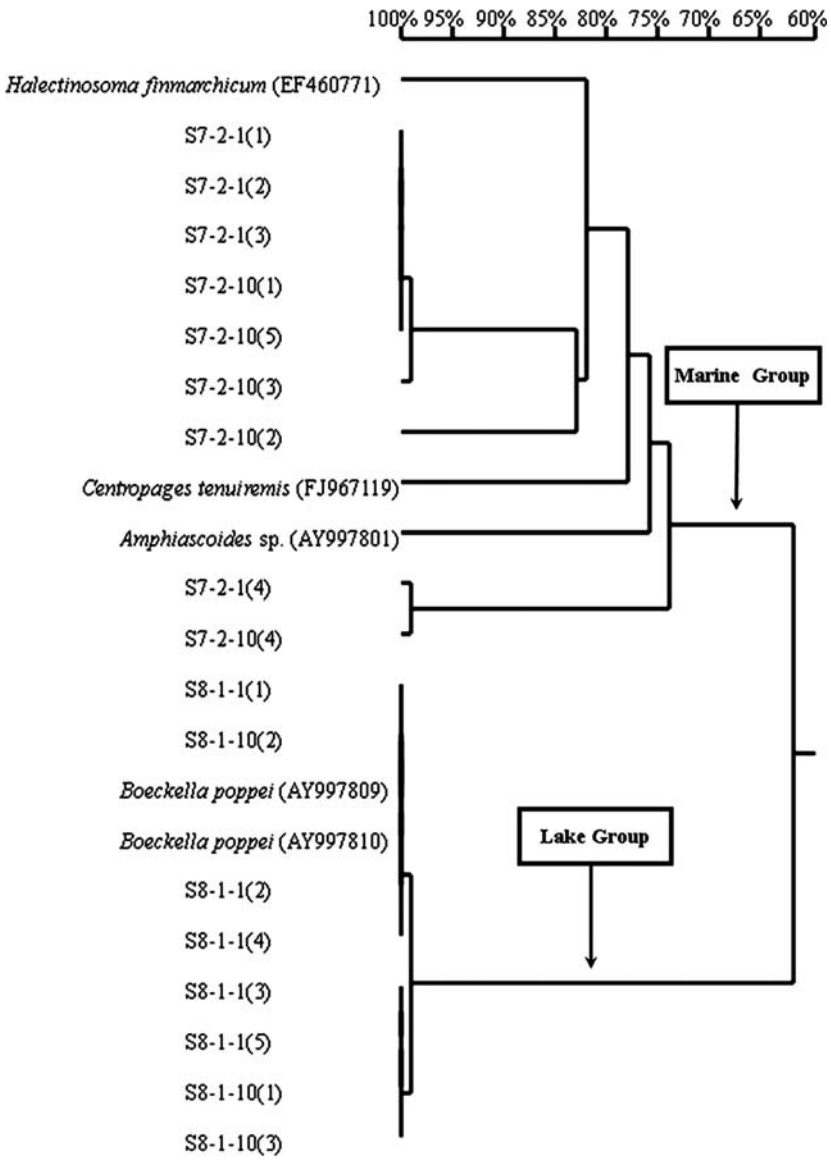

Fig. 3. A homology tree of the five sequences from GenBank and 17 sequences obtained in this study.

purified and recovered owing to the small amount of DNA. On the basis of our observations, we believed that the relatively low DNA concentration had a negative impact on the efficiency of the CsCl density centrifugation. The three commercial DNA extraction kits obtained high-purity DNA extracts, especially the E.Z.N.A.^{TM} Soil DNA Kit method, which had steady values of the 260/280 ratio near to 1.85.

DNA degrades with time (Haile *et al.*, 2007) resulting in higher concentrations of biomolecules in the upper sediment layers than the lower ones (Lindahl, 1993; Poinar *et al.*, 1996; Coolen and Overmann, 1998; Willerslev *et al.*, 2004; Coolen *et al.*, 2006). Even under ideal circumstance such as the constant low temperature in Antarctica, amplifiable DNA is only expected to survive for a maximum of 1 Myr (Willerslev and Cooper, 2005). The DNA degradation process was also observed in our study in that surface layers had relatively higher DNA concentrations than the 10-cm depth layers from S7-2 and S8-1, but the difference was not significant ($P = 0.083$), which may have been due to the short time-span of the 10-cm depth sediment samples. Moreover, DNA degradation can also be caused by spontaneous hydrolysis and oxidation, in which case small-size DNA fragments would appear (Pääbo, 1989; Handt *et al.*, 1994; Höss *et al.*, 1996). Thus, it was not surprising that we observed small DNA fragments in each DNA extract (Fig. 2). However, many more small

DNA fragments were observed from the three commercial soil DNA extraction kits than the two manual soil DNA extraction methods on the S7-2-1 and S7-2 10 sediment samples (Fig. 2). This may be due to the two manual soil DNA extraction methods preventing DNA fragmentation during the extraction, but the three commercial soil DNA kits used beads to homogenize the sediment samples before extraction really started. The contrived manipulations during the DNA extraction increase the possibility of DNA fragmentation which lead to much small DNA fragments being observed in DNA extracts from the three commercial soil DNA extraction kits.

Although DNA degradation occurred spontaneously and naturally, this is likely to be due to the existence of naturally fragmented DNA (Coolen and Overmann, 1998; Coolen *et al.*, 2006). However, constant low temperature seems to be important in prolonging the survival time of ancient DNA. In our study, DNA was successfully isolated from both the top and the deeper layers of S7-2 and S8-1 and the DNA extracts were PCR amplifiable for further analysis which suggests that the Antarctic may be an ideal place for analyzing ancient DNA and could provide a rich source of information on past environmental conditions (Coolen and Overmann, 2007; Haile *et al.*, 2007; Coolen *et al.*, 2009).

The amount of time taken is another important factor in manipulating DNA extraction procedures. The three commercial kits have rapid extraction procedures: the FastDNA Spin Kit for soil takes only half an hour and the E.Z.N.A.™ Soil DNA Kit and PowerMax® Soil DNA Isolation Kit methods take around 3 h; and the two manual procedures (Tsai and Olson, 1991; Coolen and Overmann, 1998) take even more time, especially the latter method of which takes nearly 110-fold the time of the FastDNA Spin Kit for soil method (Table II). Compared with the manual sediment DNA extraction methods (Ogram *et al.*, 1987; Tsai and Olson, 1991; Jacobsen and Rasmussen, 1992; Coolen and Overmann, 1998), the commercial DNA extraction kits used in our study had advantages in terms of the time spent on extraction. The cost for each method is also a consideration. The costs per extraction for the two manual extraction methods and for the E.Z.N.A.™ Soil DNA Kit method were much lower than those for the FastDNA spin kit for soil and the PowerMax® Soil DNA Isolation Kit methods (Table II). On the basis of a comprehensive comparison of DNA extraction efficiency, purity of DNA extracts, time spent and cost per extraction, we considered that the E.Z.N.A.™ Soil DNA Kit method was the most suitable one for analyzing sediment-buried copepod DNA in Antarctica. We applied the DNA extracts from this method for further DNA cloning and sequencing.

Table II: General evaluation of the five DNA extraction methods

Methods	Time-spending (hours)	Cost per extraction (USD)	Rank of extraction efficiency[a]
Coolen and Overmann (1998)	55	<1.5	5
Tsai and Olson (1991)	26	<1.5	4
FastDNA™ Spin Kit for Soil	0.5	9.3	3
E.Z.N.A.™ Soil DNA Kit	2.5	2.6	1
PowerMax Soil DNA Isolation Kit	3.5	32	2

[a]Extraction efficiency was scored 1–5 as a decrease trend using DNA concentration in Table I.

Sequence variations of the 28S rRNA target portion proved to be useful in identifying the distribution of copepod genes in the sediments. Our previous studies showed that if the differences in sequences from this 28S rRNA partial were larger than 2% (i.e. the similarity rate was lower than 98%), two different copepod species could be confirmed (authors' unpublished data). This is also confirmed in the work of Bissett *et al.* (Bissett *et al.*, 2005) which infers that sequences from the same species share as high as a 100% similarity rate in the neighbor-joining tree. In our study, two distinct groups were observed from the homology tree (Fig. 3), as well as obviously different species composition in the marine and lake sediments, which could be attributed to the distinct habitats of the copepods. In the lake group, *B. poppei* was the dominant species and species composition was simple. Furthermore, the lake group had only two subclades, and the inter subclade and inter clade similarity rate was above 98% (Fig. 3). Our results for this lake group were thus in accord with Bissett *et al.* (Bissett *et al.*, 2005) that most sequences from five different Antarctic lakes and various depths of sediment layers are closely associated with *B. poppei*. However, the marine clade (S7-2) had more subclades than the lake group and shared a relatively lower inter subclade similarity rate (Fig. 3), which suggests a much more diverse species composition in the Antarctic marine environment than in the lake environment. Bayly and Eslake (Bayly and Eslake, 1989) proposed that zooplankton is more diverse in a lake connected with the sea than in a lake cut off from the sea, and this may explain why copepods were more diverse in the marine (S7-2) than in the lake (S8-1) sediment in our study. The sequences from the surface and the 10-cm depth layers at given locations (S7-2 and S8-1) shared a

similarity rate as high as 98%, which indicated that the copepod community composition in both the marine and the lake environments were steady and balanced. We hypothesized that copepod communities in those two habits were rarely affected by predator pressure, biotic environmental changes or other changing factors.

Traditional taxonomic identification of copepod species usually involves recognizing complete structures and certain detectable specific characteristics. However, sediment-buried copepods appear in various forms and always lack obvious detectable characteristics and a complete structure. Compared with traditional taxonomic methods, molecular techniques resolved molecular systematic and phylogenetic relationships among the 17 sequences obtained from this study and five reported copepod sequences from GenBank. The result indicated the validity of 28S rRNA as a diagnostic biomarker in identifying species in the marine and the lake sediments of Antarctica.

In summary, this study has revealed the most suitable DNA extraction method (the E.Z.N.A.^{TM} Soil DNA Kit method) for studying sediment-buried copepod genes in Antarctic marine and lake sediments. To our knowledge, this is the first report of DNA extraction method comparison for analyzing sediment-buried copepod genes. By cloning and sequencing the ~300-bp fragment of 28S rRNA, we have shown that the general species composition and distribution of copepods in Antarctic marine and lake environments can be studied. We believe that this will be a new start to reconstruct past copepod diversity and distribution using molecular techniques.

ACKNOWLEDGEMENTS

We thank professor John Hodgkiss of The University of Hong Kong for his assistance with English edits.

FUNDING

This research was supported by the National Science Foundation of China (NSFC No. 40506002 and No. 40576065), and the Polar Science Foundation of China (HAIJKEF2006-39).

REFERENCES

Adamowicz, S. J., Menu-Marque, S., Hebert, P. D. N. *et al.* (2007) Molecular systematics and patterns of morphological evolution in the Centropagidae (Copepod: Calanoida) of Argentina. *Biol. J. Linn. Soc.*, **90**, 279–292.

Bayly, I. A. E. and Eslake, D. (1989) Vertical distributions of a planktonic harpacticoid and a calanoid (Copepoda) in a meromictic Antarctic lake. *Hydrobiologia*, **172**, 207–214.

Bissett, A., Gibson, J. A. E., Jarman, S. N. *et al.* (2005) Isolation, amplification, and identification of ancient copepod DNA from lake sediment. *Limnol. Oceanogr. Meth.*, **3**, 533–542.

Braga, E., Zardoya, R., Meyer, A. *et al.* (1999) Mitochondrial and nuclear rRNA based copepod phylogeny with emphasis on the Euchaetidae (Calanoida). *Mar. Biol.*, **133**, 79–90.

Bucklin, A., Frost, B. W. and Kocher, T. D. (1995) Molecular systematics of six *Calanus* and three *Metridia* species (Calanoida: Copepoda). *Mar. Biol.*, **121**, 655–664.

Bucklin, A., Frost, B. W., Bradford-Grieve, J. *et al.* (2003) Molecular systematic and phylogenetic assessment of 34 calanoid copepod species of the Calanidae and Clausocalanidae. *Mar. Biol.*, **142**, 333–343.

Caudill, C. C. and Bucklin, A. (2004) Molecular phylogeography and evolutionary history of the estuarine copepod, *Acartia tonsa*, on the Northwest Atlantic coast. *Hydrobiologia*, **511**, 91–102.

Coolen, M. J. L. and Overmann, J. (1998) Analysis of subfossil molecular remains of purple sulfur bacteria in a lake sediment. *Appl. Environ. Microbiol.*, **64**, 4513–4521.

Coolen, M. J. L. and Overmann, J. (2007) 217000-year-old DNA sequences of green sulfur bacteria in Mediterranean sapropels and their implications for the reconstruction of the paleoenvironment. *Environ. Microbiol.*, **9**, 238–249.

Coolen, M. J. L., Muyzer, G., Rijpstra, W. I. C. *et al.* (2004) Combined DNA and lipid analyses of sediments reveal changes in Holocene haptophyte and diatom populations in an Antarctic lake. *Earth Planet. Sci. Lett.*, **223**, 225–239.

Coolen, M. J. L., Boere, A., Abbas, B. *et al.* (2006) Ancient DNA derived from alkenone-biosynthesizing haptophytes and other algae in Holocene sediments from the Black Sea. *Paleoceanography*, **21**, PA1005. doi:10.1029/2005PA001188

Coolen, M. J. L., Saenz, J. P., Giosan, L. *et al.* (2009) DNA and lipid molecular stratigraphic records of haptophyte succession in the Black Sea during the Holocene. *Earth Planet. Sci. Lett.*, **284**, 610–621.

Gray, J. S. and Elliott, M. (2009) Chapter 4: diversity. In *Ecology of Marine Sediments: From Science to Management*, 2nd edn. Oxford University Press, New York, pp. 52–69.

Haile, J., Holdaway, R., Oliver, K. *et al.* (2007) Ancient DNA chronology within sediment deposits: are paleobiological reconstructions possible and is DNA leaching a factor? *Mol. Biol. Evol.*, **24**, 982–989.

Hales, B. A., Edwards, C., Ritchie, D. A. *et al.* (1996) Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis. *Appl. Environ. Microbiol.*, **62**, 668–675.

Handt, O., Höss, M., Krings, M. *et al.* (1994) Ancient DNA: methodological challenges. *Experientia*, **50**, 524–529.

Hirche, H. (1983) Overwintering of *Calanus finmarchicus* and *Calanus helgolandicus*. *Mar. Ecol.-Prog. Ser.*, **11**, 281–290.

Hofreiter, M., Jaenicke, V., Serre, S. *et al.* (2001) DNA sequences from multiple amplification reveal artifacts induced by cytosine deamination in ancient DNA. *Nucleic Acids Res.*, **29**, 4793–4799.

Höss, M., Jaruga, P., Zastawny, T. H. *et al.* (1996) DNA damage and DNA sequence retrieval from ancient tissues. *Nucleic Acids Res.*, **24**, 1304–1307.

Jacobsen, C. S. and Rasmussen, O. F. (1992) Development and application of a new method to extract bacterial DNA from soil based on separation of bacteria from soil with cation-exchange resin. *Appl. Environ. Microbiol.*, **58**, 2458–2462.

Limburg, P. A. and Weider, L. J. (2002) 'Ancient' DNA in the resting egg bank of a microcrustacean can serve as a palaeolimnological database. *Proc. R. Soc. Lond. Ser. B*, **269**, 281–287.

Lindahl, T. (1993) Instability and decay of the primary structure of DNA. *Nature*, **362**, 709–715.

Machida, R. J., Miya, M. U., Nishida, M. *et al.* (2006) Molecular phylogeny and evolution of the pelagic copepod genus *Neocalanus* (Crustacea: Copepoda). *Mar. Biol.*, **148**, 1071–1079.

Marcus, N. H. and Boero, F. (1998) Production and plankton community dynamics in coastal aquatic systems: the importance of benthic-pelagic coupling and the forgotten role of life cycles. *Limnol. Oceanogr.*, **43**, 763–768.

Miller, D. N., Bryant, J. E., Madsen, E. L. *et al.* (1999) Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. *Appl. Environ. Microbiol.*, **65**, 4715–4724.

Ogram, A., Sayler, G. S. and Barkay, T. (1987) The extraction and purification of microbial DNA from sediments. *J. Microbiol. Meth.*, **7**, 57–66.

Pääbo, S. (1989) Ancient DNA: extraction, characterization, molecular cloning and enzymatic amplification. *Proc. Natl Acad. Sci. USA*, **86**, 1939–1943.

Pääbo, S., Higuchi, R. G. and Wilson, A. C. (1989) Ancient DNA and the polymerase chain reaction. *J. Biol. Chem.*, **264**, 9709–9712.

Pinto, F. L., Thapper, A., Sontheim, W. *et al.* (2009) Analysis of current and alternative phenol based RNA extraction methodologies for cyanobacteria. *BMC Mol. Biol.*, **10**, 79.

Poinar, H. N., Höss, M., Bada, J. L. *et al.* (1996) Amino acid racemization and the preservation of ancient DNA. *Science*, **272**, 864–866.

Qu, J. H., Yuan, H. L., Wang, E. T. *et al.* (2008) Bacterial diversity in sediments of the eutrophic Guanting Reservoir, China, estimated by analyses of 16S rDNA sequences. *Biodiv. Conserv.*, **17**, 1667–1683.

Rogers, A. D. (2007) Evolution and biodiversity of Antarctic organisms: a molecular perspective. *Phil. Trans. R. Soc. Lond. B*, **362**, 2191–2214.

Tsai, Y. L. and Olson, B. H. (1991) Rapid method for direct extraction of DNA from soil and sediments. *Appl. Environ. Microbiol.*, **57**, 1070–1074.

Wuyts, J., De Rijk, P., Van de Peer, Y. *et al.* (2001) The European large subunit ribosomal RNA database. *Nucleic Acids Res.*, **29**, 175–177.

Willerslev, E. and Cooper, A. (2005) Ancient DNA. *Proc. R. Soc. Lond. Ser. B*, **272**, 3–16.

Willerslev, E., Hansen, A. J., Binladen, J. *et al.* (2003) Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science*, **300**, 791–795.

Willerslev, E., Hansen, A. J. and Poinar, H. N. (2004) Isolation of nucleic acids and cultures from fossil ice and permafrost. *Trends Ecol. Evol.*, **19**, 141–147.

Willerslev, E., Cappellini, E., Boomsma, W. *et al.* (2007) Ancient biomolecules from deep ice cores reveal forested southern Greenland. *Science*, **317**, 111–114.

Yebra, L., Hirst, A. G. and Hernández-León, S. (2006) Assessment of *Calanus finmarchicus* growth and dormancy using the aminoacyl-tRNA synthetases method. *J. Plankton Res.*, **28**, 1191–1198.

Zeng, J., Yang, L. Y., Li, J. Y. *et al.* (2009a) Vertical distribution of bacterial community structure in the sediments of two eutrophic lakes revealed by denaturing gradient gel electrophoresis (DGGE) and multivariate analysis techniques. *World J. Microbiol. Biotechnol.*, **25**, 225–233.

Zeng, Y. X., Zheng, T. L. and Li, H. R. (2009b) Community composition of the marine bacterioplankton in Kongsfjorden (Spitsbergen) as revealed by 16S rRNA gene analysis. *Polar Biol.*, **32**, 1447–1460.

Zervoudaki, S., Christon, E. D., Nielsen, T. G. *et al.* (2007) The importance of small-sized copepods in a frontal area of the Aegean Sea. *J. Plankton Res.*, **29**, 317–338.

Zhang, W., Song, L. S., Ki, J. S. *et al.* (2008) Microbial diversity in polluted harbor sediments II: Sulfate-reducing bacterial community assessment using terminal restriction fragment length polymorphism and clone library of *dsrAB* gene. *Est. Coast Shelf Sci.*, **76**, 682–691.

Zhao, J., Zhang, R., Lin, N. W. *et al.* (2003) Small-scale DNA extraction from deep sea sediment and application. *Oceanol. Limnol. Sinica*, **34**, 313–321 (in Chinese with English abstract).