Antarctic Science 16 (1): 29–36(2004) © Antarctic Science Ltd Printed in the UK DOI: 10.1017/S0954102004001786

Molecular phylogeny and divergence time of the Antarctic sea urchin (*Sterechinus neumayeri*) in relation to the South American sea urchins

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Abstract: *Sterechinus neumayeri* is an abundant regular sea urchin that lives in the shallow Antarctic waters. This organism has been used as a model system in many fields of the Antarctic biology. Yet, understanding of the evolutionary identity of the species, such as its phylogenetic relationships and divergence time, remains limited. Here, we reconstructed the molecular phylogenies of the species together with two sea urchin species in southernmost South America (*Loxechinus albus* and *Pseudechinus magellanicus*), a parechinid species (*Paracentrotus lividus*) and three strongylocentrotid species (*Strongylocentrotus purpuratus*, *S. intermedius*, and *Hemicentrotus pulcherrimus*) using mitochondrial DNA sequences of 12S rDNA-tRNA(gln) region (877nt) and cytochrome oxidase subunit I (COI, 1079nt). The rate of sequence evolution and the divergence time of the species were then estimated from the trees. The phylogenetic trees reveal that *S. neumayeri* is a sister group to the lineage of *L. albus* and *P. lividus*, and separated from the lineage 24–35 million years ago (m.y.a.). The divergence between *S. neumayeri* and *L. albus* coincides with the separation of Antarctica from South America, suggesting that the tectonic event must have provoked the cladogenesis of the species through vicariance.

Received 4 June 2003, accepted 3 October 2003

Key words: classification, evolution, *Loxechinus albus*, *Pseudechinus magellanicus*, vicariance

Introduction

The Antarctic sea urchin, *Sterechinus neumayeri*, is the most abundant regular sea urchin in shallow Antarctic waters (Brey *et al*. 1995, Sahade *et al*. 1998) and plays an important role in the benthic trophic web of McMurdo Sound (Brey *et al.* 1995). The species shows circum-Antarctic distribution down to about 400 m water depth and also occurs at the South Sandwich Islands and South Georgia (Mortensen 1936, Pawson 1969, Brey & Gutt 1991). Because of its abundance, wide distribution and easy availability, this organism has been used as a model system in many fields of the Antarctic biology such as reproductive biology (Pearse & Giese 1966), embryology (Bosch *et al.* 1987, Pearse *et al.* 1991), ecology (McClintock 1994, Brey *et al.* 1995, Amsler *et al.* 1999, Brockington *et al.* 2001), physiology (Marsh *et al.* 2001), and toxicology (King & Riddle 2001). Yet, little is known about the evolutionary identity of the species, such as its phylogenetic relationships and when it diverged from its closest relatives.

There are five or six species identifiable in the genus *Sterechinus*, *S. agassizii*, *S. antarcticus*, *S. diadema*, *S. dentifer*, *S. neumayeri* and *S. bernasconiae*, all of which inhabit the Antarctic and sub-Antarctic regions (Mortensen 1936, Pawson 1969, Larrain 1975). Mortensen (1936) considered that *S. antarcticus*, *S. agassizii*, and *S. diadema* are very closely related and represent only local phenotypes of one species, and that *S. neumayeri* is a distinct species. However, putative hybrids between *S. neumayeri* and *S. antarcticus* (or *S. agassizii*), which are difficult to identify as either of the species, are also found (Mortensen 1936).

For the study of evolution of the Antarctic sea urchins, the sub-Antarctic sea urchins in southernmost South America should be considered because it is the continent from which Antarctica was separated last (Lawver *et al*. 1992, Crame 1999). There are two echinoid species, *L. albus* and *Pseudechinus magellanicus*, abundant in shallow waters of the Magellan region. Phylogenetic relationships of the two species with *S. neumayeri* are not yet established. Based on morphological characters, Mortensen (1936) and Larrain (1975, 1995) placed *L. albus* in Echinidae to which *S. neumayeri* belongs, but Smith (2003) placed it in Strongylocentrotidae (Table I). *Pseudechinus magellanicus* was assigned to Echinidae by Mortensen (1936), but later to Temnopleuridae by Larrain (1975, 1995) and Smith (2003). No studies have been done on the phylogeny of these echinoids based on molecular characters.

In the present study, an attempt was made to infer phylogenetic relationships between the Antarctic sea urchin, *S. neumayeri,* and the two southernmost South America sea urchins, *L. albus* and *P. magellanicus*, by reconstructing

Table I. Change of the family level classification for the species discussed in this paper

	Mortensen (1936)	Larrain (1975, 1995)	Smith (2003)[a]
Loxechinus albus (Molina, 1782)	Echinidae	Echinidae	Strongylocentrotidae
Pseudechinus magellanicus (Philippi, 1857)	Echinidae	Temnopleuridae	Temnopleuridae
Sterechinus neumayeri (Meissner, 1900)	Echinidae	Echinidae	Echinidae

[a] The echinoid directory, Natural History Museum of London (www.nhm.ac.uk/palaeontology/echinoids)

molecular phylogenies with mitochondrial DNA sequences of 12S rDNA-tRNA(gln) region (877nt) and COI gene (1079nt). The analysis included not only the three species but also a species of Parechinidae, *Paracentrotus lividus*, and three species of Strongylocentrotidae, *S. purpuratus*, *S. intermedius*, and *H. pulcherrimus*. The divergence time between the Antarctic and the South America sea urchins were then estimated from the phylogenetic trees after the rate of sequence evolution was calibrated using the time of separation between Parechinidae and Strongylocentrotidae (35–50 m.y.a.; Smith 1988) as a reference time frame.

Materials and methods

Samples and sequences

The Antarctic sea urchin, *S. neumayeri*, was sampled from Marian Cove, King George Island, South Shetland Islands in 15 m water depth. The two South America sea urchins, *L. albus* and *P. magellanicus*, were sampled from the Bahia Mansa, Magellan Strait in 10 m water depth. The voucher specimens are stored in Korea Ocean Research and Development Institute. DNA was isolated from the gonad tissue using the Qiagen blood and cell culture DNA kit (Hilden, Germany) according to the manufacturer's instructions. Two mitochondrial genes, 12S rDNA-tRNA(gln) region (877nt) and COI (1079nt), were amplified from the total genomic DNA using the polymerase chain reaction (PCR). PCR primers were designed from the conserved regions of the genes with reference to the complete mitochondrial DNA sequence of *S. purpuratus* (Jacobs *et al.* 1988). For the 12S rDNA-tRNA(gln) region, the forward primer was from the middle of 12S rDNA gene (L12S, 5'-AAACCAGGATTAGATACCC-3') and the reverse primer from the tRNA-gln gene (HtRNA(gln), 5'-GGAAAAAC GARGARCTTTGA-3'). This primer pair amplified the region containing the 3'-half of 12S rDNA, control region, and tRNAs-glu, -thr, -pro and -gln, that corresponds to positions 491–1330 of the sequence of *S. purpuratus* (Jacobs *et al.* 1988). Primer pairs of LCOI1490N (5'-TCTACAAACCACAARGAYATTGG-3') and HCOIN (5'-CCCATTGAAAGAACGTAGTGAAAGTG-3'), and LCOI1490ERCH (5'-ACACTATATTTGATTTTTGG-3') and HCOINERCH(5'-CGACTACGTAGTATG TGTCA-3') were used for the COI gene (positions 5809–6935 of the sequence of *S. purpuratus*; Jacobs *et al.* 1988).

PCR reactions were carried out in the T-gradient thermocycler (Whatman, Germany) using HotStar Taq DNA polymerase (Qiagen). Three or four reaction mixes of a sample were prepared and different annealing temperatures were applied to each reaction. The 50 µl of reaction mix was made of 5 µl 10XPCR buffer (Qiagen), 2.5 µl 10mM each dNTPs, 25 pmoles of both forward and reverse primers, 0.5 µg DNA, 2.5 unit Taq polymerase. After 10 min of initial heating at 95°C, amplification was done in 35 repetitions of a three-step cycle (denaturation, 95°C for 1 min; annealing, 48–55°C for 1min; extension, 72°C for 1.5 min). PCR products were then cloned into pCRII-TOPO vector using TOPO TA cloning kit (Invitrogene, Carlsbad, CA) and at least three clones were sequenced. The sequences newly obtained in the present study have been deposited in GenBank under accession numbers AY275548-53.

Other than the above sequences of the three species, sequences of *P. lividus* and *S. purpuratus* were obtained from the complete mitochondrial sequences (Cantatore *et al.* 1989, Jacobs *et al.* 1988; GenBank accession numbers, NC_001572 and NC_001453), respectively. The sequences of *S. intermedius* and *H. pulcherrimus* were obtained from Lee (2003) (12S rDNA-tRNA(gln), AF525769 and AF525768; COI, AF525455 and AF525453).

Phylogenetic analyses

The sequences were aligned using Clustal W in MacVector (version 6.5.3, Oxford Molecular). Indel sites in the aligned sequences of the two genes were ignored between the affected pairwise comparisons in the following phylogenetic analyses. Phylogenetic trees of 12S rDNA-tRNA(gln), COI and the combined sequences of the two genes were reconstructed by minimum evolution (ME), maximum parsimony (MP), maximum likelihood (ML) methods using PAUP* program (version 4.0b10; Swofford 1998) and also by the method of Bayesian inference using MRBAYES (Huelsenbeck & Ronquist 2001). *Pseudechinus magellanicus* was used as an interim outgroup considering its phylogenetic position in Temnopleuridae from morphological characters (Table I). The ME analysis of 12S rDNA-tRNA(gln) used Log-determinant distances among the sequences (Lake 1994, Lockhart *et al.* 1994) since the branch length of *S. purpuratus* was exceptionally long in this gene tree (relative rate test, $P < 0.01$; Tajima 1993). The ML analysis, a model-based method, was carried out after

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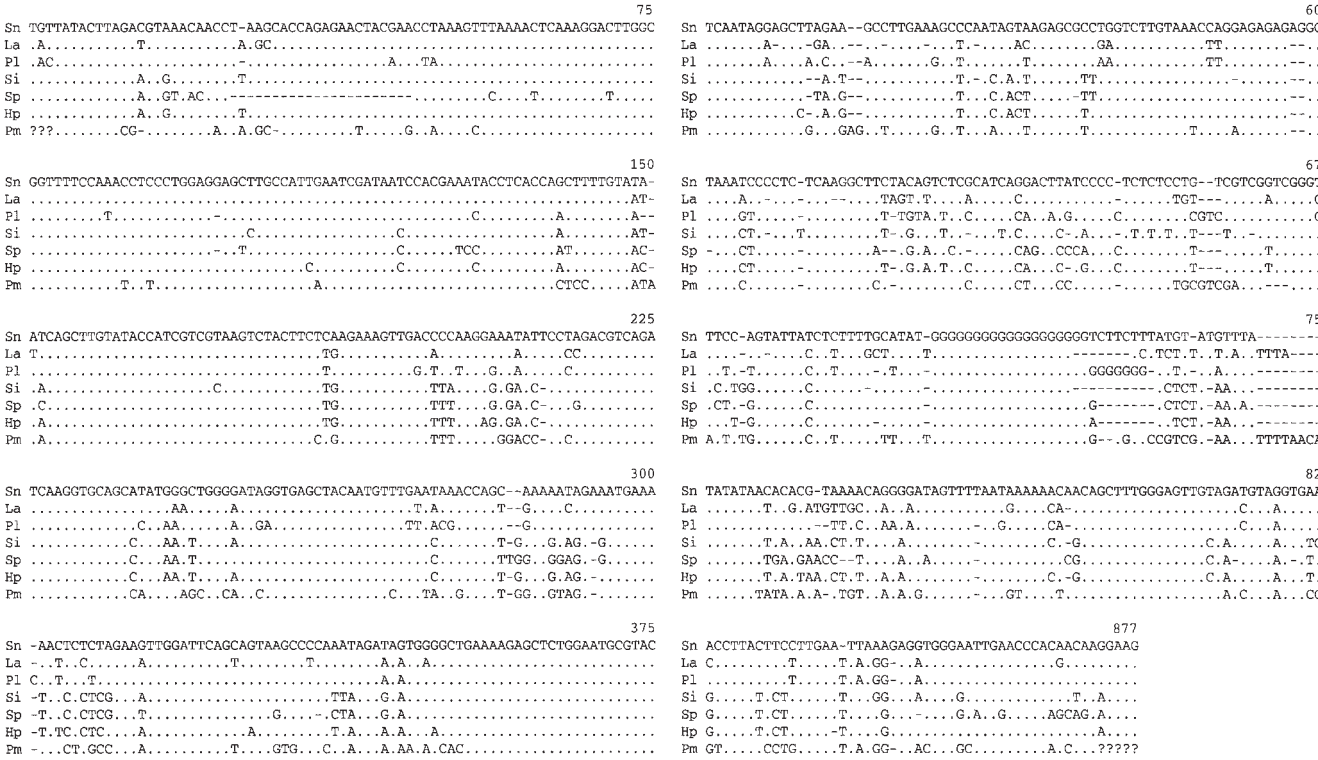

Fig. 1. Aligned DNA sequences of the 12S rDNA-tRNA(gln) regions from the Antarctic, *Sterechinus neumayeri* (Sn), and the South America, *Loxechinus albus* (La) and *Pseudechinus magellanicus* (Pm), sea urchin species as well as a species of Parechinidae, *Paracentrotus lividus* (Pl), and three species of Strongylocentrotidae, *Strongylocentrotus intermedius* (Si), *Strongylocentrotus purpuratus* (Sp) and *Hemicentrotus pulcherrimus* (Hp). Dots denote identical nucleotides to the first sequence and dashes are inserted for alignment. Question marks represent missing data.

an appropriate model of sequence evolution and its parameters were inferred from ModelTest (Posada & Crandall 1998). For 12S rDNA-tRNA(gln), ModelTest chose a TrN+G model with alpha = 0.3265 and relative frequencies of A-G change = 3.5864 and C-T change = 5.4571 (-lnL = 3036.6924). For COI sequences, ModelTest chose a TrN+G model with alpha = 0.1631 and relative frequencies of A-G change = 6.7478 and C-T change = 6.6927 (-lnL = 4214.1914). For the combined sequences of the two genes, 12S rDNA-tRNA(gln) and COI, a GTR+G model with parameters of alpha = 0.2392 and relative frequencies of A-C change = 3.1603, A-G = 9.1021, A-T = 4.1940, C-G = 0.6768, C-T = 16.0302, and G-T = 1.0 was chosen by ModelTest (-lnL = 7277.2354). In using PAUP*, exhaustive searches were performed for ML and MP analyses. For Bayesian analysis, the general time-reversible model (GTR) was applied and MRBAYES was run in 50,000 generations with four chains, sampling trees at every 10 generations. The likelihood scores had reached stationarity by 10 000 generations so that the first 1000

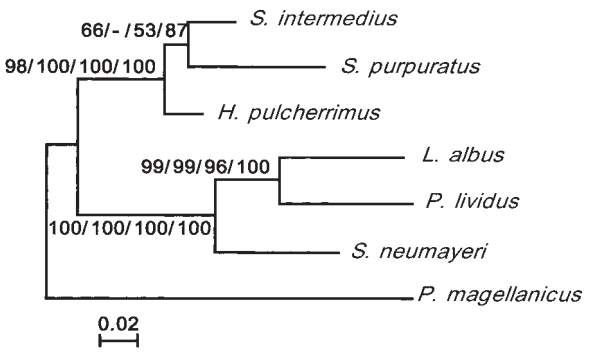

Fig. 2. The maximum likelihood (ML) tree of the 12S rDNA-tRNA(gln) sequences from the seven regular sea urchins. The phylogeny is reconstructed by PAUP* program (version 4.0b10; Swofford 1998). Parsimony analysis (MP; RCI = 0.55, ti/tv = 2.26), Bayesian analysis by MRBAYES program (BI; Huelsenbeck & Ronquist 2001), and the minimum evolution (ME) method based on LogDet distances identify the same topology tree as the ML tree. The branch support values are from ML, MP, and ME (LogDet distances) bootstrap analyses with 1000 repetitions and from BI posterior probabilities.

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sampled trees were discarded ("burnin" = 1000) and the parameters and a 50% majority consensus tree were obtained from the last 4000 trees. Robustness of each branch in the trees was evaluated by the bootstrapping method with 1000 replicates in ME, MP and ML analyses. Heuristic searches were carried out for bootstrapping.

Estimation of the rate of sequence evolution and the divergence time

Molecular clock-enforced ML trees were used to estimate the rate of sequence evolution and the divergence time of each species. Heterogeneity of evolutionary rates among the branches was checked by the log likelihood ratio test between the clock-enforced ML tree and the non-enforced ML tree. If the test failed, any taxa that showed exceptionally long or short branch lengths were excluded and new tests were carried out. *S. purpuratus* was excluded in the clock-enforced ML tree of the 12S rDNA-tRNA(gln) sequences because of its long branch. The rates of sequence evolution were calibrated by dividing the branch length to the midpoint between the strongylocentrotid species and *P. lividus* by a reference time of 35–50 m.y.a. for the split between these species (Smith 1988). Divergence time of each internal node in the trees was then estimated by dividing the branch length to that node with the calibrated evolutionary rate.

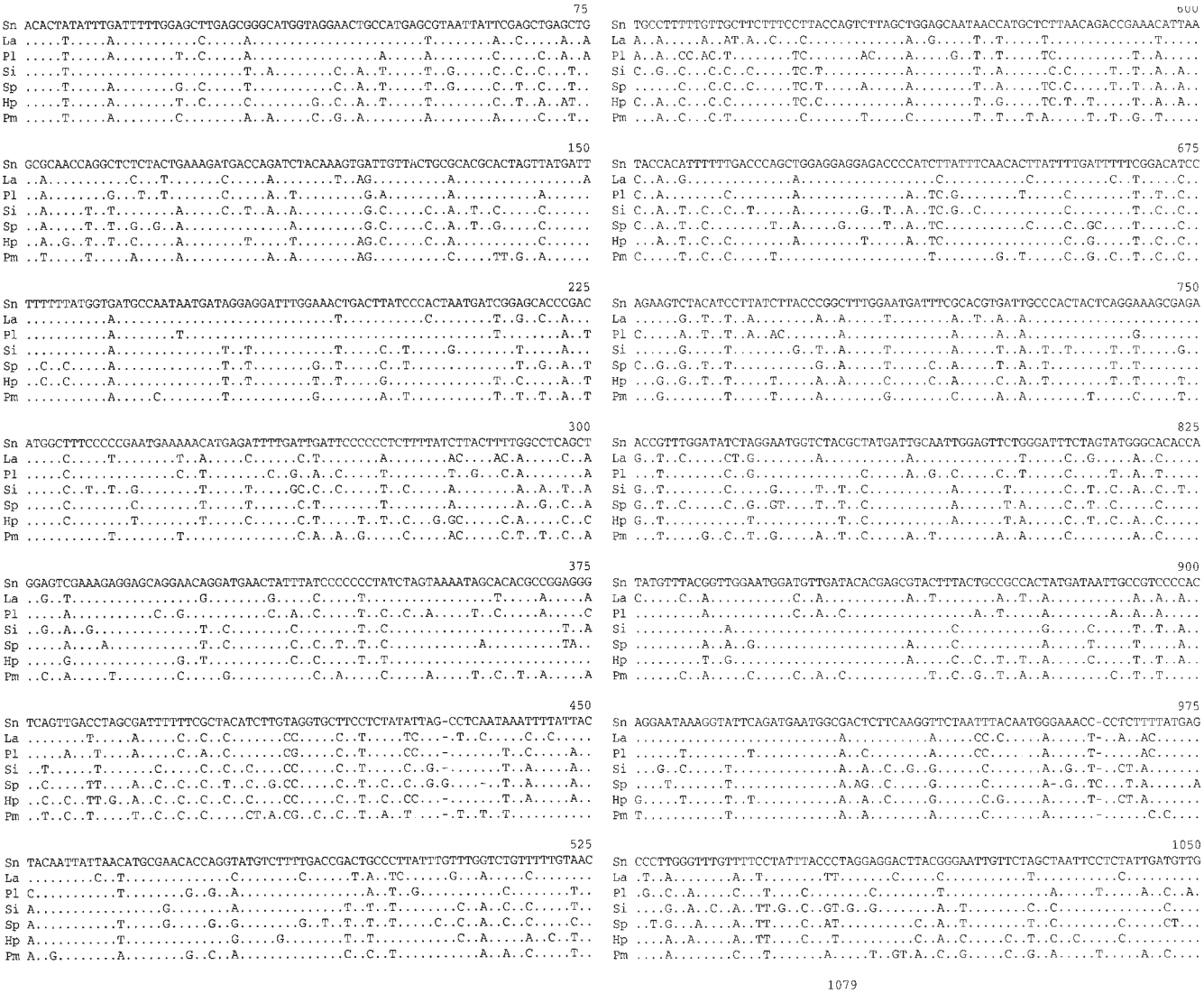

Fig. 3. Aligned DNA sequences of the COI gene from the sea urchins. Dots are for identical nucleotides and dashes for gaps, question marks for missing data. Sn = *S. neumayeri*, La = *Loxechinus albus*, Pl = *P. lividus*, Si = *S. intermedius*, Sp = *S. purpuratus*, Hp = *H. pulcherrimus*, Pm = *P. magellanicus*.

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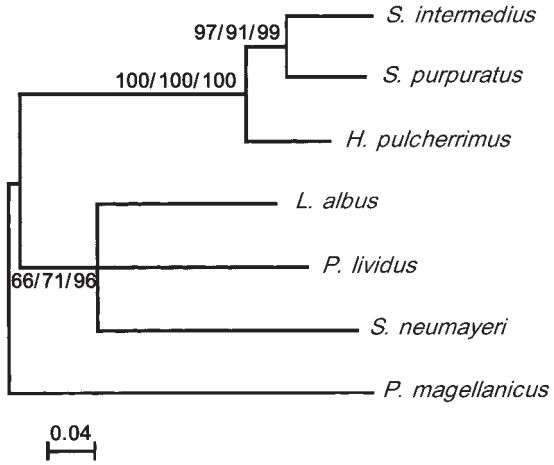

Fig. 4. The maximum likelihood (ML) tree of the COI sequences from the seven sea urchin species. MP (RCI = 0.39, ti/tv = 3.36) and BI analyses result in the same topology tree as the ML tree. The internal node for *L. albus*, *P. lividus*, *S. neumayeri* remains unresolved. The branch support values are from ML and MP bootstrap (1000 repetitions) and BI analyses.

Results

Phylogenetic relationships

The obtained DNA sequences of 12S rDNA-tRNA(gln) region aligned in 877 sites among the seven species, *S. neumayeri*, *L. albus*, *P. magellanicus, P. lividus, S. intermedius*, *H. pulcherrimus,* and *S. purpuratus* (Fig. 1). Of the 877 aligned nucleotide sites, 273 were variable and 128 were parsimony informative. Comparison of the sequences showed that there are dozens of indels, most of which are single or double nucleotide insertions or deletions. Maximum likelihood analysis (ML) of the sequences with the ModelTest parameters resulted in a tree that distinguishes two major clades, one consisting of the three strongylocentrotid species and the other of *L. albus*, *P. lividus*, and *S. neumayeri* (Fig. 2). *Pseudechinus magellanicus* comes as the most distantly related species from either of the two clades with the longest branch. In the latter clade, *L. albus* and *P. lividus* form the closest species pair and *S. neumayeri* comes as a sister taxon to this species pair (Fig. 2). MP (ti/tv = 2.26; RCI = 0.55) and ME (LogDet distances) analyses by PAUP* and Bayesian inference by MRBAYES all produced the same topology tree as the ML tree with similar branch lengths. The branching pattern of the tree other than the clade of *S. intermedius* and *S. purpuratus*, in which the branch of *S. purpuratus* is exceptionally long ($P < 0.01$, Fig. 2), is strongly supported by bootstrap values higher than 96% in ML, MP, and ME analyses and by Bayesian credibility values higher than 99% (Fig. 2).

The obtained COI sequences from the seven species aligned in 1079 sites (Fig. 3). Of the 1079 aligned nucleotide sites, 348 were variable and 219 were parsimony informative. There were only a couple of single nucleotide indels among the sequences. ML analysis of the sequences with the parameters chosen by Modeltest resulted in a tree that is congruent with the tree of 12S rDNA-tRNA(gln) (Fig. 4). The COI tree consists of two major clades, one of the three strongylocentrotid species and the other of *L. albus*, *P. lividus* and *S. neumayeri*, having *P. magellanicus* as the most distantly related species. In the latter clade, however, relationships among the three species are not resolved (Fig. 4). Both MP (ti/tv = 3.36; RCI = 0.39) analysis and Bayesian inference of the COI sequences also produced the same topology tree as the ML tree with similar branch lengths. In Bayesian analysis of the COI sequences, both site-specific rate estimation rendering the first two codon positions and the third codon position to have separate rate estimates and site-nonspecific rate estimation were performed, which resulted in the same tree with similar branch lengths as in Fig. 4. The clade of three strongylocentrotid species is strongly supported by bootstrap or Bayesian credibility values of 100% in all the three analyses and the other clade of *L. albus*, *P. lividus* and *S. neumayeri* is moderately supported (66–96%) although the relationships among the three species are not resolved any further (Fig. 4).

When the two sequences of 12S rDNA-tRNA(gln) and COI are combined and analysed together, the phylogenetic relationships among the seven species turns out to be (*P. magellanicus*, (*S. neumayeri*, (*L. albus*, *P. lividus*)), (*H. pulcherrimus*, (*S. intermedius*, *S. purpuratus*))), which constitutes the same tree topology as the tree of 12S rDNA-tRNA(gln) gene (Fig. 2). ML analysis of the sequences with the parameters chosen by Modeltest, MP analysis (ti/tv = 2.78; RCI = 0.43), and Bayesian inference all produced the same topology tree. Separation of the two major clades in these relationships, (*S. neumayeri*, (*L. albus*, *P. lividus*)) and (*H. pulcherrimus*, (*S. intermedius*, *S. purpuratus*)), are strongly supported by bootstrap and Bayesian credibility values of more than 99% in all the three analyses. The cluster of *L. albus* and *P. lividus* in the former clade is also well supported by more than 80% of bootstrap and posterior probability values.

Table II. Divergence time estimations for separation among the sea urchins (internal nodes) based on the mitochondrial gene genealogies (m.y.a.).

Internal nodes		12S rDNA-tRNA(gln)	COI	COI-12S rDNA-tRNA(gln)
Sn-La/Hp-Si	(A)[a]	35–50	35–50	35–50
Sn/Pl-La	(B)	24–34	24–34	25–35
Pl/La	(C)	18–26		21–30

Species names same as in Fig. 1.
[a] Labels of the internal nodes same as in Fig. 5.
(A) = the reference time point, 35–50 m.y.a., from the separation between Strongylocentrotidae and Parechinidae (Smith 1988).

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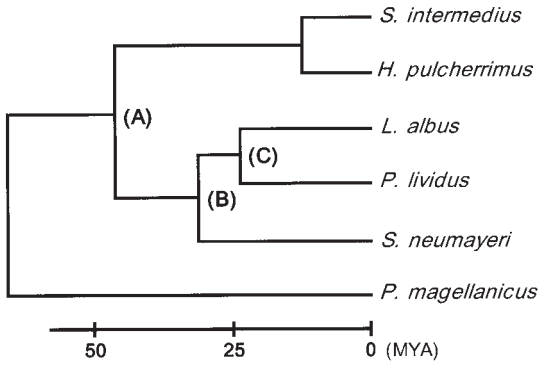

Fig. 5. The molecular clock-enforced maximum likelihood (ML) tree of the 12S rDNA-tRNA(gln) sequences. The sequence of *S. purpuratus* was not included in this tree because of its significantly long branch (relative rate test, $P < 0.01$). The scale bar represents the divergence time calibrated by the use of separation between Strongylocentrotidae and Parechinidae as the reference time point (35–50 m.y.a.; Smith 1988). The internal nodes for the Antarctic species and the South American species are labelled (A) through (C).

Divergence times

The molecular clock-enforced ML trees of 12S rDNA-tRNA(gln), COI, and the combined sequences of the two genes gave rise to similar estimations with each other for the divergence times of the Antarctic and the South America sea urchins (Table II). In the 12S rDNA-tRNA(gln) sequences, the assumption of clock-like evolution was rejected by the log likelihood ratio test when all the seven sequences were included. Without the sequence of *S. purpuratus* (which had a long branch), the six sequences passed the test of molecular clock assumption. The clock-enforced ML tree calibrates the rate of sequence evolution being 0.23–0.33% Myr^{-1} when the time of split between Strongylocentrotidae and Parechinidae, 35–50 m.y.a. (Smith 1988), is applied to the midpoint of the branch between *S. intermedius* and *P. lividus* (node A in Fig. 5). Based on this evolutionary rate, the time of separation between *S. neumayeri* and the lineage of *L. albus* and *P. lividus* is estimated to be 24–34 m.y.a. (node B, Table II and Fig. 5). Divergence time between the latter two species is then calculated to be 18–26 m.y.a. (node C). For the other South America sea urchin, *P. magellanicus*, the time of separation of the species from all the other species included in the present study is estimated to be 50–71 m.y.a. when the evolutionary rate of 12S rDNA-tRNA(gln) is applied by extrapolation to its branch.

The seven COI sequences passed the molecular clock assumption: difference between the likelihoods of the clock-enforced ML tree and the non-enforced ML tree (-lnL = 4214.1914 and 4216.0498) was not significant. From the clock-enforced ML tree, the rate of COI sequence evolution is calibrated to be 0.51–0.72% Myr^{-1} when the reference time is applied to the internal node of *S. intermedius* and *P. lividus* as was done in the 12S rDNA-tRNA(gln) tree. This evolutionary rate calculates the time of separation among the three species, *S. neumayeri*, *L. albus* and *P. lividus*, to be 24–34 m.y.a. (node B, Table II). The combined sequences of 12S rDNA-tRNA(gln) and COI passed the molecular clock assumption without the sequence of *S. purpuratus*: the likelihoods of the clock-enforced ML tree and the non-enforced ML tree are -lnL = 6722.2975 and –lnL = 6721.1599, respectively. The evolutionary rate of the sequences is calibrated to be 0.33–0.47% Myr^{-1} and the divergence time of *S. neumayeri* from the clade of *L. albus* and *P. lividus* is estimated to be 25–35 m.y.a. (Table II). The separation between the latter two species then seems to have occurred 21–30 m.y.a..

Discussion

The phylogenetic relationships between the Antarctic sea urchin, *S. neumayeri* and the southernmost South America sea urchins are presented in this study based on the analyses of the mitochondrial DNA sequences. Although it is assumed that *S. neumayeri* might have an evolutionary sister group in South America since Antarctica was separated from this continent last (Lawver *et al.* 1992, Crame 1999), no species has been identified unequivocally as a close relative of the genus *Sterechinus*. According to Larrain's classification (1977, 1995), *L. albus*, a regular sea urchin in the Magellan region, could be a sister group of *Sterechinus* since it belongs to the family Echinidae to which *Sterechinus* also belongs. However, such relationship is doubted by Smith (2003), who places *L. albus* in Strongylocentrotidae. The molecular phylogenies of 12S rDNA-tRNA(gln) and COI support the hypothesis that *L. albus* and *S. neumayeri* are closely related species: the two species together with *P. lividus* form a distinct clade clearly separated from the clade of strongylocentrotid species (Figs 2, 4 & 5). Another regular sea urchin in the Magellan region, *P. magellanicus*, turns out to be the most distantly related to any species of Echinidae, Parechinidae, and Strongylocentrotidae, in the molecular phylogenies. Such a relationship is consistent with its classification as a temnopleurid species based on morphological characters (Table I; Larrain 1975, 1995, Smith 2003).

The phylogenetic position of *P. lividus*, a parechinid species inhabiting the Mediterranean and the Atlantic shallow waters, is notable in that the species clusters with *L. albus* as a sister taxon, being located within the clade of family Echinidae (Figs 2 & 5). In fact, the monophyly of Parechinidae has been doubted and the morphological similarity between *P. lividus* and *L. albus* has been pointed out (Smith 2003), although the two species have been classified into two different families. It would be relevant to mention here that a Mediterranean–Atlantic euphausiid species, *Euphausia krohni*, is a sister taxon of two sub-Antarctic species, *E. longirostris* and *E. similis* in the

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molecular phylogeny of the combined sequences of 16SrRNA and NDI (Zane & Patarnello 2000). The estimates based on the molecular clock indicate the divergence time between *P. lividus* and *L. albus* to be 18–26 m.y.a. in the 12S rDNA-tRNA(gln) tree and 21–30 m.y.a. in the combined sequence tree of 12S rDNA-tRNA(gln) and COI (Table II). The divergence time suggests that the two species might have diverged shortly after the separation between the lineage of the two species and the Antarctic sea urchin *S. neumayeri*.

Divergence between *S. neumayeri* and *L. albus* at 24–35 m.y.a. (Table II) coincides with the opening of Drake Passage. It has been suggested that the seaway existed as a shallow connection as early as 37 m.y.a. or even earlier, and that it became a true deep-water connection with Polar Frontal Zone in the middle by the time 32.8 m.y.a. as South America and Antarctica continued to spread out (Crame 1999, Latimer & Filippelli 2002). The estimated time of divergence, therefore, implies that the common ancestor of the two species inhabited the shallow waters of Drake Passage at its early stage and then speciation occurred by vicariance as a result of the tectonic events in this region. Although sea urchin species have planktonic larvae, establishment of the Antarctic Polar Front (APF) after the complete opening of Drake Passage might have prevented interchange of larvae between populations on either side of the seaway. In addition, strong selection might have exerted on the population on the side of Antarctica because of cold environment, leading to speciation. Following this hypothesis, the current presence of *S. neumayeri* in South Georgia and in the South Sandwich Islands (Mortensen 1936, Pawson 1969) and the occurrence of its relative species, *S. agassizii* in South Georgia (Mortensen 1936) could be explained by evolution through dispersal from Antarctica northwards along the Scotia arc, consistent with suggested origins of the Southern Ocean fauna (Knox & Lowry 1977, Clarke & Crame 1989). In this sense, it is worth mentioning that Larrain (1975) reported the occurrence of another species of *Sterechinus*, *S. bernasconiae*, in the southern region of Chile and that examination of its phylogenetic relationship with *S. neumayeri* and *S. agassizii* would be a reasonable test for the above explanation.

There are other examples of speciation of the Antarctic marine fauna either by vicariance resulting from the opening of Drake Passage and formation of APF or by dispersal across APF. Molecular phylogenetic studies of 16S rRNA (Patarnello *et al.* 1996) indicated that two Antarctic species, *E. superba* and *E. crystallorophias,* are sister taxa which are distinctly separate from the sub-Antarctic species *E. vallentini*. The time of separation between the Antarctic and the sub-Antarctic species was estimated to be 19–20 m.y.a., suggesting their speciation by dispersal. Zane & Patarnello (2000) extended the study and suggested the possibility of euphausiid speciation by dispersal across APF in the case of divergence between *E. vallentini* and *E. frigida*. For the notothenioid fish, both vicariance and dispersal are attributed as factors contributing to speciation in explanation of species radiations in the Southern Ocean (Bargelloni *et al.* 2000). Divergence between *Eleginops maclovinus* and non-bovichtid notothenioids was explained by establishment of APF, and the sub-Antarctic distributions of *Dissostichus eleginoides*, *Patagonothen tessellata*, *Notothenia angustata*, and *Paranotothenia magellanica* were explained by dispersal across APF (Bargelloni *et al.* 2000). For speciation of the Southern Ocean bivalve *Limatula*, dispersal was invoked as a factor leading to divergence from the analyses of 18S rDNA, 16S rDNA and ITS-1 (Page & Linse 2002). A molecular study of the marine algae *Phaeocystis* with the 18S rDNA dated the time of split between warm- and cold-water taxa to be 25–50 m.y.a. and attributed their divergence to the opening of Drake Passage (Medlin *et al.* 1994).

The time of separation between *P. magellanicus* and the other sea urchin species could be estimated from the molecular phylogeny of 12S rDNA-tRNA(gln) (Fig. 2), if extrapolation of the rate of sequence evolution is applied to the midpoint of the branch to *P. magellanicus* beyond the reference time point. Such extrapolation resulted in 50–71 m.y.a. for the divergence of the suborder Echinina from the suborder Temnopleurina. This estimation is close to the proposition of Smith (1988) for this split, 45–65 m.y.a., from fossil records of the echinoid sea urchins (fig. 3 in Smith 1988).

The results of this study provide information on the time and the lineage of evolution of *S. neumayeri*, the most abundant sea urchin in the shallow Antarctic waters. The species appears to have diverged from the lineage of a southernmost South America sea urchin by vicariance as Antarctica and South America separated. There are five or six species identified in the genus *Sterechinus* although inter-species hybrids are found. Phylogenetic relationships among these species and the speciation factors for their radiation in the Southern Ocean are yet to be investigated.

Acknowledgements

We thank Drs S.H. Kang, J.S. Kang, D.H. Kang, Mr W.S. Chung, and Mrs H. Park for their technical help, and the reviewers for their comments on the manuscript. This work was supported by KORDI grant PE83500 and Korea Ministry of Maritime Affairs and Fisheries grant PM11100. The comments of two referees helped to improve this paper.

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