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# Evolutionary pathways among shallow and deep-sea echinoids of the genus *Sterechinus* in the Southern Ocean

A. Díaz<sup>a,\*</sup>, J.-P. Féral<sup>b</sup>, B. David<sup>c</sup>, T. Saucède<sup>c</sup>, E. Poulin<sup>a</sup>

<sup>a</sup> Instituto de Ecología y Biodiversidad (IEB), Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

<sup>b</sup> Université de la Méditerranée-CNRS, UMR 6540 DIMAR, COM-Station Marine d'Endoume, Marseille, France

<sup>c</sup> Biogéosciences, UMR CNRS 5561, Université de Bourgogne, Dijon, France

## A B S T R A C T

Antarctica is structured by a narrow and deep continental shelf that sustains a remarkable number of benthic species. The origin of these species and their affinities with the deep-sea fauna that borders the continental shelf are not clear. To date, two main hypotheses have been considered to account for the evolutionary connection between the faunas: (1) either shallow taxa moved down to deep waters (submergence) or (2) deep-sea taxa colonized the continental shelf (emergence). The regular sea urchin genus *Sterechinus* is a good model to explore the evolutionary relationships among these faunas because its five nominal species include Antarctic and Subantarctic distributions and different bathymetric ranges. Phylogenetic relationships and divergence times among *Sterechinus* species were established using the COI mitochondrial gene by assuming a molecular clock hypothesis. The results showed the existence of two genetically distinct main groups. The first corresponds exclusively to the shallow-water Antarctic species *S. neumayeri*, while the second includes all the other nominal species, either deep or shallow, Antarctic or Subantarctic. Within the latter group, *S. dentifer* specimens all formed a monophyletic cluster, slightly divergent from all other specimens, which were mixed in a second cluster that included *S. agassizi* from the continental shelf of Argentina, *S. diadema* from the Kerguelen Plateau and *S. antarcticus* from the deep Antarctic shelf. These results suggest that the deeper-water species *S. dentifer* and *S. antarcticus* are more closely related to Subantarctic species than to the shallow Antarctic species *S. neumayeri*. Thus, for this genus, neither the submergence nor emergence scenario explains the relationships between Antarctic and deep-sea benthos. At least in the Weddell quadrant, the observed genetic pattern suggests an initial separation between Antarctic and Subantarctic shallow species, and a much later colonization of deep water from the Subantarctic region, probably promoted by the geomorphology of the Scotia Arc.

## 1. Introduction

The Southern Ocean comprises the southernmost waters of the World Ocean south of 60°S latitude, as designated by the International Hydrographic Organization in 2000. It also marks the Antarctic Treaty System. However, we consider as a more likely biological (or biogeographical) limit the Antarctic Circumpolar Current (ACC) which is the largest wind-driven current on Earth. It goes all the way around the globe and connects the Atlantic, Pacific and Indian Oceans. The ACC is a massive flow of water that acts as a barrier separating the Southern Ocean from more northern oceans. The current extends from the sea surface to depths of 4000 m and can be more than 200 km wide. Usually, the ACC encompasses all water masses from the ocean surface down to the sea floor (Witworth, 1988). It is a very cold current with temperatures

ranging from  $-1$  to  $5^{\circ}\text{C}$  depending on the time of the year, and with speeds up to 3.7 km per hour. However, the ACC is not a uniform current. It consists of a series of fronts that are associated with deep jets distributed in the meridian direction and encircling the Antarctic continent. The Southern Ocean has several unique environmental characteristics, such as presenting little stratification within the water column and being the main source of cold water for the deep zones of all the oceans (Brandt et al., 2007).

The marine fauna of the Southern Ocean substantially differs from that of other regions due to the geological history of the Antarctic continent and the extreme environmental conditions to which species are subjected (Clarke, 1983; Aronson and Blake, 2001; Peck, 2002). The isolation of the fauna began with the fragmentation and dispersion of the blocks that formed the Gondwana supercontinent and finished with the opening of the Drake Passage. Lawver and Gahagan (2003) stated that the Drake's Passage was definitely open by 28.5 Ma. It is also stated that it was probably open to deep-water circulation as early as 30 Ma (Barker and Burrell, 1977; Barker and Thomas, 2004) or  $31 \pm 2$  Ma

(Lawver and Gahagan, 2003). Other recent estimates place this opening at approximately 24 Ma, near the time of the Oligocene-Miocene boundary (Pfuhl and McCave, 2005; Lyle et al., 2007). After the effective separation of South America from the Antarctic Peninsula, three types of barriers were installed around the Antarctic Continent: geographic, oceanographic and bathymetric (Lawver et al., 1992; Crame, 1999; Clarke and Barnes, 2005; Pfuhl and McCave, 2005; Tripathi et al., 2005). These barriers have helped produce a rich endemic fauna on the continental shelf and slope that surrounds the Antarctic continent (Poulin et al., 2002; Clarke and Johnston, 2003; Clarke et al., 2005; Thatje, 2005). Griffiths et al. (2009) considered it as a differentiated biogeographic province, with only a few internal differences between the Eastern and Western regions. The Antarctic continental shelf is quite narrow and unusually deep, about 450 m and up to 1000 m in some places; its macrobenthic fauna is characterized by a high diversity (Brey et al., 1994), that probably includes more than 15,000 species (Gutt et al., 2004), mainly polychaetes, bryozoans, pycnogonids, amphipods and echinoderms (Clarke and Johnston, 1996). Another notable characteristic of the fauna is that it has eurybathic species compared to the continental shelf fauna of other continents (Brey et al., 1996).

In spite of the isolation established millions of years ago, Arntz et al. (2005) suggested a strong biogeographic union between the marine benthic faunas of the Antarctic Peninsula and the Magellanic region, explained by the geologic history of the two continents. Dell (1972) recognized the possibility of interchange of species after the separation of the continents, arguing that the ACC and the Polar Front would not have acquired sufficient intensity at the beginning of the tectonic process (Barker and Thomas, 2004; Hassold et al., 2009). Also, the reports of larvae and adult individuals of a group historically absent from the Antarctic convergence in 2002 (zoa larvae of brachyuran decapods and individuals of the spider crab *Hyas araneus*) have led several authors to suggest a re-establishment of connection between the faunas of Antarctica and South America due to a weakening of the current (Thatje and Fuentes, 2003; Tavares and De Melo, 2004). Although the studies of Thatje and Fuentes (2003) and Tavares and De Melo (2004) emphasized their observations in the current climatic situation, in which anthropogenic interference may play a fundamental role (e.g., climatic change, ballast water transport), they also opened the debate about possible contacts between faunas during the interglacial periods of the Pleistocene as a consequence of oceanographic changes during these episodes. More generally, some authors have suggested that neither the ACC nor the PF are absolute barriers (Stanchowicz et al., 2002; Page and Linse, 2002), while others hypothesized the existence of cryptic species rather than a permeability of the PF barrier (Thornhill et al., 2008).

Based on the similarity between the marine benthic fauna of the Antarctic continental shelf and that of the surrounding deep areas, many authors have proposed a continuity of the benthic fauna by means of the abyss, which would allow a connection with the Subantarctic and Magellan zones (Clarke and Crame, 1997; Held, 2000; Rogers, 2000; Briggs, 2003; Gage, 2004; Clarke et al., 2005; Brandt et al., 2007; Strugnell et al., 2008). The wide bathymetric distribution of the isopod crustaceans in the Southern Ocean revealed a complex evolutionary history, which has opened a discussion on the evolutionary relationships between shallow-water and deep-water faunas in the Antarctic Ocean (Menzies et al., 1973). Two main hypotheses have been proposed to explain the evolutionary relations of these faunas: (1) evolutionary polar submergence, in which taxa moved from shallow water to deeper waters; and (2) evolutionary polar emergence, in which taxa colonized the continental shelf from the deeper waters (Menzies et al., 1973; Zinsmeister and Feldmann, 1984; Clarke and Crame, 1989; Brandt, 1991, 1992; Crame, 1999; Brandt et al., 2007; Aronson et al., 2008).

The climatic history of the Antarctica with its successive glacial periods may suggest that the submergence hypothesis would be more effective in order to explain evolutionary relationships between congeneric species belonging to the two faunas. Sedimentary registers indicated that during the glacial periods the continental ice extended farther than the border of the continental shelf (Grobe and Mackensen, 1992), thus the survival of the benthic communities would only have been possible on the slope or in the depths (Brey et al., 1996). This suggests that the wide bathymetric distribution of the species of the Antarctic continental shelf reflects the oscillations in the extension of the ice shelf, supposing that these were the species that could re-colonize the shelf when the ice receded during the following interglacial period (Thatje et al., 2005; Aronson et al., 2008). Physiological adaptations (i.e. to pressure) in order to survive during these periods of deep-water refuges for shallow-water species were hypothesized (Clarke and Johnston, 2003); these adaptations could be the key to the colonization of the deeper zones. However, the emergence hypothesis cannot be discarded, since the constant physical regime of the Antarctic Ocean, which is close to the conditions in deep water, would allow colonization of the shallow zone by benthic species of the deep ocean (Dayton and Oliver, 1977; Brandt et al., 2007). In addition, as Berkman et al. (2004) suggested, the similarity between deep-water and shallow-water habitats may have been greater during glacial periods. The presence of an extensive ice field on the shelf and continental slope would have changed the conditions of sedimentation and greatly reduced the light and primary productivity, allowing the colonization of the continental shelf by abyssal organisms (Berkman et al., 2004). Brandt et al. (2007) suggested that the advance and retreat of the shelf ice during the succession of glacial-interglacial cycles, which pushed species to the deeper ocean or caused their extinction, would have caused a combination of emergence and submergence in the margins of the Antarctic.

Spatial analyses of genetic lineages are necessary in order to reconstruct the evolutionary history of Antarctic species. However, studies of this type are relatively scarce in Antarctica, due to the logistic difficulty in obtaining representative samples of populations and species, especially from deep waters. The programs "Evolution and Biodiversity in Antarctica: the response of life to change" (EBA), "Census of Antarctic Marine Life" (CAML), and the development of the International Polar Year (2007-2009) have facilitated such studies. They contributed to the inventory of current biodiversity and improved our understanding of the Antarctic marine ecosystem, by means of multi-scale studies and promoting cooperation among investigators. These efforts have facilitated phylogenetic studies in the last few years; most efforts have been concentrated on crustaceans, fish and mollusks, and have allowed us to tackle wider questions about habitat colonization, patterns of occurrence of species and the evolution of adaptations (Stanchowicz et al., 2002). However, studies that examine the evolutionary relationships between shallow and deep-sea faunas of the Antarctic Ocean are still scarce (Held, 2000; De Mesel et al., 2006; Brandt et al., 2007; Strugnell et al., 2008).

The regular sea urchin genus *Sterechinus* (family Echinidae, order Echinoida) is represented by five nominal species distinguished by morphological and biogeographical criteria. Fertilization is external and development includes a planktonic larva (Bosch et al., 1987). Three of the five species are found all around Antarctica: *S. neumayeri* (Meissner, 1900) is the most abundant regular echinoid in shallow waters (Clarke, 1992); its depth ranges from a few meters to 810 m; *S. antarcticus* (Koehler, 1901) is dominant below 450 m, although it has a wide bathymetric range (30 – 2000 m); while *S. dentifer* (Koehler, 1926) has a much more restricted and deeper range (1200 – 1600 m) (David et al., 2005a). The Subantarctic species are *S. diadema* (Studer, 1876), which is considered as endemic to the Kerguelen shelf between 15 and

750 m, and *S. agassizii* (Mortensen, 1910) found between 55° and 35°S along the Argentinian continental shelf and characterized by a wide bathymetric distribution (10 to about 1000 m, most typically at about 200 m) (David et al., 2005a). These contrasting geographic and depth ranges make this genus a good model: (1) to determine when Antarctic and Subantarctic marine faunas loosed contact and started to follow independent evolutionary histories; and (2) to elucidate the evolutionary relationships between shallow and deep-water faunas in the Southern Ocean, testing both hypotheses of polar submergence and emergence.

## 2. Material and methods

Specimens of *S. neumayeri* were obtained from the shallow zone of the Antarctic Peninsula and the eastern region of this continent, while *S. diadema* and *S. agassizii* were obtained from the Kerguelen Archipelago and the continental shelf of Argentina, respectively. In the case of deep-water species, *S. dentifer* were dredged from Bellinghousen Sea, while *S. antarcticus* samples were obtained from both the Antarctic Peninsula and East Antarctica (Fig. 1, Table 1).

### 2.1. Extraction, amplification and sequencing

Total DNA was extracted from gonadal tissue or spines of 193 specimens using the salt method (Aljanabi and Martínez, 1997). PCR was used to amplify the mitochondrial Cytochrome Oxidase gene, using the primers described by Lee et al. (2004), LCO11490 (5'- TCTA CAA ACC ACA ARG AYA TTG G -3') and HCOIN (5'- CCC ATT GAA AGA ACG TAG TGA AAG TG -3'). A fragment of 945 bp was amplified, which corresponds to a part of the COI gene of

**Table 1**

Number of sequences obtained and analyzed of species of the genus *Sterechinus*.

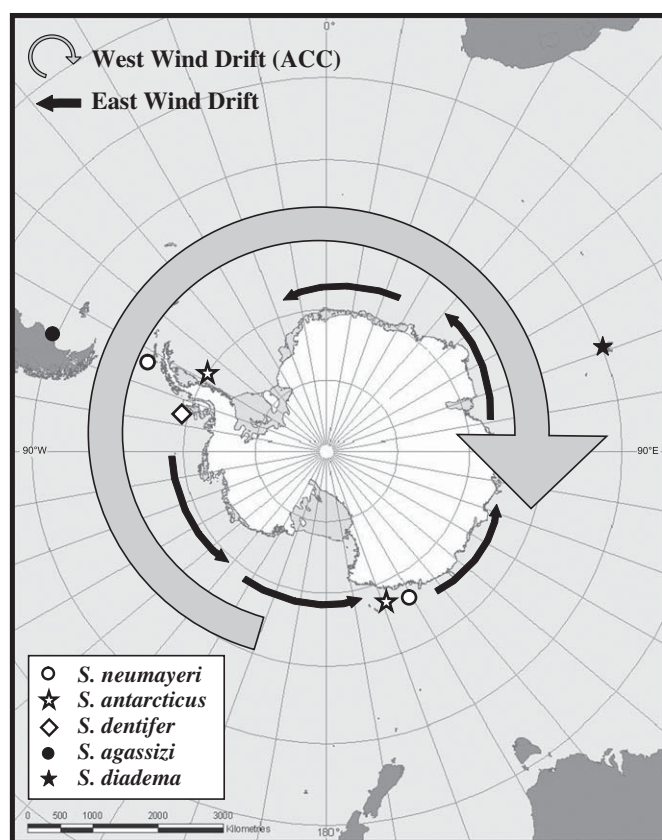
Species	Geographic origin	Number of sequences
<i>Sterechinus neumayeri</i>	Antarctic Peninsula	116
	Terre Adélie (East Antarctic)	15
<i>Sterechinus antarcticus</i>	Antarctic Peninsula	3
	Terre Adélie (East Antarctic)	2
<i>Sterechinus dentifer</i>	Antarctic Peninsula	4
<i>Sterechinus diadema</i>	Kerguelen Island	13
<i>Sterechinus agassizii</i>	Argentinian continental shelf	40

*S. neumayeri*, from nucleotide 65 to 1009 (Genbank accession AY275548, size 1077 pb). However, due to the low quality of samples from the Subantarctic region (degraded DNA), it was necessary to design intermediate primers from the sequences obtained in *S. neumayeri*; ERZin-F (5'- GAC CGA CTG CCC TTA TTT -3') and ERZin-R (5'- CTC GCT TTC CTG AGT AGT -3'). Polymerase chain reactions (PCR) were carried out in a Thermo PxE 0.5 thermocycler using Taq DNA polymerase (Invitrogen, Recombinant, 500 U) under standard amplification conditions with 25 µL of reaction volumes. Each reaction tube contained: 1 µL (approx 10 ng) of genomic DNA extract, 2.5 µL 10XPCR buffer, 1.5 Mm MgCl<sub>2</sub>, 2.5 µL 10 mM each dNTP, 10 picomoles of each primer, 0.2 U Taq polymerase. After 7 min of initial heating at 95 °C, amplification was performed in 35 repetitions of a three-step cycle (denaturation, 95 °C for 1 min; annealing, 58 °C for 1.5 min; extension, 72 °C for 1.5 min) and a final extension for 10 min. PCR products were purified with Qiagen Qiaquick columns and the sequencing was done by the Korean company Macrogen, in an ABI 3100 automatic sequencer (Applied Biosystems); the sequences obtained were aligned with the programs Proseq v. 2.91 (Filatov, 2002) and ClustalX 1.8 (Thompson et al., 1997).

### 2.2. Genealogical and phylogenetic relations among shallow and deep-water species

The genealogical relations among haplotypes of the shallow and deep-water species were graphed by median joining using the program Network 4.1.5 (Röhl, 2002). In addition, we calculated the pairwise genetic p-distances between pairs of morphospecies with the program Arlequin 3.1 (Excoffier et al., 2005).

For phylogenetic reconstruction within *Sterechinus* we used as outgroup two species of the same family Echinidae: *Gracilechinus multidentatus* (Genbank access EU869929.1) and *Loxechinus albus* (Genbank access AY275550.1). Phylogenetic trees were constructed by maximum parsimony (MP) and maximum likelihood (ML) methods using PAUP\* program (version 4.0b10; Swofford, 1999). For MP all sites were treated as unordered characters with equal weight, using the heuristic search method with the addition of ordered sequences and branch swapping (TBR Branch Swapping algorithm). ML analysis was carried out using the substitution model of sequence evolution HKY+I inferred from ModelTest (Posada and Crandall, 1998). Support for branches was estimated using bootstrap re-sampling with 1000 replicates in MP and ML analyses (Felsenstein, 1981). We also performed Bayesian phylogenetic analyses with the program MrBayes v.3.1.2 (Huelsenbeck and Ronquist, 2003), using the substitution model GTR+G as recovered by the MrModeltest program (Nylander, 2004). This method estimates the posterior probability that each taxon recovered is a monophyletic unit. All the Markov Chain Monte Carlo analyses (MCMC) were performed twice, using independent runs with random trees for a total of  $5 \times 10^6$  generations. Trees were sampled every 500 generations and the first 1000 trees discarded; the rest were used to construct a consensus phylogram with the majority rule and posterior probability for the nodes. Phylogenetic



**Fig. 1.** Sample collection sites of species of the genus *Sterechinus*. The two different arrows indicate the main oceanographic currents of the Southern Ocean.



reconstructions for all search criteria were performed with a subsample of the most representative haplotypes of each species.

### 2.3. Divergence time estimation

Divergence times among *Sterechinus* species were estimated with BEAST version 1.5.3 (Drummond and Rambaut, 2007), using the two substitution rates of COI sequence calibrated by Lee et al. (2004), 0.51 and 0.72% Ma<sup>-1</sup>, for species of the family Echinidae. This is a Bayesian Markov chain Monte Carlo method that incorporates a strict clock model and for this reason, the heterogeneity of evolutionary rates among the branches was checked by the log likelihood ratio test (Felsenstein, 1981) between the clock-enforced ML tree and the non-enforced ML tree ( $-\ln L = 2601.6390$  and  $-\ln L = 2597.9814$ , respectively), using DAMBE (Xia and Xie, 2001). We assumed a Yule speciation process, as recommended for species-level phylogenies (Drummond and Rambaut, 2007). The HKY+I substitution model was used for the two substitution rates, and we used default prior distributions for all other parameters. Each independent analysis was performed with MCMC chain runs for 50 million generations and the parameters were sampled every 1000 generations.

For each BEAST analysis, the divergence time and its confidence interval for the species of the *Sterechinus* was assessed using the software Tracer Version 1.5 (Drummond and Rambaut, 2007).

## 3. Results

### 3.1. Genealogical relations among shallow- and deep-water species from Antarctic and Subantarctic regions

The haplotype network of the shallow- and the deep-water species of the genus *Sterechinus* showed the existence of two highly divergent groups, separated by 56 fixed substitutions. The first group included all haplotypes of the shallow Antarctic species *S. neumayeri*, while the second gathered all haplotypes of shallow subantarctic species as *S. diadema* from the Kerguelen Archipelago and *S. agassizii* of the Argentinian continental shelf, as well as those species typically found in deep water, *S. dentifer* and *S. antarcticus* (Fig. 2). The network showed that haplotypes of deep-water species have a much closer relation with the shallow-water species of the Subantarctic region than with the shallow-water Antarctic species *S. neumayeri*. While *S. antarcticus* shared the most frequent

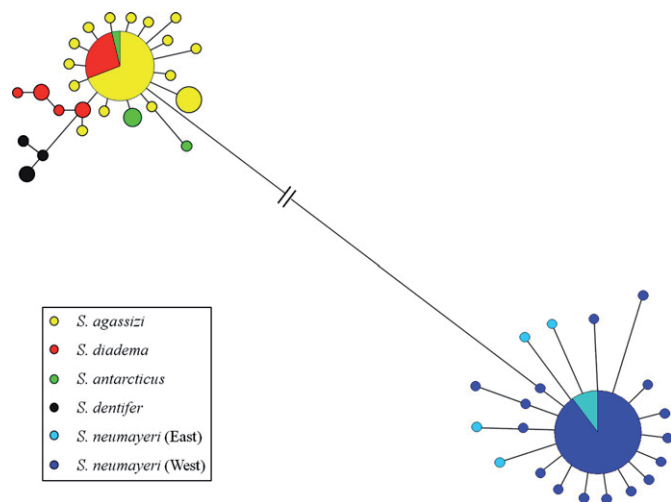


Fig. 2. Median Joining Haplotype network showing the genealogical relations among all species of the genus *Sterechinus*.

Table 2

Pairwise p-distances values (above diagonal), among species of the genus *Sterechinus*.

Species	<i>S. neumayeri</i>	<i>S. agassizi</i>	<i>S. diadema</i>	<i>S. antarcticus</i>	<i>S. dentifer</i>
<i>S. neumayeri</i>	----	5.99	5.95	5.94	5.96
<i>S. agassizi</i>	-	----	0.20	0.24	0.60
<i>S. diadema</i>	-	-	----	0.24	0.51
<i>S. antarcticus</i>	-	-	-	----	0.65
<i>S. dentifer</i>	-	-	-	-	----

haplotype with the Subantarctic group, the four specimens of *S. dentifer* clustered into a differentiated haplogroup. The p-distance values showed that *S. neumayeri* had about 5% sequence difference with all other species of the genus, while the rest of the species (deep Antarctic and shallow Subantarctic) had values of less than 0.7% (Table 2). Considering only shallow-water species, large p-distance values separated Subantarctic and the Antarctic species (about 6%), while the values between samples of *S. neumayeri* from the East and West Antarctic and between *S. diadema* and *S. agassizi* were only 0.08% and 0.03%, respectively (Table 2).

### 3.2. Phylogenetic relations among shallow and deep-water species

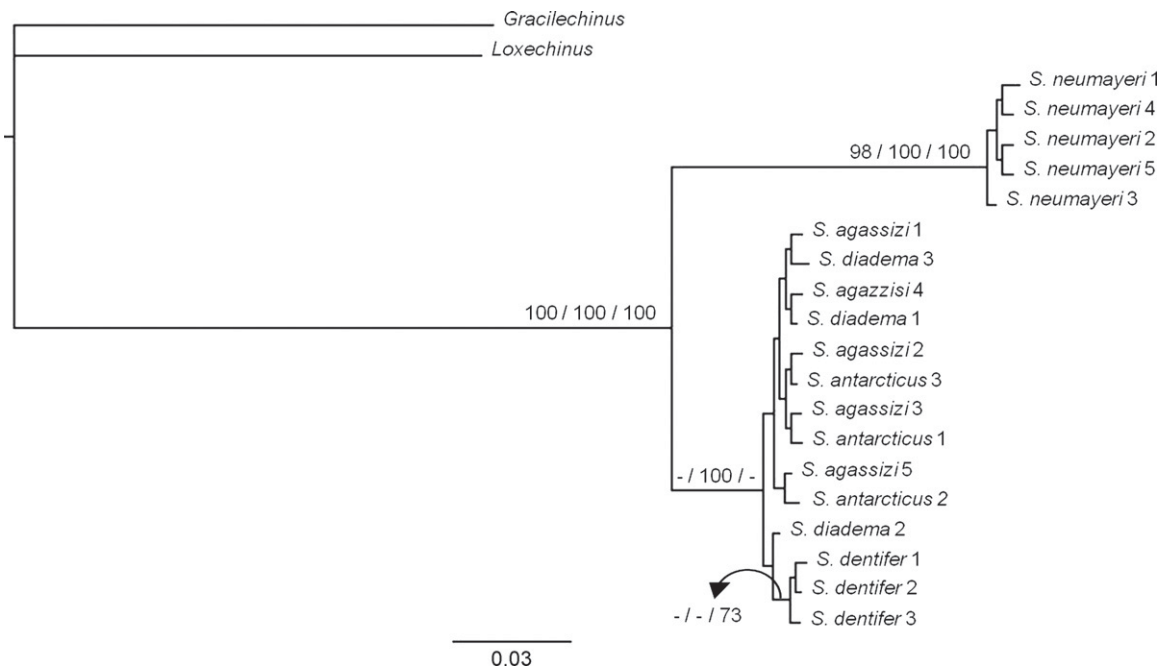
The length of the section of the COI gene obtained for the species of *Sterechinus* was 774 pb. Of these sites, 210 were variable and 110 were informative for parsimony. The nucleotide composition was 27.14% A, 21.95% C, 18.24% G and 32.67% T. The substitution saturation test showed that the COI sequences are not saturated ( $Iss = 0.2945 < Iss.c = 0.7363$ ;  $df = 463$   $p < 0.0001$ ), which validates their use in phylogeny. The maximum parsimony (MP) analysis found 10 equally parsimonious trees, with a consistency index of 0.8228, homoplasy index of 0.1772 and retention index of 0.8260. The tree topology shows two well-differentiated groups; one includes the haplotypes of *S. neumayeri* and the other has the rest of the species, corroborated by a 100% bootstrap value (Fig. 3). Within the latter group the tree shows a subgroup which includes *S. diadema*, *S. agassizi* and *S. antarcticus*, however with low bootstrap support. Maximum likelihood analysis (ML) of the sequences with the ModelTest parameters and the Bayesian analysis resulted in a tree with similar topology that distinguishes the two major clades described above. All three methods, based on different assumptions, produced similar trees in terms of branching and the robustness of the same nodes (Fig. 3). The genus *Sterechinus* appears monophyletic and, among the five nominal, formerly described, species, only *S. neumayeri* is monophyletic.

### 3.3. Divergence time estimation

Results from BEAST Program dating analyses estimated the beginning of the divergence among the two main clades between 5.6 and 7.9 M (substitution rates 0.72 and 0.51% Ma<sup>-1</sup>, respectively), in the late Miocene. The confidence interval for the greater substitution rate is 4.01 – 7.19 Ma, whereas the slower rate is 5.77 – 10.27 Ma.

## 4. Discussion

The phylogenetic analysis of the genus *Sterechinus* showed two main clades. One corresponds to all individuals of the shallow-water Antarctic species *S. neumayeri*, which fulfills the criteria of a phylogenetic species. The second clade gathers specimens belonging to the four other nominal species. Among them, only individuals of *S. dentifer* (Antarctic deep-sea species) form a haplogroup slightly differentiated from other species of the clade. Specimens of



**Fig. 3.** The Bayesian inference (BI) tree of the COI sequences obtained for the genus *Sterechinus*. The branch support values are from ML and MP bootstrap (1000 repetitions) and BI analyses.

*S. diadema*, *S. agassizii* and *S. antarcticus* do not separate into different haplogroups but form one single genetic entity and do not fulfill the criteria for distinguishing different phylogenetic species. The distinctions between *Sterechinus* species were so far grounded on architectural (plate pattern), general shape and appendages characters (Mortensen, 1943; David et al., 2005b). However, those supposedly distinctive characters are rather subtle, and their ranges of variation tend to overlap making the separation between species unclear. The most conspicuous distinction concerns *S. dentifer*, which displays characteristic pedicellariae. For the other species, part of the identification of specimens by former authors relied generally on few specimens, therefore without appraisal of variation, and sometimes also on geographic criteria (Clark, 1925). The four nominal species of the second clade appear now to be much more similar from a phylogenetic point of view, so that they could be possibly clumped into a single entity, all the more that there is few support to distinguish them as already suggested (David et al., 2005b). This absence of genetic differences challenges the status of *S. diadema*, *S. antarcticus*, and *S. agassizii* as separate species. The case of *S. dentifer* is still pending, considering the low number of specimens analyzed, due to the scarcity of deep-sea samples.

Within the first clade, the absence of phylogeographic structure of *Sterechinus neumayeri* could reflect the existence of past or present connectivity sufficient to impede any divergence processes, even at the scale of thousands of kilometers. This fact indicates that these distant populations have not been isolated, at least as regard as the mutation time scale of the COI gene (around 250.000–175.000 years, considering 0.51% and 0.74%, respectively). According to the oceanographic characteristics of the Southern Ocean, past or even present connectivity may have been facilitated by the East Wind Drift, which flows counterclockwise around the continent (Fig. 1). Such connectivity would be also explained by an important dispersion capacity and possible long permanence in the water column of its larval stages. Based on temporal samples in the Antarctic Peninsula, Bowden et al. (2009) estimated that the development time of *S. neumayeri* larvae could reach four months. Moreover, Marsh et al. (2001) showed that larvae of the same species have an extremely low metabolism,

which may allow them to remain for up to a year in the plankton (D. Manahan, personal communication). In the shallow Subantarctic, samples from the Argentinian continental shelf and from Kerguelen Plateau correspond to distinct nominal species, *S. agassizii* and *S. diadema*, albeit weakly morphologically differentiated. However, we found no evidence for an ongoing divergence process between them and this may reflect the existence of past or recent contacts between South America and Kerguelen Islands driven by the ACC. In this regard, Moyano (1999) proposed the existence of a high connectivity within the Subantarctic ecosystem, emphasizing the Magellan Straits region as a zone united from a zoogeographical point of view with the circum-austral Subantarctic region by the West Wind Drift. More recent molecular studies performed on other marine benthic organisms are in agreement with our results, showing recurrent and modern gene flow among Subantarctic regions in *Ostrea chilensis* (Ó Foighil et al., 1999), *Macrocystis pyrifera* (Coyer et al., 2001), *Mytilus* spp. (Gérard et al., 2008), and *Durvillaea antarctica* (Fraser et al., 2009).

Based on our results and recent studies of shallow Antarctic and Subantarctic benthos, we propose a model of two rings of connectivity (Antarctic and Subantarctic two-ring model) which includes two large routes of dispersion: (1) the ACC (historically referred to as West Wind Drift), which would connect the Subantarctic zones, and (2) the Antarctic Coastal Current (East Wind Drift) which surrounds the Antarctic. These rings may have maintained a degree of connectivity sufficient to avoid the process of molecular divergence between their populations (Fig. 1). Genetic relationships among Antarctic shallow and deep-sea *Sterechinus* species do not support either of the two hypotheses of submergence or emergence previously proposed to explain the biogeographic and evolutionary relationships between the two Antarctic benthos. Deep specimens identified as *S. antarcticus* and *S. dentifer* that were collected close to the Antarctic Peninsula and East Antarctica form a single haplogroup together with the specimens from Argentina and Kerguelen. Moreover, individuals recognized as *S. antarcticus* share the main haplotype with both subantarctic shallow species. These results are congruent with the suggestion of Mortensen (1936) and David et al. (2005b), who proposed, based on

morphological characters, that *S. antarcticus*, *S. agassizi* and *S. diadema* may be closely related and may correspond to local phenotypes of a single species. In the case of the four individuals collected in the deepest zone, identified as *S. dentifer*, three new haplotypes cluster in a slightly divergent clade (p-distance less than 1%). Compared with the divergence found between *S. neumayeri* and all other samples, this low value may indicate a recent divergence between *S. dentifer* and the group composed of *S. agassizii*, *S. diadema* and *S. antarcticus*. These results contrast with previous studies on the relationship between Antarctic and deep-sea fauna. While some groups such as pectinid bivalves (Berkman et al., 2004) fit the emergence hypothesis, others such as deep-water foraminiferans (*Epistominella*, Pawlowski et al., 2007), serolid isopods (Held, 2000) and octopuses (Strugnell et al. 2008) match the submergence hypothesis. Based on the extensive sampling effort of the ANDEEP project, Brandt et al. (2007) found that a combination of the processes of submergence and emergence would explain satisfactorily the evolutionary relationships among isopod families.

In this context, our work does not support either the emergence or submergence hypothesis, but provides evidence of an evolutionary relationship between deep-sea and subantarctic shallow fauna. A possible scenario to explain the evolutionary pathways between shallow and deep-sea *Sterechinus* spp. may first involve an initial separation between shallow Antarctic and Subantarctic species by the end of the Miocene, and secondly a later colonization of the deep ocean from the Subantarctic zone, probably promoted by the geomorphology of the Scotia Arc (Thompson, 2004). Then the colonization of the abyssal area extended to the slope and deep part of the Antarctic continental shelf, where the highly divergent species *S. neumayeri* and *S. antarcticus* are now found in sympatry. In this scenario and according to our divergence time estimation, the divergence between the shallow-water species of *Sterechinus* from the Antarctic and Subantarctic regions would have begun at the end of the Miocene and/or the beginning of the Pliocene. Page and Linse (2002), who used the 16S gene, indicated a similar separation time for gastropod species of the genus *Limatula* (*L. ovalis* and *L. pygmaea*); about 5.8–8 Ma. More recently, González-Wevar et al. (2010) revealed the diversification of the limpet genus *Nacella* in the Southern Ocean and estimated the appearance of the main *Nacella* lineages between  $9.3 \pm 1.5$  and  $5.4 \pm 1.1$  Ma in Antarctica, Central Chile, Magellan and Kerguelen Provinces. Divergence time between geminate Antarctic and South American species may have occurred even later, as proposed by Hunter and Halanych (2008) for the brittle star *Astrofoma*. These results strongly suggest that Antarctic and Subantarctic benthic populations of invertebrates with long-lived larvae could have maintained connectivity long after the physical separation of the continents and the formation of the ACC. Different lines of evidence show that the time period that we propose in this paper for the divergence of the faunas (Miocene-Pliocene transition) involved particular events, such as an exceptionally cold period which would have generated a new and important expansion of the ice cap in the western part of the Antarctic continent (Kennett and Barker, 1990; Zachos et al., 2001; Gersonde and Censarek, 2006). Crame (1999) suggested that the rapid development of the ice cap between 6.2 and 4.8 Ma would have produced a change in the velocity of the circum-Antarctic current (Barker and Thomas, 2004; Hassold et al., 2009). According to this interpretation, the interruption of gene flow between populations of *Sterechinus* may correspond to the installation of an effective oceanographic barrier, probably linked to an intensification of the ACC (see Thornhill et al., 2008). Additionally, our results support the absence of gene flow between the regions during the Pliocene and Pleistocene (5 Ma to present), in spite of episodes of possible weakening of the current during the repeated inter-glacial periods. The reduction in ice extension, along

with a probable homogenization of sea water temperatures, has not been sufficient to create a viable bridge for the interchange between the Antarctic Peninsula and the Magellanic region. Finally, the intensification of the oceanographic barrier, which occurred at about 5 Ma, may have produced a definitive separation between shallow species of the two regions, in spite of a high, but different dispersal capacity of the planktonic larvae.

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