Antarctic Science 12 (3): 259–268 (2000) © British Antarctic Survey Printed in the United Kingdom

Molecular zoogeography of Antarctic euphausiids and notothenioids: from species phylogenies to intraspecific patterns of genetic variation

LUCA BARGELLONI[1,*], LORENZO ZANE[1], NICOLAS DEROME[3], GUILLAUME LECOINTRE[3] and TOMASO PATARNELLO[1,2]

[1]Dipartimento di Biologia, Università di Padova, Via Ugo Bassi 58/B, I-35121 Padova, Italy
[2]Facoltà di Veterinaria-Agripolis, Università di Padova, Via Romea 16, I-35020 Legnaro, Italy
[3]Museum National d'Histoire Naturelle, 43 rue Cuvier, F-75231 Paris Cedex 05, France
**corresponding author*

Abstract: Published and unpublished data are used to investigate possible mechanisms of species diversification in two key groups of Antarctic organisms: the Notothenioidei and the Euphausiidae. Species distributions are mapped onto molecular phylogenies, and this evidence is interpreted in light of the various ecological and historical factors which characterize the Southern Ocean. The joint effect of diverse agents (vicariance, "jump" dispersal) appears to be determinant in several cases for species divergence. A review of results from population genetics studies, together with new molecular evidence, confirm the importance of physical barriers (oceanographic fronts) in reducing migration, thereby promoting speciation, at least in notothenioids.

Received 8 November 1999, accepted 21 February 2000

Key words: Euphausiidae, phylogeny, population genetics, Notothenioidei, zoogeography

This paper is dedicated to the memory of Martin White. For us, Martin was always a most kind and expert guide to the fascinating field of Antarctic biology. We will miss him.

Biogeography of Antarctic marine organisms: an introduction

Biogeography is generally defined as "the science that seeks to explain the geographic distribution of species" (Futuyma 1998). Patterns of distribution are typically ascribed either to ecological or to historical factors (Ridley 1996). Although these two categories of explanations are often presented as alternatives, biogeographers are coming increasingly to recognize the importance of considering both of them. In most empirical studies a complete account of a species distribution requires both ecological and historical knowledge (Ridley 1996). Geographic distributions are, in fact, generally affected by climate (past and present), vicariant events, and dispersal. The latter is the process which causes a species range to change, through the active or passive movement of individuals into previously unoccupied areas. The present-day range of a species is usually the final result of the action of the mentioned factors, summed over history.

The need for phylogenies and population genetic studies

As Futuyma states (Futuyma 1998) "biogeography is completely reliant on an accurate understanding of phylogenetic relationships" among the species under study. This is true, especially in the case of historical biogeography, where, in order to test alternative hypotheses of speciation, distributions of taxa are mapped onto phylogenetic trees, producing "area cladograms". For instance, vicariance biogeography predicts that different taxonomic groups might have a common vicariant origin, an event which caused a fragmentation in the species range. In this case, area cladograms are expected to be congruent, and the branching order in the phylogeny should reflect range split(s). From all the above it is therefore clear that reliable phylogenies are needed for meaningful biogeographic studies.

In addition to such a phylogenetic approach, however, it is important also to adopt a population genetics perspective of biogeography. Analysing how genetic variation is partitioned within the same species, it allows the evaluation of levels of gene flow among populations, thereby giving an indirect estimate of dispersal in the organism under study. Understanding contemporary and/or recent dispersal capacity of a species could then effectively complement the "historical" perspective derived from analyses at the interspecific level.

Molecular tools

To implement the approaches described, a solid phylogenetic framework at the interspecific level, as well as an accurate analysis of genetic variation patterns within species is necessary. For this, the use of molecular techniques might be extremely effective, giving access to DNA sequence information, and

therefore to a nearly unlimited pool of genetic variability. In recent years, a new discipline, molecular systematics, has emerged, which reconstructs species phylogenies based on molecular data (Hillis *et al.* 1996). This provides a powerful tool to infer phylogenetic trees, relying on a large number of (in most cases) independent characters. A second, potential application of DNA data is the estimation of time of divergence between taxa, by means of "molecular clocks" (Avise 1994, p.103). Although controversial, this method might be of some use especially when fossil data are completely lacking.

Owing to key technical innovations, particularly the polymerase chain reaction (PCR), DNA data have also become increasingly available for intraspecific studies, and molecular markers are now widely used in most population genetics studies, complementing the well established approach based on protein level variation (Avise 1994). A more detailed description of the use of molecular markers in population genetics, with particular regard to Antarctic species, can be found in other contributions in this issue.

The Southern Ocean: a case study for biogeography of marine organisms

In general, biogeographic analyses have focused on terrestrial organisms, while relatively less attention has been paid to marine species, with the exception of benthic invertebrates (e.g. see a series of papers on polar marine invertebrates reviewed in Crame 1993). This might be because the marine environment, especially in the pelagic realm, often lacks clear geographic boundaries, and that many marine organisms have moderate or even high dispersal abilities, either through active migration or passive transport by ocean currents. In many cases, a significant level of dispersal in marine taxa hampers the work of biogeographers, confounding historical distribution patterns. The Southern Ocean represents an extremely interesting area for biogeographic studies because of the remarkable level of endemism in several Antarctic taxonomic groups. This observation is related to the historical events that led to the formation of the Antarctic marine environment, as well as to the present day ecological and oceanographic conditions. After the separation from Australia (about 37 million years ago [m.y.a.]) and the deepening of the Drake Passage (25–22 m.y.a.), Antarctica became effectively isolated from the other continents. Deep sea conditions between South America and the Antarctic continent also led to the complete establishment of the Antarctic Polar Front (APF), 25–22 m.y.a. (Kennett 1982). This hydrographic barrier, located between 50°S and 60°S, is formed by the convergence of cold Antarctic water with warmer sub-Antarctic water, and extends to a depth of 1000 m. South of the Polar Front, a complex system of currents, particularly the clockwise Circumpolar Current, aids the passive dispersal of planktonic organisms. Moreover, during the past 55 million years (m.y.) the temperature of the Southern Ocean has declined progressively from about 20°C to the present-day minimum (-1.8°C). The cooling process was characterized by temperature fluctuations, leading to repeated expansions and retreats of ice shelves over the past 40 m.y., resulting in changes in the availability of coastal habitats. The study of biogeography of Antarctic marine taxa might help to elucidate the relative role of all the features mentioned above in the evolution of the Antarctic fauna.

Comparisons between many taxa are desirable for the detection of common biogeographic patterns and an explanation of their causes. We will focus on two large taxonomic groups: teleost fish belonging to the perciform suborder Notothenioidei, and selected species from the crustacean family Euphausiidae. Both notothenioids and euphausiids have an extremely important role in Antarctic marine ecosystems, thereby representing a subject of great interest for evolutionary studies.

The Notothenioidei

The suborder Notothenioidei, which comprises more than 120 species (Eastman 1993), represent more than 90% of the Antarctic coastal fish fauna in terms of biomass and number of species. During their evolution, the notothenioid fish underwent a remarkable diversification, filling numerous ecological niches (Clarke & Johnston 1996). Like their ancestor, most notothenioids are benthic, with a pelagic larval phase of variable length. A few notothenioid species such as *Pleuragramma antarcticum* Boulenger also live in the pelagic realm as adults. With regard to geographic distribution, although many notothenioids are endemic to the High Antarctic waters, several other species are found, often exclusively, on the shelf of peri-Antarctic islands (South Georgia Province, Kerguelen Province), or in the sub-Antarctic Region (Fig. 1).

High level of endemism, wide range of potential for dispersal, and diversity of geographic distribution therefore make nototheniods an extremely interesting target for Antarctic biogeographers.

The Euphausiidae

The crustacean family Euphausiidae comprises 85 recognized pelagic species, several of which are present in Antarctic or sub-Antarctic waters. Many species have similar characteristics, such as high abundance and swarming behaviour, that make them an important food resource for other species. Indeed, euphausiids play a key role in the ecology of the Southern Ocean because they transfer, concentrate, and buffer energy from phytoplankton and microplankton (on which they feed) to a variety of higher level heterotrophs. Among euphausiids, the most important species is *Euphausia superba* Dana, whose standing biomass of at least 500 million tons makes it possibly the most abundant multicellular species on the planet (Miller & Hampton 1989).

The distributions of many krill species span an extremely wide geographic range, often limited by transition zones between water masses (McGowan 1977) thus suggesting that

krill is incapable of active migration, at least on a large geographic scale. The distribution of euphausiids in the Antarctic–sub-Antarctic area is strongly influenced by the APF, which could be considered a strong barrier to dispersal. South of the APF, Antarctic species of krill occur on a circumcontinental scale, and seem to behave as passive drifters, dispersed by the two strong surface currents of the Southern Ocean (Fig. 1). A possible exception is *E. superba* which has a discontinuous distribution with areas of higher concentration of adult krill associated with oceanic gyres. This suggests that gyres may generate a retention mechanism that promotes the formation of separate, self-recruiting stocks of krill (Mackintosh 1973).

It seems evident then that the two groups exhibit a great diversity in terms of biology and ecology, especially with respect to dispersal (euphausiids in general drifting passively, while notothenioids having varying ability of active migration) and geographic distribution (krill being found over vast areas, whereas most notothenioid species being endemic to specific regions). A joint examination of such diverse taxa can reveal recurrent and/or discordant biogeographic patterns, thereby shedding some light on the interplay between ecology and history in promoting the formation of new species.

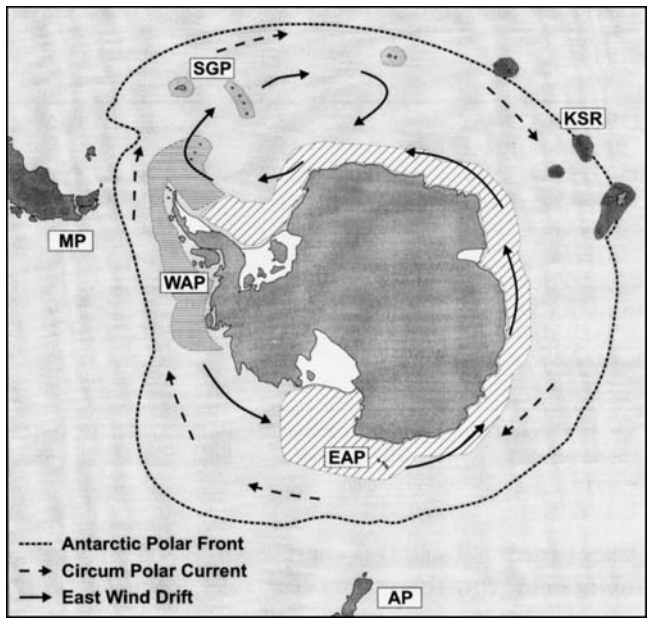

Fig. 1. For fish fauna, the Southern Ocean and the adjacent seas have been classically divided in the following zoogeographic areas: a. the Antarctic Region, formed by i) the Glacial subregion, itself divided in East Antarctic Province, West Antarctic Province, and South Georgia Province; and by a ii) Kerguelen subregion; b. the sub-Antarctic region divided in i) Magellanic Province and ii) Antipodean Province. Subdivisions are indicated by acronyms and different shaded areas. Antarctic region: East Antarctic Province (EAP), West Antarctic Province (WAP), South Georgia Province (SGP), Kerguelen subregion (KSR). Sub-Antarctic region: Magellanic Province (MP), Antipodean Province (AP). The two major currents, the Circumpolar Current and the East Wind Drift, are indicated by arrows. The location of the Antarctic Polar Front is indicated as a hatched line. From an ecological point of view, these divisions are not satisfactory and three main ecological zones have been proposed: i) the High Antarctic Zone, that is the region immediately adjacent to the continent, covered permanently by ice; ii) the Seasonal Pack-ice Zone, which extends between the seasonal limits of pack ice; iii) the Ice-free Zone, where ice is never present, and which includes also the Magellanic Province (Hureau 1994).

Molecular evidence

Species phylogenies

The Notothenioidei. Since the publication of the first notothenioid phylogeny based on DNA data (Bargelloni *et al.* 1994), a wealth of sequence information has been collected. Several different genes have been sequenced in a variety of species, including both mitochondrial (12S and 16S rRNAs, cytochrome b, D-loop) and nuclear genes (28S rRNA, globin(s), metallothionein(s), lactate dehydrogenase A (LDHA), antifreeze glycopeptide(s), beta-tubulin(s), myoglobin). Not all of these molecular data, however, have been used to construct phylogenetic trees, because of the peculiarities of the gene(s) under study or owing to the limited number of species examined. Although records for more than 60 different species appear at least once in DNA sequence databases, single studies often report data for a few species only. In this paper, relevant sequence information already available in the literature was selected (listed in Table I), and re-examined. All these data, together with some unpublished results, have been merged in order to infer the most complete hypothesis on notothenioid phylogenetic relationships to date.

So far, the most complete species sampling has been obtained for two mitochondrial genes, coding for the small (12S) and the large (16S) subunit of ribosomal RNA (Table I). To provide a phylogenetic framework to discuss, a phylogenetic tree is presented in Fig. 2. This tree summarizes results obtained from 12S and 16S rRNA sequences for notothenioids (Ritchie *et al.* 1997, Bargelloni *et al.* 2000). In the tree, specific nodes are indicated by a letter code (A, B, C). These three nodes characterize respectively three clades. Clade A includes all notothenioid taxa examined, except for two species belonging to the family Bovichtidae, *Cottoperca gobio* Günther and *Bovichtus variegatus* Richardson (Ritchie *et al.* 1997). Within clade A, a third bovichtid species, *Pseudaphritis urvillii* Cuvier turns out to be the sister group to the rest of notothenioids, which are all contained in clade B (Ritchie *et al.* 1997). Clade B contains a third clade (C) including all non-bovichtid taxa, and a single species, *Eleginops maclovinus*, Cuvier which is the sister taxon to clade C (Ritchie *et al.* 1997, Bargelloni *et al.* 2000). The latter group encompasses 31 notothenioid species, and the number of species increases if additional phylogenetic information from studies on other

https://doi.org/10.1017/S0954102000000328 Published online by Cambridge University Press

Table I. Molecular phylogenetic data on notothenioids. Notothenioid families are named according to Eastman (1993).

gene(s)	no. of species examined	notothenioid families examined	phylogenetic method	reference
12S and 16S rRNAs	18	A, Ba, Bo, C, N	NJ, MP	(Bargelloni *et al.* 1994)
12S and 16S rRNAs	13	N	NJ, MP, ML	(Ritchie *et al.* 1996)
12S and 16S rRNAs	26	A, Ba, Bo, C, H, N	MP	(Ritchie *et al.* 1997)
12S and 16S rRNAs	33	A, Ba, Bo, C, H, N	NJ, MP, ML	(Bargelloni *et al.* 2000)
cytochrome *b*, D-loop	15	Ba, C	MP	(Chen *et al.* 1998)
28S rRNA	6	A, Bo, N	MP	(Lecointre *et al.* 1997)
α- and β-globin	8	Ba, N	MP	(Bargelloni *et al.* 1994)
α- and β-globin	13	A, Ba, Bo, N	MP	(Stam *et al.* 1997)
α- and β-globin	14	A, Ba, Bo, N	MP	(Stam *et al.* 1998)
lactate dehydrogenase A (LDHA)	12	Ba, C, H, N	NJ	(Fields & Somero 1998, reporting only sequence data; phylogenetic analysis: this paper)

A = Artedidraconidae, Ba = Bathydraconidae, Bo = Bovichtidae, C = Channichthyidae, H = Harpagiferidae, N = Nototheniidae.
Abbreviations for phylogenetic methods are as follows: NJ = Neighbour-Joining, MP = Maximum Parsimony, ML = Maximum Likelihood.

genes (listed in Table I) is considered. Based on evidence from globin genes (Bargelloni *et al.* 1994, Stam *et al.* 1997), clade C should also include *Bathydraco marri* Norman, *Racovitzia glacialis* Dollo, *Artedidraco orianae* Regan, and *Notothenia angustata* Hutton. Phylogenetic analysis of α- and β-globins also gives additional support to the early divergence from clade B of *P. urvillii*, shown in Fig. 2, as well as to the position of *C. gobio* (Stam *et al.* 1998). Further independent evidence for such a cladogenetic event (node B in Fig. 2) is obtained from the analysis of the nuclear gene coding for the RNA of the large ribosomal subunit (Lecointre *et al.* 1997). The same data set (28S rRNA) provides strong support for the position of *E. maclovinus* (node C in Fig. 2) and suggests the inclusion of *Artedidraco loenbergi* Roule in clade C. Finally, when sequence data from the lactate dehydrogenase A gene (Fields & Somero 1998) are used to infer a phylogenetic tree (Fig. 3), *E. maclovinus* also comes out as sister group of all those taxa which appear in clade C (Fig. 2). The LDHA tree also indicates that *Paranotothenia magellanica* Forster and *Patagonotothen tessellata* Richardson, two sub-Antarctic species, should be included in clade C. In summary, several different data sets offer concordant evidence on early cladogenetic events (corresponding to nodes A, B, C).

In the past, hypotheses on biogeography of notothenioid fish have suffered from a lack of well-established phylogenetic relationships. Moreover, geographic distribution of species has been often used as an additional source of phylogenetic information, leading to the hypothesis (for review, see Eastman 1993, Clarke & Johnston 1996) that most sub-Antarctic notothenioid taxa predate the complete isolation of Antarctica (25 m.y.a.).

If we examine species distributions in light of the integrated phylogenetic tree shown in Fig. 2, a different scenario is apparent. Tectonic events can be invoked as vicariant factors to explain the divergence of *P. urvillii* and *E. maclovinus* from the rest of non-bovichtid notothenioids. The fragmentation of Gondwana and separation of Australia from Antarctica (38 m.y.a.) is probably the vicariant event that led to the speciation of *P. urvillii*, which is endemic to Australian waters. The successive deepening of Drake Passage and formation of Polar Front Zone (25–22 m.y.a.) might have then split the ancestral notothenioid stock, giving rise to the sub-Antarctic species *E. maclovinus* and to the common ancestor of the rest of the notothenioid species. Vicariance, on the other hand, cannot account for the divergence of other taxa like *Dissostichus eleginoides* Smitt, *Patagonotothen tessellata, Notothenia angustata* and *Paranotothenia magellanica*, which are also distributed along the coasts of South America. The tree topology that emerges from molecular data does not separate these sub-Antarctic species from Antarctic ones (Fig. 2), as it would be expected under a vicariant speciation mode. Estimates of divergence time between taxa confirm the hypothesis of a limited role of the settlement of the APF as a vicariant event. The separation of *E. maclovinus* from all species comprised in clade C appears to date back to either 27 m.y.a. (Bargelloni *et al.* 2000), or 21 m.y.a. (this paper), using either mitochondrial rRNA sequence data or LDHA data. The latter estimate (21 m.y.a.) was obtained through the analysis of LDHA sequence data from Fields & Somero (1998). A molecular divergence rate was calibrated, assuming that divergence between notothenioid taxa and *Gillichtis mirabilis* Cooper occurred at the time of the perciform radiation (60 m.y.a.). The average distance (corrected according to Kimura 1980) between notothenioid taxa and the outgroup is 11.5% and this gives a rate of 0.19% sequence divergence m.y.$^{-1}$ Applying this rate yields a estimate of 21 m.y.a. for the divergence of *E. maclovinus*, and 12–8 m.y.a. for the diversification of the remaining notothenioid taxa, which include two other sub-Antarctic species, *P. tessellata* and *P. magellanica* (distance data not shown). These taxa then appear to have diverged much later than 25–22 m.y.a., which is the estimated time for the settlement of the APF.

Caution is necessary when using molecular clocks. Variances of time estimates can be rather large, owing to the erratic

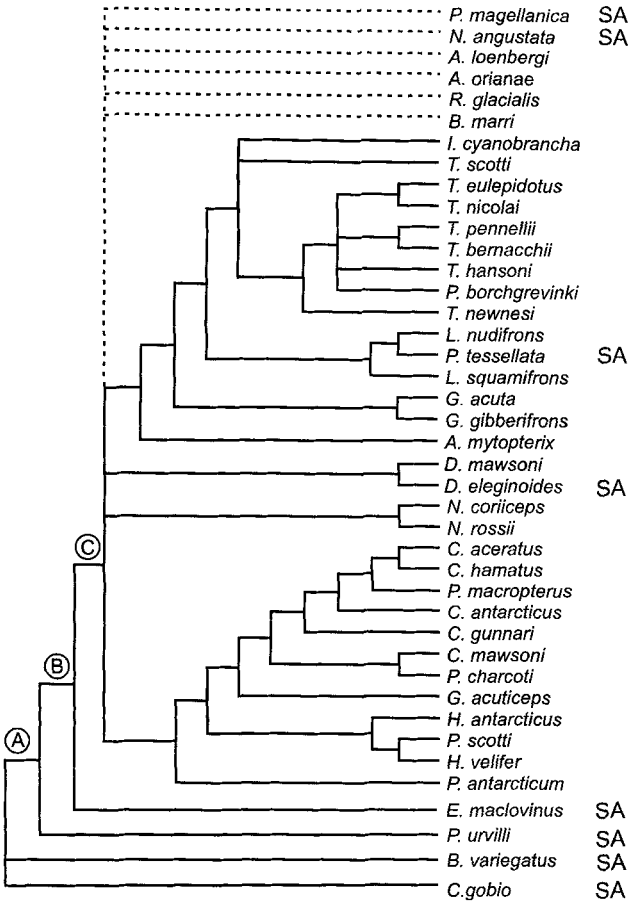

Fig. 2. Cladogram summarizing phylogenetic evidence for notothenioid species based on mitochondrial ribosomal genes (Ritchie *et al.* 1997, Bargelloni *et al.* 2000). Phylogenetic relationships within clade C are based on a maximum parsimony (MP) analysis was of 16S and 12S rRNA genes reported in Bargelloni *et al.* (2000). See this paper for further detail on phylogenetic methods and results. Dashed lines refer to unresolved relationships within clade C for those species which have been examined in other studies (see text). Circled capital letters refer to specific branching points (see text). Species with sub-Antarctic distribution are indicated with SA.

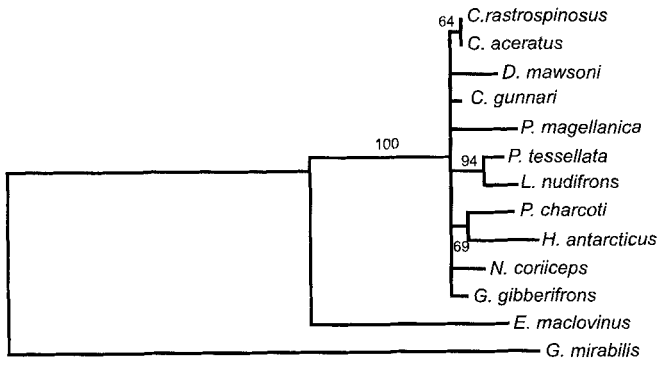

Fig. 3. Neighbour-joining (NJ) tree constructed on the basis of Kimura (1980) pairwise distances between LDHA notothenioid sequences (see Table I). Numbers at branching points refer to bootstrap values after 2000 replicates. Only values higher than 50% are shown. *Gillichtis mirabilis* (Perciformes, Gobiidae) was used as outgroup.

behaviour of the clock and to approximation in the calibration procedure. Rate heterogeneity among taxa might be observed as, for instance, in the case of some fast or slow evolving notothenioid sequences in the 12S and 16S rRNA data set (Bargelloni *et al.* 2000) or in the 28S rRNA data (data not shown). Another source of potential errors is the rate calibration. Molecular clocks are often adjusted according to ancient cladogenetic events. In cases of substantial phylogenetic distances, substitutions could have reached saturation, and this in turn might lead to underestimates in the divergence rate between recently separated taxa. In the case of the diversification of species within clade C, however, underestimating divergence rates (or overestimating divergence times) should be considered a conservative approach if the null hypothesis is that most notothenioid lineages diverged in the Oligocene (38–30 m.y.a.). Hence, failure to reject the null hypothesis might be a result of the rate underestimation, as might be the case for estimates based on globin genes where the 400 m.y. divergence time between actinopterygians and sarcopterygians is used for calibration (Stam *et al.* 1997).

Despite all limitations, both internally and externally calibrated rates appear to indicate divergence of bovichtid species, as well as of *E. maclovinus*, before the establishment of the APF. The diversification of several notothenioid lineages (included in clade C) seems to have started later than 20–15 m.y.a. (Bargelloni *et al.* 1994, 2000), with some estimates (antifreeze genes, Chen *et al.* 1997; LDHA, this paper) suggesting an even more recent date (12–5 m.y.a.).

The combined analysis of tree topology and times of divergence offers compelling evidence against the hypothesis of vicariant speciation for most of the notothenioids lineages distributed in the sub-Antarctic regions (Figs 1 & 2). The divergence of *E. maclovinus* can be explained as a consequence of passive vicariance after the establishment of the APF, whilst several other species (*D. eleginoides*, *P. tessellata*, *N. angustata* and *P. magellanica*) appear to have achieved a sub-Antarctic distribution through dispersal across the APF. Such dispersal events can be considered instances of "jump dispersal", colonizations which occur only rarely across a substantial barrier (Myers & Giller 1988). We are then left with the question: how did these species cross this hydrographic barrier? *Dissostichus eleginoides*, like its sister species, *D. mawsoni* Norman, can swim down to more than 2000 m; thus, the APF, which extends to only 1000 m in depth, does not represent a barrier for this species. *Dissostichus eleginoides*, a pelagic species with pelagic eggs like *P. magellanica*, is found south of the APF, on the shelf of several islands around the Antarctic continent. *N. angustata* and *P. tessellata*, on the other hand, are benthic, sedentary species living in coastal waters, with a much lower dispersal ability. A likely scenario

https://doi.org/10.1017/S0954102000000328 Published online by Cambridge University Press

for the origin of *P. tessellata* is through "jump" dispersal along the Scotia Arc, across the APF, and consequent speciation by isolation. According to two independent phylogenetic reconstructions (Figs 2 & 3), this species is placed as the closest sister taxon to *Lepidonotothen nudifrons* Lönnberg (Bargelloni *et al.* 2000), which is distributed from the tip of the Antarctic Peninsula to South Georgia. Such a distribution is rather intriguing. In fact, the only relatively shallow water route for migration into or out of the Antarctic waters is supposed (Eastman 1993) to pass through the islands and underwater ridges of the Scotia Arc that forms an easterly arc between South America and the Antarctic Peninsula. Therefore, similarly, dispersal over long geographic distances might be responsible also for the formation of some new notothenioid species. Several taxa which are distributed in the South Georgia Province or in the Kerguelen subregion (Fig. 1) appear, in the molecular tree (Fig. 2), to be the closest sister groups to High Antarctic species, living on the Antarctic continental shelf. Harpagiferidae, for instance, are found almost exclusively on the islands around the continent, often endemic to a particular group of islands. According to Fig. 2, however, harpagiferids are the closest sister group to Artedidraconidae, which are typically found only in the waters surrounding the continent. A similar pattern is observed, quite unexpectedly, for *Indonotothenia cyanobrancha* Richardson, which is included (Bargelloni *et al.* 2000) in the same clade of Trematominae. While the latter are all High Antarctic species (except for *Trematomus hansoni* Boulenger which is found also at South Georgia), *I. cyanobrancha* is endemic to Îles Kerguelen, in the Kerguelen subregion (Fig. 1). This evidence seems to reject the hypothesis of a simple trend of progressive adaptation to colder environmental conditions, suggesting, instead, a recurrent, rather than unique, pattern of diversification through dispersal, with a lesser role for ecological elements.

All the observations made suggest that speciation events might have repeatedly occurred across "ecological" zones (Hureau 1994), possibly indicating that historical events (periodic expansions of ice sheet, northward movement of the APF, occasional dispersal via circumantarctic currents) and geographical features (large deep water bodies between coastal habitats) might be the most important factors in promoting species diversification. Repeated transitions between the High Antarctic Zone and the Ice-Free Zone also suggest that notothenioids have a remarkable ecological plasticity.

Other notothenioid taxa, however, such as Artedidraconidae, Channichthyidae, Trematominae are sympatrically distributed in the waters surrounding Antarctica. Although the High Antarctic Zone does not represent a single habitat, there are no clear physical barriers to gene flow even between distant geographic areas, while the current system favours passive dispersal of the pelagic nototheniod larvae around the continent. On the other hand, episodes of partial deglaciation occuring during the Pliocene might have modified the Antarctic coastal habitat with formation of local basins, possibly promoting allopatric speciation (Eastman 1993). Only genetic studies at the intraspecific level, however, could allow the estimate of effective dispersal capacity, which is likely to be different for different notothenioid species and depend on biology and life-history. Species with reduced dispersal ability, if present, might then provide evidence that populations might become effectively isolated in certain areas even under the present-day ocean current conditions.

Historical factors, however, might not be the only cause of speciation. In a recent study (Chen *et al.* 1998) a few ecological features of each species were mapped onto the phylogenetic tree of channichthiids. The authors observed that changes in either feeding behaviour or depth range were associated with many cladogenetic events. Although such an association does not imply that the ecological shifts caused speciation, nevertheless it suggests a possible relationship between colonization of new niches and species divergence. The study also indicated the importance of carrying out phylogenetic analyses at the level of single notothenioid families. To this end, molecular data for two mitochondrial regions (D-loop and cytochrome *b*) are being collected on several species of Artedidraconidae, Harpagiferidae and Bathydraconidae (Derome & Lecointre, unpublished data). These families have been poorly sampled in previous studies. We report here preliminary evidence on nine bathydraconid species, belonging to different genera (Fig. 4). Phylogenetic relationships of Bathydraconidae appear extremely problematic, as four separate lineages emerge from the basal polytomy, with no evidence for monophyly of this family based on molecular data. These new results confirm that traditional hypotheses on notothenioid taxonomy are often not reliable, considering that DNA sequence data have suggested paraphyly at the family level, as well as for sub-families and even genera (as for instance in the case of the genus *Bathydraco* in Fig. 4). As mentioned above, biogeographic studies should be based on accurate phylogenetic trees. The need for detailed and robust phylogenies of nototheniod families, in order to complete the larger framework already available from previous molecular studies, is therefore obvious.

The Euphausiids. Compared to the wealth of phylogenetic studies conducted on notothenioids, euphausiid species have received far less attention. To date, a single study has appeared in the literature (Patarnello *et al.* 1996), where phylogenetic relationships among several euphausiids were reconstructed by sequencing part of the 16S rRNA mitochondrial gene. Analysed species included the two most abundant Antarctic *Euphausia* species, namely *E. superba* and *E. crystallorophias*, and a sub-Antarctic species, *E. vallentini*. The inferred phylogenetic tree unambiguously clustered together the two Antarctic species, with *E. vallentini* as their sister-group. A molecular clock, calibrated on crustacean 16S rRNA (Cunningham *et al.* 1992), was used to date the separation of *E. vallentini* from the Antarctic *Euphausia* clade, which might have occurred 20–19 m.y.a. This estimate

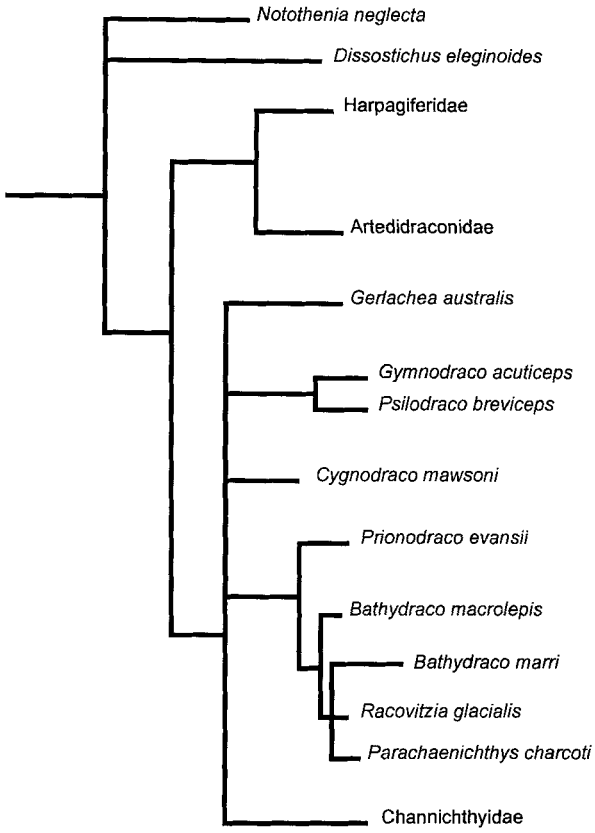

Fig. 4. Summary of the strict consensus tree obtained from MP analysis of a combination of D-loop and cytochrome *b* sequences of 34 species harpagiferids, artedidraconids, bathydraconids and channichthyids (Derome *et al.* unpublished data; tree length = 1382, consistency index = 0.488, ritention index = 0.698, total sequence length 1009 base pairs, providing 344 MP informative sites). Nodes collapsed are those of the strict consensus, however to be more conservative nodes with less than 90% bootstrap values were also collapsed. Consequently the nodes shown are considered the most reliable.

is in agreement with the time of formation of the APF (25–22 m.y.a.). Based on this evidence, vicariant speciation was proposed, suggesting that the APF might have represented a hydrographic barrier which could have promoted the separation of the ancestral lineage from which evolved the

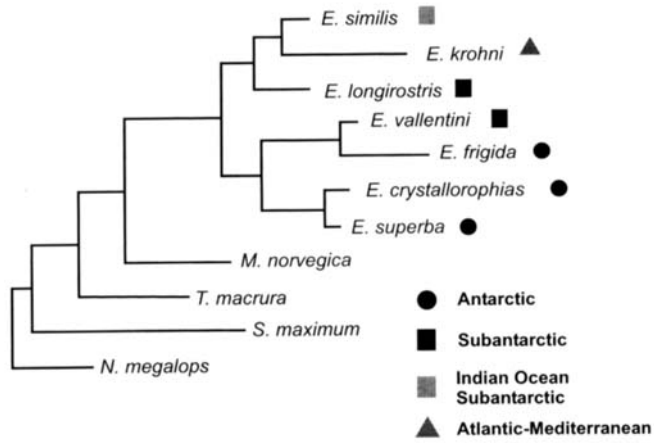

Fig. 5. Phylogenetic relationships among krill species. The tree is based on a NJ analysis of 16S rRNA sequences reported in Zane & Patarnello (in press). Distribution of species is indicated by different geometrical symbols.

Antarctic clade of the genus *Euphausia* (Patarnello *et al.* 1996). The mentioned paper, however, included only a limited number of euphausiid taxa. To obtain a more complete picture, the same region of the 16S rRNA has been sequenced in four additional species of the genus *Euphausia* from a wider geographic area, and two new outgroups have been included in the phylogenetic analysis, namely *Stylocheiron maximum* Hansen and *Nematoscelis megalops* G.O. Sars (Zane & Patarnello in press). A list of the species (and their distribution) examined is reported in Table II.

The phylogenetic tree based on the new data set shows the genus *Euphausia* to be monophyletic with all nodes having a strong bootstrap support. Within this clade two distinct lineages emerge, one grouping together *E. krohni* (a Mediterranean species), *E. similis* and *E. longirostris* (two sub-Antarctic species with distribution similar to *E. vallentini*). The other clade includes *E. superba*, *E. crystallorophias* and *E. vallentini* (these three are grouped together as in Patarnello *et al.* (1996)) and *E. frigida*, a fully Antarctic species. Interestingly, *E. frigida* is placed as sister group of the sub-Antarctic species *E. vallentini*, and not into the "Antarctic" clade (*E. superba* + *E. crystallorophias*). Euphausiid

Table II. Krill species examined, relative distribution and reference.

Species	Distribution	Reference
Euphausia superba Dana	Antarctic	Patarnello *et al.* 1996
Euphausia vallentini Stebbing	sub-Antarctic	Patarnello *et al.* 1996
Euphausia crystallorophias Holt & Tattersall	Antarctic	Patarnello *et al.* 1996
Meganyctiphanes norvegica M. Sars	Mediterranean–North Atlantic	Patarnello *et al.* 1996
Thysanoessa macrura G.O. Sars	Antarctic	Patarnello *et al.* 1996
Euphausia frigida Hansen	Antarctic	Zane & Patarnello in press
Euphausia longirostris Hansen	sub-Antarctic	Zane & Patarnello in press
Euphausia similis G.O. Sars	sub-Antarctic	Zane & Patarnello in press
Euphausia krohni Brandt	Mediterranean–Atlantic	Zane & Patarnello in press
Nematoscelis megalops G.O. Sars	Mediterranean–Atlantic	Zane & Patarnello in press
Stylocheiron maximum Hansen	Mediterranean–Atlantic	Zane & Patarnello in press

https://doi.org/10.1017/S0954102000000328 Published online by Cambridge University Press

phylogenetic relationships from Zane and Patarnello (in press) are summarized in Fig. 5.

Assuming the same molecular clock previously applied to krill 16S rRNA (that is a transversional rate of 0.155% per million year (Patarnello *et al.* 1996)), the genetic distance (0.0117) between *E. vallentini* and *E. frigida* (calculated on transversions only for the 16S rRNA) can be used to infer that the split between these two species occurred *c.* 7 m.y.a., therefore considerably later than the settlement of the APF. When the same calculation is applied for estimating the separation between the "Antarctic clade" (*E. superba* + *E. crystallorophias*) and the sister clade (*E. vallentini* + *E. frigida)* the result (*c.* 20 m.y.a.) is still compatible with the period of formation of the APF.

On the basis of these results, if the Antarctic clade originated by vicariance, the present day distribution of *E. frigida* (south to the Antarctic Convergence) should be interpreted as the consequence of a dispersal event. In summary, the speciation processes could have started about 20 m.y.a. with a first split, possibly promoted by the formation of the APF, which separated the Antarctic lineage (the ancestor of *E. superba* and *E. crystallorophias)* from a sub-Antarctic one. A later split (7 m.y.a.) occurred within the sub-Antarctic stock, giving rise to *E. vallentini* and *E. frigida.* The latter species, during the course of its evolution, was able to disperse southward, crossing the APF. Alternatively, the four species (*E. superba, E. crystallorophias, E. vallentini* and *E. frigida*) might have an Antarctic common ancestor and *E. vallentini* may have originated after dispersal across the APF into sub-Antarctic waters. This evidence is similar to that observed for notothenioid species, indicating that in any case vicariance could not be the only force responsible for range separation of Antarctic and sub-Antarctic euphausiids or nothenioids.

Intraspecific studies

The Notothenioidei. To understand speciation processes in the nototheniod fish population genetics studies are needed, complementing evidence from phylogenetic analyses. Intraspecific data on notothenioid species, however, are extremely scarce. The few reports available in the literature in general seem to indicate lack of genetic structure in the species examined (*Notothenia rossii* Richardson and *Champsocephalus gunnari* Lönnberg, allozymic data, (Duhamel *et al.* 1995); *C. gunnari,* mitochondrial DNA data (Williams *et al.* 1994)).

Some indirect evidence of genetic differentiation, however, was reported for *Dissostichus eleginoides* at the biochemical level (Diano 1989), and for *Trematomus hansoni* in the form of caryotypic polymorphism (Pisano *et al.* 1998). Moreover, in a recent paper on intraspecific allozyme variation in channichtids (Clement *et al.* 1998), genetic differentiation in two species, *Neopagetopsis ionah* Nyebelin and *Chionodraco myersi* Tyler, was reported between population samples collected in Prydz Bay and the Weddell Sea, respectively. While the limited number of sampling sites and of specimens examined suggests some caution, analyses conducted on mitochondrial DNA intraspecific variation in two other notothenioid taxa (*Pleuragramma antarcticum*, and *Chionodraco hamatus* Lönnberg and *C. rastrospinosus*, Bargelloni & Patarnello unpublished data) seem to confirm that, despite the large potential for dispersal, gene flow might be indeed reduced among populations. Quite interestingly, *P. antarcticum* shows a significant differentiation between samples collected in the Weddell Sea and on the west side of the Antarctic Peninsula, respectively. As these two sites are geographically close, other mechanisms rather than geographic distance, for instance the presence of the Weddell–Scotia Sea confluence, might be responsible for the observed reduction of genetic exchange. In *P. antarcticum*, a truly pelagic species, some genetic structure is evident between populations sampled in two different zoogeographic provinces (East Antarctic Province (EAP) and West Antarctic Province (WAP), Fig. 1). Even stronger is the genetic differentiation observed in the genus *Chionodraco*. *Chionodraco rastrospinosus*, Dewitt & Hureau distributed only in the WAP and *C. hamatus*, generally found in the EAP, although genetically close, represent two distinct phylogenetic units, mirroring the separation between EAP and WAP detected in *P. antarcticum*. Moreover, population samples of *C. hamatus* collected in the Weddell Sea and in the Ross Sea show significant genetic heterogeneity (Bargelloni & Patarnello unpublished data), suggesting reduced migration between these two areas. The observed evidence is not surprising considering that these two *Chionodraco* species have a benthic habit, which might decrease the potential for dispersal compared to a pelagic species as *P. antarcticum*. Although preliminary, these results, together with the observation reported by Clement *et al.* (1998), seem to suggest a genetic discontinuity between populations in species distributed around the continent. This might represent a partial answer to the open question about the possible mechanisms of diversification among High Antarctic, co-distributed species.

The Euphausiids. Contrasting with the strong pattern of differentiation that is becoming increasingly clear for notothenioids, euphausiids or more precisely, *Euphausia superba*, the only species for which intrapopulation data are present in the literature, seems to be more genetically homogeneous, as expected for planktonic organisms. Despite reports of genetic heterogeneity more than 20 years ago (Ayala *et al.* 1975), only weak genetic differentiation among populations has been detected so far. In particular, one of the first studies (Fevolden & Ayala 1981), based on allozymes found statistically significant differences in allele frequencies at the phosphoglucose isomerase (PGI) locus between samples collected near Anvers Island, and samples from South Orkney Islands and Bouvet Island. This first result, though followed by a concordant result of genetic differentiation between Anvers Island samples and specimens from west of the Antarctic

Peninsula (Anderson 1982,cited in (Fevolden & Schneppenheim 1989)), was not to gain further support from allozymic data. Analyses of these, together with several other population samples from Antarctica, failed to detect any other deviation from genetic homogeneity (Fevolden & Schneppenheim 1989), and the first reports were explained as technical or statistical vagaries, or being due to localized non-random mating and selection acting on particular genes (Fevolden & Schneppenheim 1989).

Only recently another finding of genetic differentiation has been reported (Zane *et al.* 1998). This study, based on mtDNA sequence data of the NADH dehydrogenase subunit 1 (ND1) gene, analysed four population samples from different sectors of the Southern Ocean (Zane *et al.* 1998). The samples collected at South Georgia and the one from east Weddell Sea were found to be significantly different and the overall probability distribution suggested that krill are not genetically homogeneous.

The difference between the South Georgian samples and those from the east Weddell Sea appears intriguing considering that these two locations are separated by the Scotia–Weddell Confluence (see above), which could restrict the gene flow and create two isolated populations.

A comparison of intraspecific data on genetic variation in krill and notothenioids seems to reveal shared patterns as well as marked differences. Genetic differentiation is associated in all species with the transition between East and West Antarctic Province, in the area where the Scotia–Weddell confluence is present. This might suggest that the same oceanographic feature is able to influence the genetic population structure of extremely diverse marine organisms. However, the observed genetic differentiation is very weak for *E. superba*, more pronounced in *P. antarcticum*, while for *Chionodraco* species it represents a phylogenetic break. Such differences are in good agreement with the different population sizes and dispersal capacities of the species examined.

Concluding remarks

In summary, the study of biogeography in Antarctic organisms might profit greatly from the application of molecular tools, both at the interspecific and intraspecific level. Reliable phylogenies have been obtained for notothenioids and euphausiids, often with concordant evidence from independent data sets. This has provided a phylogenetic framework on which to map species distributions, thereby allowing the testing of alternative biogeographic hypotheses. Although a more complete molecular taxonomy is needed, especially for the notothenioids, where "only" half of the recognized species have been examined, molecular trees have already provided convincing evidence on some long-standing evolutionary questions.

Much less is known about how genetic variation is distributed within the single species. Intraspecific data, however, are becoming increasingly available, and the day is not too distant when the macroevolutionary and microevolutionary approaches will be fully integrated.

Acknowledgements

We thank all the participants in the SCAR workshop on Evolutionary Biology of Antarctic Organisms for fruitful discussions, and the organizers for inviting this contribution. Part of the samples for the population genetic studies mentioned in the text were collected during the "ANT XII Cruise 1996" (sponsored by Alfred Wegener Institute), and the "Gene Flow Cruise 1997" sponsored by British Antarctic Survey. The Italian Antarctic Research Program (PNRA) is acknowledged for financial support to L.B., L.Z. and T.P. We thank the referees, L. Hauser and A. Rogers for their helpful comments.

References

AVISE, J.C. 1994. *Molecular markers, natural history and evolution.* New York: Chapman & Hall, 5–15.

AYALA, F.J., VALENTINE, J.W. & ZUMWALT, G.S. 1975. An electrophoretic study of the Antarctic zooplankter *Euphausia superba*. *Limnology and Oceanography*, **20**, 635–640.

BARGELLONI, L., MARCATO, S., ZANE, L. & PATARNELLO, T. 2000. Mitochondrial phylogeny of notothenioids: a molecular approach to Antarctic fish evolution and biogeography. *Systematic Biology*, **49**, 114–129.

BARGELLONI, L., RITCHIE, P.A., PATARNELLO, T., BATTAGLIA, B., LAMBERT, D.M. & MEYER, A. 1994. Molecular evolution at subzero temperatures: mitochondrial and nuclear phylogenies of fishes from Antarctica (suborder Notothenioidei), and the evolution of antifreeze glycopeptides. *Molecular Biology Evolution*, **11**, 854–863.

CHEN, L., DEVRIES, A.L. & CHENG, C.H. 1997. Evolution of antifreeze glycoprotein gene from a trypsinogen gene in Antarctic notothenioid fish. *Proceedings of the National Academy of Sciences of the USA*, **94**, 3811–3816.

CHEN, W.-J., BONILLO, C. & LECOINTRE, G. 1998. Phylogeny of the Channichthyidae (Notothenioidei, Teleostei) based on two mitochondrial genes. *In* DI PRISCO, G., PISANO, E. & CLARKE, A., *eds. Fishes of Antarctica.* Milan: Springer Verlag, 287–298.

CLARKE, A. & JOHNSTON, I.A. 1996. Evolution and adaptive radiation of Antarctic fishes. *Trends in Ecology and Evolution*, **11**, 212–218.

CLEMENT, O., OZOUF-COSTAZ, C., LECOINTRE, G. & BERREBI, P. 1998. Allozymic polymorphism and phylogeny of the family Channichthyidae. *In* DI PRISCO, G., PISANO, E. & CLARKE, A., *eds. Fishes of Antarctica.* Milan: Springer Verlag, 299–309.

CRAME, J.A. 1993. Latitudinal range fluctuations in the marine realm through geological time. *Trends in Ecology and Evolution*, **8**, 162–166.

CUNNINGHAM, C.W., BLACKSTONE, N.W. & BUSS, L.W. 1992. Evolution of king crabs from hermit crab ancestors. *Nature*, **355**, 539–542.

DIANO, M. 1989. Enzymatic data as a criterion for characterizing two populations of *Dissostichus eleginoides* (Notothenioidei). *Marine Biology*, **103**, 416–420.

DUHAMEL, G., OZOUF-COSTAZ, C., CATTANEO-BERREBI, G. & BERREBI, P. 1995. Interpopulation relationship in two species of Antarctic fish *Notothenia rossii* and *Champsocephalus gunnari* from the Kerguelen Islands: an allozyme study. *Antarctic Science*, **7**, 351–356.

EASTMAN, J.T. 1993. *Antarctic fish biology.* San Diego: Academic Press, 322 pp.

FEVOLDEN, S.E. & AYALA, F.J. 1981. Enzyme polymorphism in Antarctic krill (Euphausiacea); genetic variation between populations and species. *Sarsia,* **66**, 167–181.

FEVOLDEN, S.E. & SCHNEPPENHEIM, R. 1989. Genetic homogeneity of krill (*Euphausia superba* Dana) in the Southern Ocean. *Polar Biology,* **9**, 533–539.

FIELDS, P.A. & SOMERO, G.N. 1998. Hot spots in cold adaptation: localized increases in conformational flexibility in lactate dehydrogenase A4 orthologs of Antarctic notothenioid fishes. *Proceedings of the National Academy of Sciences of the USA,* **95**, 11476–11481.

FUTUYMA, D. 1998. *Evolutionary biology.* 3rd ed. Sunderland, MA: Sinauer Associates, 201–226.

HILLIS, D.M., MORITZ, C. & MABLE, B.K. 1996. Applications of molecular systematics. *In* HILLIS, D.M., MORITZ, C. & MABLE, B.K., *eds. Molecular systematics.* Sunderland, MA: Sinauer Associates, 515–543.

HUREAU, J.-C. 1994. The significance of fish in the marine Antarctic ecosystems. *Polar Biology,* **14**, 307–313.

KENNETT, J.P. 1982. *Marine geology.* Englewood Cliffs, NJ: Prentice-Hall, 401–420.

KIMURA, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution,* **16**, 111–120.

LECOINTRE, G., BONILLO, C., OZOUF-COSTAZ, C. & HUREAU, J.C. 1997. Molecular evidence for the origins of Antarctic fishes: paraphyly of the Bovichtidae and no indication for the monophyly of the Notothenioidei (Teleostei). *Polar Biology,* **18**, 193–208.

MACKINTOSH, N.A. 1973. Distribution of post larval krill in the Antarctic. *Discovery Reports,* **36**, 95–156.

MCGOWAN, J.A. 1977. What regulates pelagic community structure in the Pacific? *In* ANDERSEN, N.R. & ZAHURANEC, B.J., *eds. Oceanic sound scattering predictions.* New York: Plenum Press, 423–443.

MILLER, D.G. & HAMPTON, I. 1989. Biology and ecology of the Antarctic krill (*Euphausia superba* Dana): a review. *Biomass Scientific Series*, No. 9, 1–166.

MYERS, A.A. & GILLER, P.S. 1988. *Analytical biogeography.* London: Chapman Hall, 120–125.

PATARNELLO, T., BARGELLONI, L., VAROTTO, V. & BATTAGLIA, B. 1996. Krill evolution and the Antarctic ocean currents: evidence of speciation as inferred by molecular data. *Marine Biology,* **126**, 603–608.

PISANO, E., OZOUF-COSTAZ, C. & PRIRODINA, V. 1998. Chromosome diversification in Antarctic fish (Notothenioidei). *In* DI PRISCO, G., PISANO, E. & CLARKE, A., *eds. Fishes of Antarctica.* Milan: Springer Verlag, 275–285.

RIDLEY, M. 1996. *Evolution.* 2nd ed. Cambridge, MA: Blackwell Science, 508–533.

RITCHIE, P.A., BARGELLONI, L., MEYER, A., TAYLOR, J.A., MACDONALD, J.A. & LAMBERT, D.M. 1996. Mitochondrial phylogeny of trematomid fishes (Nototheniidae, Perciformes) and the evolution of Antarctic fish. *Molecular Phylogenetics and Evolution,* **5**, 383–390.

RITCHIE, P., LAVOUÉ, S. & LECOINTRE, G. 1997. Molecular phylogenetics and the evolution of Antarctic notothenioid fishes. *Comparative Biochemistry and Physiology,* **118A**, 1009–1025.

STAM, W.T., BEINTEMA, J.J., D'AVINO, R., TAMBURRINI, M., COCCA, E. & DI PRISCO, G. 1998. Evolutionary studies on teleost hemoglobin sequences. *In* DI PRISCO, G., PISANO, E. & CLARKE, A., *eds. Fishes of Antarctica.* Milan: Springer Verlag, 356–359.

STAM, W.T., BEINTEMA, J.J., D'AVINO, R., TAMBURRINI, M. & DI PRISCO, G. 1997. Molecular evolution of hemoglobins of Antarctic fishes (Notothenioidei). *Journal of Molecular Evolution,* **45**, 437–445.

WILLIAMS, R., SMOLENSKI, A.J. & WHITE, R.W.G. 1994. Mitochondrial DNA variation of *Champsocephalus gunnari* Lönnberg (Pisces: Channichtyidae) stocks on the Kerguelen Plateau, southern Indian Ocean. *Antarctic Science,* **6**, 347–352.

ZANE, L., OSTELLARI, L., MACCATROZZO, L., BARGELLONI, L., BATTAGLIA, B. & PATARNELLO, T. 1998. Molecular evidence for genetic subdivision of Antarctic krill (*Euphausia superba* Dana) populations. *Proceedings of the Royal Society London,* **B265**, 2387–2391.

ZANE, L. & PATARNELLO, T. In press. Krill: a possible model for investigating the effects of ocean currents on the genetic structure of a pelagic invertebrate. *Canadian Journal of Fisheries and Aquatic Sciences.*