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# Global genetic homogeneity in the deep-sea foraminiferan *Epistominella exigua* (Rotaliida: Pseudoparrellidae)*

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## Abstract

*Epistominella exigua* is one of the most common deep-sea foraminiferal morphospecies and has a world-wide distribution. A recent molecular study revealed high genetic similarity between Arctic, Atlantic and Antarctic populations of this species. Here, we show that the small-subunit (SSU) and internal transcribed spacer (ITS) rDNA sequences of an *E. exigua* population from Pacific are almost identical to those reported previously from the other three oceans. This result confirms the genetic homogeneity of *E. exigua*, which contrasts with the prevalence of highly differentiated populations in planktonic and shallow-water benthic foraminiferans. We discuss special features of diversifications mechanisms in the deep sea that may be responsible for the lack of genetic differentiation and global distribution of some meiofauna species.

**Keywords:** deep-sea diversity, meiofauna, Foraminifera, SSU rDNA, ITS rDNA, geographic distribution

## Introduction

At a local scale, deep-ocean sediments contain some of the most species rich communities on Earth (Grassle & Maciolek 1992). Recently, there has been an increasing emphasis on the relationship between local diversity and diversity at larger (regional to global) scales (Levin *et al.* 2001). However, there is still little information available about how widely species are distributed or about biodiversity at the genetic level (Etter *et al.* 1999, 2005). There is a particular need to increase the genetic database on small size deep-sea benthic organisms in order to learn whether the biodiversification mechanisms operating there are different from those in shallow-water.

Deep-sea benthic foraminiferans provide particularly good models to tackle these questions. They occur in all marine environments and their rich fossil record reveals their morphological evolution over geological time. Molecular studies, focused mainly on planktonic and shallow-water foraminiferal species, have demonstrated considerable cryptic diversity (reviewed in Pawlowski and Holzmann 2008, Darling *et al.* 2008). In striking contrast, three deep-sea species showed very low levels of genetic differentiation between populations in the Arctic and Antarctic sectors of the Atlantic, separated by a distance of up to 17,000 km (Pawlowski *et al.* 2007).

To explore whether this surprising degree of genetic homogeneity along a north-south axis was indicative of a global distribution for some deep-sea benthic meiofauna, we examined molecular diversity in *Epistominella exigua* from the Pacific Ocean and compared it to the earlier data on Arctic, Atlantic and

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Southern Oceans populations of this well-known and widely-distributed foraminiferal morphospecies (Pawlowski *et al.* 2007). Our analyses were based on sequences of the ITS rDNA, which have already revealed cryptic species of benthic foraminiferans (Tsuchiya *et al.* 2003). We found that the Pacific population is genetically similar to those from the other three oceans. This represents strong evidence that *E. exigua* is globally distributed and points to some major differences between biodiversification mechanisms occurring in deep-sea and shallow-water environments.

## Material and methods

*Sampling*: Pacific *Epistominella exigua* were collected off Japan during RV *Hakuho-Maru* cruise KH06-03. Our study includes also new sequences of *E. vitrea* and two unidentified species of genus *Epistominella* (morphologically close to *E. vitrea*) collected in Admiralty Bay (King George Island, Antarctica) (Majewski *et al.* 2007) and off Ushuaia (Argentina), one unidentified specimen collected off Japan, as well as two specimens of *E. arctica* collected during ANDEEP III cruise to Weddell Sea and during ARKXXI1b cruise to Arctic Ocean. Detailed information for each specimen is compiled in Supplementary Material, Table 1.

Samples were collected using a multiple corer equipped with tubes of 8.2 cm diameter. The upper 2 cm layer of selected cores was sliced off, sieved on a 63-micron mesh screen, and foraminiferans picked out individually by hand under a binocular microscope. *Epistominella* specimens were either directly fixed in guanidine DNA extraction buffer or stored frozen at -80°C.

*Sequencing, phylogenetic and statistical analyses*: The partial SSUrDNA and complete ITS rDNA region were amplified and sequenced as described in Pawlowski et al. (2007). Thirty-three new DNA sequences were obtained for partial SSU and 22 for ITS (1 to 6 clones were sequenced for each amplified product). They were deposited in the EMBL/GenBank under accession numbers listed in Supplementary Material, Table 1. The sequences were aligned using Seaview software and sequence divergence was calculated using PhyloWin (Galtier et al., 1996) with Kimura 2 parameters and pair wise comparison. SSU rDNA tree was built according to the Maximum Likelihood (ML) method using PhyML program (Guindon and Gascuel, 2003), with the GTR + I + G model (suggested by Mr Modeltest, Posada and Crandall 1998) with 6 categories of substitution rates and 1000 replicates for bootstrap analysis. Bayesian phylogenetic analyses were also performed on the same dataset with the same model using MrBayes program via Bioportal (*http://www.bioportal.uio.no*).

Haplotype ITS network was established with *E. exigua* sequences using TCS software, with gaps considered as 5$^{th}$ state (Clement et al., 2000). Population genetic analysis was performed with Analysis of Molecular Variance (AMOVA, Excoffier et al., 1992). We defined four hypothetic groups of different geographic origin (Arctic, Antarctic, Atlantic and Pacific) and tested their relevance by computing Fixation Indices $F_{SC}$ and $F_{CT}$ with Arlequin ver 3.01 (Excoffier et al., 2005). Significance tests were performed with 10,100 permutations.

## Results

*SSU rDNA*: New SSU rDNA sequences were obtained for 5 specimens of *Epistominella exigua*, 2 specimens of *E. vitrea*, 2 specimens of *E. arctica*, 3 specimens of *Epistominella* sp. 1, 2 specimens of *Epistominella* sp. 2 and 1 specimen of *Epistominella* sp. 3. The sequences were compared to 11 specimens of *E. exigua* from the Arctic Ocean, Weddell Sea and North Atlantic and to 7 specimens of *E. vitrea* from Weddell Sea and McMurdo Sound. Because we were interested in comparing specimens from extremely distant locations, our study focused on *E. exigua*. There were almost no differences between partial SSU rDNA sequences derived from this species, The only exceptions were four specimens from Weddell Sea (WED5127, WED5191, WED5222, WED3623), all from sites deeper than 4650 m, which differed from all other, including the Pacific

ones, by a single substitution (C – T). The total length of the sequenced SSU fragment was 1021 nucleotides (nt).

Phylogenetic analyses (Fig. 1) show *E. exigua* branching as sister group to *E. vitrea*, a species found at depths down to 1000 m in the Southern Ocean (Pawlowski et al. 2007). Four other clades of *Epistominella* can be distinguished, including *E. arctica* (depth ~ 2600 m), *Epistominella* sp. 1 (depth ~ 100 m), *Epistominella* sp. 2 (depth 20-100 m), and *Epistominella* sp. 3 (depth 1110 m). All of these species (except *E. arctica*) form well supported monophyletic clades, but the relationships between these clades are not well resolved.

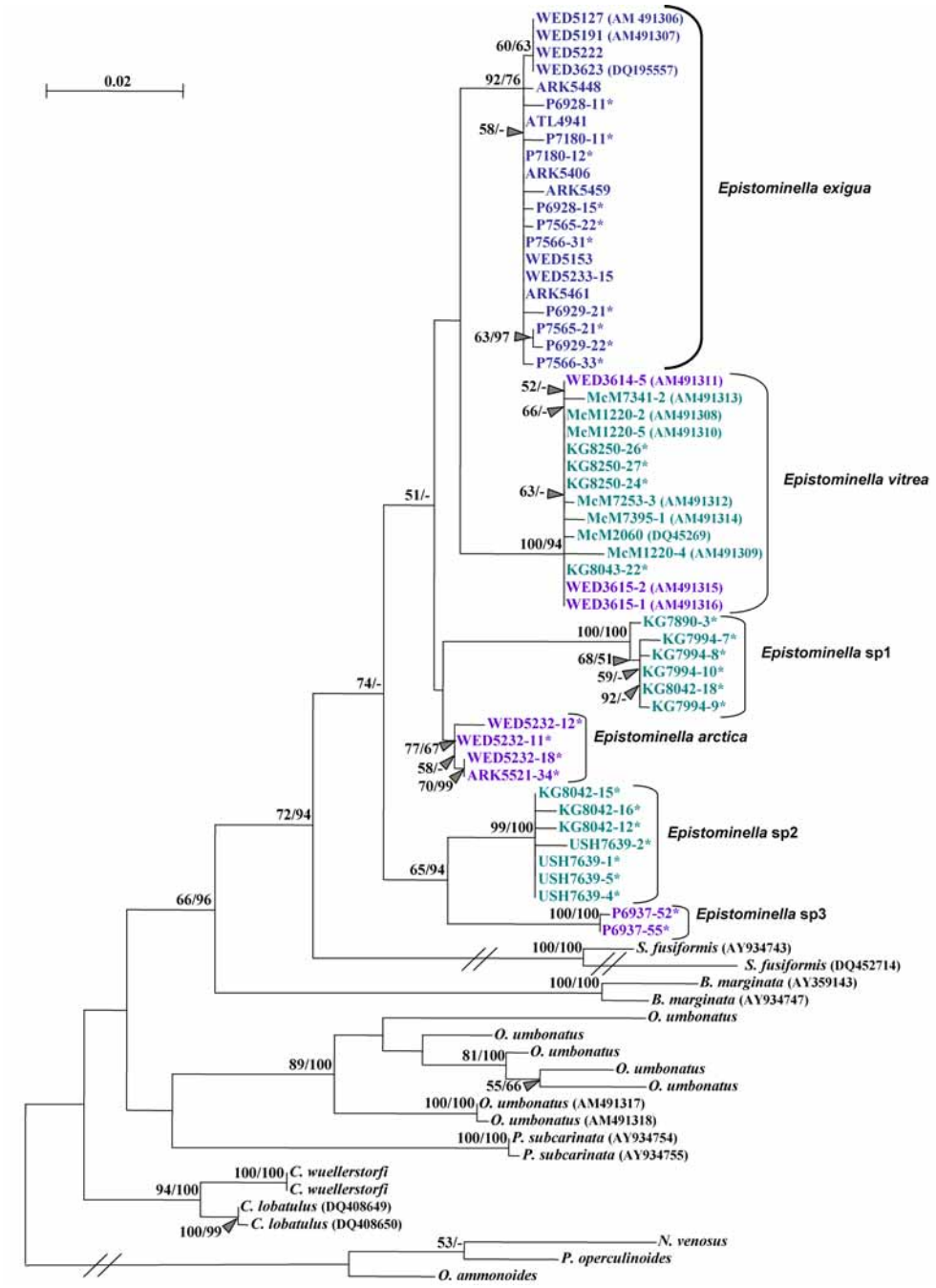


**FIGURE 1.** Phylogenetic tree inferred from SSU rDNA sequences showing the position of *E. exigua* among other *Epistominella* species and the lack of differentiation between populations of *E, exigua* from different regions. This tree has been obtained using the ML method with 1000 bootstrap replicates. The support values at internal nodes correspond to ML (left) and Bayesian (right) analyses. Only values over 50% were indicated. Within the genus *Epistominella*, first letters of the *Epistominella* sequence names indicate their geographical origin (ARK: Arctic, WED: Weddell Sea, ATL: North Atlantic, P: Pacific, McM: Mc Murdo Sound, USH: Ushuaia, KG: King George Island) and colour indicates a range of depths (purple: 0-200 m; green: 200–1200 m; dark blue: >1200 m). New sequences are indicated by a star (*).

*ITS rDNA*: To evaluate the genetic diversity within *E. exigua*, we analysed the complete ITS rDNA. We compared our 26 new Pacific sequences to 92 previously published sequences of *E. exigua* from the other three oceans (Pawlowski et al., 2007). The total length of sequenced fragment varied between 992 and 1009 nucleotides. There was a quite high number of variable sites (141) but most of them differed by single nucleotide polymorphisms (SNP) or insertion/deletion events. The maximum percentage of intra-individual polymorphism (1.2%) was almost as high as the variability between individuals (1.5%).

We then performed a population genetics analysis to better understand the internal structure of the *E. exigua* "global" population. The TCS network (Supplementary Material, Fig. 3) includes 104 haplotypes for a total of 118 sequences. Every haplotype is connected with one to nine others, which means that they are all extremely close. The lack of any biogeographic pattern in this network indicates that there is no cryptic speciation among the collected specimens. Sequences originating from different geographic regions are often closer to each other than to sequences from the same region.

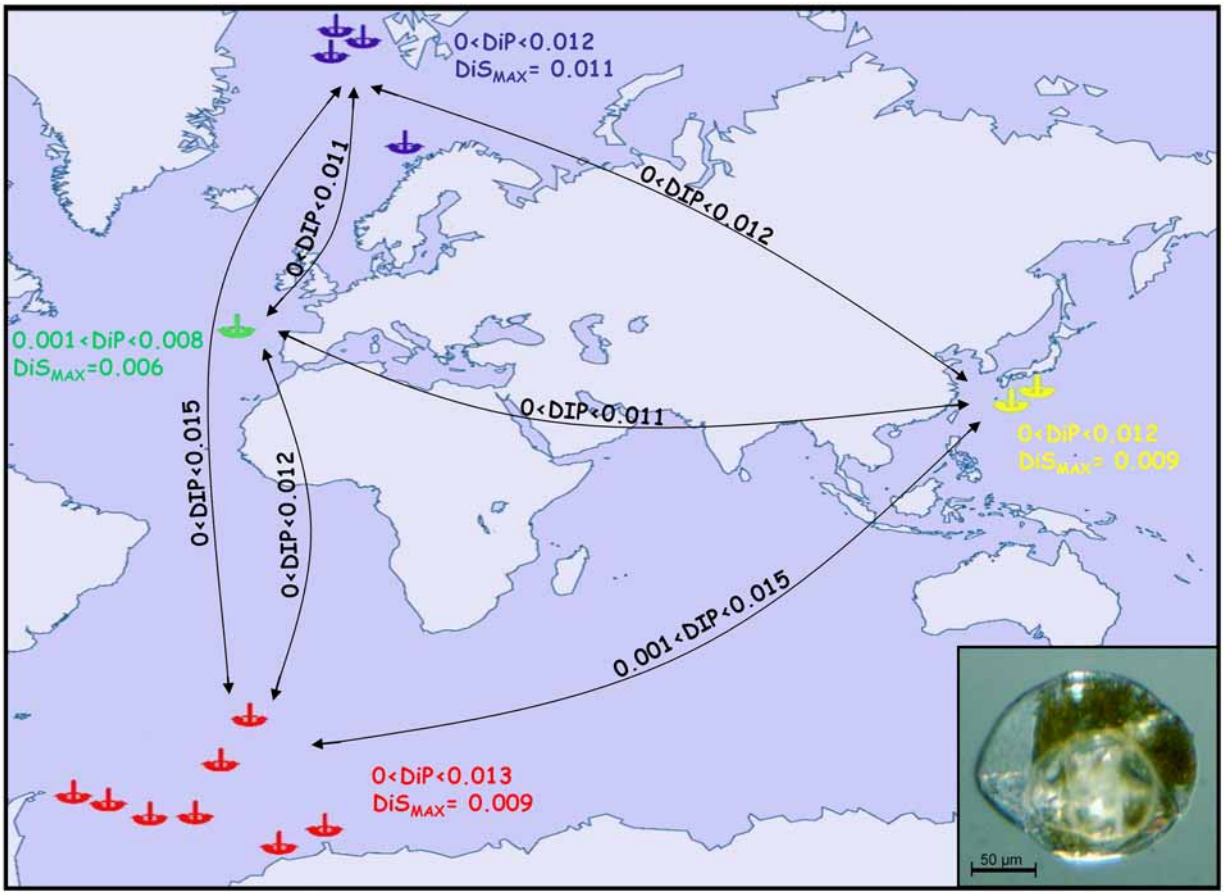


**FIGURE 2.** *In Background.* Map with position of *Epistominella exigua* sampling sites (red anchors for Antarctic, green for Atlantic, blue for Arctic and yellow for Pacific). *In Foreground. E. exigua* ITS rDNA sequence divergences. Minimum and maximum values of divergences within each geographic population (DiP) as well as maximum values of intraspecimen divergences (DiS) are indicated in color according the sampling sites. Minimum and maximum values of divergences between each geographic population (DIP) are indicated above black arrows. *Inset. Epistominella exigua* specimen collected off Japan.

Finally we performed an AMOVA analysis to quantify the different components of the genetic variations. By computing the Fixation Indices, we evaluated the part of the variation due to the differences inside each specimen, those due to differences between specimens and finally those due to the differences between the "hypothetic" geographic populations: Arctic, Antarctic, Atlantic and Pacific. We found an $F_{CT}$ value of 0.11 (p= 0.0023) and an $F_{SC}$ value of 0.38 (p= 0.0000). This means that a significantly greater part of the genetic variation is due to differences between specimens within each population rather than differences between populations. $F_{SC} > F_{CT}$ indicates that the subdivision of the four geographic populations are not relevant and

thus *E. exigua* specimens form one big genetically homogenous population. This lack of geographic pattern is also obvious in Fig.2, which shows sequence divergences within different clones from the same specimen (intraspecimen divergence "DiS"), within each region (intrapopulation divergence "DiP"), and between regions (interpopulations divergence "DIP"). In each case, the minimum value found for DIP is smaller than the maximum value found for DiP and DiS. This means that some sequences from the same site are more different from each other than they are from sequences obtained at other sites. Moreover, for each site some intraspecimen divergences exceed some divergences between sequences from different locations.

## Discussion

The present study confirms our previous analyses showing a lack of genetic differentiation between populations of *E. exigua* from the Arctic, Atlantic and Southern Oceans, with the exception of an abyssal subpopulation from Weddell Sea that may be undergoing speciation (Pawlowski et al. 2007). The SSU phylogenetic tree shows that the diversity within the deep-sea clade of *E. exigua* from different oceans is as high as the diversity within the shallow-water clades of *Epistominella vitrea*, *Epistominella* sp. 1, and *Epistominella* sp. 2 from the same region (Southern Ocean). We also observe very low diversity between populations of *E. arctica* from Arctic and Antarctic, but this species is represented in our material by only two specimens. Although the SSU rDNA is known to be quite conservative, our analyses show clearly that this gene is able to distinguish different species of *Epistominella.* Therefore the lack of divergence between populations of *E. exigua* is not an artifact caused by slow evolutionary rates. Although the samples from distant localities are not yet available for the shallow water *Epistominella* species, we predict that their diversity will be much higher than in *E. exigua.*

The genetic homogeneity of *E. exigua* in the SSU and especially in the ITS supports morphological evidence for a global distribution of some small (meiofauna-sized), abyssal foraminiferans and clearly contrasts with the high genetic diversification usually observed in shallow-water benthic species (Pawlowski and Holzmann 2008, Pawlowski et al. 2008). We therefore advance the hypothesis that global genetic homogeneity is more common in the abyssal deep sea than in shallow water. This could be explained by differences in dispersal opportunities and/or in biodiversification mechanisms.

Compared to shallow-water habitats where strong environmental gradients and seafloor topography create ecological barriers, the deep-sea floor provides a more uniform environment with a greater potential for global gene flow, particularly across abyssal plains. *Epistominella exigua* is a predominantly abyssal species (Murray, 1991). It has an opportunistic life style, exploits ephemeral patches of phytodetritus derived from surface production (Gooday, 1988, 1993), and tends to be abundant where primary production is strongly seasonal (Sun et al., 2006). Thus, metapopulations of *E. exigua* could comprise spatially separated, relatively dense, temporally fluctuating populations of different sizes, living wherever a suitable habitat exists, and with some degree of gene flow occurring between them. *Epistominella exigua* also occurs over a much wider range of organic flux rates (spanning three orders of magnitude) than other deep-sea foraminiferans (Altenbach et al., 1999). Dispersal and gene flow therefore may be mediated by the sparse populations living in more oligotrophic regions (Gooday, 1996) where phytodetrital deposits are likely to be confined to occasional small patches, as well as by the ability of foraminiferans to survive long periods of starvation.

The small size of foraminiferans such as *E. exigua* (typically 100-200 μm) may also help to ensure their wide dispersal. Because body size is inversely correlated to population size, microbial eukaryotes and small metazoans (< 1 mm) occur in sufficient numbers to ensure ubiquitous distributions (Finlay et al., 2002). Many other deep-sea benthic foraminiferans, particularly those living at abyssal depths, have very wide distributions (Murray, 1991; Pawlowski and Holzmann 2008) and may be dispersed globally. A similar conclusion has been suggested in the case of hydrothermal vent flagellates (Atkins et al., 2000) and small eukaryotes from the water column (Slapeta et al., 2006). Although many marine species (benthic or planktonic) may be dispersed worldwide it seems nevertheless that deep-sea species are more likely to be cosmopolitan.

A greater prevalence of global genetic homogeneity in the deep sea than in shallow waters could also result from different speciation mechanisms. Abyssal species may evolve more slowly than shallow-water species. The high intra-individual divergence and the important single nucleotide polymorphism (SNP) of *E. exigua* suggest that it has preserved old mutations. Indeed, big metapopulations will be more likely to conserve selectively neutral genetic polymorphisms because they present higher initial diversity. One could ask whether the ITS rDNA homogeneity accurately reflects evolutionary rates. We cannot totally discount the possibility that the ITS of *E. exigua* has an atypically slow substitution rate, but we consider this unlikely and expect that the analysis of other parts of the genome will confirm its genetic homogeneity.

Our evidence for the ubiquity of *E. exigua* could be interpreted as an argument that global benthic diversity in the deep ocean has been overestimated (Lambshead and Boucher, 2003). However, *E. exigua* most likely constitutes an exceptional case. The other two deep-sea species, *Cibicides wuellerstorfi* and *Oridorsalis umbonatus*, included in our preceding study (Pawlowski et al. 2007) have not been found during the sampling off Japan and seem to be absent or rare in Pacific Ocean. A very large database on the distribution of modern deep-sea benthic foraminiferans lists a relatively limited number of ubiquitous deep-sea morphospecies (Murray 2006). Therefore, it is possible that globally-dispersed species do not constitute the majority of those living at the abyssal depths, even if they are more common here than in shallow water.

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**Supplementary material:**

**TABLE 1.** List of DNA extractions indicating clones sequenced and their accession number in GenBank, sampling station, depth and location. a. ITS DNA extractions. b. SSU DNA extractions.

| a | DNA | Clones (GenBank access number) | Depth | Coordinates | Station |
|---|---|---|---|---|---|
| ITS | **P6928** ***E. exigua*** | -12 (EF653496)<br>-14 (EF653497)<br>-16 (EF653498)<br>-17 (EF653499)<br>-19 (EF653500)<br>-28 (EF653501) | 1905 m | N33°51’26<br>E136°29’96 | PC12 |
| | **P6929** ***E. exigua*** | -22 (EF653503)<br>-23 (EF653504)<br>-24 (EF653505)<br>-29 (EF653506)<br>-30 (EF653507) | 1905 m | N33°51’26<br>E136°29’96 | PC12 |
| | **P7156** ***E. exigua*** | -31 (EF653508)<br>-34 (EF653509)<br>-35 (EF653510) | 1990 m | N33°49’00<br>E137°08’70 | PC10 |
| | **P7180** ***E. exigua*** | -41 (EF653511)<br>-45 (EF653514) | 1905 m | N33°51’26<br>E136°29’96 | PC12 |
| | **P7165** ***E. exigua*** | -61 (EF653515)<br>-63 (EF653516) | 1990 m | N33°49’00<br>E137°08’70 | PC10 |
| | **P7166** ***E. exigua*** | -71 (EF653518)<br>-72 (EF653519)<br>-73 (EF653520)<br>-75 (EF653521) | 1905 m | N33°51’26<br>E136°29’96 | PC12 |

**TABLE 1b.**

| b | DNA | Clones (GenBank access number) | Depth | Coordinates | Station |
|---|---|---|---|---|---|
| SSU | **P6928** ***E. exigua*** | -11 (FJ185806)<br>-15 (FJ185804) | 1905 m | N33°51’26<br>E136°29’96 | PC12 |
| | **P6929** ***E. exigua*** | -21 (FJ185805)<br>-22 (FJ185803) | 1905 m | N33°51’26<br>E136°29’96 | PC12 |
| | **P7180** ***E. exigua*** | -11 (FJ185798)<br>-12 (FJ185797) | 1905 m | N33°51’26<br>E136°29’96 | PC12 |
| | **P7565** ***E. exigua*** | -21 (FJ185801)<br>-22 (FJ185802) | 1990 m | N33°49’00<br>E137°08’70 | PC10 |
| | **P7566** ***E. exigua*** | -31 (FJ185800)<br>-33 (FJ185799) | 1905 m | N33°51’26<br>E136°29’96 | PC12 |
| | **KG8043** ***E. vitrea*** | -22 (FJ185825) | 100 m | S62°09’29<br>W58°29’43 | KG14 |
| | **KG8250** ***E. vitrea*** | -24 (FJ185822)<br>-26 (FJ185824)<br>-27 (FJ185823) | 40 m | S62°11’05<br>W58°23’00 | KG19 |
| | **WED5232** ***E. arctica*** | -11 (FJ185793)<br>-12 (FJ185794)<br>-18 (FJ185795) | 2609 m | S63°40’99<br>W50°44’29 | 121/4 |
| | **ARK5521** ***E. arctica*** | -34 (FJ185796) | 2633 m | N7855’05 E50’13 | 273 |
| | **KG7890** ***E.* sp1** | -3 (FJ185807) | 107 m | S62°09’58<br>W58°34’28 | KG10 |
| | **KG7994** ***E.* sp1** | -7 (FJ185808)<br>-8 (FJ185809)<br>-9 (FJ185811)<br>-10 (FJ185810) | 108 m | S 62°09’46<br>W58°29’73 | KG13 |
| | **KG8042** ***E.* sp1** | -18 (FJ185812) | 100 m | S62°09’29<br>W58°29’43 | KG14 |
| | **KG8042** ***E.* sp2** | -12 (FJ185813)<br>-15 (FJ185815)<br>-16 (FJ185814) | 100 m | S62°09’29<br>W58°29’43 | KG14 |
| | **USH7639** ***E.* sp2** | -1 (FJ185817)<br>-2 (FJ185816)<br>-5 (FJ185818)<br>-4 (FJ185819) | 20 m | S54°51’50<br>W68°27’00 | 4 |
| | **P6937** ***E.* sp3** | -52 (FJ185820)<br>-55 (FJ185821) | 1110 m | N34°11’97<br>E137°45’73 | MC16 |

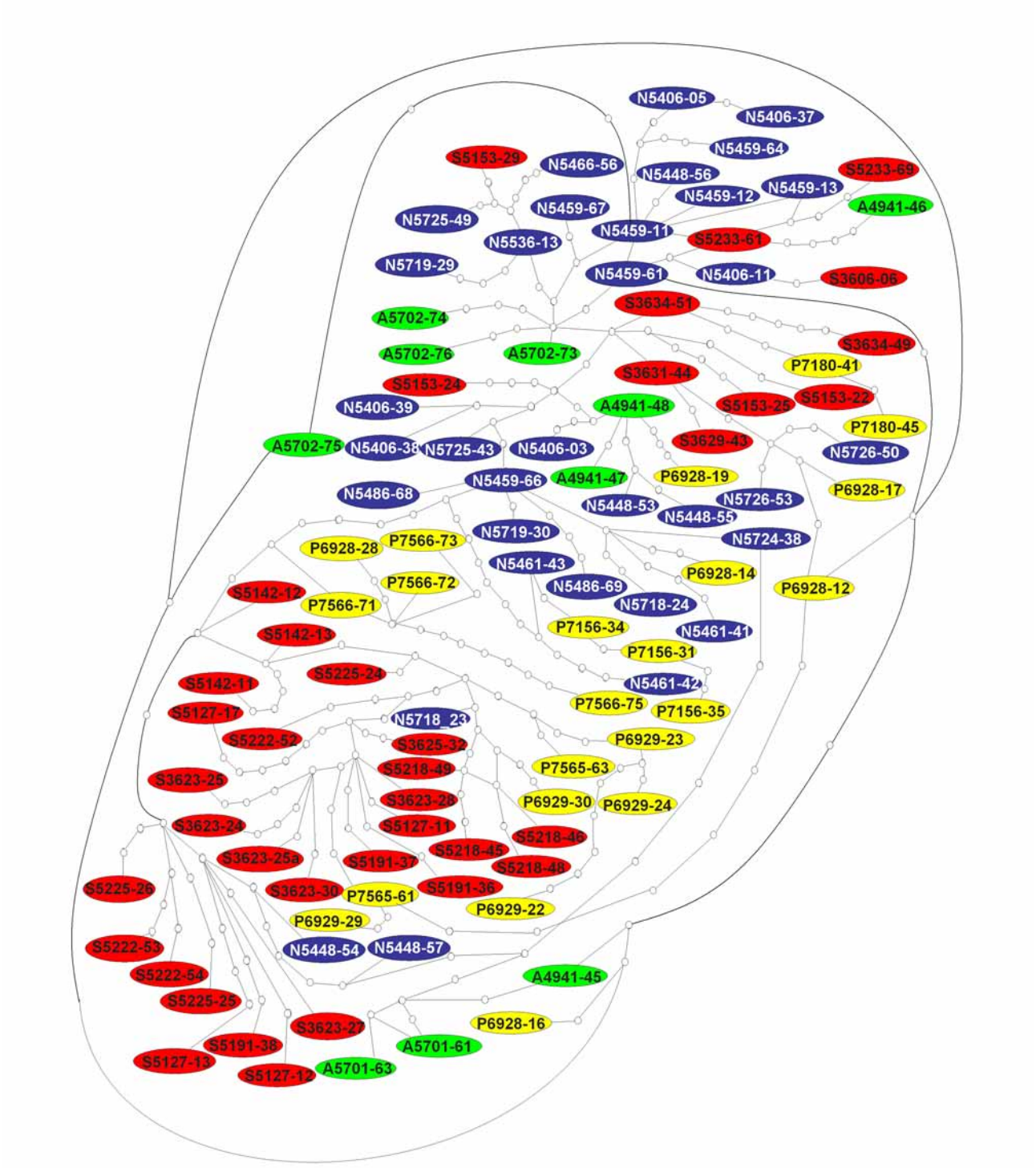


**FIGURE 3.** ITS sequences network performed by TCS software: schema of genetic relationships between each haplotype (1 node represents a difference of 1 nucleotide or indel). Geographic origin of haplotypes is indicated by the color of the boxes: yellow for Pacific, red for Antarctic, blue for Arctic and green for North Atlantic.