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Ribosomal DNA shows extremely low genetic divergence in a world-wide distributed, but disjunct and highly adapted marine protozoan (*Virgulinella fragilis*, Foraminiferida)

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ABSTRACT

Virgulinella fragilis can be mainly observed in different, separated, oxygen-depleted and sulfide-enriched environments around the world and seems to be well adapted to such extreme habitats. Dispersal mechanisms behind this geographical distribution pattern are not yet understood. To analyze the genetic differentiation of geographically isolated populations, we conducted molecular phylogenetic analyses of the small subunit (SSU) ribosomal DNA (rDNA) and internal transcribed spacers (ITS) of rDNA nucleotide sequences in populations of *V. fragilis* collected in the South Atlantic (upwelling area off Namibia) and in the Pacific (Wellington Harbor, New Zealand, and Namako-ike, Japan). Our molecular analyses revealed SSU rDNA and ITS sequences strikingly similar or identical among these three disjunct populations. Such a low molecular genetic differentiation, a fixation rate converging to zero, could either arise from rapid dispersal, ultraslow mutation rates due to a strictly asexual mode of reproduction, unlimited genetic exchange between populations or the existence of a resting stage for survival under unfavorable conditions. We discuss each explanation and conclude that *V. fragilis* might possibly represent a protozoan trapped in evolutionary stasis.

1. Introduction

The benthic foraminifer *Virgulinella fragilis* Grindell and Collen, is often distributed in oxygen-depleted and sulfide-enriched environments and seems to be tolerant to nearly anaerobic conditions (Bernhard, 2003; Takata et al., 2003; Erbacher and Nelskamp, 2006). In deeper water depths, it can coexist with bacterial mats of sulphur-oxidizing bacteria like *Beggiatoa* and *Thioplaca*. Additionally, potential symbiotic sulphur-oxidizing bacteria and sequestered chloroplasts were observed in the cytoplasm of *V. fragilis* (Bernhard, 2003), which may enable them to survive under dysoxic conditions.

Modern *V. fragilis* was first described in Wellington Harbor, New Zealand (Grindell and Collen, 1976), where both dead and living specimens were recovered from muddy bottom sediments at 16 to 31 m water depth. Central and western areas of Wellington Harbor experienced eutrophication induced mainly by organic waste contamination from a local freezing works (Johanneson and Martin, 1955a,b), and therefore sediments became organic-rich but oxygen-poor and sulfidic during the 1960s and 1970s. The specimens of *V. fragilis* explosively increased during this period. Environmental improvement has been done nowadays; limited number of specimens can be found in the sediment although overlying oxic water is available. The oxygen concentration within the sediment decreased in relation to the sediment depth, still, the oxygen concentration in the sediment in Wellington harbor is higher than under dysoxic conditions found both in Namako-ike and Walvis Bay. Numerous investigations have recovered both fossil and living specimens of *V. fragilis* and related species from low-oxygen oceanic environments around the world and today's records of living *Virgulinella* spp. are summarized in Fig. 1. Live specimens have been observed at a range of depths and temperatures and in different oceanic regimes, such as coastal upwelling off Namibia (Altenbach et al., 2002; Ertan et al., 2004), strong stratification of the water column in Cariaco Basin, Venezuela (Bermudez and Seiglie, 1963; Bernhard, 2003; Sellier de Civrieux, 1977), the oxygen minimum zone off the Pakistan margin in the North Arabian Sea (Erbacher and Nelskamp, 2006), or meromictic lakes in Japan (Takata et al., 2003, 2005). Most of these environments showed euxinic characteristics.

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Fossils assigned to genus *Virgulinella* have been reported in Miocene formations of the United States, Germany, the Netherlands, Italy, Algeria, and Egypt (Todd and Brönnimann, 1957; Loeblich and Tappan, 1964), and in Miocene and Pliocene formations of Belgium (Reuss, 1861) and Indonesia (Yabe and Asano, 1937). *Virgulinella* deposits from upper Oligocene Chattian formations found in the Ciscaucasus, Volga-Don, and Mangyshlak regions indicate sedimentation under anoxic and sulfidic water conditions (Stolyarov, 2001; Stolyarov and Ivleva, 2004).

The genus *Virgulinella* seems to appear in hypoxic environments rich in hydrogen sulfide and therefore is a good proxy for oxygen-depleted and sulfide-enriched environments. However, the discontinuous distribution of *V. fragilis* populations linked to different oxygen-depleted environments around the world and the dispersal mechanisms behind this geographical distribution pattern are not yet understood. Basic aspects of the natural history of *V. fragilis* remain unclear, including details of the life cycle, genetic structures and adaptive ecological strategies. Molecular genetic analyses of living *V. fragilis* specimens can be a valuable tool in evaluating the relationships among disjunctive populations in similar environments, and phylogenetic analyses based on small-subunit (SSU) ribosomal DNA (rDNA) and, in particular, the internal transcribed spacers (ITS) of rDNA sequences can clarify whether disjunct populations are isolated and evolve independently or exchange genetically (Tsuchiya et al., 2003; Grimm et al., 2007; Pawlowski et al., 2007).

Here, we performed molecular genetic and phylogenetic analyses of *V. fragilis* based on SSU rDNA and ITS sequences to assess the genetic divergence among distant and disjunct populations in the Atlantic (upwelling area off Namibia) and in the Pacific (Wellington Harbor, New Zealand, and Namako-ike, Japan) to ascertain whether genetic exchange occurs among these disjunctive populations.

2. Materials and methods

2.1. Sampling sites and sample collection

Samples of *V. fragilis* were collected from three widely separated localities: the landlocked lake Namako-ike, Kagoshima, Japan; Wellington Harbor, Wellington, New Zealand; and Walvis Bay, Namibia (Table 1). Sediment samples were collected with an Ockelmann-type bottom sledge net by hand both in Namako-ike lagoon and Wellington Harbor. In Wellington Harbor, sediments were taken from research vessel *Raukawa Challenger*, Island Bay Marine Laboratory, Victoria University of Wellington. At Walvis Bay, Namibia, sediment samples were taken with a GEOMAR-type multiple corer during *FS Meteor* cruise M57-3 (Ertan et al., 2004).

Sediments that contained living *V. fragilis* were placed in screw-cap glass bottles, which were sealed and transported to laboratories under in situ temperature conditions.

Environmental data were measured for each locality; depth, dissolved oxygen, water temperature, conductivity, salinity, pH by a calibrated CTD unit (Quanta, Hydrolab Inc.) that measured from surface to the bottom at 1 m intervals. In addition, hydrogen sulfide concentration was determined at the depth where the oxygen

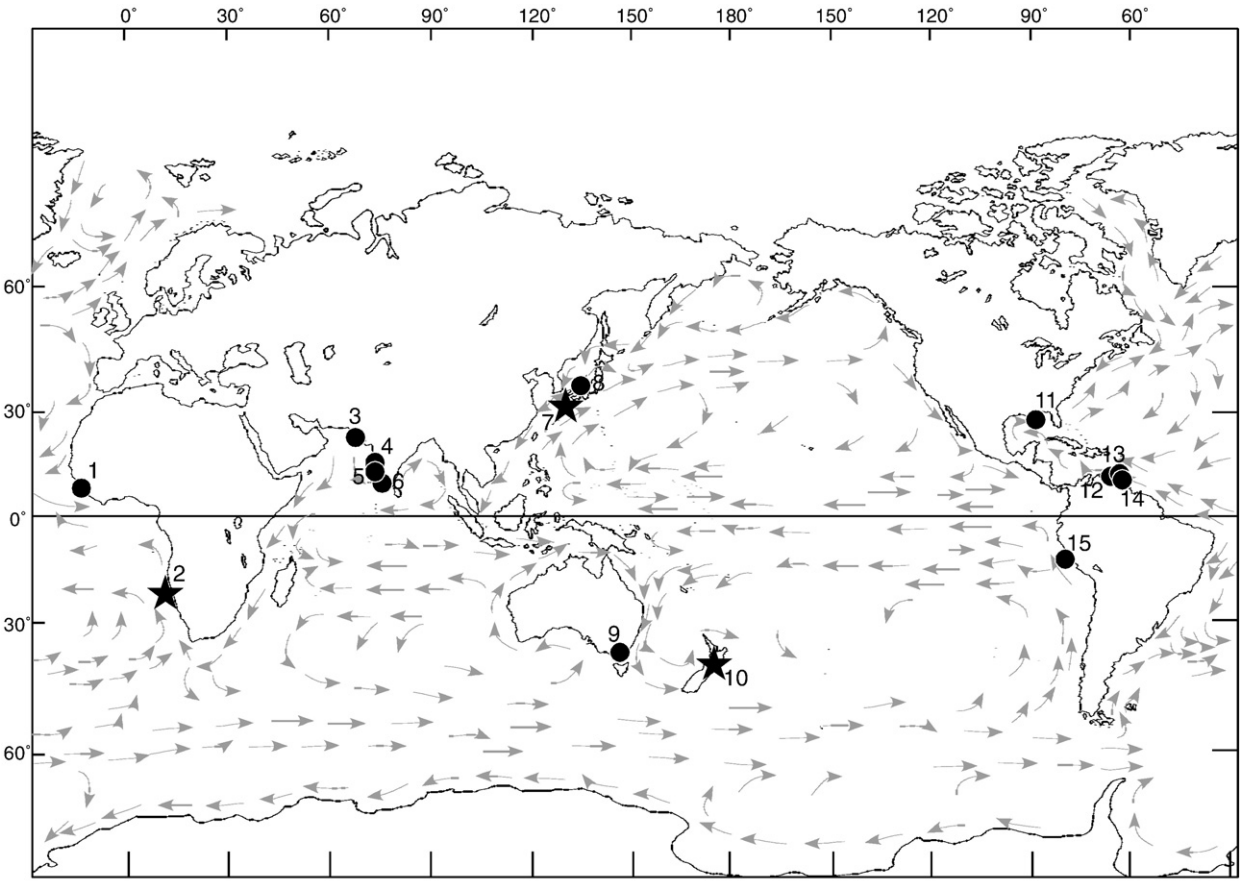

Fig. 1. Global distribution of living *Virgulinella* species, including *V. fragilis*, *V. pertusa*, *V. gunteri*, and *Virgulinella* sp. Sampling localities in this study are marked with stars, and those of previous studies are marked with black circles. Surface ocean currents are indicated by arrows. The numbers in the figure are corresponds to the references (1: Seibold, 1975; 2: Altenbach et al., 2002, Ertan et al., 2004, this study; 3: Erbacher and Nelskamp, 2006; 4: Zobell, 1973; 5: Nigam and Setty, 1982; Bhatia and Kumar, 1976; 6: Seibold, 1975; 7: this study; 8: Takata et al., 2003, 2005; 9: Apthorpe, 1980; 10: Grindell and Collen, 1976, this study; 11: Lankford, 1959; Culver and Buzas, 1981; 12: Seiglie, 1966, 1967; 13: Bermudez and Seiglie, 1963; Bernhard, 2003; 14: Sellier de Civrieux, 1977; Todd and Brönnimann, 1957; 15: Revets, 1991).