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Phylogenetic relationship of *Alexandrium monilatum* (Dinophyceae) to other *Alexandrium* species based on 18S ribosomal RNA gene sequences

John E. Rogers [a,*], Jeffrey D. Leblond [b], Cynthia A. Moncreiff [c]

[a] *United States Environmental Protection Agency, Gulf Ecology Division, National Health Effects and Environmental Research Laboratory, 1 Sabine Island Dr., Gulf Breeze, FL 32561, USA*
[b] *Department of Biology, P.O. Box 60, Middle Tennessee State University, Murfreesboro, TN 37132, USA*
[c] *Gulf Coast Research Laboratory, Department of Coastal Sciences, The University of Southern Mississippi, Ocean Springs, MS 39566, USA*

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Abstract

The phylogenetic relationship of *Alexandrium monilatum* to other *Alexandrium* spp. was explored using 18S rDNA sequences. Maximum likelihood phylogenetic analysis of the combined rDNA sequences established that *A. monilatum* paired with *Alexandrium taylori* and that the pair was the first of the *Alexandrium* taxa to diverge, followed by *Alexandrium margalefii*. All three are members of the *Alexandrium* subgenus *Gessnerium* Halim nov. comb.

Keywords: *Alexandrium monilatum*; Phylogeny; 18S rDNA

1. Introduction

Alexandrium monilatum (Howell) (Balech, 1995) is a ''chain forming'' dinoflagellate that periodically blooms in coastal and estuarine waters of the Gulf of Mexico. Blooms have been reported for areas near Galveston Bay, Texas (Connell and Cross, 1950; Gates and Wilson, 1960), in the coastal waters of Mississippi, and as Far East as Mobile Bay, Alabama and Pensacola Bay, Florida (Perry et al., 1979). Not limited to the Gulf of Mexico, *A. monilatum* has also been documented in the Western Atlantic (Williams and Ingle, 1972; Owen and Norris, 1982) and Caribbean (Halim, 1967). *A. monilatum* was first identified and described from waters collected near Melbourne on the east coast of Florida (Howell, 1953).

Because many of these blooms are associated with fish mortality, a number of research efforts have been initiated to examine the toxicity of *A. monilatum* to fish and a number of other organisms. *A. monilatum* cultures were toxic to mullet, *Mugil cephalus* (Gates and Wilson, 1960) and guppy, *Lebistes reticulates* (Aldrich et al., 1967); crude cell extracts were toxic to mice (Erker et al., 1982). Clemons et al. (1980) found that crude cell extracts were hemolytic, and Bass and Kuvshinoff (1982) observed a neurotoxic response. The toxic components were later shown by Erker et al. (1985) not to be saxitoxin, the gonyautoxins, and related toxins known to occur in other toxic *Alexandrium* spp., such as *Alexandrium tamarensis*, *Alexandrium catenella*, or *Alexandrium minutum*. In addition, filter-

* Corresponding author. Tel.: +1 850 934 9326; fax: +1 850 934 2401.
E-mail address: rogers.johne@epa.gov (J.E. Rogers).

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feeding shellfish did not accumulate the toxins of *A. monilatum*.

A. monilatum is a chain former similar to *A. catenella*. However, *A. monilatum* is generally larger (Balech, 1995) with a protoplasmic continuum between cells in chains (Walker and Steidinger, 1979). Balech (1995) placed *A. monilatum* in the subgenus *Gessnerium* Halim nov. comb. of the genus *Alexandrium*; *A. catenella* was placed in the subgenus *Alexandrium*. A more recent morphotype grouping scheme (Dr. Makoto Yoshida, personal communication in Usup et al., 2002) identifies *A. catenella* as a Type C along with other *Alexandrium* spp. producing toxins that cause paralytic shellfish poisoning. *A. monilatum* was not included in this scheme nor has it been included in the many phylogenetic analyses of the *Alexandrium* genus (Destombe et al., 1992; Adachi et al., 1994, 1996; Scholin et al., 1994; Scholin and Anderson, 1994; Hirashita et al., 2000; Sako, 2000; Usup et al., 2002; Hansen et al., 2003; John et al., 2003; Montresor et al., 2004). Exclusion in the latter analyses occurred because sequence data was not available. Here we report the phylogenetic relationship of *A. monilatum* to other *Alexandrium* spp. based on 18S rDNA analysis.

2. Materials and methods

2.1. Dinoflagellate cultures and growth conditions

Nuclear 18S rDNA sequences of 12 taxa were included in the phylogenetic analysis. The sequences for *A. monilatum* JR07 (AY883005), *Alexandrium minutum* CCMP 113 (AY883006), and *Alexandrium tamarense* UTEX 2521 (AY883004) were determined from unialgal cultures maintained at the Gulf Ecology Division. The sequences were deposited in GenBank. *A. monilatum* JR07 was isolated from a bloom sample collected on 12 September 2000, just south of the Mississippi barrier islands (30°10.6′N, 88°46.4′W). At the time of collection the surface was visibly discolored, the *A. monilatum* cell count was 550 ml^{-1}, salinity was 35, and the temperature was approximately 28 °C. A clonal culture was developed from a single cell isolated from a mixed culture maintained on L1 medium. The clonal culture was unialgal but not axenic. *A. minutum* CCMP 113 (AY883006) was obtained from the Provasoli–Guillard Center for the Culture of Marine Phytoplankton (CCMP), and *A. tamarense* (AY883004), listed as *Gonyaulax tamarensis* UTEX 2521, was obtained from The Culture Collection of Algae at the University of Texas at Austin (UTEX). Both organisms were cultured in f/2 medium at 21 °C and a 14-h light:10-h dark cycle, with a light intensity of 45 $\mu E\ m^{-2}\ s^{-1}$.

In addition, the following 18S rDNA sequences (with GenBank accession numbers) were used: *Alexandrium affine* (AJ535375), *A. catenella* (AJ535392), *Alexandrium fundyense* (U09048), *Alexandrium margalefii* (U27498), *A. minutum* (U27499), *Alexandrium ostenfeldii* (AJ535384 and U27500), *A. tamarense* (AF022191 and X54946), *Alexandrium tamutum* (AJ535376), *Alexandrium tamiyavanichi* (AF113935), *Crypthecodinium cohnii* (M64245), *Gonyaulax spinifera* (AF022155), *Pyrocystis noctiluca* (AF022156), and *Sarcocystis muris* (M64244).

2.2. DNA extraction and PCR conditions

DNA was extracted from cell pellets derived from 15 mL of culture according to the method outlined by Amann et al. (1992). Polymerase chain reaction (PCR) amplification of the gene for 18S rRNA was amplified using a minor variation of the primers specified by Medlin et al. (1998) and Ready-To-Go PCR Beads (Amersham Pharmacia Biotech Inc., Piscataway, NJ). The forward (1F) and reverse (1800R) primers were 5′-AACCTGGTTGATCCTGCCAGT-3′ and 5′-TCCTT CTGCAGGTTCACCTAC-3′, respectively. PCR was initiated by holding at 94 °C for 4 min. The initial hold was followed by 36 cycles that included for each cycle: denaturation at 94 °C (1 min), annealing at 60 °C (1 min), and elongation at 72 °C (1 min). Each dinoflagellate DNA sample yielded one PCR product (approximately 1800 bp), that was purified using Ultrafree-MC® centrifugal filter units (Millipore, Bedford, MA).

2.3. Sequencing and alignment

Sequencing of purified PCR products was performed at the University of Florida ICBR Core Lab using the fluorescent dideoxy terminator method of McCombie et al. (1992). In addition to the external primers mentioned above, six internal primers were used: 5′-CGGTAATTCCAGCTCC-3′ (560F), 5′-GGAGCTGGAATTACCG-3′ (560R), 5′-TTTGACT-CAACACGGG-3′ (1170F), 5′-CCCGTGTTGAGT-CAAA-3′ (1170R), 5′-CAGGTCTGTGATGCCC-3′ (1420F), and 5′-TCCTTCTGCAGGTTCACCTAC-3′ (1420R). The resulting partial sequences were assembled into contiguous sequences, edited and aligned using the Lasergene99 software package (DNASTAR Inc., Madison, WI). External primer sequences were excluded from the analysis.