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[Note]

Phylogenetic Analysis of Dinoflagellate *Gonyaulax polygramma* Stein Responsible for Harmful Algal Blooms Based on the Partial LSU rDNA Sequence Data

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This study carried out phylogenetic analysis of dinoflagellate *Gonyaulax polygramma* which was responsible for a harmful algal bloom episode in Korea in 2004. Molecular phylogenetic tree inferred from the partial LSU rDNA data showed that *G. polygramma* came up among the monophyletic *Gonyaulax* clade, but did not have apparent genetic affiliation to other *Gonyaulax* species. This result appears to be consistent with characteristic morphological features of *G. polygramma* such as epitheca sharply tapering to the apex and thecal plates ornamented with numerous longitudinal striations.

Key Words: *Gonyaulax polygramma*, harmful algal bloom (HAB), LSU rDNA, molecular phylogeny

INTRODUCTION

Dinoflagellate *Gonyaulax polygramma* Stein blooms have been reported in Japan and South Africa, and sometimes caused mass fish mortalities from abrupt oxygen deficiency (Grindley and Taylor 1962; Koizumi *et al*. 1996; Morton and Villareal 1998). The harmful algal bloom (HAB) episode observed in Korean water in 2004 showed a different phenomenon from those in the previous years in that a *Cochlodinium polykrikoides* Margalef bloom occurred transiently offshore the southern coast of Korea, terminated rapidly after Typhoon Maegi, and was subsequently succeeded by an unprecedented *G. polygramma* bloom (The 2004 HAB Report, National Fisheries Research and Development Institute, Korea). In this study, the partial LSU rDNA sequence analysis of *G. polygramma* was carried out to elucidate its phylogenetic position within dinoflagellate lineages.

MATERIALS AND METHODS

A clonal culture of *Gonyaulax polygramma* (NF-D-GP-1) was established from Tongyeong, Korea by a micropipette method and maintained under general laboratory culture conditions (Kim *et al*. 2005). Vegetative cells were photographed with a light microscope (Olympus BX50, Olympus, Japan) equipped with the differential interference contrast (DIC) optic.

Genomic DNA extraction, PCR mixtures and conditions to amplify the LSU rDNA D1-D3 region, and sequencing were performed as Kim *et al*. (2005) did. The sequences analyzed in this study can be obtained from GenBank (http://www.ncbi.nlm.nih.gov/) under the accession number, DQ162802.

For phylogenetic analysis, the partial LSU rDNA sequence of *G. polygramma* was aligned using Clustal W with the representative gonyaulacalean sequences retrieved from GenBank. The LSU rDNA sequences, excluding primer regions and the hypervariable D2 domain, were used for the final phylogenetic analysis. Apicomplexan *Toxoplasma gondii* and dinoflagellate *Prorocentrum micans* Ehrenberg were used as outgroups.

Akaike Information Criterion (AIC) in Modeltest 3.7 (Posada and Crandall 1998) was used to determine the best-fit evolutionary model for maximum likelihood (ML) analysis. ML tree for the partial LSU rDNA data was reconstructed with the GTR + I + Γ model in PAUP*

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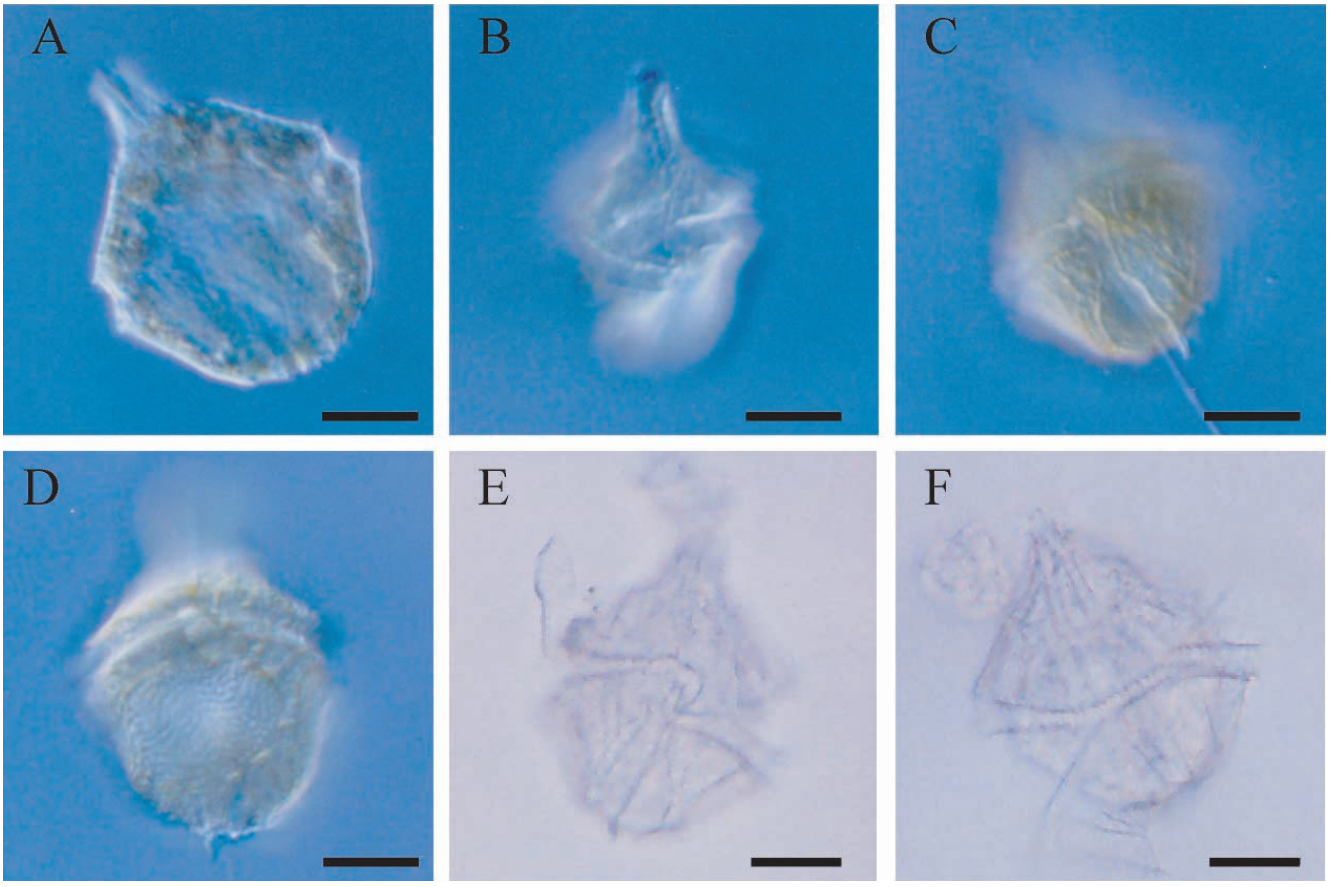

Fig. 1. Microphotographs of dinoflagellate *Gonyaulax polygramma* by a light microscope. A motile cell in ventral (A-C) and dorsal (D) views, and an empty theca in ventral (E) and dorsal (F) views. E: an inverted image. Scale bar = 10 μm.

4.0b10 (Swofford 2002) with the likelihood settings determined from Modeltest. Heuristic searches with random sequence addition and tree-bisection-reconnection (TBR) branch rearrangements were done with 100 replicates. Bootstrap analysis with 1,000 replicates was conducted to determine the robustness of clades (Felsenstein 1985).

A phylogenetic tree was also reconstructed in MrBayes 3.1.1 (Huelsenbeck and Ronquist 2001) using the same model in ML analysis. Bayesian analysis was run for 1,000,000 generations in four chains, sampling trees every 100 generations. The likelihood scores had reached stationarity by 10,000 generations, and so the first 100 sampled trees were discarded ("burnin" = 100). The majority-rule consensus tree and posterior probabilities were obtained using PAUP* from the last MCMC trees after convergence.

RESULTS AND DISCUSSION

Cells of *Gonyaulax polygramma* NF-D-GP-1 were 34-42 μm long and 29-34 μm wide. The epitheca was triangular and sharply tapered to the apex, whereas the hypotheca was trapezoidal with the round antapex (Fig. 1A). There were two short spines in the antapex (Fig. 1C-E). The deeply incised cingulum was postmedian and displaced by 1-1.5 times of girdle width without offset of the two ends (Fig. 1B-E). The inwardly dented sulcus stretched to the antapex with slight widening (Fig. 1C, E). Thecal plates were fairly thick and ornamented with numerous longitudinal striae (Fig. 1E, F). Cells were yellow-brown, and a nucleus was located in the hypotheca (Fig. 1A-D). The combination of these morphological characteristics of Korean *G. polygramma* was consistent with those of previous descriptions (Kofoid 1911; Taylor 1962; Dodge 1989).

ML and Bayesian trees inferred from the partial LSU rDNA data showed that *Gonyaulax* species formed a monophyletic clade with 96% ML bootstrap support and 1.00 Bayesian posterior probability (Fig. 2). Within *Gonyaulax* clade, *Gonyaulax baltica* Ellegaard, Lewis *et* Harding emerged at the most basal position followed by *Gonyaulax elongata* (Reid) Ellegaard *et al*. In accordance with Ellegaard *et al*. (2003), it was unexpected for *G. baltica* to form the primitive branch of the *Gonyaulax* clade

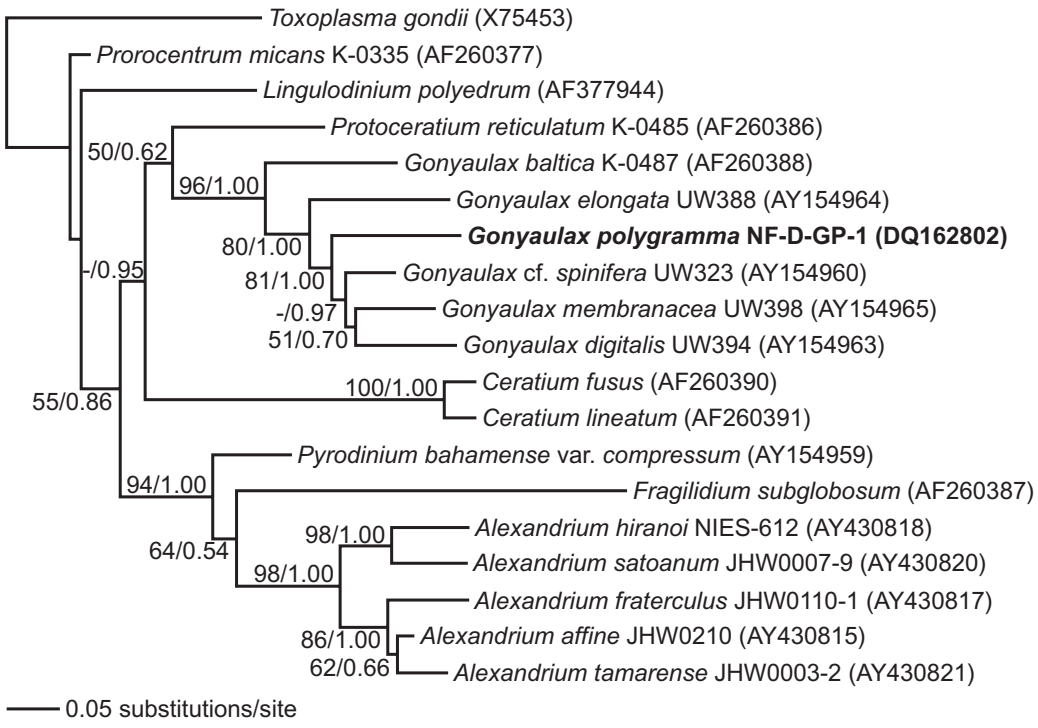

Fig. 2. Bayesian tree of gonyaulacalean species inferred from the partial LSU rDNA sequence data with the GTR + I + Γ model. The sequence of *Gonyaulax polygramma* in boldface letter was determined in this study. ML bootstrap values (> 50%) and Bayesian posterior probabilities (> 0.50) were indicated at each branch node. Apicomplexan *Toxoplasma gondii* and dinoflagellate *Prorocentrum micans* were used as outgroups. The number in parenthesis indicates the GenBank accession number for each species.

because of its similar morphological features to *Gonyaulax membranancea* (Rossignol) Ellegaard *et al*. and *Gonyaulax spinifera* (Claparède *et* Lachmann) Diesing (Ellegaard *et al*. 2002). Meanwhile, *G. elongata* possessing a characteristic triangular antapical flange (Ellegaard *et al*. 2003) branched afterward giving rise to a cluster composed of *G. polygramma*, *G.* cf. *spinifera*, *G. membranacea* and *Gonyaulax digitalis* (Pouchet) Kofoid supported by 81% ML bootstrap value and 1.00 Bayesian posterior probability (Fig. 2). The interspecific relationships among the four species were poorly resolved in ML tree, but *G. polygramma* formed an independent basal branch of the later three species in Bayesian tree with 0.97 posterior probability. This result appears to be consistent with the combination of distinct morphological features of *G. polygramma* such as epitheca sharply tapering to the apex and thecal plates with numerous longitudinal striations. Likewise, Kofoid (1911) separated the *polygramma* group (*Gonyaulax kofoidii* Pavillard, *Gonyaulax pacifica* Kofoid, *G. polygramma*, *Gonyaulax scrippsae* Kofoid and *Gonyaulax turbynei* Murray *et* Whitting) from the *polyedra*, *sphaeroidea* and *spinifera* groups of the subgenus *Gonyaulax* Kofoid by its predominant longitudinal striae on surface.

For the first time, this study presented the molecular sequence of the LSU rDNA D1-D3 region and phylogenetic analysis of *G. polygramma*, which occasionally forms HABs worldwide and is also one of HAB-causing species in Korea.

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