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IDENTIFICATION OF SOME STRAINS OF DINOFLAGELLATES BASED ON MORPHOLOGY AND MOLECULAR ANALYSIS

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Abstract

Dinoflagellates are the important primary producers in aquatic environments. In oceans, they play interesting role in ecological functions such as red tide forming organisms, symbiont of coral reef or sea anemone and DSP (Diarrhetic Shellfish Poisoning) or PSP (Paralytic Shellfish Poisoning) producing organisms. Morphology and molecular analysis of dinoflagellates were conducted on November 2002 to March 2003. The phylogenetic studies based on 18S rDNA analyses, sequence have begun to appear more frequently in the literature, as attention has turned to relationships within the major eukaryotic lineages, particular importance for the taxonomy of the armored and unarmored genera of dinoflagellates (*Gyrodinium* sp., *Cachonina* sp., *Gymnodinium* sp., *Amphidinium* sp.), because many of the genera cause extensive plankton blooms, fish kills and other harmful events, were studied used to amplify 18S rDNA, present in the total DNA extracted from algal pellet. The amplify approximately 1400 bp of the nuclear-encoded LSU rDNA gene using terminal primeirs DIR, products were cheked by 1.0 % agarose gel electrophoresis, then cloning with TA cloning KIT. Sequencing were analyzed by the GENETIX Mac Software, Homology search by Blast and Phylogenetic analysis. Results of hylogenetic analysis of 18S rDNA are: Strain no. 10893 (un identified) from the genera, it is belonging *Gymnodinium* or *Polarella.* Strain no. 10795 is closely related other species *Cachonina hallii*. We tentatively named strain no 11151 and 11160 similar to *Gyrodinium* or *Gymnodinium* based on morphology, but these strain indepently position in this tree and is not a real of *Gymnodinium* sensu stricto. It is possible, we can establish the new genera for strain no. 11151; 11160 because this not cluster any other unarmored species.

Keywords: Morphology, molecular analysis, dinoflagellates

1. Introduction

Dinoflagellates are primarily single organism that possess, firstly, a nucleus lacking histones and having chromosomes that remain condensed throughout the cell division cycle and, secondly, at least one life-cycle stage involving cells with two characteristic flagella. As in related protist, such as ciliates, dinoflagellates possess a layer of vesicles towards the periphery of the cell. In dinoflagellates, these vesicles commonly contain cellulosic plates arranged in consistent patterns (tabulation patterns). These patterns provide the primary basis for determining evolutionary relationships within the group.

About half of living dinoflagellates species are photosynthetic, others are heterotrophic; and some species have both nutritional modes, underlining the futility of attempting to classify these relatively simple organisms as plants or animals. Dinoflagellates are today most diverse in continental shelf environments, but also occur in oceanic and freshwater habitats. Some are parasitic and one group, popularly known as zooxanthellae, live symbiotically in the soft tissue of invertebrates such as corals, giving these animals their bright colors. Dinoflagellates are of major economic importance, being at or near the base of the marine food-chain; they are also primary causal agents of paralytic shellfish poisoning and related toxic phenomena (red tides) [1].

Molecular biology has provided new tools to decipher genetic information and can be used in attempts to reconstruct the evaluation of organism and improve their taxonomy. Molecular biology of cyanobacteria was to summarize more than a decade of progress in analyzing the taxonomys biochemistry, physiology, and cellular differentiation and development biology of cyanobacteria by modern molecular methods and especially by molecular genetics and molecular biology also had an important impact to the taxonomy of cyanobacteria and the origins of chloroplasts in algae and higher plants. Further advances in this area are expected in the future.

There has been a virtual explosion of studies in recent years that use relatively new molecular biological techniques that permit characterization of the communities without the need to cultivate the organism, which opens up a much larger fraction of the community to identification, but they also currently are limited in what we know about these newly-identified organisms. Among the new techniques, there is cloning and sequencing of taxonomically useful genes [2].

2. Materials and Methods

Dinoflagellates isolation. The Dinoflagellates MBIC 10795 (*Cachonina* sp.); MBIC 11151 (*Gyrodinium* sp.); MBIC 10893 (un identified); MBIC 11160 (*Gymnodinium* sp.) and MBIC 11135 (*Amphidinium* sp.). All strains of Marine Biotechnology Institute Culture Collection (MBIC) (Japan isolated) were grown on IMK (Ikemoto Miyashita Kawachi) medium.

Volume of 10 – 15 ml of exponentially growing cultures were collected by centrifugation at room temperature (1500 rpm for 10 minute). Prior to extraction of total genomic DNA, the pellet was kept frozen (-20^{o}C) for a minimum of 2 days. DNA was extracted using the CTAB method [3] and precipitated using ethanol, as described in [4], Extracted DNA was used as a template to amplify approximately 1400 bp of the nuclear as – encoded LSU rDNA gene using terminal primers DIR [5] and 28 – 1483R (5' – GCTACTACCACCAAGATCTGC-3′). Internal primers used to determine the LSU rDNA gene sequences and conditions for polymerase chain reaction (PCR) amplification and thermal cycling are outlined in [6]. The QIAquick PCR Purification Kit (Qiagen) was used to purify PCR products, and nucleotide sequences were determined using the Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). The sequence reactions were run on an ABI PRISM ™ 377 DNA sequencer (Perkin Elmer), following the recommendation of the manufacturer. Software used for Phylogenetic analysis by BLAST

3. Results

Prior to the analysis of some of the major genera of based on Morphology, we tentantively name some of genera of dinoflagellates are MBIC 10795 *Cachonina* sp. (armored); MBIC 11151 *Gyrodinium* sp., MBIC 10893 un identified, MBIC 11160 *Gymnodinium* sp., MBIC 11135 *Amphidium* sp. (unarmored) (Figure 1).

The Electrophoresis of 18S rDNA analysis of the 5 dinoflagellates were detected in each the dinoflagellates, such as Lane 1 (Size marker/Lamda/Hind III); lane 2 strains no. 10795 (*Cachonina* sp.); lane 3 strains no. 11151 (*Gyrodinium* sp.); lane 4 strains no 10893 un identified; lane 5 strains no. 11135 (*Amphidinium* sp.) and lane 6 strains no. 11160 (*Gymnodinium* sp.), respectively (Figure 2).

The result of Homology by blast, we show top ten of species. The sequences of these strains was similarities to those of dinoflagellates. Strains no. 10795 especially this strain similar to *Heterocapsa* sp and *Cachonina* sp. Strain no. 11151, this strain seem to be similar to another unarmored member. Strain no. 10893, we could not identified this strain based of morphology but the member the older SUESSIALES. Strain no. 11160 like strain no. 11151 seem to be similar to other unarmored members (Figure 3).

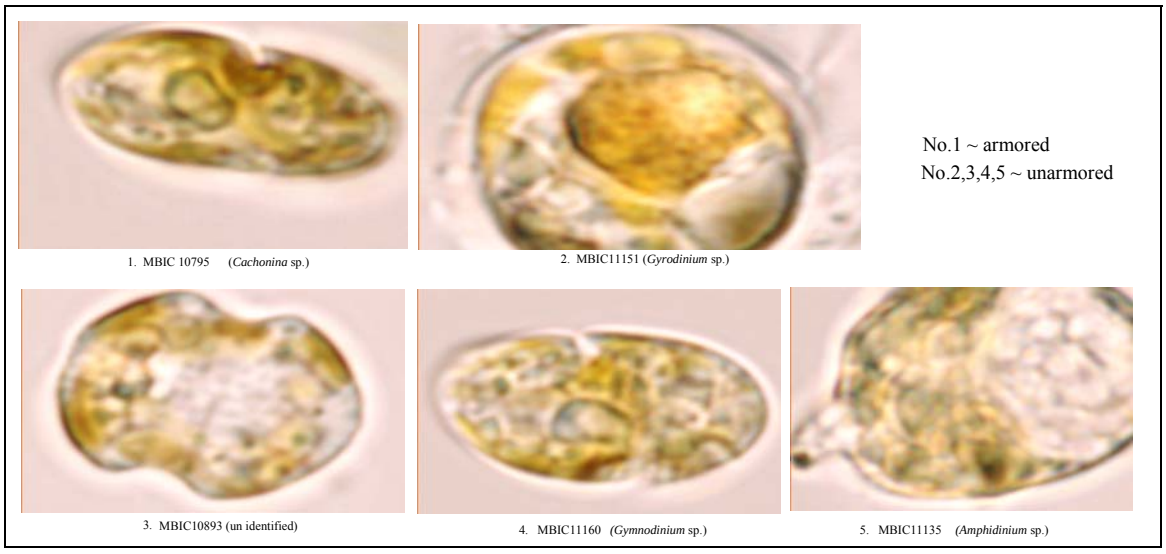

Figure 1. Some genera of dinoflagellates

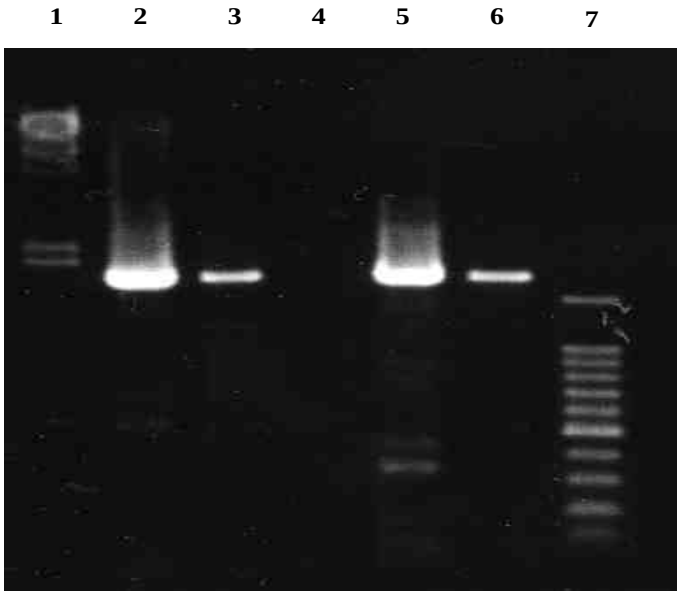

Figure 2. Lane 5 strains no. 11135 (*Amphidinium* sp.) and lane 6 strains no. 11160 (*Gymnodinium* sp.), respectively

Strain no. 10795 (*Cachonina* sp)

AF274265|AF274265.1 Heterocapsa niei strain CCMP 447 small subu...
3364 0.0

AF033865|AF033865.1 Cachonina hallii small subunit ribosomal RN...
3346 0.0

AF022198|AF022198.1 Heterocapsa triquetra 18S ribosomal RNA gen...
3311 0.0

AF274267|AF274267.1 Heterocapsa rotundata strain CCCM 680 small...
3307 0.0

AF274266|AF274266.1 Heterocapsa pygmaea strain CCCM 681 small s...
3297 0.0

AF022201|AF022201.1 Pentapharsodinium tyrrhenicum 18S ribosomal...
3188 0.0

AF274270|AF274270.1 Pentapharsodinium sp. CCMP771 strain CCMP 7...
3174 0.0

AF274260|AF274260.1 Gymnodinium sp. small subunit ribosomal RNA...
3150 0.0

AF272049|AF272049.1 Gymnodinium galatheanum small subunit ribos...

Figure 3. The result of Homology by BLAST top 10

The phylogenic tree of nuclear 18S rDNA from some dinoflagellates, that tree include wide ranging species. Many strains of our MBIC culture collection are indicated by the blue and the red color is sequences from this study. Strain no. 10893 (un identified), this strain closely related *Gymnodinium* or *Polarella.* So, I tentatively name *Gymnodinium* or *Polarella* but this group is not real *Gymnodinium*. In the future the taxonomy of this group will be analyzed and genus names will be change. At the time, I have to change for this strain. Strain no. 10795 is genus closely related of *Cachonina hallii*. I previously identified *Cachonina* sp. base on morphology, So, this is results is reasoanable.

Strain no. 11151 and 11160, result of cluster which MBIC strains no. 11130; 11150; 11159 and this cluster independently position in this tree and we previously tentatively name are *Gyrodinium* or *Gymnodinium* but is not a real of *Gymnodinium*. I think our identification base on morphology is not reasonable (Figure 4). After detail ultra structure study, it is possible we can establish a new genera name of these strains (11151; 11160) because this not clustered with any other unarmored species [7].

According [4], The molecular phylogeny based on partial LSU rDNA is basically similar to the phylogeny based on SSU rDNA sequences. The bootstrap support for the branching pattern is generally greater, however, indicating that LSU rDNA sequences are more suitable for studies of phylogenetic relationship at the generic and species level than are SSU rDNA sequence data. The molecular reconstructions have provided support for conclusions based on ultrastuctural features, notably features associated with the flagellar apparatus, and biochemical features, notably photosynthetic pigments. The combination of these different approaches has enabled us to reach conclusions on taxonomy and phylogeny of the dinoflagellates that would have been difficult to reach if only one of the techniques had been employed. Future studies based on LSU rDNA should include heterotrophic dinoflagellates in order to allow us to better understand the systematics and evolutionary history of this highly diverse assemblage of protests.

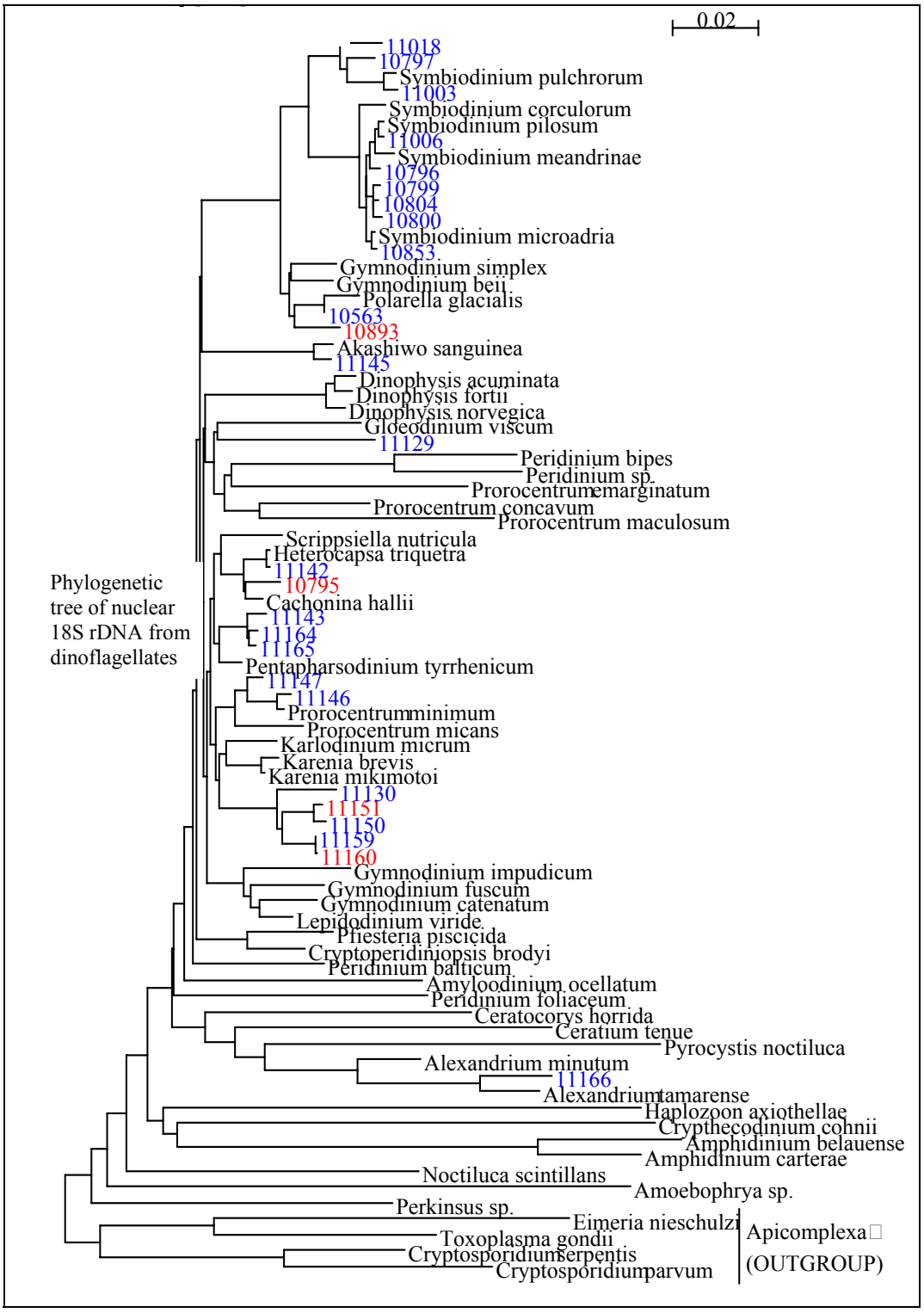

Figure 4. Phylogenetic tree of Nuclear 18S rDNA from MBIC strains of Dinoflagellates. The bootstrap values shown above internal nodes are inferred from MP analysis using a weighted rescaled consistency index over an interval of 1 – 1000. The bootstrap values below the internal nodes are inferred from distance analyses of the some data set and are based on a maximum likehood model to calculate dissimilarities (the Felsenstein 1984 model available in PAUP*) and are used as input for NJ analyses.

3. Result and Discussion

According [8] reported that small organic scales 0.2 to 0.4 µm in diameter have been observed to form a layer on the surface of three species of marine dinoflagellates. Two of the species were originally described as members of the genus *Cachonina*; the third is *Heterocapsa pygmaea* [9]. The body scales are very similar to those previously described covering cells of *Heterocapsa triquetra* .These scales are freely shed from the cells of the above four species and can be found in the culture medium; no scales were observed in the culture medium of 35 other dinoflagellates species representing 18 genera and 11 families. The complete thecal plate pattern of *H. triquetra* , type species of *Heterocapsa*, has been determined, and on the basis of similarities in body scales morphology and thecal plate tabulation, the genus *Cachonina* is placed in the synonomy of *Heterocapsa*. *Cachonina illdefina* and *Cachonina niei* are transferred to *Heterocapsa*. [8] Recently [10] analyzed the type of culture of *Cachonina illdefina* and concluded on the basis of thecal plate pattern that it is synonymous with *C. niei*. [10]. According [4] The genus *Gyrodinium* is presently circumscribed as containing those gymnodinioid dinoflagellates in which the two ends of the cingulum are separated in the longitudinal direction of the cell by a distance exceeding one fifth of the cell length. That this generic circumscription is unsatisfactory has been known for a long time. Several gymnodinioid dinoflagellates possess a cingulum whose ends are separated by approximately this distance. In some cells of a clonal culture, the two ends may be separated by slightly less than one fifth of the cell length; in others, the two ends may be separated by slightly more than one fifth of the cell length [11]. A study on the ultrastructure of the type species of *Gyrodinium*, *G. spirale*, is being published separately. Based on this work and on SEM micrographs by [12], it now clear that *Gyrodinium* is readily distinguished in the SEM. [13]. According, [12], **t**he characteristic feature of *Gyrodinium* is not so much the cingulum displacement as the morphology of the apical groove system. The apical groove is an elliptical structure situated around the apical end, perpendicular to the longitudinal axis of the cell. The ellipse is bisected into two equal parts by central line. The long axis of the apical groove is mid dorsal to mid ventral. If present, an anterior extension of the sulcus extends toward one end of the apical groove.

According [4]. In addition to the type species of *Gymnodinium*, *G. fuscum*, which is a species of oligotrophic freshwaters, the clade revealed by LSU r DNA data comprises six marine species (*G. aureolum*, *G. catenatum*, *G. chlorophorum* (with green choloroplasts), *G. impidicum*, *G. nolleri*, and *G. cf.placidum*) and one freshwater species (*G. palustre*). Both light microscopical and ultrastuctural characters confirm that these species form a natural group. The apical grooves of *G. palustre* and *G. cf. placidum* are not known, but all of the other species have a delicate horseshoe-shaped apical groove running anticlockwise around the apex of the cell. In *G. fuscum* this groove is situated further away from the apex, and it is very faint and only visible in SEM [3]. However, we believe it to be essentially the same as in other species of the clade. A phylogeny based on SSU rDNA sequences revealed *Noctiluca Suriray* as the earliest lineage of the taxa analysed [14], and indicated that it was apparently not closely related to G. sensu stricto or other "Gymnodinioids". The phylogenetic significance of the nuclear chambers is therefore uncertain at present [7]. In the phylogenetic tree of RuBisCO LSU since *G. mikimotoi* was not positioned within the heterokonts group and did not reveal philogenetic affinity with *P. subviridis*, the plastid of *G. mikimotoi* is not likely to have originated from *P. subviiridis*. This result conflicts with the hypothesis proposed by [15], *Gymnodinium mikimotoi* and six haptophytes constituted a monophyletic lineage with 73% and 76% bootstrap values in the NJ and MP analysis, respectively, and *G. mikimotoi* was positioned most basally within this lineage. Furthermore, the *G. mikimotoi*/haptophytes group clustered with the rhodophytes group with 66% bootstrap value in the NJ analisys. This phylogenetic status *G. mikimotoi* coincides well with that of the pl-SSU rDNA tree [16]. These findings strongly suggests that the plastid of *G. mikimotoi* and haptophytes are related to each other.

Traditionally, *Amphidinium* has been grouped within the gymnodinioids, more formally in the order Gymnodiniales [1]. However, the molecular data indicated that Amphidinium is not closely related to other gymnodinioids. Interestingly, [7,17], Classified *Amphidinium* together with *Dinophysis* in the subfamily Dinophyta, whereas *Gymnodinium* and *Polykrikos* were placed in *Gymnodinia.* The present data provide some support for this idea, and [18], also discussed whether the Amphidinioid morphotype has given rise to the Dinophysoid type. Unfortunately, the phylogenetic position of Dinophysis acuminate, based on the molecular data presently available in GenBank, is only indicate and is not well supported.

4. Conclusions

Results of phylogenetic analysis of 18S rDNA are: Strain no. 10893 (un identified) from the genera, it is belonging *Gymnodinium* or *Polarella.* Strain no. 10795 is closely related other species *Cachonina hallii*. We tentatively named strain no 11151 and 11160 similar to *Gyrodinium* or *Gymnodinium* based on morphology, but these strain indepently position in this tree and is not a real of *Gymnodinium* sensu stricto. It is possible, we can establish the new genera for strain no. 11151; 11160

because this not cluster any other unarmored species. The molecular reconstructions have provided support for conclusions based on ultrastuctural features, notably features associated with the flagellar apparatus, and biochemical features, notably photosynthetic pigments.

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