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沖縄県沿岸域から分離した海産底生性渦鞭毛藻類数種の形態・分子系統と分類

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ABSTRACT

Currently, less than 10% of described marine dinoflagellates inhabiting the benthic environment. However, the classification of marine benthic dinoflagellates is far from being resolved. Many of these species were described long before the advent of modern light and electron microscopy or the implementation of molecular analyses. Additionally, their distribution has not been completely assessed or studied. As a consequence, their clarity with respect to identity, relationship, biodiversity, distribution and potential for utilization is lacking. Hence, comprehensive studies of marine benthic dinoflagellates are important. The aim of my study was to provide a comprehensive investigation on the taxonomy, morphology, molecular phylogeny and diversity of several marine benthic dinoflagellate strains isolated from the coastal areas of Okinawa Prefecture, Japan (Chapter 1). The results of this investigation are presented in Chapter 2-6 of the total seven chapters of this thesis.

In Chapter 2, the morphological and genetic diversity of numerous heterotrophic dinoflagellate strains of the genus *Crypthecodinium* from Okinawa Island, Ishigaki Island and Iriomote Island were investigated. Although all strains shared morphological characters conforming to the description of *Crypthecodinium cohnii* (Seligo) Javornický , molecular phylogenies based on SSU, ITS regions and LSU rDNA D1-D3 divided them into two genetically distinct clades (Clade 1 and Clade 2). These clades were closely associated with other sequences of *Crypthecodinium cohnii* obtained from GenBank, but largely distinct from *Crypthecodinium* sp. strain CAAE-CL2. The SEM investigations further revealed the presence of two cingulum types in both clades from Okinawa: complete and incomplete cingulum. The presence of complete cingulum in *Crypthecodinium cohnii* has never been reported in any study of this genus. Although this finding is interesting in term of morphological variation, it presents a problem for the taxonomic characterization of this dinoflagellate. In this study, the "cingulum reduction" hypothesis was proposed as a possible explanation for this phenomenon. This hypothesis suggests that the presence of two cingulum types might actually represent different stages of cell development / growth from young cells to mature cells, in which the right end of the cingulum was progressively reduced and completely disconnected from the sulcal furrow. The results of this study have revealed hidden diversity within the *Crypthecodinium cohnii* strains isolated from Okinawa Prefecture, Japan and suggests the further evaluation of the morphology, molecular phylogeny and genetic diversity of other *Crypthecodinium cohnii* strains (sibling species) reported elsewhere.

Chapter 3-5 focused on the unarmored and phototrophic genus *Moestrupia* (Larsen *et* Patterson) Hansen *et* Daugbjerg. First, on the basis of morphology and molecular phylogenetic analysis, the genetic diversity of *Moestrupia oblonga* strains from Okinawa Island and one strain reported from Tenerife, Canary Island, Spain was investigated (Chapter 3). All isolated strains shared similar morphologies such as unarmored cells with a rice grain-like shape, a cingulum displacing about one third of the body length, a distinctive ventral flange, a conspicuous central pyrenoid, and a nucleus was in the hypocone. The results of the LSU rDNA D1-D3 molecular phylogenetic analyses suggested the presence of at least three pseudo-cryptic species of *Moestrupia oblonga* (namely Clade A, B and C) from Okinawa Island, which are distantly related to the strain from Tenerife. Additionally, the ultrastructure, haplontic life cycle and intracellular lipid bodies of *Moestrupia oblonga* from Okinawa Island were observed and its potential utilization was briefly discussed (Chapter 4). Subsequently, a new clade (Clade D) was revealed from Miyako Island based on LSU rDNA D1-D3 molecular phylogeny (Chapter 5). Intra-clade sequence divergences in Clade A, B, C and D was less than 2% in LSU rDNA, suggesting the conspecifity of strains within respective clade. Despite the morphological similarity of the four Okinawan clades and *Moestrupia oblonga* from Tenerife, the inter-clade LSU rDNA D1-D3 phylogeny showed sequence divergence of about 5.62-13.37%, indicating that each clade represent one distinct species. Moreover, the distinction between Okinawan clades was consistent with morphological differentiation based on cell size. Clade D in particular, possessed the largest cell size among them. This among other features, led to the proposal of describing Clade D as a new species: *Moestrupia enormis* sp. ined. Furthermore, because the geographical distance between Australia and Japan is less than between Tenerife to Japan or Australia, the remaining three clades from Okinawa Island have more change of being closely related or even conspecific with the type material and other specimens from Australia. This study reveals that the genus *Moestrupia* is much more complex than previous reports have suggested.

In Chapter 6, a comprehensive investigation of several peridinioid and armored marine benthic dinoflagellate strains from Ishigaki Island was conducted. Morphological and molecular phylogenetic analyses of the LSU rDNA D1-D3 sequences revealed that all strains were conspecific with isolates from Indonesia and the Philippines. The key characters of this species are: (1) plate tabulation formula: Po, x, 4', 2a, 6", 6c, 4s, 5"', 2""; (2) a type B eyespot (*sensu* Moestrup and Daugbjerg 2007); and (3) the presence of girdle-lamella-like thylakoids in the chloroplast lobes. Furthermore, LSU rDNA D1-D3 phylogeny revealed that this species formed a sister clade to the type species of *Peridiniopsis*: *Peridiniopsis borgei* (Lemmermann). However they diverged about 10.40-10.58% in LSU rDNA sequences. Comparative morphology suggested that this dinoflagellate was not only distinct to *Peridiniopsis borgei* but also to any other species of this genus (e.g. having two anterior intercalary plates, roundish cell outline, smooth cell surface) or other described dinoflagellate species. This led to the proposal of describing this peridinioid dinoflagellate as a new genus and new species: *Bovistadinium arenicolum* gen. *et* sp. ined. In addition to this, its haplontic life cycle was postulated (Chapter 6).

Finally, the significant and novel findings of these investigations were summarized in the last chapter of this thesis (Chapter 7). In conclusion, the results of this study have added novel and substantial contribution to our knowledge in understanding the taxonomy, morphology, molecular phylogeny, diversity, and to some extent, the life cycles and distribution of several marine benthic dinoflagellates presently described under the genus *Crypthecodinium, Moestrupia* and *Bovistadinium* gen. ined. from Okinawa Prefecture, Japan.