Abstract of Doctoral Dissertation

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Morphological and molecular phylogenetic study of tidal pool dinoflagellates
(タイドプール性渦鞭毛藻類の形態学的および分子系統学的研究)

Dinoflagellates are an extraordinarily diverse group of bi-flagellate eukaryotic microbes that occupy a wide range of niches in aquatic environments. Most dinoflagellates inhabit freshwater ponds, lakes and marine neritic/open ocean environments. However, there are some dinoflagellate species that specifically inhabit tidal pools on rocky beaches. These species form dense blooms and show characteristic diurnal vertical migratory behavior depending on tidal movement. During day time, the motile cells actively swim, forming a bloom at low tide. Before seawater is introduced into the pools at high tide, the dinoflagellates migrate down to the bottom of the pool and attach themselves firmly to the substratum so that the cells can avoid being washed out from the tidal pools. Despite their showy presence of characteristic blooms on rocky shores, studies on tidal pool dinoflagellates are scarce. All the previous descriptions of tidal pool dinoflagellates were based on external morphology and limited molecular work has been done. Therefore, the use of more contemporary methods such as molecular phylogenetics is needed in order to confirm many of their taxonomic positions. Due to their characteristic lifestyle to prevent being washed away from the pools, it was hypothesized that the dispersal ability of these dinoflagellates is small. However, this has not been tested so far. Thus, I conducted research to obtain a better understanding of: 1) species biodiversity of bloom forming tidal pool dinoflagellates in Japan and South Africa; 2) their taxonomic positions, based on molecular phylogenetics; and 3) the genetic distances between populations (= tidal pools) within the same species from different localities.

Following the general introduction in Chapter I, in chapter II of my thesis, I described a new species of the genus *Bysmatrum*, *B. austrafrum* Dawut *et al*. sp. nov. from South Africa. Detailed morphological observations were made by light microscopy, as well as scanning and transmission electron microscopy (SEM and TEM). This was the second report of the internal ultrastructure of any member of this genus. The phylogenetic position of this species was determined using small-subunit ribosomal DNA (SSU-rDNA) sequence data. Based on the distinct morphology of the first apical plate (1') and its isolated position in the phylogenetic tree in relation to the other five members of this genus, I concluded that this species is a new member of this genus. Moreover, I provided the first report of the SSU rDNA sequence for *Bysmatrum arenicola*.

In chapter III, I have examined 30 *Bysmatrum* strains isolated from tidal pool water samples collected from three different prefectures, Kanagawa, Chiba and Okinawa, in Japan. The morphological analysis was done using LM and SEM. In general, the external morphology was almost identical to those of *B.*

subsalsum, *B. gregarium* and *B. austrafrum*, but there were some features unique to those isolates, such as the cell size, the position of the nucleus, the degree of the displacement of the cingulum, and the habitat. I refer to them as the *Bysmatrum gregarium* species complex. All 30 strains formed three separated clades, with moderate support, distinct from any previously reported species (here, designated as Group I, II and III). It was revealed that Group I contains strains from Okinawa, Kanagawa and Chiba prefecture, while Group II and III consisted of strains from Kanagawa and Chiba. It was also demonstrated that even a single tidal pool can contain three species (genotypes) at the same time. In this study, I demonstrated that the distributional pattern of *B. gregarium* species complex is quite complex and further studies with a wider range of global sampling is merited. Based on the current available data, I concluded that those isolates (Group I to III) represent cryptic species.

In chapter IV, a small photosynthetic dinoflagellate was isolated from tidal pool water collected in South Africa, designated as strain HG246. Based on detailed inter/external morphological observations and molecular analysis, it was confirmed as a new *Symbiodinium* species. *Symbiodinium stagnumense* differed from the other members of *Symbiodinium* by having an extra intercalary plate on the epicone. It also formed a robust isolated clade, with moderate support, in the phylogenetic tree inferred from the LSU rDNA sequences, separating it from the morphologically similar species, *S. natans*. This was the first report of a free-living bloom forming *Symbiodinium* species, inhabiting tidal pools.

In chapter V, I proposed a new combination, *Ansanella natalensis* (Horiguchi & Piennar) Dawut et al. comb. nov. This species was originally described as, *Gymnodinium natalense*. Later, it was reclassified in the genus *Biecheleria*, as *B. natalensis*, based on re-observation of the original photographs, but without molecular data. Recently, a small dinoflagellate was isolated from the same tidal pool at the type locality of *G. natalense* in South Africa. The detailed cell surface information, which was not available in the first and second reports, along with the strongly supported phylogenetic tree inferred from SSU sequence data, revealed that this species should belong to the newly erected genus *Ansanella*. Furthermore, the presence of a 51-base pair fragment of the LSU domain 2 (D2) in our species, which is characteristic of the type species of *Ansanella* and absent in *Biecheleria* species, gives further strong support to our conclusion.

In chapter VI, I proposed a new combination, *Peridiniopsis hexapraecingula* (Horiguchi & Chihara) comb. nov. This species was originally described as, *Scrippsiella hexapraecingula* in 1983, but no molecular data were available until today. In this study, I was able to collect this species again from Chiba Prefecture and able to conduct molecular phylogenetic analysis as well as morphological examinations. The phylogenetic tree indicated that this species is closely related to the type species of the freshwater dinoflagellate genus *Peridiniopsis*. Based on genetic similarity, I proposed to transfer the species to the genus *Peridiniopsis*.

In conclusion, this thesis contains the description of two new species, demonstrated the presence of three cryptic species, and proposed two new combination, unveiled the genetic structure of tidal pool dinoflagellate species. These results will contribute to further understanding of the biodiversity and phylogeny of tidal pool dinoflagellates.