論文の内容の要旨

水圏生物科学専攻

平成 18 年度博士課程　入学

氏名　ニュエン　バン　ニュエン

指導教員名　福代　康夫

論文題目 Taxonomic study on dinoflagellates belonging to the order Dinophysiales (Dinophyceae)

（ディノフィシス目（渦鞭毛藻綱）に属する渦鞭毛藻類の分類学的研究）

Dinophysiales, known as dinophysoids, are among the most diverse group of dinoflagellates in terms of morphology. Their fine morphological gradients implicate evolutionary sequences, and are attractive to investigators. During the last few decades, the attention on this group rapidly increased, since some of the member species were found to produce toxins responsible for diarrheic shellfish poisoning.

The taxonomy of dinophysoids is, however, very problematic. Currently their classification is mainly based on cell size and shape, the presence of chloroplast, and cell ornamentations such as the presence and structure of lists, ribs, protrusion and spines. But, as the intraspecific variation is strong, these morphological characteristics are often overlapping among species. Species boundaries are thus ambiguous and specific assignment usually cannot be made with certainty.

A constraint for taxonomic study of these dinoflagellates is that most of them are rare and mainly distributed in pelagic waters. The sampling therefore faces many difficulties. Another problem is that the original diagnoses, which were mostly made 50 years or even more than a century ago, were usually very simple and not clear enough for specific separation. These difficulties hinder the understanding on the nature of species, making the classification system very artificial. Many taxonomists considered it is necessary to re-construct the classification of the order more natural. And DNA sequences are suggested to be a useful supporting tool.

This study aims to make the classification of the order clearer. By collecting material of a wide spectrum of taxa from various places and subjected to detailed morphological examination, including the plate patterns, and compared with the original description; and by analyzing their DNA sequences, I try to grasp the ranges of variations and figure out their specific boundaries, so as to make the classification of the order to be more robust.

The study area was in the pelagic waters of central and western Pacific Ocean collected during the two cruises of R/V MIRAI, MR07-01 and MR07-06, and some coastal waters of Japan, Thailand and

Vietnam. Samples were collected by plankton net with a mesh size of 20 µm, either by towing net - in the case of coastal waters, or by filtering the seawater pumped up from vessel bottom while cruising - in the case of open ocean. Live cells of Dinophysiales were individually isolated under microscope, examined for their morphology, photographed and then transferred each to PCR tube, where they were thermally broken by several frozen-melt cycles, before directly subjected to DNA amplification. The rDNA of the D1-D2 domains of the large subunit were amplified by two rounds of PCR reaction. The products were purified and directly sequenced for both strands. The phylogenic trees were conferred by the neighbour-joining and the minimum evolutionary methods. Cells after analyzed for DNA were harvested for plate pattern examination. Through this process, the same cells were used for both the DNA analysis and the detailed morphological analysis.

A total of 57 species of Dinophysiales belonging to 9 genera, *Dinophysis* (25 species), *Pseudophalacroma* (2), *Metaphalacroma* (1), *Metadinophysis* (2), *Ornithocercus* (10), *Citharistes* (2), *Histioneis* (8), *Amphisolenia* (6), and *Oxyphysis* (1) were recorded, including many rare species. The existence and morphology of *Dinophysis acutissima* and, particularly, the genus *Metadinophysis*, were confirmed for the first time since their first description.

There are 3 morphotypes, *Dinophysis* sp., *Histioneis* sp. and *Metadinophysis* sp., do not fall into any previous descriptions, and thus must be established as new species. Each of the genera *Metadinophysis* and *Pseudophalacroma* consists of more than one none-conspecific morphotypes, indicating that these two genera are not monospecific.

Among the 38 species belonging to 9 genera examined for plate pattern, 20 species were observed for the first time. These observations resulted in some different conclusions from what previous taxonomists made.

(a) In all dinophysoids examined, the four sulcal plates were found to surround the flagellar pore. This is in contrast to conclusion made by previous study that the left sulcal plate of the genus *Pseudophalacroma* does not contact the flagellar pore, which was thought to be the distinct characteristic of the genus.

(b) Examination of plate pattern of *Amphisolenia* showed that the left sulcal plate in this genus was located right beside the flagellar pore, and never reached the far-away cingulum as it was assumed by previous authors. The two ventral hypothecal plates were arranged vertically, one after another, not horizontally as they were believed before.

(c) The genus *Citharistes* evidently had 4 cingular plates, not 6 plates as it was reported. This lead to an updated conclusion that all species of dinophysoids shared the similar plate pattern: 4 epithecal, 4 cingular, 4 sulcal and 4 hypothecal plates, in addition to an apical complex, which contained one or, usually, two pore platelets/plates.

Polymorphism – the formation of cells with different size and shape - was confirmed in 4 genera. Among them, genera *Ornithocercus*, *Metadinophysis* and *Histioneis* were for the first time observed to show this phenomenon. These recordings indicated that this phenomenon is very common in dinophysoids,

and it was probably the main source of intraspecific variation in morphology of these dinoflagellates. The general rules of cell deformation during depauperating division were pointed out: cells always remained the anterior ventral part and discharged the dorsal posterior part. These understandings gave clues to guess the possible shape of small cell of certain species, once depauperating division undergoes.

The height of epitheca, the depth of cingulum, the deflection of body axis, thecal surface structure and the relative distance of rib 2 compared to rib 1 and rib 3 and the capability of hosting chloroplast were proved to be stable and specific for species, and they are therefore reliable criteria for classification. However, the judgment of these characteristics must be taken with full awareness of the effect of depauperating division on morphology of new cell, as mentioned above. In contrast, some characteristics, such as the structures of cingular list, the left sulcal list, were evidently not reliable characters, although in many cases they could provide hints for classification. This is because these characteristics could easily change according to the age of the cell and due to depauperating divisions.

Sequence of the D1-D2 domains turned out to be rather specific for species of Dinophysiales. The difference in DNA sequence usually correlated well with the morphological difference, indicating that this is a good criterion supporting the species differentiation in Dinophysiales.

Considering the above understandings, two trends of treatment on the classification of Dinophysiales were proposed, namely merging and splitting of taxa. Each of the species *O. magnificus* (with 3 morphotypes), *D. fortii* (2), *D. infundibular* (2) and *D. hastata* (5) , *D. doryphora* (2) contained several morphotypes, which were striking different in both morphology and genetic, and must be separated into new species. Merging was proposed to some other species that were differences in morphology, but those differences appeared to be resulted from polymorphism. *H. hippoperoides*, *H. dolon* and *H. helenae* should be considered as synonymous with *Histioneis megalocopa*, while *O. steinii*, *O. skogsbergii*, and probably also *O. quadratus*, may be conspecific with *O. thumii*; and various species of *Dinophysis* with minor differences in morphology, such as *D. anabilis*, *D. lapidistrigiformis* and *D. microstrigiformis*, may be conspecific with *D. acuminata* or *D. fortii*.

There had been morphological and molecular biological evidences for these treatments in the cases of *D. infundibular*, the *D. hastata* complex and the above mentioned *Histioneis* spp., but for other cases, further evidences are needed before such treatment could be made.

A well-known morphotype of *Dinophysis*, which is frequently referred as *Dinophysis rotundata* world wide, turned out to be not conspecific with the type specimen of *D. rotundata* Claparede et Lachmann, since they showed striking differences in thecal surface, cell shape and the height of epitheca, which are, as mentioned above, important specific characteristics. A new name therefore should be given to this morphotype. The same recommendation was raised for other two species, *D. odiosa sensu* Tai et Skogsberg, and *D. elongata sensu* Abe.

The study also revealed several important characteristics that were specific for genera or species, but were constantly overlooked or were not considered to be important by previous taxonomists. These included the position of the left ventral epithecal plate in *Pseudophalacroma*, the dissimilarity in convexity

of the two main hypothecal plates of *Metadinophysis*, the deflection of body axis and the dorsal concavity of cingulum in *Ornithocercus*, and the epitheca depth in *D. caudata-D. tripos* complex.

The definition of *O. quadratus* was confined to morphotypes that corresponded to the type specimen only. Those were with different cell shape, which were mostly belonging to *O. quadratus* var. *assimilalis*, were excluded from this taxon and transferred to *O. galea*.

The three species, *O. biclavatus*, *O. carolinae* and *O. galea*, which had been repeatedly considered as conspecific with *O. heteroporus*, *O. francescea* and *O. quadratus*, respectively, were confirmed here to be valid independent species, since they showed difference in various important morphological criteria.

Most of the 79 sequences of 43 species of dinophysoids obtained in this study were for the first time to be read. Phylogenetic tree based on the obtained DNA sequence showed that the order Dinophysiales was monophylic. The combination of morphological and genetic evidences suggested that this group may have been evolved from some primitive forms like *Phalacroma* or some microcephalic group, such as *Metaphalacroma* or *Metadinophysis* etc. Among dinophysoids, the genus *Amphisolenia*, which were well defined in morphology and monophylic in genetic, appeared to diverge very early, in a separated direction. The rest of dinophysoids fell in 12 clades, the detailed arrangement of which was uncertain. The photosynthetic species of the genus *Dinophysis sensu stricto* evidently evolved independently. These species, which showed heavy thecal surface and had capability of holding chloroplast, formed a solid clade that was clearly separated from the rest of dinophysoids, including the rest of *Dinophysis*. Within this clade, the closely related groups *D. caudata - D. tripos – D. miles* formed their own evolutionary branch, with fine gradients in both genetic and morphology. Both morphological and genetic data supported the assumption by some previous taxonomists that *Citharistes* might have been evolved from the *Ornithocercus* stock, which might have been sprung from a certain form of *Dinophysis*, likely *D. hastata*. The participation of the genus *Histioneis*, which were likely monophylic in genetic, in this evolutionary sequence was not clear, despite their obvious intermediate morphology between O*rnithocercus* and *Citharistes*. Relationship of other groups, including the non-photosynthetic *Dinophysis sensu stricto*, the former members of *Phalacroma* and genus *Oxyphysis*, was uncertain.

In summary, with the results from examination of morphology and molecular biology of 57 dinophysoid species in Pacific region, the rules of morphological variation were understood, and the importance of some morphological and genetic characteristics for classification was realized. With these understandings, some confusion in classification of the order was clarified. Considering these approaches and understandings, by further study on other species of dinophysoids, as well as on materials from other areas, such as the Atlantic and the Indian Ocean regions etc., a more robust classification of the order can be obtained.