Supplementary Material 4

Taxonomical and ecological issues of major aquatic palynomorphs

Organic-walled microfossils have been known to be present in samples obtained by palynological processing for a long time. However, except for dinoflagellate cysts, most of them have been not well investigated regarding the organisms from which they originate and their utility for paleontological studies. Recently, McCarthy et al. (2021), Mudie et al. (2021), and Shumilovskikh et al. (2021) published reviews of the usefulness of these organic microfossils for enhancing paleoenvironmental studies. However, as suggested by McCarthy et al. (2021) and Mudie et al. (2021), many insoluble taxonomical and ecological issues including dinoflagellate cysts are waiting for clarification. In this section, several taxonomical and eco-physiological issues of aquatic palynomorphs found in sediments of Beppu Bay are discussed, because these palynomorphs will be useful tools for reconstructing paleoenvironments after deeper biological studies in future.

Dinoflagellate cysts (Fig. 4, 1-11)

Spiniferites bulloideus sensu Wall 1965 (Fig. 4,1): This species was originally described as a fossil species, *Hystrichosphaera bulloidea* Deflandre and Cookson (thereafter, *S. bulloideus*), from Australian Miocene sediments (Deflandre and Cookson 1955). Wall and Dale (1968) identified the corresponding motile cells as *Gonyaulax scrippsae* based on germination experiments using live cysts of this species collected from modern sediments. However, Ellegaard et al. (2002) suggested that the planktonic stage corresponding motile form of *S. bulloideus* is identical to the cyst of *Gonyaulax baltica* rather than *G. scrippsae*, based on molecular phylogenetic analysis. In addition to the morphological differences between the originally described fossil species (spherical cysts) and *S. bulloideus* (ovoidal cysts) from Quaternary sediments, this species recovered from coastal surface sediments has been pointed out to have variations in the length of processes, diameter of cyst body and the development of parasutural septa (Mertens et al. 2018). This circumstance indicates that the cysts identical to *S. bulloideus* are derived from different motile species. Further research is needed to identify the plankton corresponding to this species.

Lingulodinium machaerophorum (Deflandre and Cookson 1955) Wall 1967 (Fig. 4, 4): The processes of this species become shorter in low-salt environments (Mertens et al. 2009), and the species is abundant in upwelling and river mouths in tropical to temperate waters (Zonneveld et al. 2013). Also, Dale et al. (1999) and Dale (2009) argued that the increased appearance of this species in Oslo fjord reflects cultural eutrophication (Oslo fjord signal). In Tokyo Bay (Matsuoka 1999), Ariake Sound and Gamak Bay (Shin et al. 2010 a, b), and Apponagansett Bay (Pospelova et al. 2002), however, this species was not related to cultural eutrophication. In Beppu Bay, the increase of this species seems to be related to climatic changes rather than eutrophication, because lower abundance of this species was observed in BP- D Unit.

Identification of brownish peridinioid cysts is also confusing, in particular for *Quinquecuspis concreta* and *Lejeunecysta sabrina*. In the paleontological sense, these species are classified into different genera; however, their corresponding motile forms were reported as *Protoperidinium leonis* for *Q. concreta* by Wall and Dale (1968) and *P. leonis* for *L. subrina* by Dale (1983), respectively. Careful re-investigation of these cysts and also motile forms is needed regarding both their morphology and molecular phylogenetic position.

Microforaminiferal linings (Fig. 7, 2-4)

Microforaminiferal linings are roughly classified into a single chamber type, uniserial type, biserial type, coiled type and composite type (coiled + uniserial; coiled + biserial) according to the morphology of the membrane formed in the chamber of foraminifera (Stancliffe 1989, 1996; Mudie and Yanko-Hombach 2019).

Sugaya and Nakao (1983) investigated the composition of foraminiferal communities in the Bungo Channel area, including Beppu Bay, and found that the pelitic sediments distributed in the inner part of Beppu Bay were characterized by the *Hopkinsina-Textularia* community. Based on the fact that this assemblage contained agglutinative *Textularia* cf. *tenuissima* and *Reophax* sp., and calcareous *Buccella frigida*, a biserial type might be derived from *Textularia* cf. *tenuissima*, a uniserial type from *Reophax* sp., and a coiled type from *Buccella* sp. and/or *Ammonia* sp.

The benthic foraminifera assemblage in the inner part of Beppu Bay, including *Texturalia*, *Reophax*, and *Buccdella*, corresponds to type D of Kitazato (1981) and is consistent with the inference that it reflects sandy to muddy sediment environments. These foraminiferans have a lifestyle of feeding by moving around slightly from the surface of the sediment (Kitazato 1981). Moreover, the prolific production of *Texturalia* and *Reophax*, which are hyaline type and aggregated type, respectively, is consistent with Brasier (1980), suggesting the sedimentary environment of mostly shelf area. In the Beppu cores, where the uniserial type and the biserial type foraminifera predominate because of the significance of hyaline and agglutinated types respectively, the biserial type began to decrease after yr 1744, and the average appearance status was 1281 linings / g, but by yr 2003 it decreased sharply to 195 linings / g. The Uniserial type also dropped from an average of 495 linings / g before yr 1744 to 159 linings / g by yr 2003. The benthic foraminifera reflected in the microforaminiferal lining assemblages of the core samples were derived from the *Hopkinsina-Textularia* assemblage of Sugaya and Nakao (1983).

Crustacean remains (Fig. 7, 7-10)

Belmonte and Rubino (2019) listed resting (dormant) cysts and/or eggs of coastal plankton, including diatoms, dinoflagellates, ciliates, and crustaceans (9 species of Cladocera and 48 species of Copepoda). Thus, many crustacean resting eggs can be preserved in sediments of Beppu Bay. However, further morphological observations of these resting eggs are also needed for clear species identification.

In Cladocera, resting eggs are known in the genera *Evadne, Penilia, Podon,* and others (Croce and Bettanine 1969). The resting eggs of *Penilia avirostri* and *Podon intermedius* have been found in the sediments of Seto Inland Sea (Onbé 1972, 1985). A resting egg with a dark brown egg membrane and an irregular fine network structure on the surface was confirmed from Beppu Bay, and such morphological features suggest that this is a resting egg of *P. avirostri*.

In Copepoda, species that produce resting eggs have been mainly known in the genera *Acartia, Centropages, Labidocera, Tortanus*, and others (Belmonte and Rubino 2019). Resting eggs such as *A. clausi, A. pacifica, A. tsuensis, Centropages abdominalis, C. yamadai, Labidocera bippinnata, L. trispinosa,* and *Tortanus forcipatus* are also known to be present in sediments in Seto Inland Sea (Koga 1968, Kasahara et al. 1974, 1975, Uye 1985, 2005). These resting eggs could be roughly classified into thick-film type and protrusion type according to their morphological characteristics. Thick resting eggs have been known in *Acartia clausi* and *Calanopia thosoni*, and protrusion-type dormant eggs have been known in *Acartia omorii, A. steueri, Calanus finmarchicus, Centropages abdominalis, C. yamadae,* and *Tortanus discaudatus* (Koga 1968, Kasahara et al.1974, Onoue et al. 2004). However, since the morphologies of these resting eggs are similar to each other and their outer shape in chemically treated samples is often deformed, it is difficult to identify them to the species level at present. Interestingly, copepods that produce resting eggs are taxonomically known only to the order Calanoidea (Belmonte and Rubino 2019), and many of them live in coastal areas (Uye 2005).

In the core sediments of Beppu Bay, the occurrence pattern of Crustacean resting eggs was different from that of dinoflagellate cysts and microforaminiferal linings. From yr 1665 to yr 1944, it increased (1005 eggs /g) compared to before. This may be due to an increase in prey organisms, that is, phytoplankton, primary consumer ciliates, and heterotrophic flagellar algae, as a weak but positive correlation ($r = 0.302$, $p > 0.05$) was found between the appearance of Crustacean resting eggs and dinoflagellate cysts. The majority of Crustacean resting eggs observed in our samples were copepods. The Copepod as filter feeder consumes various particles suspended in water by squeezing them with jaw stabs, and various particles including not only diatoms but also dinoflagellates, raphydophytes, prasinophytes, and others. So, phytoplankton, protozoan plankton, organic detritus, and others are also included as foods (Ohtsuka and Nishida 1997, Uye and Takamatsu 1990). Therefore, it is acceptable that they could show a positive correlation with the dinoflagellate cysts, one of the groups consistent with the ecology of copepods.

However, at present, it is impossible to discuss the paleoenvironment only based on the mode of occurrence of the crustacean resting eggs observed as palynomorphs. The reason is that the presence of crustacean remains, especially copepods in sediments, can be confirmed not only by resting eggs but also by the appearance of fragmented bodies and appendages. However, copepods undergo 11 molts from hatching to complete adults. This indicates that the body after molting may remain in sediments. Therefore, at present, it is difficult to use crustacean remains as a counting method like other palynomorphs. This is also one of the important issues to be examined in Crustacean palynomorphology.

Turbellarian egg capsules (Fig. 7, 11-13)

This palynomorph has been treated as a tintinnomorph together with ciliate lorica and cyst (e.g., van Waveren 1993), but it is recommended to treat it separately because the significance of different forms has recently been confirmed (Matsuoka and Ando, 2021). Turbellarian egg capsules have often been reported from freshwater sediments (e.g., Haas 1996), but marine turbellarian funnel, bell, pitcher, or cup-shaped eggs are also known to be preserved in marine sediments (Matsuoka and Ando 2021, Mudie et al. 2021). Actually, various turbellarian egg capsules were found in the core samples of Beppu Bay. However, research on Turbellarian egg capsules as a palynomorph has not progressed, and elucidation of their paleoenvironmental significance remains an issue for the future.

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