

Siliceous Nanoplankton. II. Newly Discovered Cysts and Abundant Choanoflagellates from the Weddell Sea, Antarctica

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Abstract

Siliceous choanoflagellates and previously undescribed, siliceous cyst-like spheroids (in the size range 2.5 to 15 μm) were found below sea-ice in the Weddell Sea and studied with scanning electron microscopy. Cell counts from water samples obtained over a 10,000 km area indicated that both spheroids and choanoflagellates were abundant in the upper 100 m of the water column, averaging about 10^5 cells l^{-1} . The large numbers of phagotrophic choanoflagellates suggest that bacterioplankton-picoplankton food chains are important and may indicate the presence of longer, more complex trophic networks than previously considered in Antarctic waters. Circumstantial evidence suggests that the spheroids may be the cysts of choanoflagellates; if so, the production of siliceous cysts by members of this enigmatic taxon may be of considerable interest in discussions of the phylogenetic position of the group in relation to the algae and the metazoans. Similar cysts were found simultaneously in the North Pacific Ocean (see preceding paper: Booth *et al.* 1980), and recently we have discovered the cysts in the upwelling area of the eastern tropical Pacific Ocean. The occurrence of the cyst in such geographically distant localities suggests that they have a worldwide distribution. Since the minute cysts are easily overlooked on the light microscope, their recognition and further study undoubtedly require the electron microscopic examination of plankton samples.

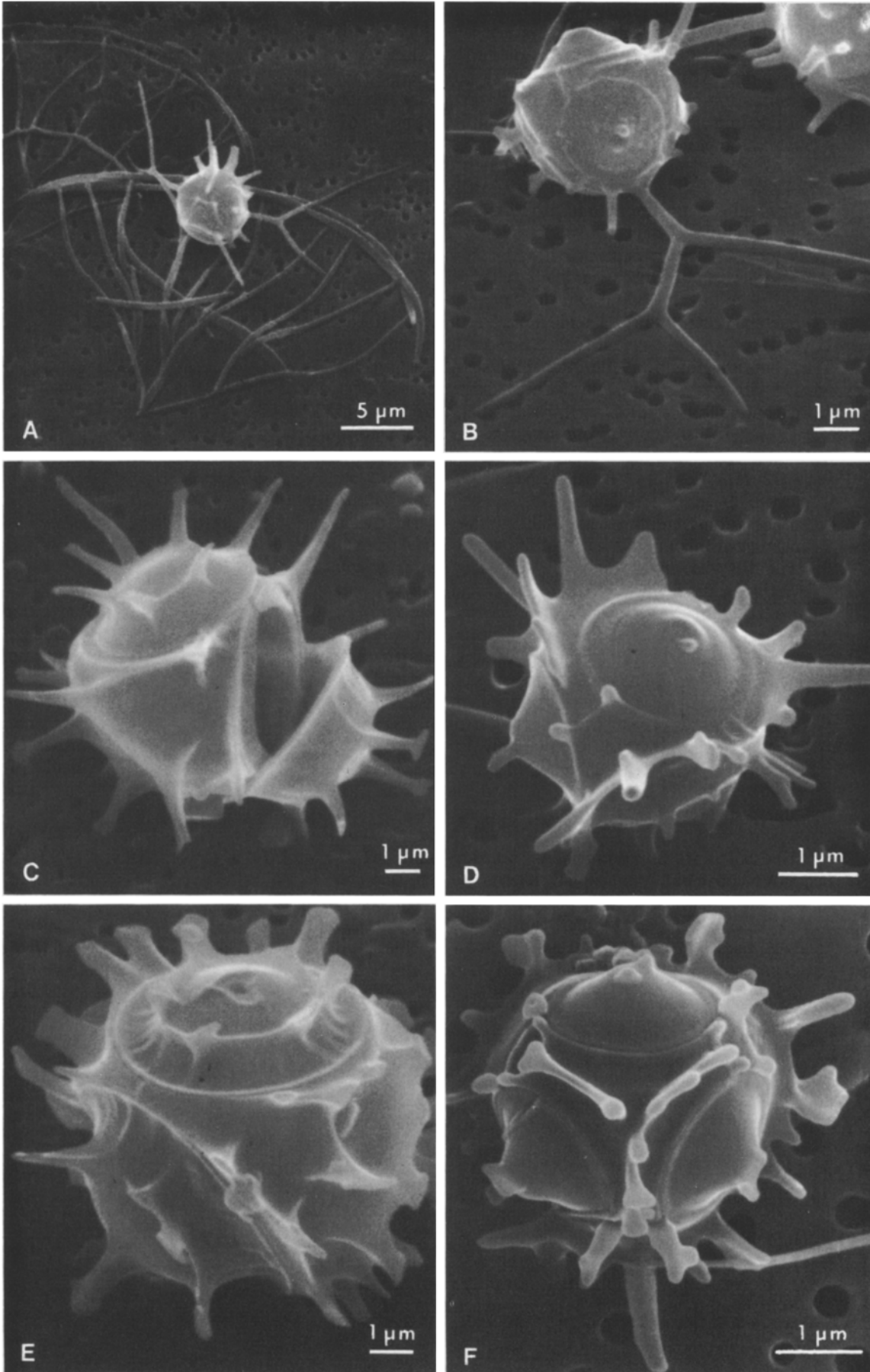
Introduction

Siliceous forms of unicellular plankton have dominated Antarctic waters since the Miocene (Kennett, 1978), and the fossil and living members of these groups have been of interest to both paleontologists and plankton biologists. Paleontologists have studied a variety of large and small siliceous unicells from Antarctic sediments, using

both light and electron microscopy (e.g. Perch-Nielsen, 1975; Haq, 1976). Biologists have generally been concerned with the large unicells, particularly the diatoms that appear to dominate pelagic production in Antarctica (Zernova, 1970; El-Sayed and Turner, 1977; Holm-Hansen *et al.*, 1977). The emphasis of most biological studies on large phytoplankton is the unavoidable consequence of sampling with nets, which do not retain most nanoplankton (cells $\leq 20 \mu\text{m}$; see Sieburth, 1979 for size fraction designations). The few studies that have used alternative methods of collecting plankton indicate the presence on occasion of large numbers of very small autotrophs and heterotrophs in open water (Hentschel, 1932; Hasle, 1969) and frequently in areas of sea-ice (Bunt, 1968; El-Sayed, 1971; Horner, 1976). The Antarctic nanoplankton are poorly known, and many of the taxa require the increased resolution of electron microscopy for recognition and study. This paper presents the first such study of nanoplankton from Antarctic waters.

Materials and Methods

Water samples were collected during the International Weddell Sea Oceanographic Expedition on the US Coast Guard ice-breaker "Glacier" in February 1978 (Foster and Middleton, 1978). We examined material from 45 stations in water depths from 490 to 1980 m over a 10,000 km² area of the southern Weddell Sea centered around 74°S, 38°W. Water samples were obtained under sea-ice (7 to 9 tenths coverage) from the upper 100 m of the water column, and the water averaged -1.8°C and 34.3‰ S. One liter aliquots from the 5 l Niskin water bottles were filtered through 47 mm Nuclepore polycarbonate filters (0.4 μm pores), rinsed with distilled water, air-dried and stored at -20°C . This treatment produced excellent preparations of skeletal and wall materials of algae and protozoans, but resulted in loss or collapse of protoplasts; thus, we could not study protoplasmic features in our electron microscopic surveys of the



material. However, a few of the samples were backwashed from the filters at the time of collection and preserved in 4% formaldehyde. These allowed examination of intact cells by light microscopy. Portions of the dried filters were mounted, gold- and carbon-coated, and studied on a JEOL JSM-2 scanning electron microscope (SEM). Counts were made by surveying transects of known field width at appropriate magnifications, with transect selection accomplished by using coordinates designated by a random-number table. Replicate counts on the same filter indicated that individuals were randomly distributed, with standard errors for replicates averaging $\pm 10\% \bar{x}$. Mineral composition of the various forms was confirmed by examining individual specimens with a KEVEX x-ray energy dispersive spectrometer coupled to an ISI 3A SEM.

Results and Discussion

Protozoan flagellates with siliceous loricae (Fig. 1A) were present at all stations and usually were the most abundant forms in our samples, generally outnumbering the diatoms. All were choanoflagellates of the family Acanthoecaceae, as shown by characteristic structure and siliceous composition of the lorica (Norris, 1965; Thomsen, 1973; see recent choanoflagellate review in Sieburth, 1979). Abundances averaged 3.6×10^5 (standard error = 0.7×10^5) cells l^{-1} , with a maximum of at least 2.14×10^5 cells l^{-1} ; at these higher densities, lorica enumeration became difficult because specimens overlapped extensively, especially the colonial *Parvicorbicula socialis*. [Details of distribution and oceanographic relationships will be presented by Mitchell (in preparation).] The acanthoecacean fauna was diverse, and included representatives of the genera *Parvicorbicula* (*P. socialis*), *Calliacantha* (*C. simplex*, Manton and Oakes, 1979), *Pleurasiga*, *Crinolina*, *Acanthoecopsis*, and *Diaphanoecca*. At the present time we are not assigning species names to most of our lorica types but are awaiting taxonomic confirmation by specialists; our material contains hemisphere range extensions in some cases and possibly new species in other cases. Total lorica lengths ranged from about 20 to 150 μm , but individuals inside were $< 10 \mu m$, as reported for this family of protozoans (Norris, 1965). Choanoflagellate protoplasts were evident in our formaldehyde-preserved material, but the loricae were quite inconspicuous on the light microscope because of the open spacing and small diameter (0.5 μm)

of the individual lattice ribs (costae) in the baskets. Given the delicacy of the lorica, the diagnostic feature of this group of zooflagellates could easily be overlooked in light microscopy, and thus the identity of the flagellates may be obscured in routine surveys of plankton material.

The next most abundant form in the nanoplankton was an array of unusual, walled spheroids (Fig. 1), morphologically unlike any microorganism or cyst reported in the biological literature. We consider it unlikely that these spheroids are the products of currently known cyst producers from the Antarctic Ocean; the resting spore wall of diatoms is a shell composed of 2 pieces, and the walls are morphologically distinctive; dinoflagellate cysts, including forms called "acritarchs" are organic-walled and often obtained by hydrofluoric acid treatment, which destroyed our specimens (see below); chrysophyte statospores and prymnesiophyte cysts possess a pore and neck structure not found in our specimens; xanthophyte cysts have a 2-part structure not evidenced by our material. When intact, the spheroids lacked perforations or defined openings larger than 10 nm (the limit of our resolution); without such apertures, a metabolically active internal protoplast could not contact the external environment, and thus we consider the spheroids to be cysts. (Definitive proof of the nature of this stage must await life-history studies and culture of the organisms producing the spheroids; we use this term only as a convenient descriptive name.)

The siliceous cysts were present at all stations and their average abundance was 8.4×10^4 (standard error = 1.7×10^4) cells l^{-1} , with a maximum of 58.6×10^4 cells l^{-1} . According to the x-ray analyses, cysts were constructed of silicon, with other minerals constituting less than 1% of the wall material. Since the x-ray system does not detect atomic species with atomic weights in the carbon and nitrogen range, some additional organic materials may have been present in the wall and remained undetected. However, the dissolution of the cysts after a 5 min, 1% hydrogen fluoride treatment indicated that organic material (e.g. sporopollenin) was not a major wall constituent. Cysts varied in diameter from 2.5 to 15 μm and consisted of a framework that had one or more circular openings. The openings were occupied by tightly fitting dome-shaped plates; broken specimens showed that the circular plates rested on an internal flange (Fig. 1C). The most abundant forms of cysts were those with 3, 4 or 6 circular plates of equal diameter, which had trigonal prismatic, tetrahedral, and cubic geometries respectively. Ornamentation varied considerably

- ◀ Fig. 1. Siliceous cysts from Weddell Sea, presumed to be product of pelagic microorganisms, possibly acanthoecacean choanoflagellates. Scanning electron micrographs of specimens on Nuclepore polycarbonate filters, 0.4 μm pore size. (A) Cyst 5 μm in diameter bearing 2 long bifurcated spines and other ornamentation; it lies in the basket-shaped lorica (exoskeleton) of the choanoflagellate *Parvicorbicula socialis*; lorica has flattened and cell body has been lost during preparation. (B) Cyst, similar to that in (A), having a long spine with 2 branches. Both cysts in (A) and (B) have 6 circular plates, each occupying one of the faces of a cube. (C) Cyst which has been broken open, probably as it settled onto filter; one of circular plates is displaced, showing its form and the flange on which it rests; this cyst type has the form of a trigonal prism, with 3 circular plates occupying the 3 square faces of the prism; diameter is about 8 μm , excluding ornamentation. (D) Cyst exhibiting tetrahedral symmetry, with 4 circular plates; about 3 μm in diameter. (E) Another tetrahedral cyst, larger (8 μm diameter) than (D) and with different ornamentation. (F) Elaborately ornamented cyst, about 3 μm diameter (photo by V. Selway, Semco Instrument Co.)

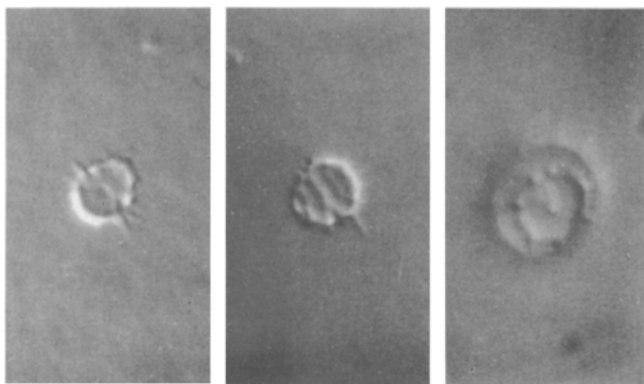


Fig. 2. Light micrographs of siliceous cysts from Weddell Sea; Nomarski optics. All specimens approximately $7\ \mu\text{m}$ diameter

in our material. Spines, wings, or blunt projections, or a combination of these, arose from both framework and circular plates. Some cubic forms had spines up to $15\ \mu\text{m}$ long, with one or more bifurcations along their length (Fig 1B). Since the ornamentation was obviously fragile, and breakage occurred either in the water column or during specimen preparation or both, we do not yet know whether the ornamentation was symmetrical. Appearance, ornamentation and size varied within a given geometric type; we have not yet attempted to determine how many different cyst forms could be classified on the basis of these characteristics. Cysts were evident in our light microscope preparations, but their distinctive features were not conspicuous, even after examination with a high-resolution, oil-immersion lens (Fig. 2). It is obvious that these cysts are readily overlooked in light microscopy preparations and that scanning electron microscopy is required for their study.

The cysts we have observed appear to include forms that closely resemble those described by Booth *et al.* (1980) in the preceding paper (c.f. present Fig. 1F with Fig. 1: 1 in Booth *et al.*, 1980). The basic arrangement

of circular plates set on a framework composed of individual plates is in agreement with the construction of cysts from the North Pacific Ocean. However, the North Pacific specimens always possessed 4 circular plates, whereas our samples contained forms with other numbers as well (e.g. 3, 4, 6 plates and additional, less common types, such as a form with just 1 plate). Moreover, our specimens possessed circular plates of similar diameter, and the dorsal and ventral asymmetry described in the Gulf of Alaska material was not evident in the specimens we have examined carefully to date. Our specimens were smooth-walled and did not show the surface ornamentation found on most of the Gulf of Alaska cysts. The long bifurcating spines were a unique feature of some of the Antarctic cysts and longer projections seemed to be common in the southern hemisphere material. The existence of such differences in ornamentation and circular plate number in the two areas suggests that different oceanic regions may contain forms with considerable endemism.

We have recently examined samples of pelagic detritus collected by opening-closing nets towed at 2800 m (approximately 10 m above the bottom) in the equatorial Pacific ($0^{\circ}33'S$; $35^{\circ}33'W$). The detritus contained abundant surface material, primarily siliceous diatoms and radiolarians, and was heavily pigmented, suggesting recent descent from the euphotic zone. Scanning electron microscopic examination of the material revealed the presence of cysts of symmetrical tetrahedral (4 circular plates) construction with smooth surface texture (Fig. 3), a form related to the spheroids described in the present and in Booth *et al.*'s (1980) paper. The occurrence of these new cysts in regions as divergent as the equatorial Pacific, the extreme south Atlantic (Weddell Sea), and the Alaskan Gulf of the North Pacific suggests that the cysts occur widely in the ocean. As scanning electron microscopy becomes used more generally in nanoplankton studies, we predict that such forms will be found in additional locations and that these new finds will include additional morphological variants.

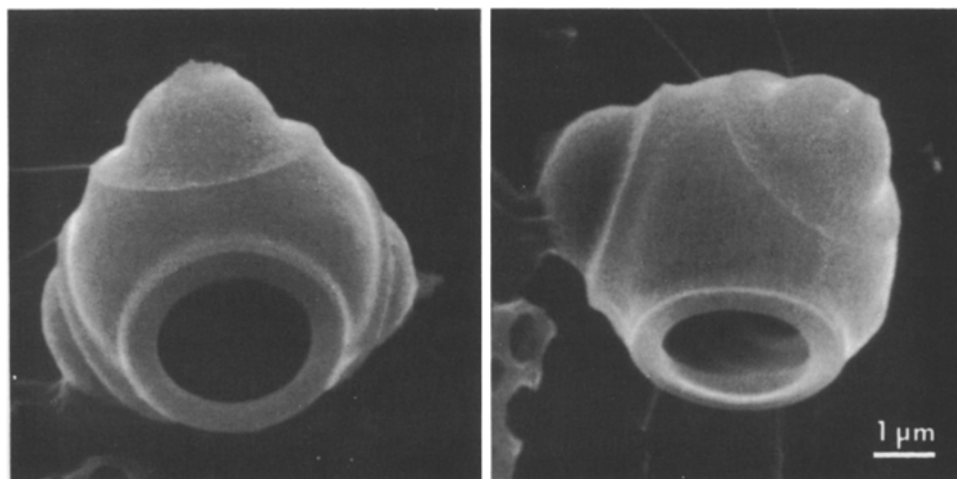


Fig. 3. Two views of siliceous cyst from 2900 m depth in equatorial Pacific Ocean. This $6\ \mu\text{m}$ diameter cyst has symmetrical tetrahedral form; one of the 4 circular plates is missing; note lack of ornamentation. Obtained from pelagic detritus 10 m above sea floor. (Scanning electron micrographs)

The marked dissimilarity of the spheroids to previously described forms suggests to us that they are members of a taxon whose cysts are either completely unknown or not yet adequately characterized by high resolution microscopy. Our examination of the Antarctic material implicates acanthoecacean choanoflagellates. We did not observe direct protoplasmic or siliceous connections between the cysts and the collar flagellates, but six lines of evidence suggest such relationship. (1) Cysts and choanoflagellates were the two most abundant nanoplankters in our samples and had similar distributional patterns; further, acanthoecaceans were also abundant in the North Pacific material that contained the spheroidal cysts (Booth *et al.*, 1980). (2) Several obviously related types of cysts were present in our samples, suggesting production by a group of related species; at the same time, at least 6 species of acanthoecaceans were present in our material. (3) Acanthoecaceans utilized silicon in skeletal construction (i.e., in the lorica), and thus may have the metabolic capability for producing other siliceous wall products; costal composition (i.e., pure silicon) and molecular density, as revealed by our x-ray analyses, were identical with those values for the cysts. (4) The cysts were sufficiently large to accommodate the protoplasts of acanthoecaceans. (5) No other taxa were evident in our samples with protoplasts of appropriate size or abundance to suggest an association with the new cysts. (6) Cysts are known to occur in choanoflagellates; these reports are to be found in the earliest papers on these protozoans but apparently have been overlooked in modern discussions of the group.

Although recent literature on choanoflagellates either does not mention cysts, or suggests that such forms may not occur in choanoflagellates (Bourelly, 1957), the earliest descriptions of these protozoans contain frequent mention and numerous drawings of cysts in the choanoflagellates (Saville-Kent, 1880-1882; Rezsö, 1897). Griessmann (1914) observed the formation of cysts in the laboratory in response to desiccation, and later germinated the cysts again to produce choanoflagellates. [In our study, cyst production occurred at the end of the austral summer in an area soon to be completely ice-covered, perhaps indicating an overwintering function here: such a role has been suggested by Meguro *et al.* (1967) for resting spores in diatom ice communities.] In some earlier accounts (Rezsö, 1897), cyst formation appeared to be a part of the life cycle; for example, in some cases germination of the cysts produced naked swarm stages. These early accounts reference cyst formation in non-siliceous species that are assigned today to the families Codoniscidae and Salpingoecidae. The acanthoecaceans were not frequently seen by the early workers, and the family was erected rather recently (Norris, 1965). Thus, cyst formation has not yet been reported in this family of choanoflagellates. Cysts seen by the early observers of the group were reported to be spherical or oval, and descriptions indicate that the walls were not mineralized, with one exception: Saville-Kent (1880-1882) noted that the marine *Codosiga cymosum* (Boucaud-Camou, 1967) possessed a "hardened" cyst

wall. Since siliceous loricae are only known in the species belonging to the marine family Acanthoecaceae, perhaps siliceous cysts also are to be found only in this family.

In previous phylogenetic speculations, choanoflagellates have been linked with both the algae and the invertebrates. Most of the older literature considered choanoflagellates to be colorless members of the golden algae (chrysophytes), on the basis of similarity to lorica-producing algae in that taxon (Bold and Wynne, 1978). One of the diagnostic features of this algal group is the formation of siliceous cysts (Bourelly, 1957). Like so many chrysophyte cysts, the cyst-like spheroids we describe are small, circular in outline, and siliceous. However, the new spheroids appear to differ fundamentally from the algal cysts in their lack of a defined opening closed by a plug and often surrounded by a collar, and in their possession of characteristic plate structure and unique geometries. Leadbeater (1972) and Hibberd (1975) have emphasized that choanoflagellates differ markedly from algae in their ultrastructure; therefore, these flagellates have been removed to the protozoans. Zoologists, on the other hand, have long considered choanoflagellates to be potential ancestors to sponges, and the increasing list of metazoans that contain collar cells (e.g. anthozoans, echinoderms) has led some workers to suggest that such forms may have been ancestral to the major invertebrate line (Taylor, 1978). With the possible rediscovery of the cysts in choanoflagellates, a new life-history phase becomes available for comparison with stages from algal and metazoan taxa, one that may clarify the systematic position of this enigmatic group.

We predict that the spheroidal cysts will be found as nanofossils in siliceous sedimentary assemblages, although such forms are not yet reported from Antarctic sediments or in the general paleontological literature. The new cysts are approximately the same size and possess a wall of similar thickness to siliceous chrysophyte cysts, which are found in marine fossil deposits and known as archeomonads (Tynan, 1971; Hibberd, 1977). These similarities to archeomonads indicate that the wall-structure of the spheroidal cysts will be sufficiently resistant for preservation in the sediments. Moreover, our finding of the cyst near the bottom of the eastern tropical Pacific suggests that these reach depths where siliceous assemblages are presently being deposited.

In this paper, we present the first data on choanoflagellate abundance in the Antarctic, supplementing three previous reports on their occurrence here (Deflandre, 1960; Hasle, 1969; Buck, 1977). Choanoflagellates are well known from the northern hemisphere, particularly from cold-water environments where they occasionally have been recorded in numbers and conditions similar to those noted in our own work (Thronsdon, 1969; Manton *et al.*, 1975). Aside from the choanoflagellate data presented here, comparable information on heterotrophic nanoplankton (i.e., protozoans) is very limited for the Antarctic Ocean. One early report, however, indicated the regular presence of unidentified protozoan flagellates in abundances of 10^3 to 10^6 cells l^{-1} (Hentschel, 1932).

The presence of such large numbers of heterotrophs, including the rapidly growing choanoflagellates (Leadbeater and Morton, 1974), is of consequence for polar food chains.

Little is known about production rates of the probable food sources (Laval, 1971) of choanoflagellates in the Antarctic: bacteria and photosynthetic picoplankton (cells $< 2 \mu\text{m}$; Johnson and Sieburth, 1979; Waterbury *et al.*, 1979). The first measurements of bacterial productivity in Antarctica suggest that a significant fraction of the photoautotrophic production may sometimes be rerouted through bacterioplankton (Fuhrman and Azam, in preparation). Production rates of photosynthetic picoplankton are not known in these (or other) areas, but Fay (1973) has shown that autotrophs smaller than $10 \mu\text{m}$ may be responsible for more than 60% of the primary production in Antarctic waters. Many previous studies of open-water plankton communities have emphasized the dominant role of diatoms in Antarctica, whereas studies in sea-ice environments have pointed to rich and complex communities of minute algae, bacteria, and heterotrophic protozoans as well as the larger diatoms (Horner, 1976). In our study, the diatoms, although abundant, were too large to be consumed directly by the choanoflagellates, and thus these other food sources must have supported their populations. Previous studies of Antarctic pelagic systems has emphasized the importance of diatom-crustacean food chains, but the highly productive ice-edge pelagic zones may have coexisting food chains of greater complexity. Whether subsisting on bacterial secondary producers or on picoplankton-sized primary producers, the choanoflagellates abundance indicates that substantial fractions of the primary production are rerouted or contained in size fractions other than those that could be utilized directly by crustacean grazers. Our results, then, suggest the importance of food chains with multiple trophic levels in this Antarctic pelagic community, and provide another example of the importance of minute heterotrophic plankton in pelagic environments (Beers and Stewart, 1970; Sieburth *et al.*, 1978).

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