

The recently established diatom *Coscinodiscus wailesii* (Coscinodiscales, Bacillariophyta) in Brazilian waters. I: Remarks on morphology and distribution

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SUMMARY

Coscinodiscus wailesii Gran et Angst is a large centric diatom (280–500 µm diameter) from marine phytoplankton, characterized by a cylindrical frustule with flat valvar surface, two marginal rings of rimoportulae on the mantle, and two macrorimoportulae. Cells from cultured and natural populations collected in Paranaguá Bay, Paraná, southern Brazil were observed under light and scanning electron microscopes to verify the populations' correct identity and morphology. In both populations, a typical central rosette or a hyaline area was found in the valvar center. The species' distribution in Brazilian waters was revised, and a discussion on possible vectors of transport was made. Blooms of the species occur sporadically in the coast of Paraná, seeming to affect the local trophic chain.

Key words: *Coscinodiscus wailesii*, diatom, distribution, harmful algae, Southern Brazil, taxonomy.

INTRODUCTION

In recent decades, blooms of marine phytoplankton have attracted the attention of public organizations in many countries due to their negative consequences on human health, seafood safety and marine trophic chains (Hallegraeff 1993; Hallegraeff 1995). As a result, an international Harmful Algal Bloom Programme was implemented in 1992 through the initiative of Intergovernmental Oceanographic Commission of UNESCO, aiming to 'understand causes of algal blooms, predict their occurrences, and mitigate their effects'.

Among the diverse algal groups (dinoflagellates, diatoms, cyanophytes, raphidophytes) composing the 3400–4100 microalgae species, it was estimated that 300 of them could produce blooms (Smayda 1997). Diatoms, when growing intensively, may cause mass mortality of marine organisms through (i) the production of toxins, as domoic acid (*Pseudonitzschia* spp.); (ii) respiratory diseases in fishes due to gill

damage caused by setae or spines present on the frustules of *Chaetoceros* spp.; (iii) decreasing of other phytoplankton species as a result of competition for nutrients; and (iv) oxygen depletion in the water column, from intense algal respiration and the incomplete diatom decomposition by bacteria near the sea bottom (Hasle and Fryxell 1995).

Since 1980, the centric diatom *Coscinodiscus wailesii* Gran et Angst has been recorded worldwide, developing blooms and damaging shellfish and macroalgae cultivations, and commercial fishery areas (Nagai *et al.* 1995a; Nehring 1998). Its distribution, first restricted to the tropical Pacific and west Atlantic oceans, has extended to Europe, the USA and Japan in recent years (Lange *et al.* 1992; Rick and Dürselen 1995; Nehring 1998). Indeed, in some areas, it has become an important component of the phytoplanktonic community. In Brazil, despite the various taxonomic works published since 1960, the species was only reported recently in the southern region (Valente-Moreira 1987; Moreira-Filho *et al.* 1990; Fernandes 1992; Souza-Mosimann *et al.* 1993).

In this work, we analyze the morphology of *C. wailesii* from cultured and natural populations sampled in Paranaguá Bay, Paraná State as well as discussing its distribution and potential vectors of transport to Brazilian waters.

MATERIALS AND METHODS

Material for analyses was obtained in May 1999 from the waters of Paranaguá Bay (25°25'S to 25°35'S and 48°20'W to 48°45'W), Paraná State, Southern Brazil (Fig. 1), an estuarine complex of 117 km² with an average depth of 4.3 m. The Bay is influenced by the subtropical climate (Cfa) with two well-defined seasons: rainy in summer and dry in winter, with annual average

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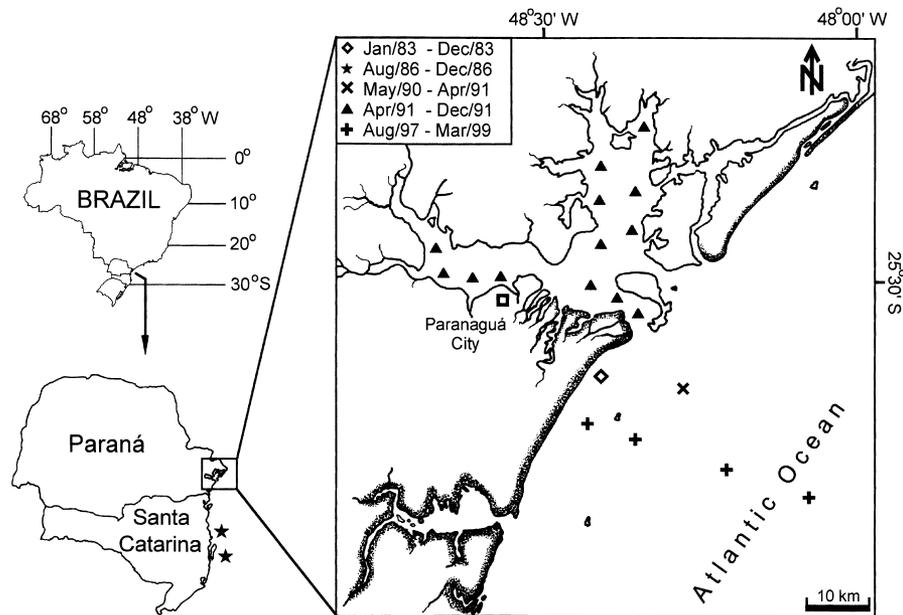


Fig. 1. Maps showing the sampling locations in Paranaguá Bay and the historical distribution records of *Coscinodiscus wailesii* in Brazil, as well as the sampling periods in which the species has been found.

rainfall of 1988 mm. Salinity varies from 12 psu to 34 psu, range of water temperature is 18–30°C, and the annual average of semidiurnal tides is 2.2 m. Mangroves, salt marshes of *Spartina* spp., and tidal flats are the main environments bordering the estuary.

Field samples were collected with a plankton net (200 μm mesh size), and separated into two aliquots; one for the study of natural populations, and another for the preparation of clonal cell cultures in Guillard F/2 medium (Guillard 1975), where salinity was set at 30 psu, temperature at 25°C and a photon flux of approximately 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in 8:16 LD cycle.

Permanent slides were prepared for light microscopy (LM) according to the method of Hasle and Fryxell (1970), using Naphrax (Northern Biological Supplies, Ipswich, UK) as mounting medium. An Olympus BX40 light microscope (Tokyo, Japan) was used for the frustule observations and photomicrographs. Some cleaned samples were prepared for observations in a Phillips LX30 scanning electron microscope (SEM) (Amsterdam, Netherlands), under 20–25 kV accelerating voltage. Descriptive terminology of valve structures follows that recommended by Ross *et al.* (1979) and Round *et al.* (1990).

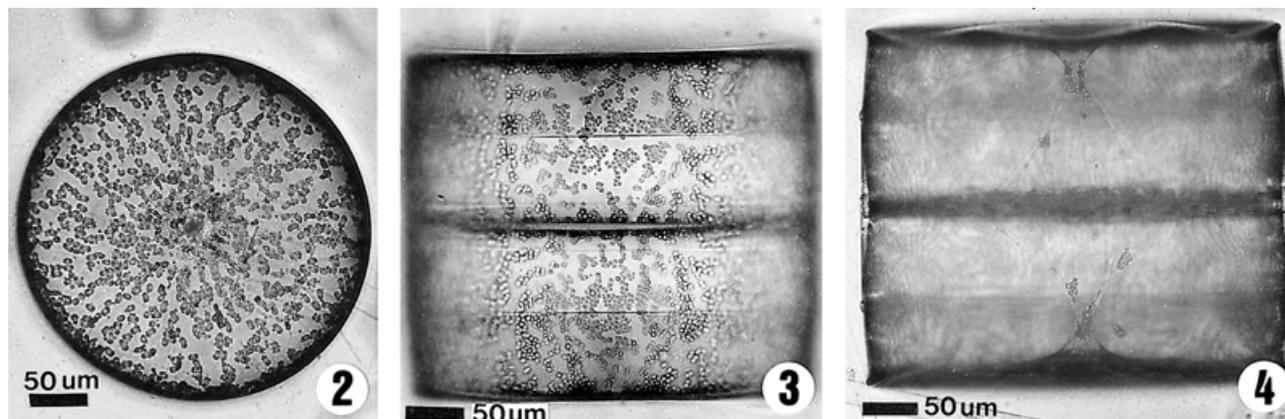
RESULTS

Species morphology has already been investigated by others (Cupp 1943; Schmid and Volcani 1983; Rincé and Paulmier 1986; Schmid 1990; Hasle and Lange 1992; Nagai and Manabe 1994; 1995b; Hasle and Fryxell 1995; Nagai *et al.* 1995a; Hasle and Syvertsen 1996; Nagai and Imai 1997), and only diagnostic features and complementary information are described here.

The cells cultivated in this work showed 268–306 μm valvar diameters, and a 112–210 μm pervalvar axis (Figs 2–4). Natural populations had 195–385 μm valvar diameters and a 96–420 μm pervalvar axis. The valve dimensions found in the literature were 230–500 μm valvar in diameter and a 44–350 μm pervalvar axis (Cupp 1943; Schmid and Volcani 1983; Schmid 1990; Hasle and Lange 1992).

The valvar surface had 32–35 hexagonal areolae in 10 μm , with sharp radial disposition (Figs 5,6). The areolae had a complex structure, with 10–13 cribral pores surrounded by larger pores (each one crossed by a silica bar), and a large internal circular foramen (Figs 13–16). Regarding the external central region, three basic variations could be recognized: (i) a well-developed hyaline area; (ii) a central rosette surrounding a small circular hyaline area; and (iii) a typical central rosette composed by 9–11 larger elongated areolae (Figs 7–12). These features are commonly observed in the valves during their successive size reduction after the cell division (Schmid 1990). It is important to emphasize that in some valves a small hyaline area also occurs on the inner side, and that a rosette may appear on its external side (Fig. 13) or a circular hyaline area replacing the rosette (Figs 14,15). Many labiate processes (rimoportulae) are scattered on the surface (Figs 16,20). In internal view, each rimoportula is projected through a tube (i.e. not sessile), and the labiate structure (semicircular and slightly concave) is turned in a 60–90° angle (Figs 19,20). Externally, there is one simple circular opening (Hasle and Lange 1992).

The mantle is orthogonal to the surface and straight, showing two marginal rings of rimoportulae (Figs 17–19). One is located on the connection between the mantle and the surface, and the other at the mantle edge (Figs 17,18). The latter has two macrorimoportulae,



Figs 2–4. Light microscopy of *Coscinodiscus wailesii* collected in Paranaguá Bay, Paraná in May 1999. 2. Valve view of a living cell. 3,4. Lateral views. Note flat valvar surface, orthogonal mantle, discoid chloroplasts, and nucleus suspended in the center by cytoplasm strands.

120° apart, with a characteristic morphology (Fig. 17). That is, the inner opening is composed of two silica spiral-like projections. Many hyaline interstriae occur on the mantle, arising from the edge rimoportulae and reaching the mantle corner (Figs 17,18).

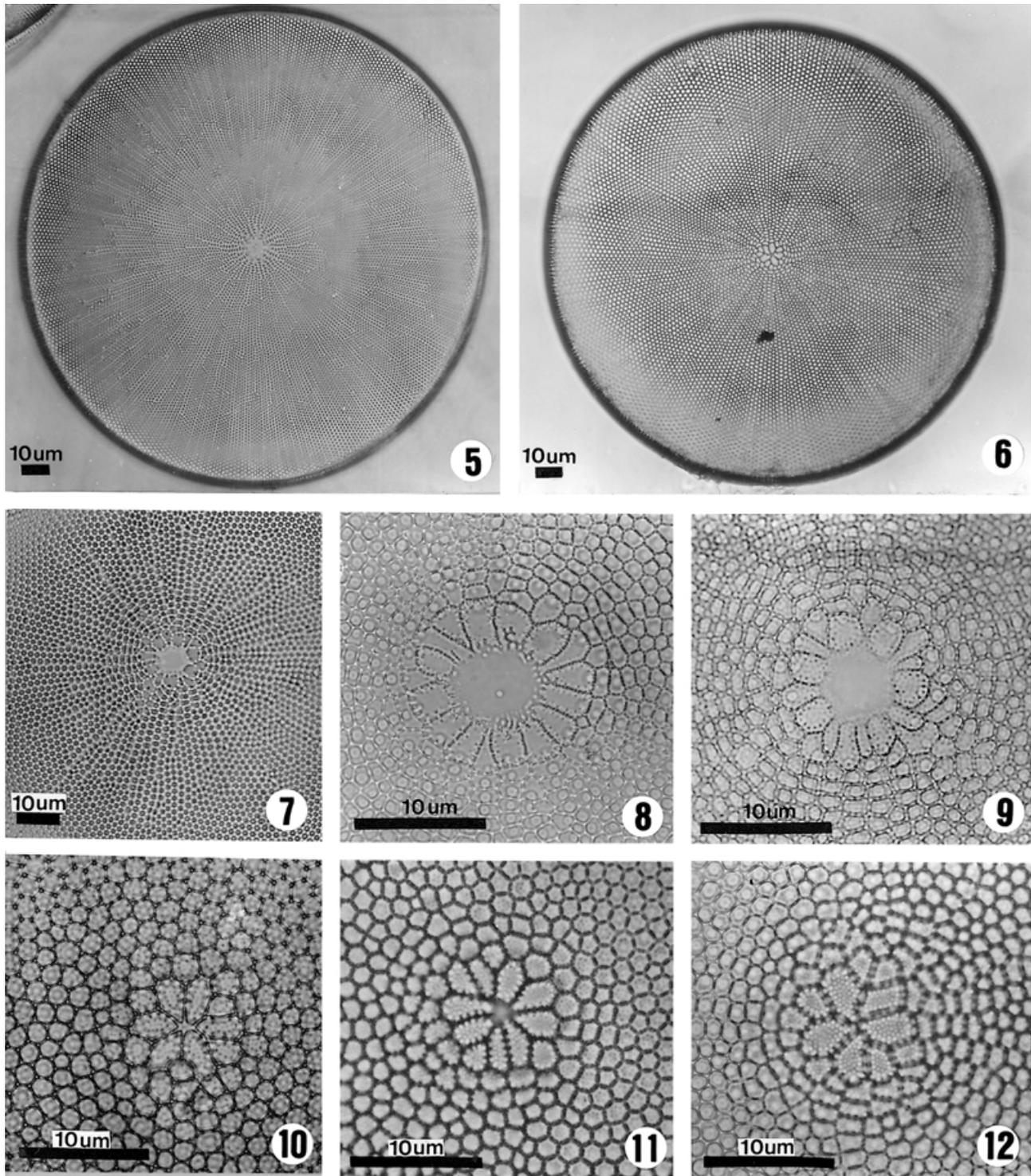
The cingulum is areolated, and is composed of two to three bands in each theca, ligulate.

DISCUSSION

The precise identification of a phytoplankton species is a crucial step in the investigation of harmful algal blooms, as the species may potentially produce toxins or affect the functioning of plankton communities. In this work, we found cells bearing well-developed rosettes or lacking that structure in both natural and cultivated populations from Paranaguá Bay. The studies on harmful algae published up to now describe *C. wailesii* as not having rosette, besides a series of diagnostic characteristics (Hasle and Lange 1992; Hasle and Fryxell 1995; Hasle and Syvertsen 1996). Such findings emphasize the necessity for high caution in identifying *C. wailesii*, as it could be easily mistaken with *Coscinodiscus concinnus* W. M. Smith and *Coscinodiscus centralis* Ehrenberg. The valve size, the number of areolae in 10 µm, the distributional pattern of areolae and the presence or absence of a central rosette are features that overlap in the three mentioned species. Additionally, their macrorimoportulae are similar to each other when viewed in SEM. When a rosette is present, *C. wailesii* becomes more related to *C. centralis*. In her comprehensive work on frustule intraclonal variation of cultivated *C. wailesii*, Schmid (1990) pointed out that the central rosette gradually replaces the hyaline area, following the successive valve reduction. However, this author and colleagues thereafter reported the phenomenon only for cultivated cells, but not for natural populations, as observed in the present work.

Despite this confusion, some characteristics which are easily viewed under LM allow the species' distinction (Hasle and Lange 1992; Hasle and Fryxell 1995). The valvar surface of *C. wailesii* is flat, and the mantle is at a right angle, while in *C. concinnus* and *C. centralis* the valve is convex, including the mantle. The marginal region of the valve in *C. wailesii* shows two rows of rimoportulae: one at the limit between the mantle and the surface; and another in the edge of the mantle. In the last two species, there is only one row, located at the mantle edge. Small rimoportulae also occur scattered on the valvar surface of *C. wailesii*, but are lacking in *C. concinnus* and *C. centralis* (Hasle and Lange 1992; Hasle and Fryxell 1995; Hasle and Syvertsen 1996).

The species has been observed in estuarine or neritic waters of many localities from temperate to tropical Pacific zones (Rincé and Paulmier 1986; Rick and Dürselen 1995). Its distribution in Brazil is showed in Fig. 1. The species was recorded for the first time by Valente-Moreira (1987), but this author did not supply measures or photographs, hampering any further comparison or confirmation of its identity. In the same sample, Valente-Moreira found *C. concinnus* and *C. centralis*, closely related to *C. wailesii*. Souza-Mosimann *et al.* (1993; figs 10,11 as '*Coscinodiscus asteromphalus*') also registered it in the coasts of Santa Catarina State, near estuaries. In Paranaguá Bay, the species has been reported in low abundance since 1991 in all months of the year, and sporadically producing blooms (Fernandes 1992; F. P. Brandini and H. L. Spach, unpubl. data). In these bloom periods, a sharp decrease of phytoplankton, zooplankton and ichthyoplankton organisms were observed (R. M. Lopes and H. L. Spach, unpubl. data). Though the relationship between the two events has not been satisfactorily explained, the simple possibility of negative effects on the pelagic chain motivates taxonomic and autoecological studies of the diatom. A similar effect was

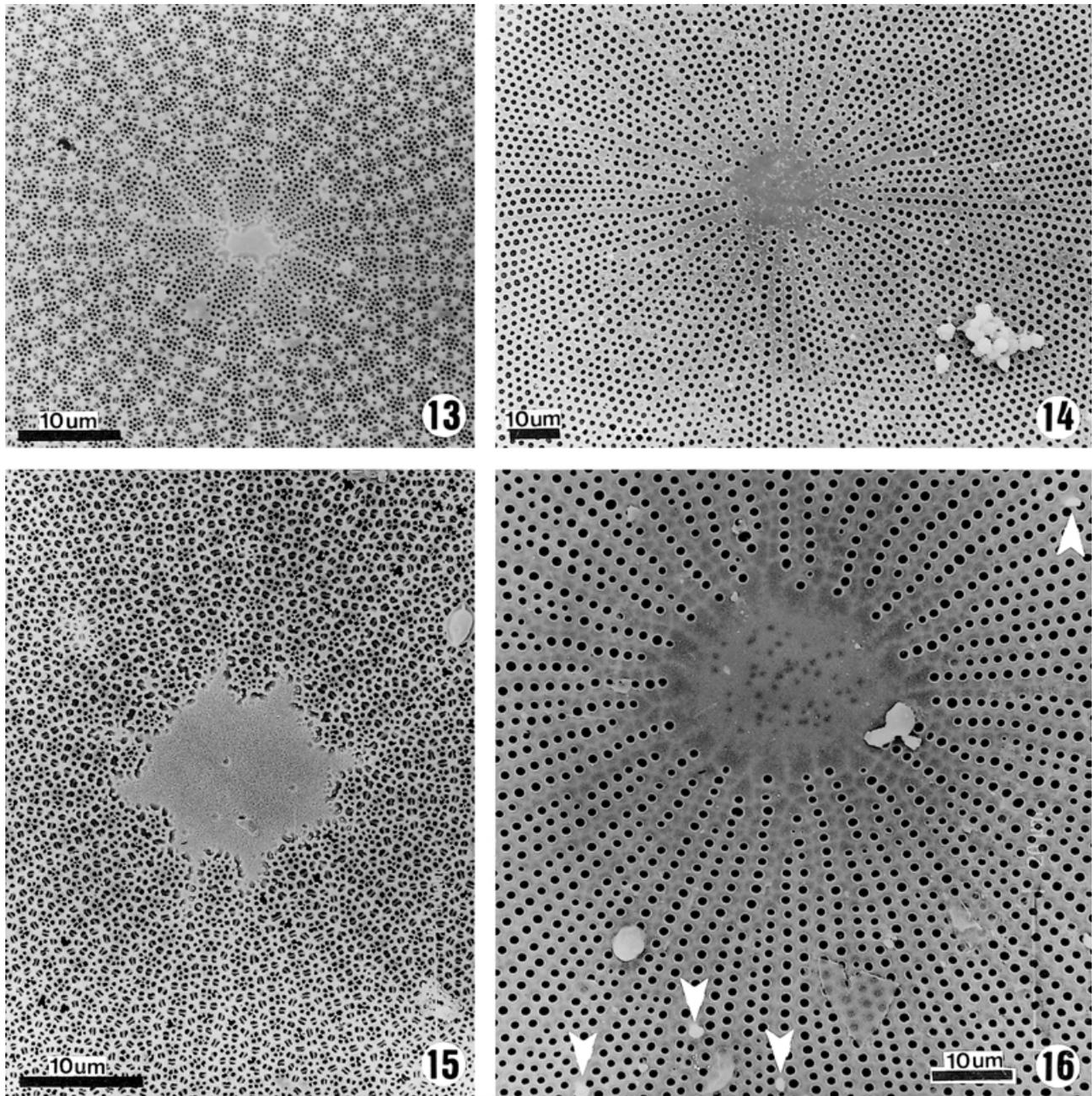


Figs 5–12. Light microscopy of *Coscinodiscus wailesii*, Paranaguá Bay, Paraná. Acid cleaned valves. 5,6. General valve views showing the central region with hyaline areas and interstriae, or a typical central rosette. 7–12. Close-up of central regions, illustrating different kinds of rosettes. Note the small hyaline area surrounded by rosette areolae in Figs 7–9.

observed when the species was cultivated with the copepods *Temora longicornis* Müller and *Calanus helgolandicus* Claus, which showed inefficient grazing and seemed to avoid feeding on *C. wailesii* (Roy *et al.* 1989). Such a reduced grazing by zooplankton may

also explain the success of *C. wailesii* after the invasion of a new environment.

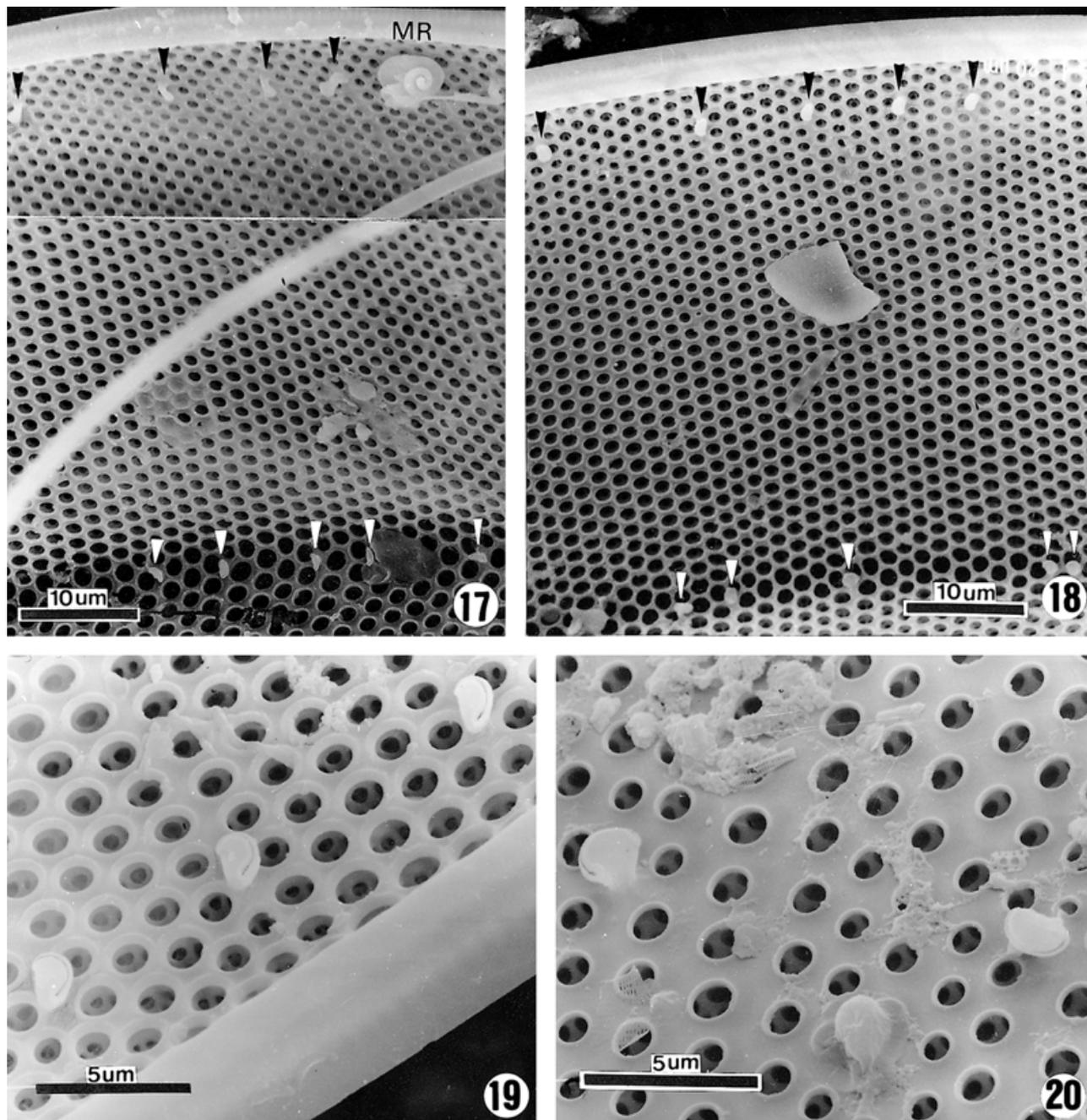
Some reports associate periods of intensive growth of *C. wailesii* with the plumes formed from the confluence of the shelf currents with the lower-salinity,



Figs 13–16. Scanning electron microscope images of central regions of *Coscinodiscus wailiesii*, Paranaguá Bay, Paraná. 13,14. External views, showing the presence and the absence of central rosettes. 15,16. Internal views of hyaline areas, from which many hyaline interstriae arise. Some labiate processes (rimoportulae) in the valvar surface also are indicated (arrowheads).

nutrient-rich waters of estuaries and bays (Rincé and Paulmier 1986). In these environments, abundances of *C. wailiesii* are low (3.0×10^2 cells L^{-1} to 18.2×10^2 cells L^{-1}), sometimes reaching 1.3×10^4 cells L^{-1} . In Paranaguá Bay and adjacencies, the diatom also appeared in low densities, except in periods of more intensive growth from September to March, reaching 4.7×10^3 cells L^{-1} (Fernandes 1992; L. F. Fernandes unpubl. data). In other months, its importance in the plankton was discrete, lower than 1% of phytoplankton densities.

In a synthesis of the global distribution of *C. wailiesii*, Rincé and Paulmier (1986) recorded its higher frequencies in waters with salinities between 30 psu and 36 psu, and temperatures from 1°C to 13°C. In Paranaguá Bay, the species has been found in less saline waters (8–35 psu), but with temperatures ranging from 18.5°C to 28.5°C (Fernandes 1992; H. L. Spach unpubl. data). The cells found in the bay were healthy, despite the low salinities. In contrast to our field observations, Nagai and Imai (1999a) recorded few viable cells in laboratory cultures at low salinity (15 psu).



Figs 17–20. Scanning electron microscopy images of *Coscinodiscus wailesii*, Paranaguá Bay, Paraná. Internal views of valve edge and mantle, and of valvar surface. 17,18. Rings of small rimoportulae in the corner of the mantle (white arrowheads) and in the mantle edge (black arrowheads). A microrimoportula is also indicated (MR) in Fig. 17. Note also interstriae arising from the rimoportulae. 19. Detail of three rimoportulae on the mantle edge. Note their direction to the same side. 20. Detail of two rimoportulae located on the valvar surface.

These authors also suggested the salinity is an important environmental factor in regulating the cell size restoration (by means of pseudoauxospore production) of *C. wailesii* cells. Other reports of *C. wailesii* in southwest Atlantic waters were not found in the literature but, judging from its presumable eurithermy, eurihaloby and cosmopolite distribution (except for cold regions), the species has the potential to be introduced either northwards or southwards in the next years.

Some hypotheses have been proposed to explain the worldwide spread of *C. wailesii* in recent years (Lange *et al.* 1992; Rick and Dürselen 1995; Nehring 1998). It seems more probable the species has been transported to other latitudes through ship ballast water, as has been pointed out for other nocive phytoplankton (Hallegraeff 1993, 1995). Another alternative is the contamination resulting from the introduction and cultivation of exotic commercial invertebrates which are

maintained in waters bearing a non-indigenous phytoplankton community (Rincé and Paulmier 1986). The capacity of *C. wailesii* to produce benthic resting cells still viable after being maintained in the dark for over 3 months (Nagai and Manabe 1994; Nagai *et al.* 1995a; Nagai *et al.* 1996; Nagai and Imai 1999b) supports the hypothesis of transportation via ballast water. Such an adaptation could allow for its survival in ballast tanks during the 8–20 days trip under adverse conditions. Paranaguá Port, in Paranaguá Bay, receives ships from many countries in different latitudes, making the hypothesis of ballast water translocation more attractive. Moreover, there is no cultivation of exotic marine organisms in the region, but only native ones, such as shrimps, oysters and mussels.

One could question the absence of previous records of *C. wailesii* in other Brazilian portuary regions with international routes. Perhaps this is because there are no long-term programs of phytoplankton monitoring in the embayments and in the ship ballast waters, which would prevent a permanent potential contamination source of local waters. Of course, in some regions the environmental conditions are not yet suitable for the species' growth.

Public health organizations should pay more attention to the problem of the introduction of exotic species in Brazilian marine waters, with the purpose of taking preventive measurements, and to mitigate their effects, as has occurred in several countries. In some instances, the species could be nocive or toxic, with negative consequences on public health, and to the commercial fishery and aquiculture.

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