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LEAFLET NO. 184

Potentially Toxic Phytoplankton

3. Genus *Prorocentrum* (Dinophyceae)

by

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Prorocentrum Ehrenberg, 1834

Introduction

The genus *Prorocentrum* was described by Ehrenberg (1834) with *P. micans* as the only species; hence *P. micans* is the type of the genus. Since then, more than 70 species of *Prorocentrum* and *Exuviaella* Cienkowski, 1881 have been described. *Exuviaella* was considered a synonym of *Prorocentrum* by Abé (1967), and this view has been generally accepted. Major taxonomic and floristic accounts include: Paulsen (1908), Pavillard (1916), Lebour (1925), Schiller (1933), Bursa (1959), Abé (1967), Dodge (1975), Fukuyo (1981), Sournia (1986), Faust (1990a), and Fukuyo *et al.* (1990).

Prorocentrum (including *Exuviaella*) has been revised by Bursa (1959) and Dodge (1975), and this has rendered many species names into synonymy (Dodge, 1975; Steidinger and Tangen, 1996). Most species have been described from the marine plankton, but there is growing recognition of *Prorocentrum* as an important and diverse constituent of marine benthic habitats (Faust, 1990a, 1993a–c, 1995). McLachlan *et al.* (1997) proposed to separate marine *Prorocentrum* species that are benthic in habitat, and split the genus *Prorocentrum* by reinstating the genus *Exuviaella*. However, further information is needed on the cytological, biochemical, and genetical nature of these *Prorocentrum* species before they can be separated into a separate genus based on lack of trichocysts, presence of mucocysts, synthesis of complex polyether secondary metabolites (DSP-type toxins) that are unknown in other prorocentroids, and benthic habitat. Species have also been recognized in freshwater environments (Croome and Tyler, 1987). With several new species having been described recently from the benthos (Faust, 1990a, 1993a, d, 1994, 1996) and with certain planktonic species still not being clearly delimited, there is a call for a modern revision of the genus.

Although several planktonic species of *Prorocentrum* may form extensive blooms, "red tides" (Lassus, 1988), rather few are reported to have caused damage to other flora and/or fauna. Therefore, only *P. balticum*, *P. micans*, and *P. minimum* are considered here. Amongst the benthic species, however, there are several toxin producers. *Prorocentrum lima*, a known toxic species, may even produce several toxins of entirely different chemical nature (Yasumoto, 1990), but it appears that the toxins enter the food chain as DSP, diarrhetic shellfish poisons (Yasumoto, 1990) rather than as ciguatera, a tropical fish-borne human disease (Banner, 1976; Withers, 1982; Juranovic and Park, 1991).

Symptoms of DSP are diarrhea, nausea, vomiting, abdominal pain, and chills (Krogh *et al.*, 1985). Symptoms last for only a few days.

DSP toxins can be classified into lipid soluble polyether compounds and water soluble compounds: (1) okadaic acid (OA), (2) methyl–okadaic acid, called dinophysistoxin (DTX-1), (3) prorocentrolide, and (4) water-soluble fast-acting toxins (FAT). For an overview see Bomber and Aikman (1991) and Yasumoto (1990).

Bioassays are used to detect toxin contaminations. Assays include: (1) Measurement of lethality and dose response to mice directly injected with purified extracts (Yasumoto *et al.*, 1984b). The results are expressed as LD_{50} concentration of toxin/kg mouse that kills a 20-g mouse in 24 hours. (2) Growth inhibition of *Aspergillus niger* and *Penicillium funiculosum* by OA and DTX-1. Inhibition is measured on paper discs in the range of 10 mg/disc of OA and DTX-1 (Nagai *et al.*, 1990). (3) Growth sensitivity of *Candida albicans* to OA. This can be used to test the presence of OA in toxin extracts (Dickey *et al.*, 1990). (4) In a radioimmunoassay, developed for polyether toxins (Baden *et al.*, 1985), the percentage tritiated bound toxin is estimated in the presence of increasing concentration of competitive toxin extracted from algal cells. An immunoassay kit for quick detection of OA and DTX-1 has been developed by UBE Industries, Japan (UBE, 1988), but practical experience with this kit is still limited. Chemical tests are also being developed for OA and DTX-1 using fluorimetry in combination with high performance liquid chromatography (HPLC) (Yasumoto, 1985; Stabell and Cembella, 1990). An improved HPLC-fluorimetric determination of OA in phytoplankton and shellfish has been used successfully to analyse naturally incurred OA residues between 0.1 and 100 ng of OA in seafood (Dickey *et al.*, 1992). In future research and monitoring programmes, the assays based on chemical methods are likely to improve and therefore would be the preferred methods. The first-mentioned bioassays may, however, still be useful in laboratories which do not have the necessary equipment to carry out sophisticated chemical analyses.

Toxins causing DSP are produced by dinoflagellates (Steidinger, 1983; Yasumoto *et al.*, 1984a). Okadaic acid and its derivative, DTX-1, were isolated from *Prorocentrum lima* (Murakami *et al.*, 1982), *P. concavum* (Dickey *et al.*, 1990), *Dinophysis fortii* (Yasumoto *et al.*, 1980b; Lee *et al.*, 1989), *D. hoffmannianum* (Aikman *et al.*, 1993) (*P. hoffmannianum* formerly was *P. concavum*) and *P. belizeanum* (Morton *et al.*, 1998). These are the only chemically characterized toxins known to be associated with DSP. Several other toxins from benthic dinoflagellates remain to be characterized.

It is difficult to establish a relationship between specific algal species and DSP. It is especially difficult to trace the symptoms of DSP to a given species such as *P. lima* or *P. concavum*. The only connection between the

above diseases and certain *Prorocentrum* species is that the toxins were extracted from shellfish as well as from algal cells (Tachibana *et al.*, 1981). It may be reasonable to conclude that OA and its derivatives have multiple sources and more algal species are involved in these diseases than previously suspected. OA and related toxins do not appear to be concentrated in fish (Lewis and Holmes, 1993).

Description of the genus

Species in the genus *Prorocentrum* have two laterally compressed valves, anteriorly inserted flagella, and cell shapes ranging from ovate to rotundate and pyriform. The left and right anterior ends can be identified by features unique to each valve. The left valve is flat, whereas the right valve has a V-shaped depression where the flagellar pore structures are fitted. The intercalary band has a well-defined appearance. All known species of *Prorocentrum* have chloroplasts.

The possible taxonomic importance of the surface morphology of the valves and the architecture of the flagellar pore area and intercalary band has received little attention until recently. Details of the V-shaped flagellar pore area containing small platelets held together by tightly fitted sutures were first illustrated by electron microscopy by Faust (1974). Subsequently, plate details of a number of species have been added (e.g. Dodge, 1975; Taylor, 1980; Steidinger, 1983; Faust, 1990a). It appears that species of *Prorocentrum* possess 5–14 apical platelets which surround the flagellar and apical pores.

In species taxonomy, the ornamentation of the apical area has attracted particular attention. For example, *P. micans* is distinguished by the presence of an apical spine on the apical plate (Dodge, 1975), *P. lima* by a curved apical collar (Faust, 1990a), and *P. cassubicum* by the absence of any ornamentation (Loeblich, 1976). Valve morphology is also important. A recent scanning electron microscope study revealed surface morphological details of six *Prorocentrum* species based on differences in ornamentation of thecal plates and the architectural detail of the periflagellar area and intercalary band. These details, though not apparent in previous studies, are useful for identification of benthic species (Faust, 1990a).

Reports on *Prorocentrum* resting cysts are limited. Early reports indicate two types of *Prorocentrum* cysts. One type from marine samples is the brown, spherical resting cyst of *P. micans* (Bergh, 1881; Breemen, 1905) and *P. lima* (Bütschli, 1885), also described as aberrant forms inside valves of old cultures (Braarud and Rossavik, 1951; Bursa, 1959).

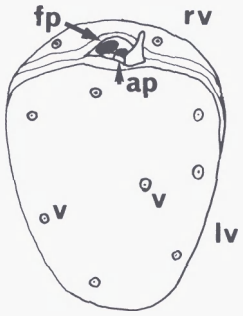

Figure 1. Schematic diagram of *Prorocentrum* cell (redrawn after Loeblich *et al.*, 1979); ap: auxiliary pore (arrowhead); fp: flagellar pore (arrow); lv: left valve; rv: right valve; v: valve pore.

The second type is the thin cyst of *P. lima* (as *Exuviaella marina*) in which the development of two daughter cells was noted (Lebour, 1925; Wood, 1954), enlarging in length and width inside the cyst (Bursa, 1959). More recently, the sexual life cycle of *P. micans* was described in actively growing cultures by Bhaud *et al.* (1988), and the existence of a hypnozygote in *P. lima* was suggested in old cultures (Steidinger, 1983) and natural populations (Faust, 1993b). Cysts of *P. foraminosum* (as *P. marinum*) containing a circular archeopyle were found in mangrove floating detritus (Faust, 1990b, 1993a). Recently, an alternative mode of asexual reproduction of *P. lima* in culture was also observed (Faust, 1993c). In general, the life cycle events appear to be unique for the *Prorocentrum* species examined so far.

Benthic *Prorocentrum* species are widely distributed in the Atlantic and Pacific Oceans. They are photosynthetic, rarely form red tides, and are associated with sediments (Fukuyo, 1981), detritus (Faust, 1996 and references therein), sand (Lebour, 1925; Drebes, 1974; Faust, 1994), coral rubble (Yasumoto *et al.*, 1980a), macroalgal surfaces (Fukuyo, 1981; Steidinger, 1983; Carlson and Tindall, 1985; Anderson and Lobel, 1987; Morton and Faust, 1997), and drift algae (Bomber *et al.*, 1988). All benthic species examined for toxicity have been shown to be toxin producers. The toxicity of several recently described benthic species, *P. emarginatum*, and *P. ruetzlerianum* (Faust, 1990a), *P. foraminosum* and *P. formosum* (Faust, 1993a), *P. elegans* and *P. caribbaeum* (Faust, 1993d), and *P. sabulosum*, *P. sculptile* and *P. arenarium* (Faust, 1994) has not been determined, but these species are associated with other known toxin-producing benthic species and are therefore included here. Previous reports on toxic *P. concavum* (SIU 882A) isolates from the US Virgin Islands (Carlson and Tindall, 1985; Dickey *et al.*, 1990), may represent *P. hoffmannianum* (Zhou and Fritz, 1993).

Description of the species

Benthic species

Prorocentrum concavum Fukuyo, 1981 Fig. 2a–g

Description: The cells are broadly ovate, pyriform in valve view (Fig. 2a–c), and convex in side view with a flattened center on both valves (Fig. 2e). Cells are 50–55 µm long and 38–45 µm wide. This species is the largest among benthic *Prorocentrum*. Cells have a centrally located pyrenoid (Fig. 2a) and a posterior nucleus (Fig. 2c). Valve surface is covered with shallow areolae (1000–1100 per valve) (Fig. 2d) with no marginal pores (Fig. 2f–g). Left valve is slightly indented (Fig. 2b). The apical area is a narrow triangle in the right valve (Fig. 2f), void of valve spines (Fig. 2f). The intercalary band is granulated and horizontally striated (Fig. 2e).

Taxonomic remarks: In 1981 Fukuyo described *P. concavum* from coral reefs of French Polynesia, New Caledonia, and the Ryukyu Islands, Japan. *Prorocentrum concavum* cells were also present in a mangrove habitat at Twin Cays, Belize (Faust, 1990a). They are difficult to differentiate from *P. lima* at the light microscope level, the shapes being very similar (Fukuyo, 1981; Dickey *et al.*, 1990). However, *P. concavum* possesses ca. 1000 areolae per valve and no marginal pores (Fig. 2d–e), while *P. lima* has ca. 100 valve pores and ca. 80 marginal pores (Fig. 6d, f–g). The apical area of *P. concavum* is a narrow triangle without ornamentation (Fig. 2f), whereas the apical area of *P. lima* is a broad triangle with a curved apical collar around the flagellar pore (Fig. 6f–h). The apical area of *P. concavum* (Fukuyo, 1981) and *P. lima* (Taylor, 1980) is composed of eight platelets. The illustrations of Carlson (1984, plate 5, figs. n–s) and Tindall *et al.* (1984; fig. 3b) refer to *P. hoffmannianum*, based on a detailed scanning electron microscope study (Faust, 1990a). Steidinger's illustration of *P. concavum* (Steidinger 1983; fig. 17) is an unidentified species. Previous reports of toxic *P. concavum* may represent *P. maculosum* (Zhou and Fritz, 1994).

Ecology and distribution: *Prorocentrum concavum* is commonly associated with red and green macroalgae and sediments at both Pacific (Fukuyo, 1981) and Atlantic sites (Carlson and Tindall, 1985; Morton and Faust, 1997), in coastal areas devoid of coral reefs (Steidinger and Baden, 1984), and on floating detritus in mangrove habitats (Faust, 1990a, 1996). *Prorocentrum concavum* was present on sediments at protected inshore stations in association with *P. lima*, *P. mexicanum*, and *Scrippsiella subsalsa*, and as an epiphyte on drift algae (Bomber *et al.*, 1988). It is most abundant at 28–32°C in protected lagoons, and may form "benthic blooms" (Carlson, 1984). Macroalgal attachment is by a mucilaginous envelope, but when disturbed, cells may swim away (Bomber *et al.*, 1988). Growth is enhanced by sediment and macroalgal extracts (Bomber and Aikman, 1991). Growth rate was faster in axenic cultures, and *P. concavum* prefers low light levels (Carlson *et al.* 1984).

Toxicology: *Prorocentrum concavum* is a toxigenic species (Dickey, 1984). Four toxins have been found in extracts of *P. concavum* isolated from Caribbean waters: (1) A water-soluble fast-acting toxin, FAT, (strain SIU 364, Tindall *et al.*, 1984). This toxin fraction killed mice within 48 hours (LD_{50} of 8.3 mg/kg, i.p.). (2) Another very potent FAT (Tindall *et al.*, 1989) that killed mice within 32 minutes with the minimum lethal dose. This toxin also has a toxic effect on guinea-pig ileum preparations (Tindall and Miller, 1987), and similar effects on the ileum were caused by extracts of maitotoxin from *Gambierdiscus toxicus* (Tindall and Miller, 1985). (3) Okadaic acid (OA) was isolated and chemically characterized by Dickey *et al.* (1990). Maximum amounts of toxin occurred during mid-log growth phase of strain SIU 882a. It had a potency of 214 and 216 MU per 100 mg of cells (Aikman *et al.*, 1990). Crude lipid extracts of a *P. concavum* isolate from the Bahamas exhibited cross-reactivity in an immunoassay directed against toxins isolated from *Gymnodinium breve* (Baden *et al.*, 1985). These extracts were lethal to mice. *Prorocentrum concavum* isolated from the Pacific produced an ether-soluble fraction which was toxic to killifish and mice (130 MU per 108 cells). Ether and butanol-soluble fractions from the same isolate exhibited hemolytic activity (Nakajima *et al.*, 1981). Yasumoto *et al.* (1987) reported mouse lethality and potent ichthyotoxicity and hemolytic activity in *P. concavum* isolated from Okinawa. (4) Diarrhetic shellfish toxins, OA and Dinophysistoxin-1 (DTX-1), were isolated from toxic Irish mussels and cultures of *P. concavum*. In large-scale cultures, three new diol esters of OA have been isolated and characterized (Hue *et al.*, 1993). Diol esters 5 and 6 exhibited no inhibition of protein phosphatase 1 and protein phosphatase 2A. DTX-1 toxin is an isomer of OA and found also in cultures of *P. lima* and *Dinophysis* species.

Prorocentrum emarginatum Fukuyo, 1981 Fig. 3a–f

Description: Cells are broadly ovate (Fig. 3a–b), 35–40 µm long, 30 µm wide and possess a large kidney-shaped posterior nucleus. Both valves are concave (Fig. 3d). Apical area is deeply excavated, ending in a sharp

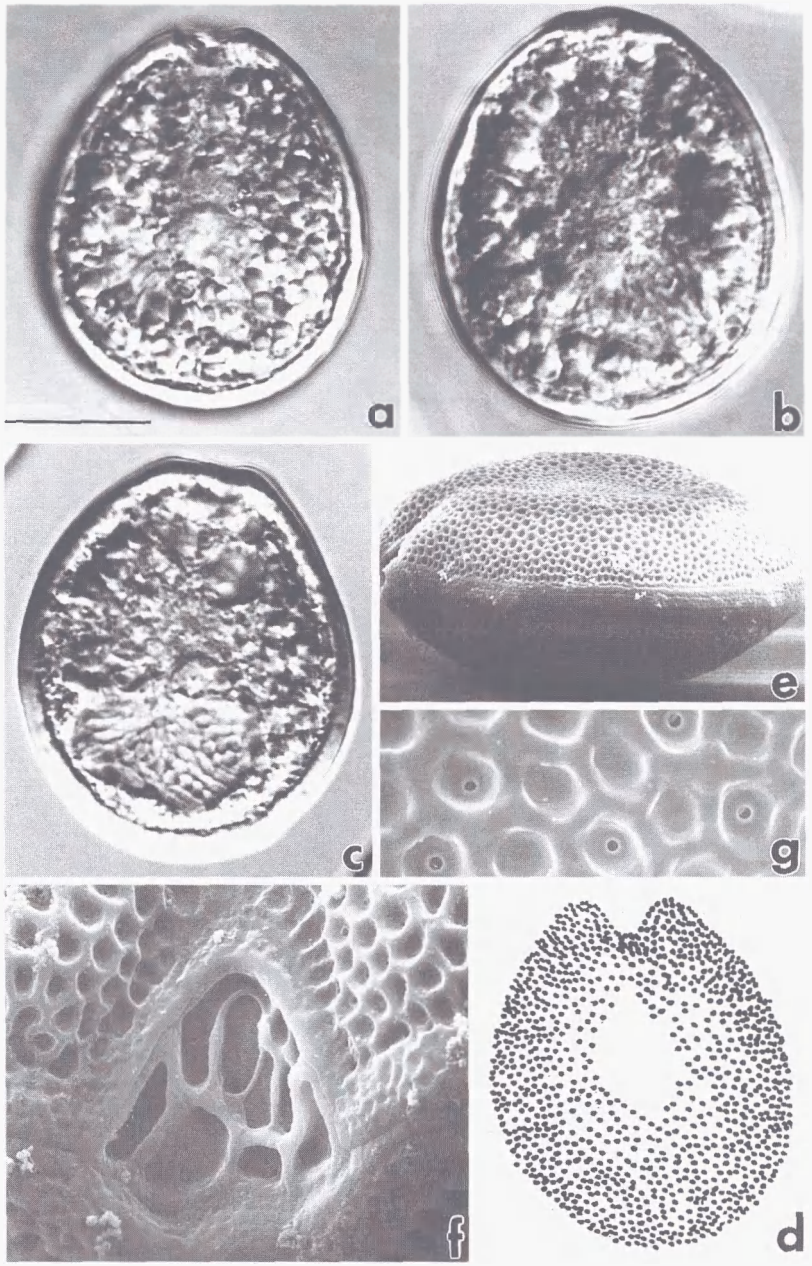

Figure 2a–g. *Prorocentrum concavum*. 2a, b: Cells are broadly ovate, right valve has a centrally located pyrenoid and (Fig. 2b) left valve has a flat apical area; 2c: nucleus is posterior; 2d: valve surface is covered with ca. *1000* areolae per valve; 2e: cell is convex and intercalary band is horizontally striated; 2f: the apical area is a narrow unornamented triangle; 2g: valve surface has many shallow areolae, some with round openings. Material from Twin Cays, Belize. Scale bars in Figure 2a–c: 20 µm; Figure 2e–g: 2 µm.

narrow point with a rectangular structure on the right valve that touches the intercalary band (Fig. 3d–e). Left valve is also deeply indented. Valve surface is smooth, each valve pore is round and situated in a depression with smooth margins (Fig. 3d–f). Valve pores (ca. 200 per valve) are arranged in radial rows spaced around the valve periphery, and marginal pores are also present (Fig. 3c). Center of valve is void of pores (Fig. 3d). Intercalary band is transversely striated and sinuous (Fig. 3d–e).

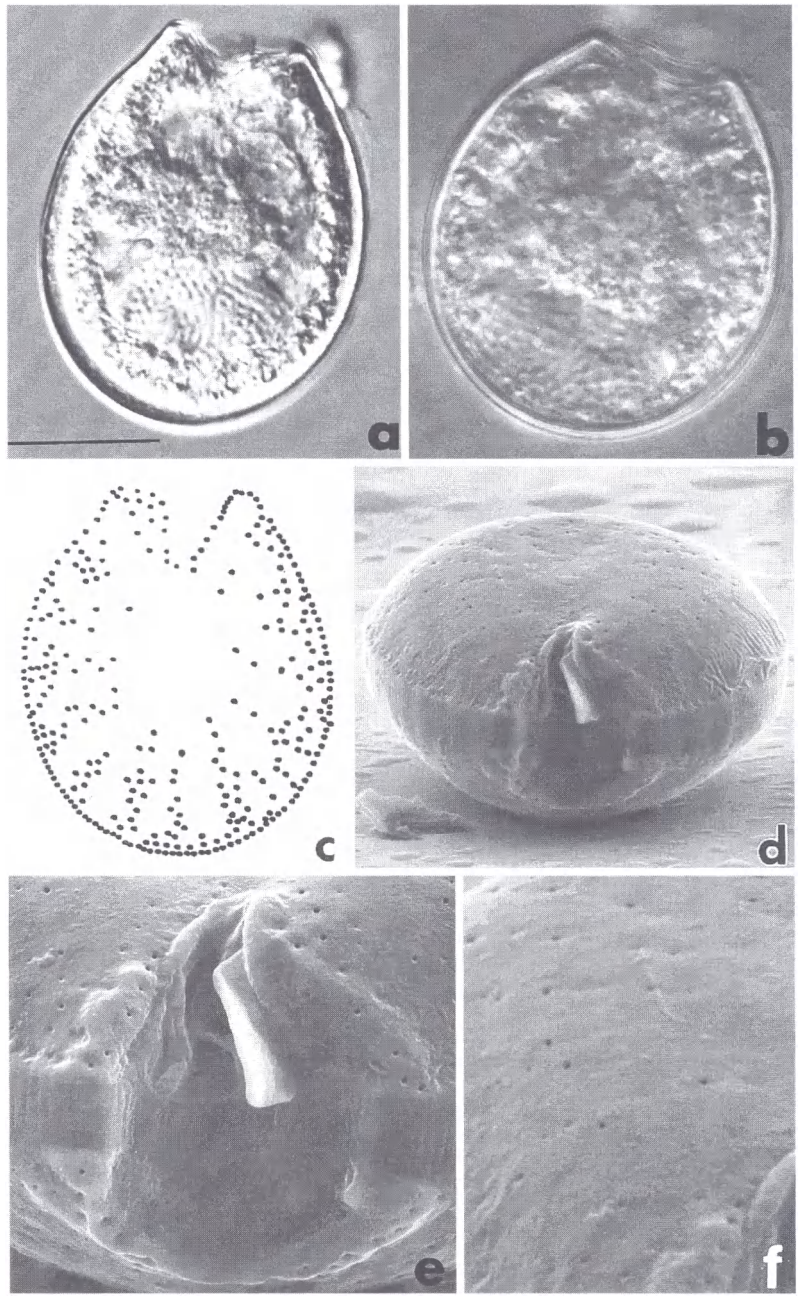

Figure 3a–f. *Prorocentrum emarginatum*. 3a, b: Cells are broadly ovate, right valve view (a) and left valve view (b) has a large kidney-shaped posterior nucleus; 3c: valve pores (ca. 200 per valve) are radially arranged and marginal pores are present; 3d: valves are concave; 3e: the apical area is deeply excavated, ending in a sharp point with a rectangular structure; 3f: valve surface is smooth with round small pores situated in deep depressions, the intercalary band is transversely striated and sinuous. Material from Twin Cays, Belize. Scale bars in Figure 3a–b: 20 µm; Figure 3d–f: 2 µm.

Taxonomic remarks: *Prorocentrum emarginatum* was described from the Ryukyu Islands by Fukuyo (1981). In profile, *P. emarginatum* resembles *P. concavum*, but it is distinguished by the smaller size and rounder body shape, and a rigid, rectangular apical plate (Fig. 3d–e). It exhibits a deep excavated apical area and pointed indentation at the apical region and a kidney-shaped nucleus. Only two *Prorocentrum* species with these features are known: *P. emarginatum* and *P. sculptile* (Faust 1994; figs. 8–12). These two species differ, however, in *P. sculptile* having ca. 910 shallow depressions per valve and a thin, inclined, apical structure, whereas *P. emarginatum* has fewer pores arranged in radial rows and a rigid rectangular plate (Faust, 1990a).

Ecology and distribution: This species has been reported from tropical Pacific coral reefs (Fukuyo, 1981), Caribbean waters (Carlson, 1984), and mangrove habitats (Faust, 1990a). It is present in low numbers in sediments and attached to macroalgae or floating detritus. Specimens were collected in shallow, protected lagoons and embayments at Twin Cays, Belize at water temperatures of 24–30°C, salinities of 28–34, and low irradiance (Faust, 1990a).

Toxicology: The toxicity of this species is unknown.

Prorocentrum foraminosum Faust, 1993 Fig. 4a–g

Description: Cells are oblong to ovate in valve view (Fig. 4a–c) and convex in side view. They are 46–66 µm long and 31–42 µm wide, and contain a small posterior nucleus and large round storage bodies (Fig. 4a–b). Valve surface is smooth, and covered with circular pores (ca. 300 per valve) (Fig. 4c). Center of valve is devoid of pores (Fig. 4c, e). The left valve is flat (Fig. 4b–c). On the right valve the apical area is narrow and unornamented with a triangular orientation composed of eight platelets (Fig. 4e, f). Marginal pores are absent and the intercalary band is smooth (Fig. 4e, g). Cells are usually embedded in mucus, although young cells are motile. The sexual life cycle of *P. foraminosum* (Faust, 1993a) includes a round thick-walled hypnozygote (Fig. 4d).

Taxonomic remarks: The type of *P. foraminosum* was described from mangrove habitats, Hidden Lake and the Lair at Twin Cays, Belize (Faust, 1993a). It is a large species, oblong in shape and the valve surface is covered with small, round, scattered pores, which are useful in differentiating this species from other benthic species in the light microscope.

In an earlier publication (Faust, 1990b), which included studies of cysts and excystment processes, *P. foraminosum* was incorrectly identified as *P. marinum*.

A thin-walled cyst of *P. marinum* (as *E. marina*) was reported by Lebour (1925), Wood (1954), and Bursa (1959). Organic-walled cysts of *P. foraminosum*, however, are different and do not survive in the environment for prolonged periods (Faust 1990b). They are also different from the thin-walled cysts described for other dinoflagellates: less storage products and no observed resting period is present (Dale, 1983), and cessation of movement is followed by a marked contraction of the protoplasts (Pfiester and Anderson, 1987).

Ecology and distribution: *Prorocentrum foraminosum* is often found attached to mangrove sediments and detritus at Twin Cays, Belize (Faust, 1993a). Maximum abundance in this mangrove was observed during winter months (January–April) at temperatures of 24–30°C, low irradiance, and salinities of 28–32. The presence of round, brown hypnozygotes with triple-layered walls and a circular archeopyle attached to floating detritus was also reported (Faust, 1990b). Growth of *P. foraminosum* in Erdschreiber's medium was enhanced by sediment extracts. In culture, *P.* foraminosum adheres to the wall of culture vessels.

Toxicology: Toxicity of *P. foraminosum* has not been reported.

Prorocentrum hoffmannianum Faust, 1990 Fig. 5a–g

Synonym: *Exuviaella hoffmannianum* (M. A. Faust) McLachlan et Boalch, 1997

Description: The cell shape is ovoid in valve view, broad in the middle region and narrow at the anterior end (Fig. 5a–d). Cells are 45–55 µm long and 40–45 µm wide with a centrally located pyrenoid (Fig. 5a) and a posterior nucleus (Fig. 5b). Valve surface is deeply areolated (ca. 700 areolae per valve) (Fig. 5c–g) and both valves are concave (Fig. 5e). The apical area is a broad triangle with a flared apical collar adjacent to the flagellar pore, and it lacks both valve spines and anterior spines (Fig. 5e–f). The left valve exhibits a flat ridge (Fig. 5e–f). The intercalary band is smooth (Fig. 5f). Cells are motile or attached to detritus by mucilage.

Taxonomic remarks: The type specimen of *P. hoffmannianum* was described from mangrove habitats at Hidden Lake and the Lair at Twin Cays, Belize, Central America (Faust, 1990a). Compared with *P. lima* (Fig. 6a–c, f–g), it is larger, broader and has an areolated valve surface (Fig. 5a–g). The apical area of *P. hoffmannianum* (Fig. 5e–f) differs from *P. lima* (Fig. 6f–h), *P.*

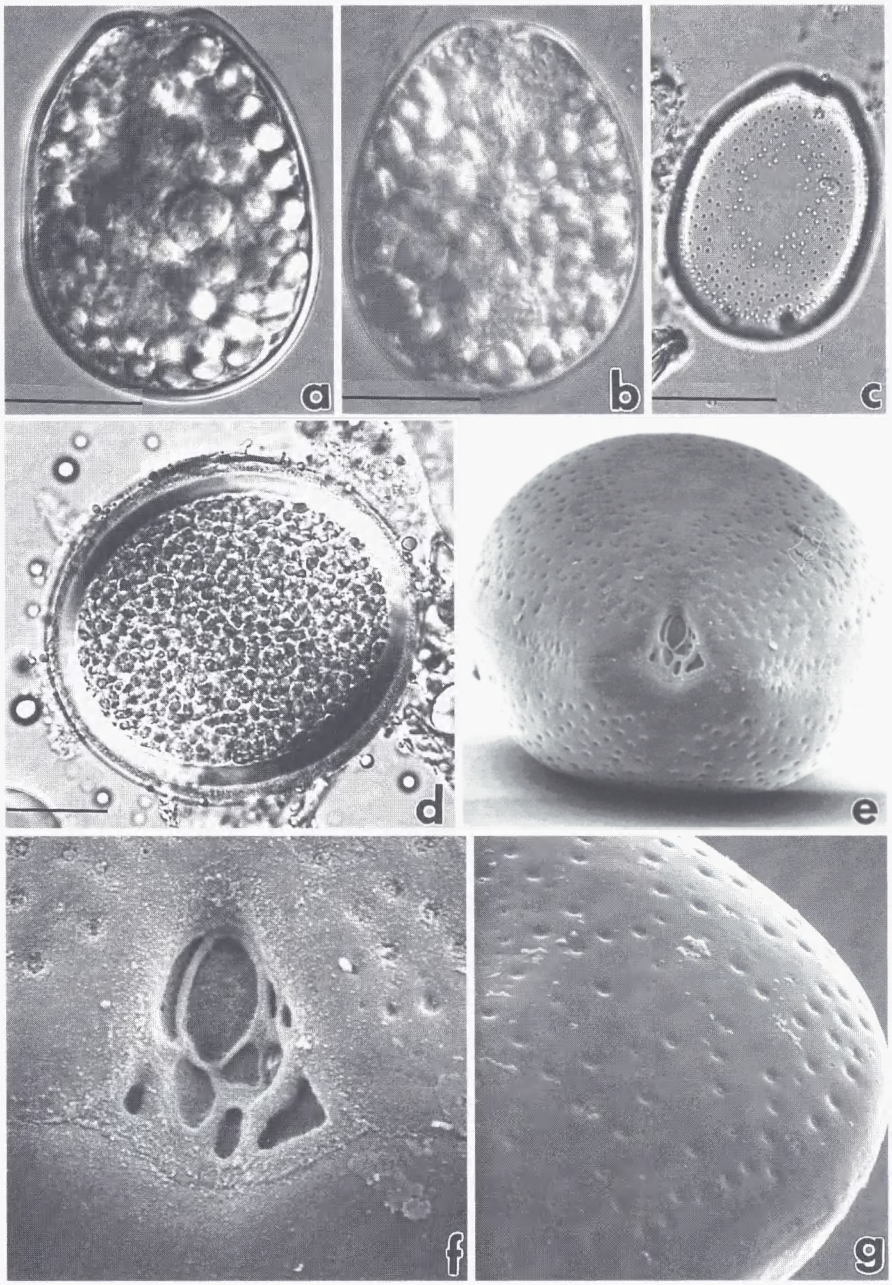

Figure 4a–g. *Prorocentrum foraminosum*. 4a, b: Cells are oblong to ovate, with a posterior nucleus; 4c: valve pores (ca. 300 per valve) are present. The center of the valve is void of pores and lacks marginal pores; 4d: hypnozygote has triple-layered cyst wall; 4e: cell is convex in side view; 4f: the apical area is triangular, narrow and unornamented; 4g: valve surface and intercalary band is smooth and valve surface covered with small, circular pores. Material from Twin Cays, Belize. Scale bars in Figure 4a–d: 20 µm; Figure 4e–g: 2 µm.

concavum (Fig. 2f) (Fukuyo, 1981), and the freshwater species, *P. playfairii* (Croome and Tyler, 1987; figs 9–11). It has a more complex platelet configuration (Fig. 5e–f) (Faust, 1990a) than *P. lima* (Taylor, 1980).

Ecology and distribution: Cells of *P. hoffmannianum* were associated with sediment and floating detritus in protected and shallow mangrove habitats in the Caribbean Sea (Tindall *et al.*, 1984; Faust, 1996 and refer-

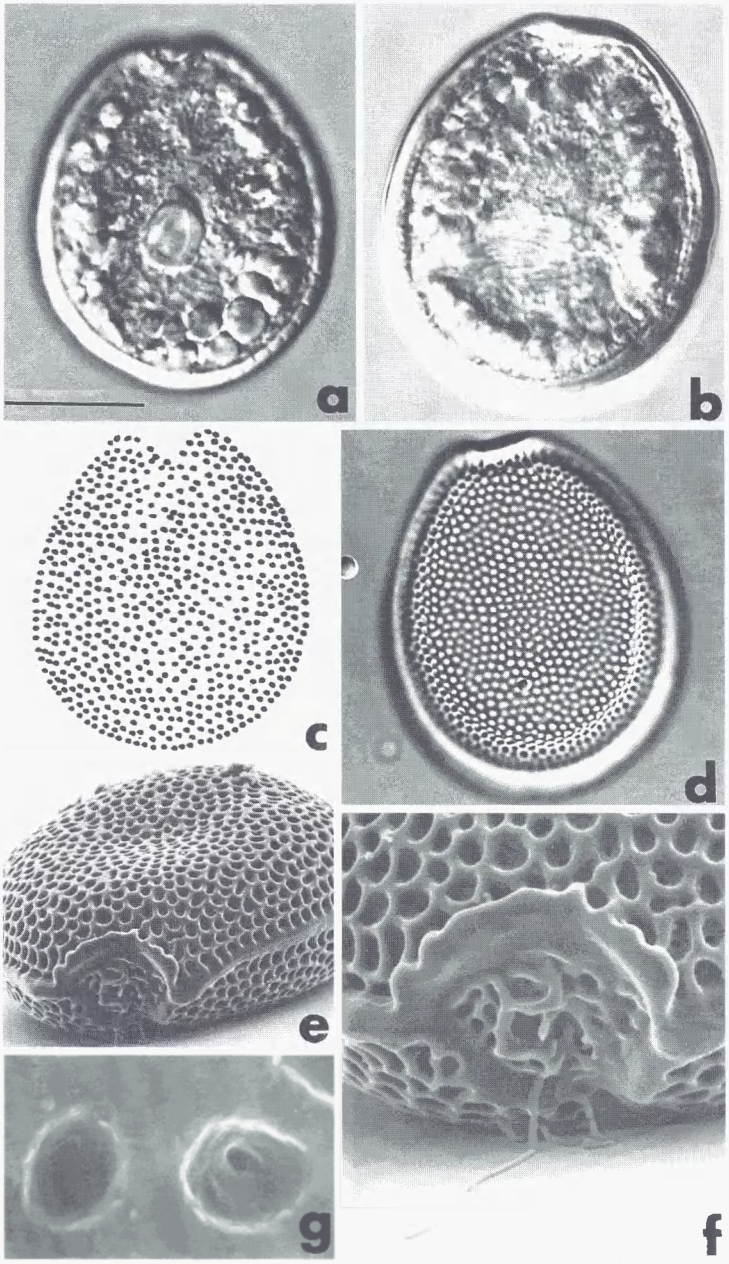

Figure 5a–g. *Prorocentrum hoffmannianum*. 5a: Cell shape is ovoid, right valve, with a centrally located pyrenoid; 5b: left valve, note the posterior nucleus; 5c, d: valve surface has ca. 700 areolae per valve and a flat, apical ridge on the left valve (d); 5e: both valves are concave and areolated and the intercalary band is smooth; 5f: apical area is a broad triangle with flared apical collar adjacent to the flagellar pore; 5g: areolae are deep with one or two round openings. Material from Twin Cays, Belize. Scale bars in Figure 5a–d: 20 µm; Figure 5e–g: 2 µm.

ences therein), and were attached to macroalgae in the Belizean barrier reef ecosystem (Morton and Faust, 1997). Specimens were collected at water temperatures of 24–30°C and salinities of 28–34. They were associated with *P. concavum*, *P. lima*, *P. mexicanum*, and *Scrippsiella subsalsa*. Growth of *P. hoffmannianum* is enhanced by low light levels and addition of sediment extract to enriched seawater medium. *Prorocentrum hoffmannianum* Clone SIU 882A grew well in K-medium (Keller and Guillard, 1985). In modified

K-medium where Tris, copper, and silica were omitted, the acclimated growth rate of *P. hoffmannianum* was maximum (k = 0.53 division day^{-1}) at 27°C and salinity 34 (Morton *et al.*, 1994).

Toxicology: The illustration of *P. concavum* by Carlson (1984) is *P. hoffmannianum* (Fig. 5d–f). Toxins identified in *P. hoffmannianum* in a Twin Cays isolate were OA (Morton, 1994), and in Clone SIU 882A isolate from US Virgin Islands, were OA and a fast-acting toxin (FAT) (Aikman *et al.*, 1993). Earlier studies of clone SIU 882A suggested the presence of six toxins (Tindall *et al.*, 1984). Okadaic acid production in axenic *P. hoffmannianum* culture was optimal (58.6 pg cell^{-1}) at 24°C (Morton and Bomber, 1994; Morton *et al.*, 1994).

Prorocentrum lima (Ehrenberg) Dodge, 1975
Fig. 6a–h

Synonyms: *Cryptomonas lima* Ehrenberg, 1860; *Exuviaella marina* Cienkowski, 1881; *Dinopyxis laevis* Stein, 1883; *E. lima* (Ehrenberg) Bütschli, 1885; *E. laevis* (Stein) Schröder, 1900; *E. cincta* Schiller, 1918; *E. ostenfeldii* Schiller, 1933; *E. caspica* Kiselev, 1940; *Prorocentrum marinum* Dodge et Bibby, 1973 comb. invalid (basionym not indicated).

Description: Cells are ovate in valve view, broad in the middle region, narrow at the anterior end, 31–47 µm long, 22–40 µm wide. Cells have a centrally located pyrenoid (Fig. 6a–b) and a posterior nucleus (Fig. 6c). Valve surface is covered with large marginal pores (ca. 80 per valve) and smaller valve pores (ca. 100 per valve) (Fig. 6d). Both valves are concave (Fig. 6f). The apical area is a wide triangle containing a curved apical collar around the flagellar and apical pores (Fig. 6h) and is void of valve spines or anterior spines (Fig. 6g). Mucocysts are present while trichocysts are absent (Zhou and Fritz, 1993). The hypnozygote is round and brown with a triple-layered wall (Fig. 6e) (Faust, 1993b).

Taxonomic remarks: In 1860, Ehrenberg described *Cryptomonas lima*, often considered identical to the species known today as *P. lima* (Ehr.) Dodge, 1975, although the first drawing published by Ehrenberg (1873) shows cells covered by spines (McLachlan *et al.*, 1997). Cienkowski (1881) presented the first line drawing of *E. marina* Cienkowski, and in 1885 Bütschli illustrated *E. lima* (Ehr.) Bütschli. He recognized the major morphological features: an excavated plate in the right valve; presence of valve pores; transverse and longitudinal flagella; nucleus; two vacuoles; chloroplasts; starch and oil bodies; and cysts. Later scattered pores on the valves of *E. lima* were observed by Paulsen (1908). This species was reported from Caribbean waters as *E. marina* var. *lima* (Margalef, 1957; Wood, 1968). Abé (1967) combined the two genera *Prorocentrum* and *Exuviaella* under the former name *Prorocentrum*. McLachlan *et al.* (1997) proposed to separate marine *Prorocentrum* species that are primarily benthic in habitat, have mucocysts, and synthesize polyether secondary metabolites (DSP-type toxins) and split the genus *Prorocentrum* by reinstating the genus *Exuviaella*.

Lebour (1925) described the presence of poroids on the valves of *E. marina* and the emergence of flagella from a slit in front between the valves. The emergence of two flagella from the same flagellar pore in *P. marinum* was described by Biecheler (1952) and Loeblich (1976). Taylor (1980) provided a line drawing of the apical area of *P. lima* ("marinum" form). Dodge and Bibby (1973) illustrated a flagellar pore plate of *P. marinum* as a single triangular unit with a large and a small pore. *Prorocentrum marinum* is distinguished from *P. lima* by the micromorphology of the valve pores, absence of marginal pores, smooth intercalary band, architecture of the apical area, larger size, and oblong shape (Faust, 1991).

Dodge (1975) recognized that size and shape alone were inadequate to identifying *Prorocentrum* species. Taylor (1980) described the apical area of *P. lima* as eight platelets, arranged in a subtriangular shape with a "fin-like crest". At high magnification the apical area of *P. lima* reveals a curved apical collar (Fig. 6h). The valves have distinct marginal pores and smaller valve pores (Faust, 1991). These morphological characteristics can be used to differentiate this species from other *Prorocentrum* (Steidinger, 1983).

The sexual life cycle of *P. lima* (Faust, 1993b) is similar to that of *P. micans* (Bhaud *et al.*, 1988). The presence of round, brown cysts in old cultures of *P. lima* was reported by Steidinger (1983), and in natural populations by Faust (1993b). A feeding tube (peduncle) of *P. lima* was also reported by Malcolm (1987) and in *P. arenarium* (Faust 1994; figs. 21–22), suggesting heterotrophy. A new type of asexual reproduction in *P. lima* was discovered in culture in which a chain of cell pairs is enclosed within a thin-walled cyst. The cells differed from vegetative cells (Faust, 1993c).

Ecology and distribution: *Prorocentrum lima* occurs in coastal areas world-wide, in temperate and tropical oceans, in benthic (incl. sand) and epiphytic habitats including the Atlantic (Lebour, 1925), the Pacific (Yasumoto *et al.*, 1980a; Faust, 1991), the Caribbean Sea (Carlson, 1984; Carlson and Tindall, 1985; Faust, 1990a), and Australia (Morton and Tindall, 1995). Epiphytic associations of *P. lima* most frequently involve rhodophytes in the Belizean reef ecosystem, where this species is associated with known toxic species; for example, *Gambierdiscus toxicus* (Carlson and Tindall, 1985), *P. belizeanum* (Morton and Faust,

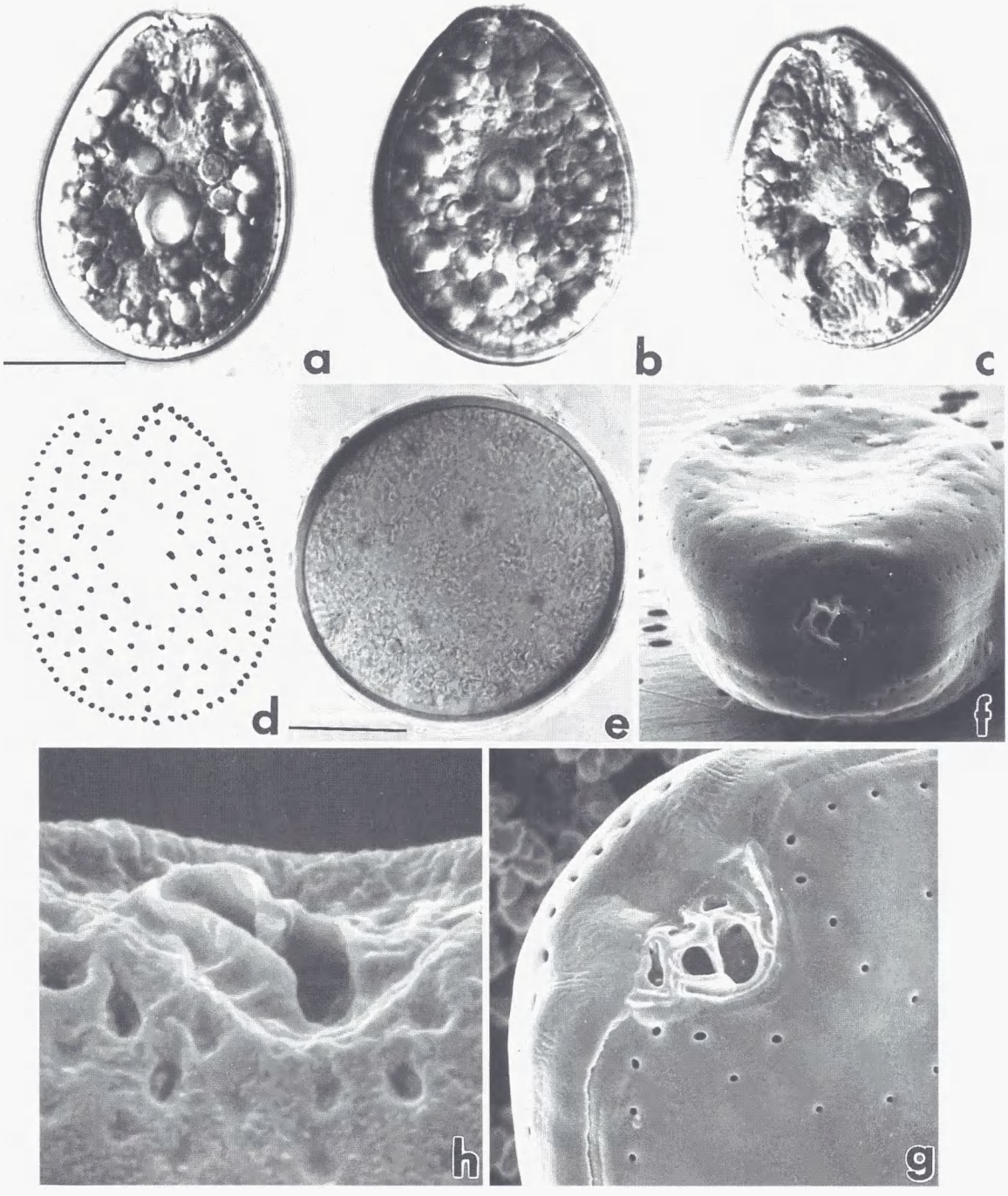

Figure 6a–h. *Prorocentrum lima*. 6a, b: Cells are ovate, right valve (a) and left valve (b) have a centrally located pyrenoid; 6c: nucleus is posterior; 6d: valve surface has valve pores (ca. 80 per valve) and marginal pores (ca. 100 per valve); 6e: the round hypnozygote has triple-layered cyst wall; 6f: valves are concave; 6g: the apical area is a wide triangle containing a curved apical collar around the flagellar and apical pores; 6h: the apical collar protrudes slightly. Material from Twin Cays. Belize, Central America. Scale bars in Figure 6a–e: 20 µm; Figure 6f–h: 2 µm.

1997), *P. hoffmannianum* (Morton *et al.*, 1994), and *P. mexicanum* (Tindall *et al.*, 1984). Mangrove-detritus-epiphytic associations may have significant impact on the ecology and life cycle of *P. lima* between proliferation and inactive populations (Faust, 1995). Floating mangrove detritus is an ideal habitat for *P. lima* (Faust, 1996). It prefers low irradiance, blue to blue–violet spectral quality and salinity of 32 for optimum growth (Morton and Norris, 1990). In the Florida Keys, maximum abundance of *P. lima* occurred in the cool water season (26°C) near channels and undisturbed coral reefs at 1–2 m depth, with indication of niche specialization (Bomber *et al.*, 1989). *Prorocentrum lima*-host relationships are complex, involving both chemical and physical characteristics (Bomber and Aikman, 1991). Macroalgae are the preferred host for *P. lima*, possibly providing beneficial exudates for growth (Carlson *et al.*, 1984) in the form of chelators and surface area for attachment (Bomber *et al.*, 1989). Antifungal activities of okadaic acid extracted from *P. lima* were also reported (Nagai *et al.*, 1990). In cultures *P. lima* adheres to the wall of culture vessels and rarely swims freely except when disturbed (Faust, personal observation). A stalk is sometimes visible at the flagellar pole in material collected in nature (Ø. Moestrup, personal observation). Morphological and biochemical variability of *P. lima* clones exist between sites, the most notable of which is toxin content, OA, and methyl–okadaic acid (DSP-1) (Morton and Tindall, 1995).

Toxicology: Several toxins were identified in *P. lima*:

(1) Okadaic acid (OA) was isolated and identified by Murakami *et al.* (1982), Lee *et al.* (1989), and Marr *et al.* (1992). The physical and symptomological properties resemble those of the partially characterized ciguatoxin from shellfish (Tachibana *et al.*, 1981), and has potent diarrhetic effects (Yasumoto *et al.*, 1987). It has been identified as the causative agent of diarrhetic shellfish poisoning (Murata *et al.*, 1982; Kumagai *et al.*, 1986). OA was derived previously from sponges causing mouse toxicity with LD_{50} of 192 mg kg^{-1} intraperitoneally (i.p.) (Tachibana *et al.*, 1981). Yasumoto *et al.* (1980a) found two more toxins related to OA, an ether soluble fraction (mouse toxicity 143×10^{-8} MU $cell^{-1}$) and a butanol soluble fraction (mouse toxicity 71×10^{-8} MU $cell^{-1}$). Both fractions caused hemolysis in mice (Nakajima *et al.*, 1981).

(2) An unnamed fast-acting water-soluble toxin (FAT) was isolated from culture extracts of *P. lima* collected from ciguatera endemic regions (Tindall *et al.* 1984, 1989). Mice injected with the minimum lethal dose (LD_{50}) either died within 32–34 minutes or recovered completely.

(3) DTX-1 was identified at various ratios and concentrations with OA in six *P. lima* isolates from Spain and Okinawa, Japan (Lee *et al.*, 1989) and from the Atlantic coast of Canada (Marr *et al.*, 1992). Its toxicity is similar to other toxins (toxicity 160 mg kg^{-1} i.p. mouse according to Tachibana *et al.* 1981).

(4) A nitrogenous macrocycle toxin, prorocentrolide, was isolated from *P. lima* (Torigoe *et al.*, 1988). OA-monoclonal antibody was localized to chloroplasts and pyrenoid in *P. lima* isolate no. 712 from Vigo, Spain (Zhou and Fritz, 1994).

Prorocentrum mexicanum Tafall, 1942 Fig. 7a–g

Synonym: *P. rhathymum* Loeblich, Sherley et Schmidt, 1979

Description: Cells are oval in valve view (Fig. 7a–c) and convex in side view (Fig. 7e–f), 30–38 µm long, and 20–25 µm wide with a posterior nucleus (Fig. 7b); a pyrenoid is absent. Apical area is a broad triangle. Ornamentation on the right valve includes a prominent curved apical plate and a smaller protruding plate (Fig. 7e–f). Under the light microscope the curved apical plate appears like a spine (Fig. 7a). Valve surface of young cells is smooth, and in older cells rugose. Valves contain radially arranged valve pores (Fig. 7d), which are round with smooth edges and at times filled or open (Fig. 7f–g). Smaller pores are also present (Fig. 7g). The intercalary band is transversely striated (Fig. 7e–f).

Taxonomic remarks: The name *P. mexicanum* is used here following Steidinger (1983) and Carlson (1984) that Tafall's (1942) description has priority. Loeblich *et al.* (1979) created the name *P. rhathymum* and considered *P. mexicanum* a synonym. *Prorocentrum mexicanum* was illustrated by Tafall (1942), who interpreted the valve pores incorrectly as spines. Tafall (1942) believed that the specimen illustrated by Böhm (1936; fig. 3a) is probably *P. mexicanum*. In profile, *P. mexicanum* is similar to *P. ovale*, as illustrated by Gourret (1883) and *P. maximum* by Schiller (1933). The latter two species were considered as synonyms by Dodge (1975), but Dodge showed a spiny valve quite different from that of *P. mexicanum*. The apical area of *P. mexicanum* is complex and apparently both flagella emerge from one flagellar pore (Loeblich *et al.*, 1979).

Ecology and distribution: *Prorocentrum mexicanum* is widely distributed in tropical regions and prefers inshore protected shallow areas of both Pacific and Atlantic Oceans (Fukuyo, 1981; Bomber *et al.*, 1985; Carlson and Tindall, 1985). It has been found in association with *P. lima*, *P. emarginatum*, *Scrippsiella subsalsa*, and *Gambierdiscus toxicus*. *Prorocentrum mexicanum* attaches to macroalgae (Carlson *et al.*, 1984), drift algae (Bomber *et al.*, 1988), sediments

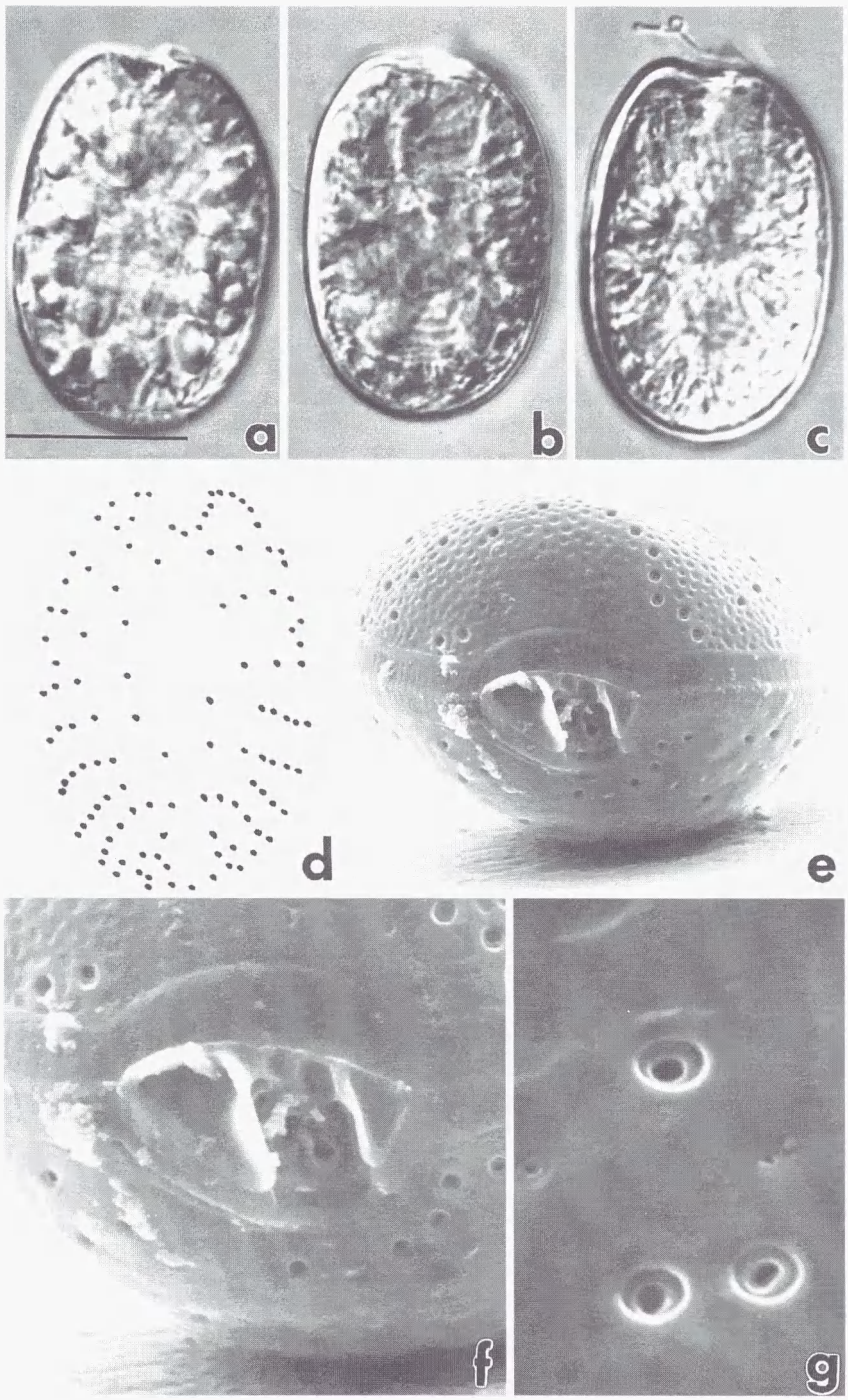

Figure 7a–g. *Prorocentrum mexicanum*. 7a: Cells are oval, right valve has a curved apical plate; 7b, c: left valve and posterior nucleus; 7d: valve surface has radially arranged valve pores; 7e, f: apical area is a broad triangle fitted into the right valve and (Fig. 7f) includes a prominent curved apical plate and a protruding plate; 7g: valve pores are large and round with a circular opening in the center, valve surface is smooth. Material from Twin Cays, Belize. Scale bars in Figure 7a–c: 20 µm; Figure 7e–g: 2 µm.

(Fukuyo, 1981) and floating mangrove detritus (Faust, 1996 and references therein).

Growth of *P. mexicanum* in bacterized cultures is inhibited by macroalgal extracts. However, artificial sea water is sufficient for growth without addition of soil extract (Carlson *et al.*, 1984). Culture filtrates of *P. concavum* contain substances that are stimulatory to growth of *P. mexicanum*.

Loeblich *et al.* (1979) considered *P. mexicanum* as an immobile species embedded in mucilage. However, in field populations it swims freely (Fukuyo, 1981; Faust, 1990a), and in cultures secretes mucilage under adverse conditions (Carlson, 1984).

Toxicology: *Prorocentrum mexicanum* produces toxins with strong hemolytic activity (nine isolates examined by Nakajima *et al.*, 1981). A water-soluble fast-acting toxin (FAT) was isolated from extracts of *P. mexicanum* by Tindall *et al.* (1989) causing death in mice within 32 minutes. The physiological action of this FAT is similar to the FAT isolated from *P. concavum* reported by Tindall *et al.* (1989). Extracts from *P. mexicanum* cross-reacted in immunoassay directed against toxins isolated from *Gymnodinium breve* (Baden *et al.*, 1985).

Prorocentrum ruetzlerianum Faust, 1990 Fig. 8a–e

Description: Cells are round to ovoid in valve view with an average diameter of 28–35 µm (Fig. 8a–b). In side view, cells are convex, with a slight indentation in the middle of both valves (Fig. 8d–e). Valves are deeply areolated over the entire valve surface (Fig. 8d–e). Each pentagonal-shaped areola has a round pore situated in a deep depression (Fig. 8e). Each valve is covered with ca. 500 areolae and ca. 70 marginal areolae (Fig. 8c). The marginal areolae are elongated depressions which in the light microscope provide the optical effect of a distinct striated pattern (Fig. 8a–b). The intercalary band is unique; it is transversally striated and possesses a sinuous groove with equally spaced waves (Fig. 8d–e). The apical area is a broad, shallow triangle within the

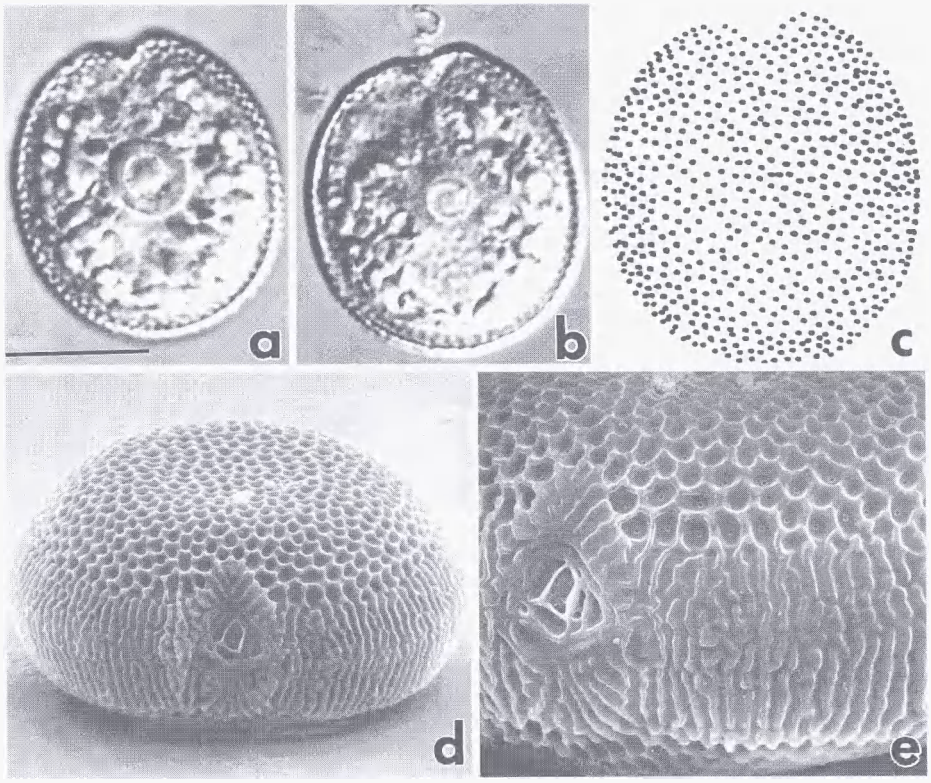

Figure 8a–e. *Prorocentrum ruetzlerianum*. 8a: Cells are round with distinctly striated valve margin and centrally located pyrenoid; 8b: left valve view with a posterior nucleus adjacent to the pyrenoid; 8c: cell surface covered with valve areolae (ca. 500 per valve) and marginal areolae (ca. 70 per valve); 8d: cell shape is convex, apical area is a broad, shallow, unornamented triangle; 8e: valve surface is covered with pentagonal areolae with a round opening situated in a deep depression. The intercalary band is transversely striated possessing a sinuous groove with equally spaced waves. Material collected from Twin Cays, Belize. Scale bars in Figure 8a–b: 20 µm; Figure 8d–e: 2 µm.

right valve. The left valve is flat (Fig. 8d–e). A pyrenoid is centrally located; the nucleus is posterior adjacent to the pyrenoid (Fig. 8b).

Taxonomic remarks: The type of *P. ruetzlerianum* was described from mangrove habitats, Hidden Lake, Boston Bay, and the Lair at Twin Cays, Belize (Faust, 1990a). It is a small benthic species. Its round shape and the striated intercalary band create an optical edge effect which is useful in differentiating this species from other benthic species.

Ecology and distribution: Cells of *P. ruetzlerianum* are attached to mangrove sediments and floating detritus (Faust, 1990a). It is present in low numbers at temperatures of 24–32°C and salinities of 28–36 at low light levels. *Prorocentrum ruetzlerianum* occurred with *Amphidinium kofoidii*, *P. emarginatum*, *P. lima*, *P. marinum*, and *P. mexicanum*.

Toxicology: The toxicity of *P. ruetzlerianum* is unknown.

Prorocentrum belizeanum Faust, 1993 Fig. 9a–f

Description: Cells are round to slightly oval in valve view with an average diameter of 55–60 µm (Fig. 9a–b). The thecal surface is areolated with ca. 950 areolae per valve (Fig. 9a–f). Each round to oval areola is deep. Not every areola has a pore. Areolae are < 1 µm in diameter. The valve margin has an array of depressions which provide the optical effect of a distinct striated pattern in the light microscope (Fig. 9c). The intercalary band is smooth at low magnification (Fig. 9b, d, e), but

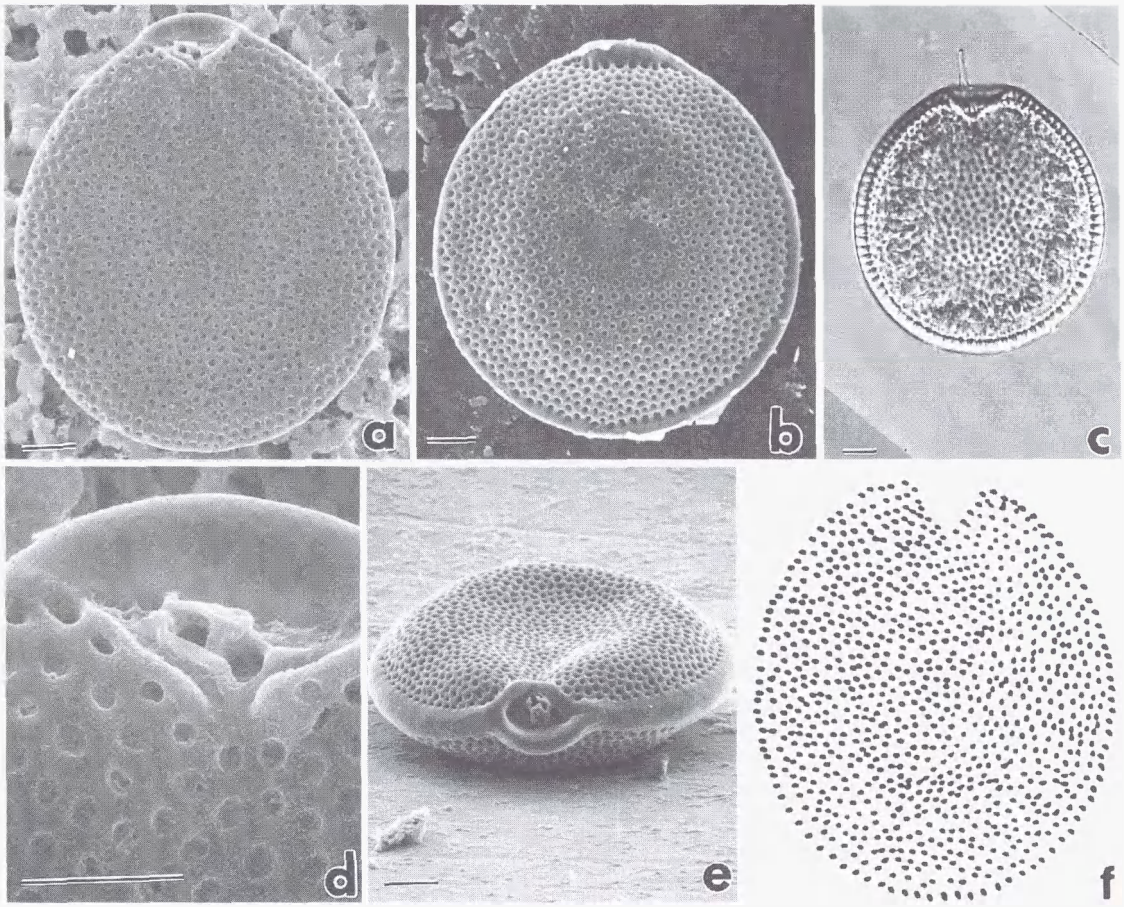

Figure 9a–f. *Prorocentrum belizeanum*. 9a: Cells are round to slightly oval, thecal surface completely areolate; 9b: left valve view with deep, round areolae; 9c: under the light microscope cell surface has evenly distributed areolae and distinct striated valve margin; 9d: the apical area is broad with a raised anterior ridge and a curved apical collar around the flagellar pore; 9e: both valves are concave and areolated and the intercalary band is smooth; 9f: right valve surface has ca. 950 areolae. Scale bars in Figures 9a–f: 10 µm.

horizontally striated at high magnification (Faust, 1993d). The apical area is a wide triangle located in the right valve (Fig. 9a, d–e). Raised anterior ridge on left valve (Fig. 9d). The flagellar and auxiliary pores are equal in size (Fig. 9a, d–e). The auxiliary pore is surrounded by a flared apical collar and void of apical spine (Fig. 9d). The pyrenoid is centrally located. The large kidney-shaped nucleus is situated posteriorly and displaced from the pyrenoid.

Taxonomic remarks: The type of *P. belizeanum* was described from mangrove habitats, the Lair, Lair Channel, and Boston Bay at Twin Cays, Belize (Faust, 1993d). It has a distinct round or near-round shape and medium size. It is larger than *P. hoffmannianum* (45–55 µm long) and P. *ruetzlerianum* (diameter ca. 32 µm) (Faust, 1990a). It is readily confused with *P. concavum* and *P. hoffmannianum*. It differs from *P. concavum* by having prominent areolae in the center of both valves and from *P. hoffmannianum* by having a periflagellar area similar to *P. lima* (Faust, 1991) and smaller but more numerous thecal areolae.

Ecology and distribution: Cells of *P. belizeanum* are a major component of benthic toxic dinoflagellate assemblages in tropical coastal marine waters in mangrove detritus (Faust, 1996 and references therein) and attached to macroalgae (Morton and Faust, 1997). It is present in floating detritus at temperatures of 24–30°C and salinities of 28–34 (Faust, 1993d). *Prorocentrum belizeanum* occurred together with 22 dinoflagellate taxa comprising a major part of the mangrove algal food web, 11 of which are considered harmful: *Gambierdiscus toxicus*, *Coolia monotis*, *Ostreopsis lenticularis*, *Amphidinium carterae*, *Dinophysis caudata*, *D. rotundata*, *Prorocentrum mexicanum*, *P. concavum*, *P. hoffmannianum*, *P. maculosum*, *P. lima*, and *Cochlodinium polykrikoides* (Faust, 1996).

Toxicology: *Prorocentrum belizeanum* produces okadaic acid and small amounts of DXT-1 toxin (Morton *et al.*, 1998).

Prorocentrum maculosum Faust, 1993 Fig. 10a–f

Synonym: *Exuviaella maculosum* (M. A. Faust) McLachlan et Boalch, 1997

Description: Cells in valve view 40–50 µm long and 30–40 µm wide, broadly ovate with a maximum width behind the middle region and narrow at the anterior end (Fig. 10a–b). Valve surface rugose with scattered poroids (Fig. 10c), 85–90 per valve, and round marginal pores 65–75 per valve (Fig. 10d). Poroids are kidney-shaped to circular or oblong and unevenly distributed on the valve surface. The center of the valves lacks poroids (Fig. 10a–b). The periflagellar area is a broad triangle with a raised margin on the right valve at the anterior end of the cell (Fig. 10a). The flagellar pore and auxiliary pore are about equal in size (Fig. 10e) and viewed from the side are surrounded by a curved and flared apical collar (Fig. 10f). The anterior end of the left valve is flat to slightly concave (Fig. 10b). A pyrenoid is centrally located, the nucleus is posterior adjacent to the pyrenoid.

Taxonomic remarks: The type of *P. maculosum* was described from floating mangrove detritus and sediment samples at Hidden Lake and the Lair in Twin Cays, Belize (Faust, 1993a). *Prorocentrum maculosum* and *P. lima* can be distinguished in scanning electron micrographs by two features: (1) the thecal surface of *P. maculosum* has large kidney-shaped valve poroids and a rugose thecal surface; (2) the apical collar surrounds round, equally sized flagellar and auxiliary pores. A similar architecture of the periflagellar area is present in *P. hoffmannianum* (Faust, 1990a), *P. compressum* (Abé, 1967; Dodge, 1975), *P. playfairii* and *P. foveolata* (Croome and Tyler, 1987). With the light microscope, *P. maculosum* is distinguished from *P. lima* by the presence of large, kidney-shaped valve poroids scattered on the thecal surface. In *P. lima* the thecal pores are round and the thecal surface smooth, the flagellar pore is larger than the auxiliary pore and surrounded by a curved apical collar, and the intercalary band has no ridge. Flask-shaped membrane-bounded mucocysts are present in *P. maculosum* (Zhou and Fritz, 1993). Okadaic acid-monoclonal antibody localizes to chloroplasts and pyrenoid, and to a lesser degree to cellular lysosomes in DSP-toxin producing *P. maculosum* (Zhou and Fritz, 1994).

Ecology and distribution: Cells of *P. maculosum* attach to mangrove sediments and detritus (Faust, 1993a) and macroalgae (Zhou and Fritz, 1993). Cells were observed in samples collected at 30–36°C and salinities of 32–36 (Faust, 1993a). *Prorocentrum maculosum* occurred together with *P. hoffmannianum*, *P. ruetzlerianum*, *P. foraminosum*, *Scrippsiella subsalsa*, and *Coolia monotis* (Faust, 1996).

Toxicology: *Prorocentrum maculosum* produces prorocentrolide B, a fast-acting toxin (Hue *et al.*, 1996). This compound produces a rapid toxic response in the mouse bioassay, a type of activity not accounted for by other diarrhetic shellfish-poisoning toxins produced by *P. maculosum*. Unlike their co-metabolites, DSP toxins, prorocentrolide B does not show phosphatase inhibition. The toxicological and pharmacological effects of the fast-acting toxins are not understood.

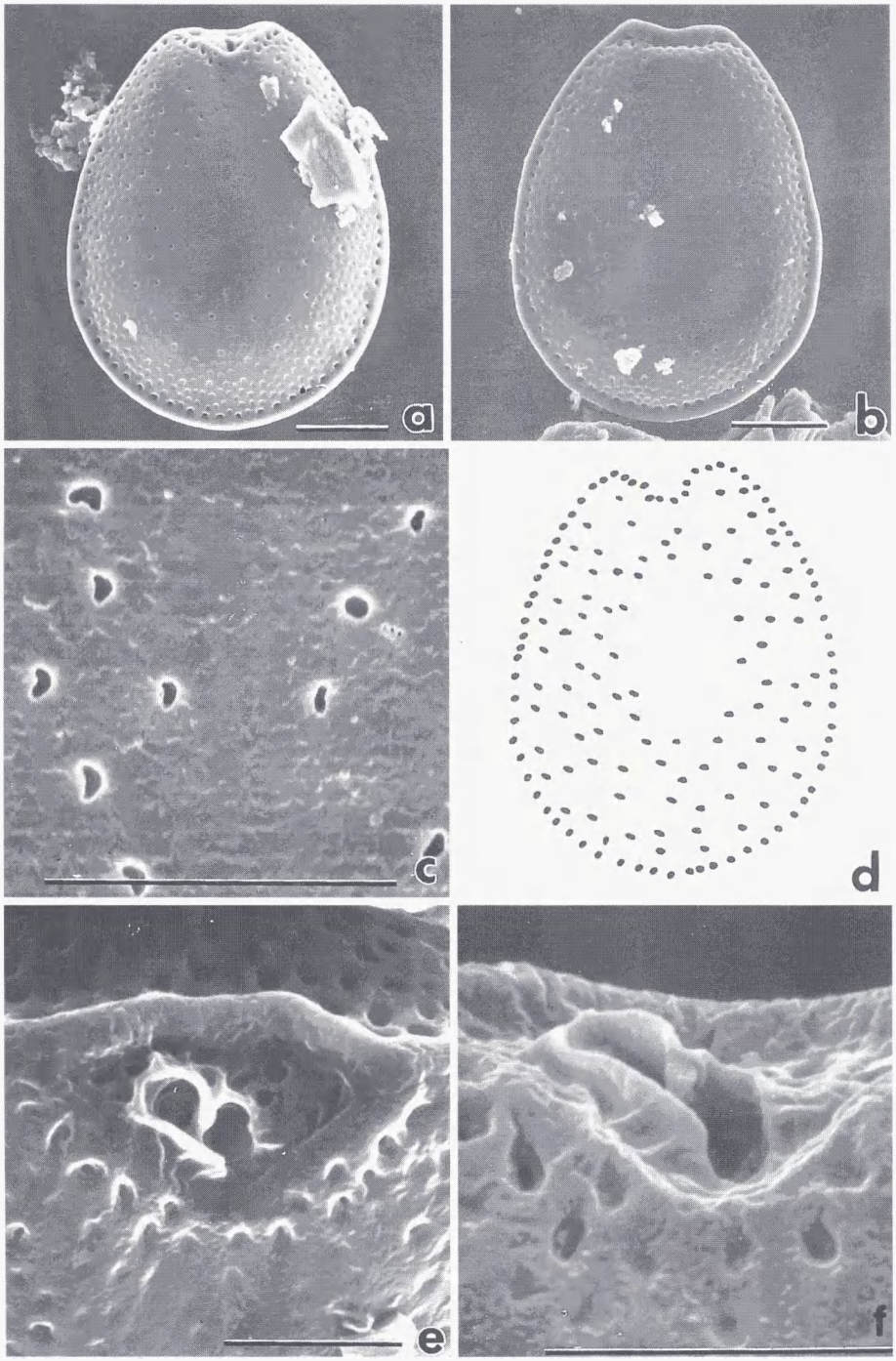

Figure 10a–f. *Prorocentrum maculosum*. 10a: Right valve including the apical area of a broadly ovate cell; 10b: valve surface is rugose with scattered poroids and round marginal pores; 10c: poroids are kidney-shaped to oblong and unevenly distributed; 10d: right valve surface has ca. 87 valve poroids and ca. 70 marginal pores; 10e: apical area is a broad triangle with flared apical collar adjacent to the flagellar pore; 10f: apical collar viewed from the side. Scale bars in Figure 10a–b: 10 µm; Figure 10c–f: 5 µm.

Planktonic species

Prorocentrum balticum (Lohmann) Loeblich, 1970
Fig. 11a–d

Synonym: *Exuviaella baltica* Lohmann, 1908; *E. aequatorialis* Hasle, 1960; *P. pomoideum* Bursa, 1959.

Description: The cells are almost circular in valve view, some slightly ovate with broad shoulders, 9–15 µm long, only slightly flattened. Two minute apical spines, which may be difficult to observe with the light microscope, are located in the pore region. The valves are covered with tiny spines which form narrow transverse rows on the intercalary band. Only a few scattered valve pores are present.

Taxonomic remarks: *Prorocentrum balticum* is not easily distinguished from *P. minimum* (see below) and a critical assessment of its taxonomic status is still needed. It is probably best identified by its small size, its almost spherical shape, and the two apical projections. An early electron microscopical study (Braarud *et al.*, 1958) revealed that the thecal plates are covered with minute spines as confirmed by subsequent authors (e.g., Dodge, 1982, 1985; Fukuyo *et al.*, 1990). The cell illustrated by Dodge (1985, p. 9) shows several irregular depressions (or holes) at the base of the spines, but such features have not been found by other workers, and their significance cannot be assessed.

Ecology and distribution: *Prorocentrum balticum* has been reported to form "red tides" in many parts of the world (see Lassus, 1988, and references therein). Many blooms have occurred in brackish-water areas (Zotter, 1979; Tangen, 1980; Edler *et al.*, 1984), in concordance with the growth experiments of Braarud (1951), who found that *P. balticum* is euryhaline, exhibiting highest growth rates at low salinities (10–15).

Toxicology: Toxicity in *P. balticum* has never been confirmed. Cells have, however, been reported in connection with toxic red tides (Silva, 1956, 1963; Numann, 1957), and Steidinger (1979) regards it as a toxic species.

Prorocentrum minimum (Pavillard) Schiller, 1933
Fig. 11e–l

Synonyms: *Exuviaella minima* Pavillard, 1916; *Prorocentrum triangulatum* Martin, 1929; *E. mariae-lebouriae* Parke et Ballantine, 1957; *P. cordiformis* Bursa, 1959; *P. mariae-lebouriae* (Parke et Ballantine) Loeblich III, 1970.

Description: The cells vary from more or less triangular to cordiform or oval, 14–22 µm long, flattened, with an apical spine which in some forms is difficult to observe in the light microscope. The valves are covered by minute spines and penetrated by scattered pores; intercalary bands are striated.

Taxonomic remarks: *Prorocentrum minimum* varies considerably and the morphological forms have been assigned to different species, as indicated in the list of synonyms. Hulburt (1965) proposed to give these varietal status, but as their basionyms were not indicated the new combinations are formally illegitimate according to the International Code of Botanical Nomenclature (Sournia, 1973). It should be noted, however, that Hulburt does not explicitly treat these organisms as plants, although this can be assumed from the context.

From our point of view, it is questionable whether the different morphological types of *P. minimum* should be given formal taxonomic status considering the variation between the different forms which form a continuous series (see Hulburt, 1965; plate 2). The intraspecific variation of *P. minimum*, as well as the taxonomic relationships with closely related species such as *P. balticum*, needs re-investigation before a sound taxonomic revision can take place. The need is illustrated by the paper of Silva (1985), who regarded earlier records of *P. balticum* blooms along the coasts of Portugal as misidentifications and denoted them as blooms of *P. minimum*.

Prorocentrum minimum may be confused with *P. balticum*, but differs by its larger size and different shape and by having only one apical spine. *Prorocentrum cordatum* (Ostenfeld) Dodge comb. illeg. (basionym is not indicated by Dodge, 1975) is very similar to *P. minimum*, but does not possess an apical spine.

Ecology and distribution: The biology of *P. minimum* was reviewed by Berland and Grzebyk (1991). Blooms have been restricted to temperate waters of the Northern Hemisphere with the possible exception of an isolated bloom in the tropical waters off the coast of Pakistan (Rabbani *et al.*, 1990). In the North Sea region, *P. minimum* was first recorded in The Netherlands in 1976 (Kat, 1979), and subsequently along the coasts of Norway (Tangen, 1980) and Denmark including the western part of the Baltic (Kimor *et al.*, 1985).

Prorocentrum minimum appears to be euryhaline and eurytherme, having been recorded within a salinity range of 5–37 and a temperature range of 4–31°C (Berland and Grzebyk, 1991). Blooms, however, seem to occur mostly in brackish water (Kondo *et al.*, 1990a; Sournia *et al.*, 1991). Under certain circumstances growth is apparently enhanced by organic compounds (Granéli *et al.*, 1985; Kondo *et al.*, 1990b).

P. minimum has recently been shown to be mixotrophic (Stoecker *et al.*, 1997).

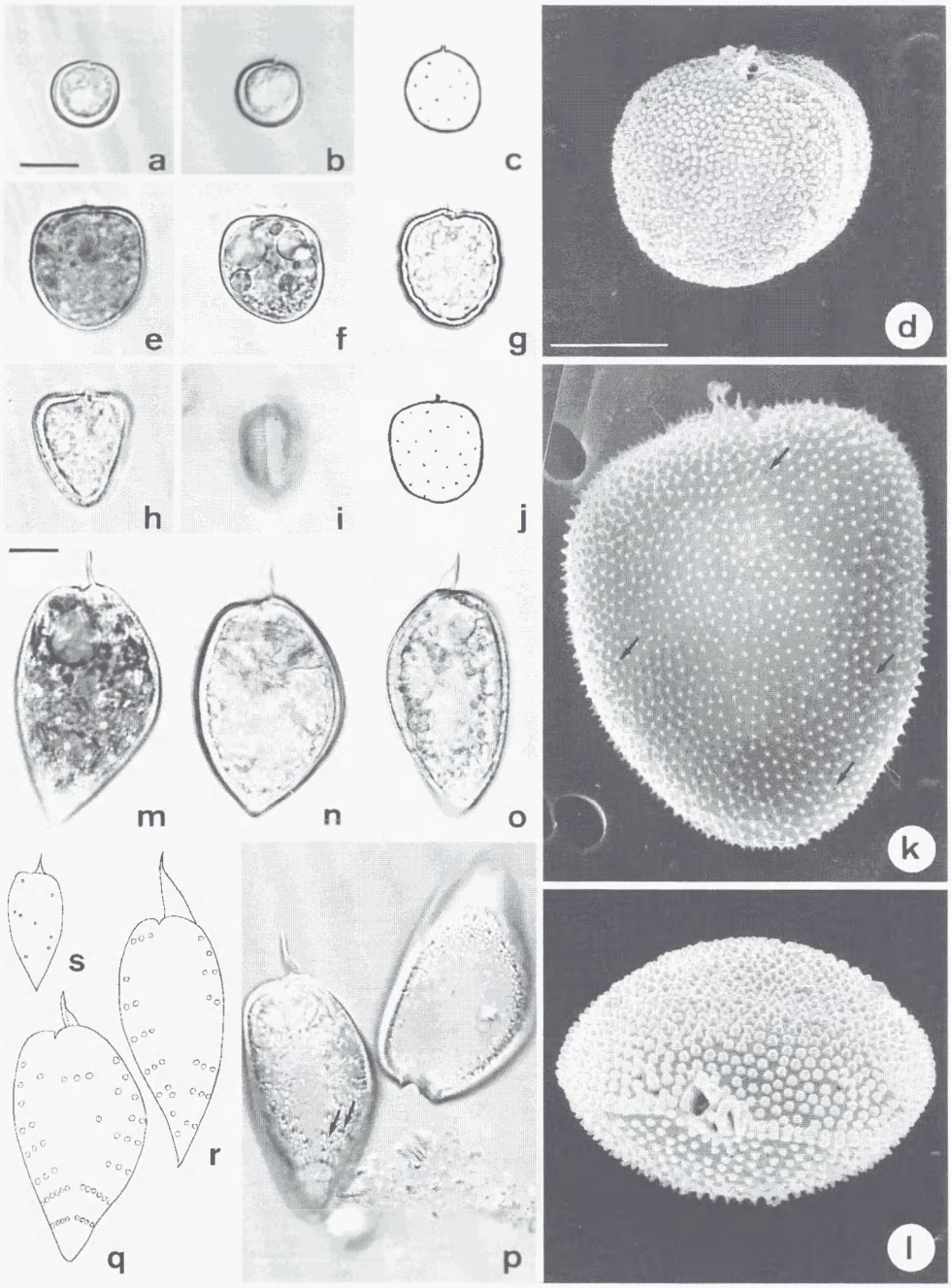

Figure 11a–s. Planktonic *Prorocentrum* species. Figure 11a–d: *Prorocentrum balticum* from the North Sea; 11a–b: cells in valve view; photographs by G. Hansen; 11c: schematic drawing; 11d: scanning micrograph by G. Hansen. Figure 11e–l: *Prorocentrum minimum* from the Kattegat, Denmark. 11e–i: Cells of different morphological types; 11e, g: live cells; 11f, h: formalin-preserved cells; 11i: empty theca; 11j: schematic drawing showing the scattered valve pores; 11k, l: scanning micrographs by G. Hansen of a cell in valve view (11k) showing the spiny surface and the scattered valve pores (arrows), 111 showing a cell in apical view. Figure 11m–o: *P. micans* from the Kattegat, Denmark; different morphological types; note the strong apical spines; 11p: empty thecae with rows of valve pores (arrows). (Figure 11q–s) Schematic drawings. (11q) *P. micans*; 11r: *P. gracile*; 11s: *P. triestinum*. Scale bar in Figure 11: 10 µm for 11a–c, e–j; 5 µm for 11d, k, l; 10 µm for 11m–s.

Toxicology: In 1942, a serious intoxication with more than 100 casualties due to shellfish consumption occurred in Japan, and also several other incidents since then (Nakazima, 1965a–c). The causative organism was first identified as *Prorocentrum* sp., and subsequently as *Exuviaella mariae-lebouriae* (Nakazima, 1968). A substance named venerupin was extracted from the shellfish, and when administered to mice the same pathological picture was observed as in humans (Nakazima, 1965a–c). It is questionable, however, whether *Prorocentrum* was responsible for this incident. Okaichi and Imatomi (1979) isolated three different toxic fractions from a culture identified as *P. minimum* var. *mariae-lebouriae*. The chemical structure of these compounds remains to be elucidated. In European waters, *P. miminum* has on a few occasions been associated with shellfish poisoning (Tangen, 1983; Silva, 1985) and a recent study has shown that senescent cultures of *P. minimum* can produce toxins (Grzebyk *et al.*, 1997).

Prorocentrum micans Ehrenberg, 1834

Fig. 11m–q

Description: Cells are oblique drop-shaped, rounded anteriorly, tapering posteriorly, 35–70 µm long, 20–50 µm wide, length:width ratio usually less than 2, strongly flattened, with a well-developed apical spine. The thecal plates are not covered with spines, but penetrated by valve pores mostly organized in short rows near and more or less perpendicular to the edge of the valve. Chloroplasts present.

Taxonomic remarks: *Prorocentrum micans* varies considerably and may be confused with closely related species, e.g., *P. gracile* and *P. triestinum*. *Prorocentrum gracile* (Fig. 11r) has a very strong apical spine and a length:width ratio usually larger than 2; *P. triestinum* (Fig. 11s) is smaller than the other species and has only few scattered valve pores, see also Dodge (1975, 1982).

Ecology and distribution: *Prorocentrum micans* is a well-known "red tide" species in many parts of the world (see Taylor and Seliger, 1979; Anderson *et al.*, 1985; Granéli *et al.*, 1990, *inter alios*). Despite its ability to form extensive blooms, *P. micans* is usually considered harmless. It may excrete substances that inhibit diatom growth (Uchida, 1977), but apparently these substances do not enter the food chain or affect organisms at higher trophic levels.

Toxicology: There are only a few reports on *P. micans* having caused problems (Pinto and Silva, 1956; Shumway, 1990), and claims for toxicity of this species need confirmation. Early reports on *P. micans* being a PSP (paralytic shellfish poison) producer as deduced from the pathogeny of the intoxication (Pinto and Silva, 1956) are unconfirmed, and recent incidents involving shellfish mortality are attributed to oxygen depletion (Lassus and Berthome, 1988).

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