

# Taxonomy, phylogeny, biogeography, and ecology of *Sabulodinium undulatum* (Dinophyceae), including an amended description of the species

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## SUMMARY

Samples of *Sabulodinium undulatum* Saunders et Dodge, the type species of the monospecific genus, were collected and characterized from Germany, Russia, Japan and Canada. This species has a laterally flattened, oval cell with a truncated apex and a dorsally pointing small episome. The dorsal margin of the hyposome has an undulating shape. A dorsal spine and/or antapical spine are sometimes present. The specimens of this heterotrophic species are 27.5–42.5 µm long and 18.5–36.0 µm wide, and have a theca with the plate arrangement apical pore complex (APC) 5' 1a 6'' 5c 4s 6''' 1'''. The shape of the dorsal theca is variable. The species distribution seems to be restricted to northern temperate regions. *Sabulodinium undulatum* occurred in all sandy eulittoral areas throughout the year and was also present in sandy sublittoral and supralittoral (beach) samples. The species can tolerate a broad range of temperatures and salinities. *Sabulodinium* occurred from –2.0 to +24.3°C and 4–35 salinity. It is likely that the observed variability in morphology and habitat reflect several varieties. We propose to establish three varieties within the species, namely *S. undulatum* var. *undulatum*, *S. undulatum* var. *glabromarginatum*, and *S. undulatum* var. *monospinum*. The systematic position of *Sabulodinium* is discussed in the context of comparative morphological and molecular phylogenetic data.

Key words: dinoflagellates, Dinophysiales, Nannoceratopsiales, *Pseudothecadinium*, *Sabulodinium*, sand-dwelling, *Sinophysis*, *Thecadinium*.

dinoflagellate found in damp sand at Holy Island, Northumberland, UK (Saunders & Dodge 1984; reproduced here as Figure 1,2). This taxon was also observed at Port Erin, Isle of Man, by Herdman (1924; figure 23; reproduced here as Figure 3) and described as a colorless variety of *Phalacroma kofoidii* Herdman (Herdman 1923). In our opinion, the circumscription of *Thecadinium petasatum* Kofoid et Skogsberg by Baillie (1971) comprised the observations of two species, namely *Sabulodinium undulatum* and *Planodinium striatum* Saunders et Dodge. Baillie's figures (pl. 4, figure 3a,b; reproduced here as Figures 4,5) illustrate *Sabulodinium undulatum*. His findings of the species in intertidal sand flats on the coast of the northeast Pacific Ocean of Canada confirmed previous observations made by one of our authors, F.J.R. Taylor (unpubl. data, year?, the original drawings are displayed here as Figures 6,7; also presented by Baillie on his pl. 5, figure 7b,c). Larsen (1985; Figs 4,5) depicted a dinoflagellate species from the Danish Wadden Sea identified as *Thecadinium kofoidii* (Herdman) Larsen. In a note added in proof, Larsen (1985, p. 37) corrected his identification to *S. undulatum* referring to Saunders and Dodge (1984). Recording marine dinoflagellates from North Sutherland, Scotland, Dodge (1989) found *S. undulatum* in benthic samples and Hoppenrath (2000a) found the species in sand samples from the North German Wadden Sea.

[3]

The original species description of *Sabulodinium undulatum* included only an incomplete plate formula: c, s, 5''', 1''' (Saunders & Dodge 1984), and it was stated that the epithecal plates have not been determined. However, Dodge and Lewis (1986) presented a plate formula including epithecal plates for

## INTRODUCTION

The genus *Sabulodinium* Saunders et Dodge was introduced with the type species *Sabulodinium undulatum* Saunders et Dodge for a marine, heterotrophic, thecate

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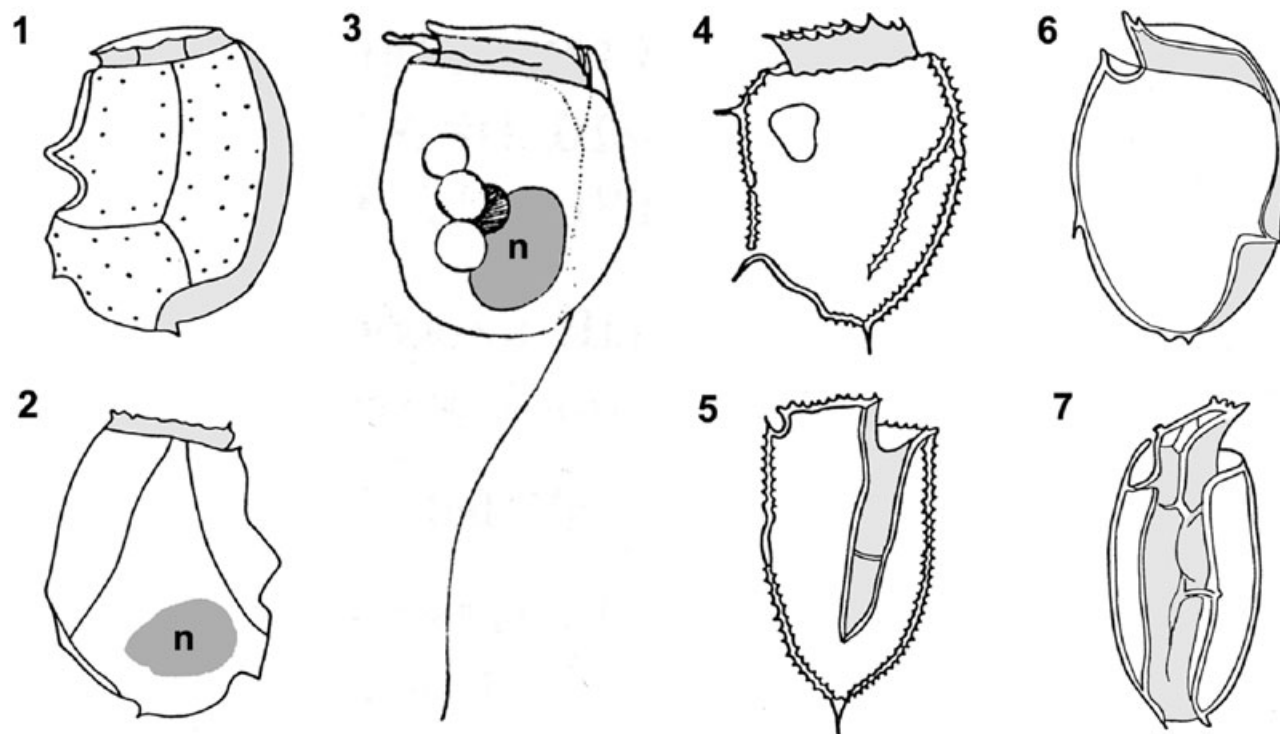
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**Fig. 1–7.** Drawings of *Sabulodinium undulatum*. 1. Right lateral view (redrawn from Saunders & Dodge 1984). 2. Left lateral view (redrawn from Saunders & Dodge 1984). 3. General view (redrawn from Herdman 1924). 4. Right lateral view (redrawn from Baillie 1971). 5. Ventral view (redrawn from Baillie 1971). 6. Right lateral view (Taylor original). 7. Ventral view (Taylor original). light gray, cingulum and sulcus; n, nucleus.

*Sabulodinium* (their table 1, p. 224: Po 3' 6'' ?c ?s 5''' 1''') referring to the Saunders and Dodge (1984) paper. Thus, because the morphology of the epithecal plates was not published, it is not clear how the information about the epithecal tabulation of *S. undulatum* was demonstrated. Hoppenrath gave a tentative plate formula for *Sabulodinium undulatum*, P 5' 1a? 8'' 5c 4s? 6''' 1''' (Hoppenrath 2000a; Hoppenrath *et al.* 2004; Hoppenrath & Selina 2006). We have revised this plate formula in the present study. Moreover, additional morphological details and variability within the species are shown for the first time. The temporal and spatial distributions of *Sabulodinium undulatum* are also presented for the first time, as well as an extended geographic distribution and molecular phylogenetic data.

Introducing their new genus *Sabulodinium*, Saunders and Dodge (1984) transferred *Thecadinium inclinatum* Balech into it as *Sabulodinium inclinatum* (Balech) Saunders et Dodge. Hoppenrath (2000b) tentatively accepted this classification, but after reinvestigating the taxon, she reclassified it as a *Thecadinium* species (Hoppenrath *et al.* 2004). This view was upheld with subsequent investigations made by Yoshimatsu *et al.* (2004).

## MATERIALS AND METHODS

### Sampling sites

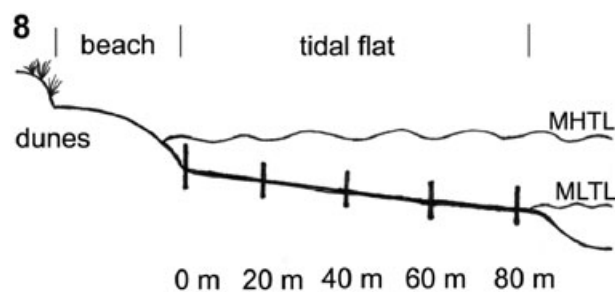
The sampling sites in Germany were located at the intertidal flats at the island of Sylt, North Frisian Wadden Sea, North Sea. Samples were collected south of List harbour (55°00.85'N, 08°06.30'E) and at the 'Oddewatt' northeast of List (55°01.80'N; 08°26.00'E). Samples in Canada were collected at the intertidal flat at Centennial Beach, Boundary Bay, BC, Canada (49°00.00'N, 123°08.00'E). The samplings in Russia were carried out in various localities of Peter the Great Bay, situated in the northwestern part of the Sea of Japan (42°17'–43°20'N, 130°40'–133°02'E) and in Aniva Bay (46°35.47'N, 142°46.37'E) and at the eastern coast of Sakhalin Island (51°24.05'N, 143°27.47'E), Okhotsk Sea. The sampling sites in Japan were located at Ishikari Beach, Hokkaido (43°13. 58'N, 141°19.56'E).

### Sampling methods

In Germany samples were collected from (not water covered) intertidal flats with a spoon during low tide

**Table 1.** Sampling dates at the different sampling sites. Parentheses indicate that *Sabulodinium undulatum* was not found

Germany, 'Oddewatt'
1997: 24.2./10.3./24.3./7.4./21.4./5.5./20.5./3.6./19.6./3.7./18.7./1.8./17.8./31.8./16.9./29.9./14.10./30.10./14.11./29.11./14.12./29.12.
1998: 12.1./27.1./10.2./26.2./16.3./13.4./13.5./29.6./27.7./24.8./23.9./23.11./23.12.
1999: 22.1./20.2./21.3.
Germany, list harbor
1997: 11.3./25.3./8.4./22.4./6.5./21.5./4.6./20.6./4.7./19.7./2.8./18.8./1.9./17.9./30.9./15.10./31.10./15.11./30.11./15.12./30.12.
1998: 13.1./28.1./11.2./17.3./14.4./14.5./30.6./28.7./25.8./24.9./24.11./24.12.
1999: 23.1./21.2./22.3.
Canada
2004: (12.9)/25.10.
2005: 16.3./30.3./25.4./9.5./6.6./13.7./4.8./5.9)/20.9./14.10.
2006: 1.2./15.5./31.5.
Russia, Peter the Great Bay, Sea of Japan (classical morphotype and variety)
2001: 18.7./26.7./16.8./28.8./10.11./22.11)
2002: 4.4./2.5./4.6./31.7./11.8./11.9./6.11.
2003: 18.1./30.3./25.5./21.6./12.7./31.7./20.8)/25.8/(14.9)/15.10.
2004: (25.4)/(2.5)/10.5./13.5./24.7./20.7.
Russia, Aniva Bay, Sea of Okhotsk
2003: 19.5. (classical morphotype)
Russia, western coast of Sakhalin Island, Sea of Okhotsk
2005: 7.10. (classical morphotype)
Japan
1997: 28.4./27.5./1.7./31.7./26.8./4.10)/2.11./5.12.
1998: 2.1./5.2.



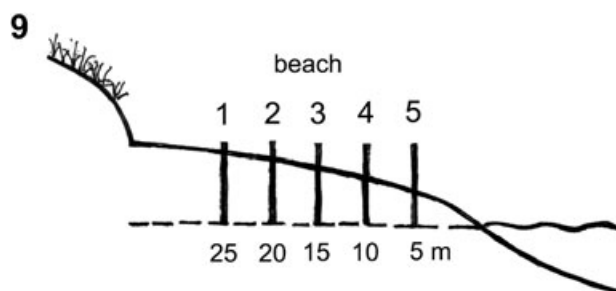
**Fig. 8.** Schematic drawing of the sampling sites of the profile through the eulittoral at the List harbor tidal flat, Germany. Every 20 m a sampling site was sampled 20 cm in depth. The 0 m site corresponded to the border between the steep beach face and the tidal sand flat. The 80 m site was close to the medium low tide line. MHTL, medium high tide line; MLTL, medium low tide line.

- 4 from February 1997 to March 1999 (Table 1). At the location south of List harbour, every 20 m a sampling site was sampled 20 cm in depth and the sample was divided into four subsamples each of 5 cm depth (Fig. 8). The 0 m site corresponded to the border between the steep beach face and the tidal sand flat. The 80 m site was close to the medium low tide line. The sand samples were transported directly to the laboratory and the flagellates were separated from the sand by extraction through a fine gauze (mesh (45 µm)) using melting seawater-ice (Uhlig 1964). The flagel-

lates accumulated in a Petri dish beneath the filter and were then identified with an inverted-microscope at 40 to 250× magnification. Cells were isolated by micropipetting for the preparations described below. The temperature range of the interstitial water was −1.6 to 22.5°C during the morning when sampling took place. The salinity range was 30–32. Recent Canadian samples were collected from September 2004 to May 2006 (Table 1) and treated in the same way as described above.

Samples from Russia were collected from the Sea of Japan from July to September 2001, from April to November 2002, from March to September 2003, from April to July 2004 and sporadically until 2005; from the Sea of Okhotsk in May 2003 and October 2005 (Table 1) during expeditions. The upper 2–3 cm sand layer was collected under water using a square reservoir at 0.5–1.0 m water depth and transported to the laboratory. The sand was rinsed with filtered seawater, it was shaken and the suspension was filtered through a 1 mm mesh and further through gauze mesh of 150 and 80 µm. The sample was then concentrated by filtration through 20 µm gauze. A 20–80 µm fraction was collected in this manner. The concentrated sample was fixed in Lugol's solution. The temperature range was −1.4 to 24.3°C over the year and the salinity range was 2–35.

Samples from Japan were collected throughout one year from April 1997 to February 1998 (Table 1).



**Fig. 9.** Schematic drawing of the sampling sites of the profile through the beach in Japan. Five sampling sites in 5 m distances were sampled as described in Materials and Methods.

Five sampling sites in a profile over the beach, from the shoreline towards inland (Fig. 9), were investigated. A hole was dug until the water level was reached. The inflowing water containing the dinoflagellates was collected and investigated by light microscopy. The temperature range was from  $-2.0^{\circ}\text{C}$  to  $22.0^{\circ}\text{C}$  and the salinity range was 1–36. During winter the beach was covered with snow and ice.

The temperature of the sediment was measured directly *in situ*. For the water salinity measurement interstitial water was collected in a hole (in the sediment) and investigated with a refractometer. At sublittoral sites the water of the collected sample was measured.

## Microscopy

Living cells were observed directly and micromanipulated with an inverted light microscope. For brightfield, differential interference contrast, and fluorescence microscopy, micropipetted cells were placed on a glass specimen slide and covered with a cover slip. Images were produced with a normal microscope camera or a color digital camera. For observations of the thecal plates, cells were stained with 0.01% Fluorescent Brightener 28 (Sigma-Aldrich, St. Louis, MO, USA) and observed with a UV-2 A ultraviolet fluorescence filter set with excitation filter wavelengths of 330–380 nm (only carried out in Japan). For a detailed examination of the thecal morphology, thecae were disassociated using 5% sodium hypochlorite solution and stained using Imamura and Fukuyo's (1987) iodine solution (only carried out in Russia). The light microscopes used in Germany, Canada, Japan, and Russia were: DMRB microscope (Leica, Wetzlar, Germany), DMIL inverted microscope (Leica, Wetzlar, Germany), Axioplan 2 imaging microscope (Carl-Zeiss, Oberkochen, Germany), BX41 microscope (Olympus, Tokyo, Japan), CK-40 inverted and BX50 microscope (Olympus, Tokyo, Japan), and OPTIPHOTO-2 fluorescence microscope (Nikon, Tokyo, Japan).

For scanning electron microscopy (SEM) mixed-extraction samples were fixed with two drops Lugol's solution over night. Fixed specimens were transferred onto a polycarbonate membrane filter, rinsed with distilled water and dehydrated with 30% ethanol followed by dimethoxypropane (in Germany) or dehydrated with a graded series of ethanol (30, 50, 70, 85, 95, 100% in Canada or 25, 50, 75, 90, 100% in Russia). The filters were air-dried (in Germany and Russia) or critical point dried with  $\text{CO}_2$  (in Canada). Filters were mounted on stubs, sputter-coated with gold-palladium (in Germany) or with gold (in Canada and Russia) before the examination in a scanning electron microscope. The scanning electron microscopes used in Germany, Canada, and Russia were DSM 940 A (Carl-Zeiss), S4700 (Hitachi, Tokyo, Japan), and LEO 430 (Carl-Zeiss, Cambridge, UK).

The measurements of cell sizes were taken by means of an ocular micrometer with the light microscope and calculated from scanning electron micrographs. More than 15 cells of each collecting site were measured.

Some SEM images were presented on a black background using Adobe Photoshop 6.0 (Adobe Systems, San Jose, CA, USA).

## DNA isolation, polymerase chain reaction, cloning, and sequencing

The cells for the multispecimen sample for the DNA isolation came from one sediment sample, from one locality (Boundary Bay, Canada), and they were all *Sabulodinium undulatum* var. *undulatum* (see *Taxonomy* in Results). Only this variety is known from this locality. Individually isolated cells were washed three times in filtered (eukaryote-free) seawater and once in distilled water. Five collected cells were placed directly into 8  $\mu\text{L}$  of distilled water in a 0.5 mL polymerase chain reaction (PCR) tube. The tube was placed in  $-20^{\circ}\text{C}$  freezer. Following 2–3 days in the freezer, distilled water was added to the sample to a volume of 24  $\mu\text{L}$  and the nuclear small subunit rRNA gene (SSU rDNA) was amplified using PCR primers and a thermocycling protocol described previously (Leander *et al.* 2003). PCR products corresponding to the expected size were gel isolated and cloned into the pCR 2.1 vector using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA). Two clones were sequenced with a BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). The genetic identity of the cloned sequences was analyzed (BLAST). These two new sequences from *Sabulodinium undulatum* (the first ones for the genus) were completely sequenced using both vector primers and two internal primers (525F: 5'-AAGTCTGGTGCCAGCAGCC-3' and 1250R: 5'-TAACGGAATTAACCAGACA-3') oriented in both



directions (GenBank accession codes: DQ975473 and DQ975474).

## Molecular phylogenetic analyses

The SSU rDNA sequences were aligned with other alveolate sequences using MacClade 4 (Maddison & Maddison 2000) forming a 71-taxon alignment. Maximum likelihood (ML), ML-distance and Bayesian methods under different DNA substitution models were carried out. The evolutionary model was selected with MODELTEST version 3.06 (Posada & Crandall 1998). All gaps were excluded from the alignments prior to phylogenetic analysis. The alpha shape parameters were estimated from the data using Hasegawa-Kishino-Yano (HKY) model for base substitutions, a gamma distribution with invariable sites and eight rate categories ( $\alpha = 0.34$ , Ti/Tv = 2.65, fraction of invariable sites = 0.18). A gamma-corrected ML tree (analyzed using the parameters listed above) was constructed from the alignment with PAUP\* 4.0 (Swofford 1999).

Maximum likelihood distances were calculated with TREE-PUZZLE 5.0 using the HKY substitution matrix (Strimmer & Von Haeseler 1996). A distance tree was constructed with weighted neighbor joining (WNJ) using Weighbor (Bruno *et al.* 2000). One thousand bootstrap datasets were also generated using SEQBOOT (Felsenstein 1993). ML distances for each bootstrap dataset were calculated with the shell script 'puzzleboot' (M. Holder and A. Roger, <http://www.tree-puzzle.de>) using the alpha shape parameter and transition/transversion ratio estimated from the original dataset and analyzed with Weighbor.

We also examined the dataset with Bayesian analysis using the program MrBayes 3.0 (Huelsenbeck & Ronquist 2001). The program was set to operate with GTR, a gamma distribution and four Monte-Carlo-Markov chains (MCMC) (default temperature = 0.2). A total of 2 000 000 generations were calculated with trees sampled every 100 generations and with a prior burn-in of 200 000 generations (2000 sampled trees were discarded). A majority rule consensus tree was constructed from 18 000 postburn-in trees with PAUP\* 4.0. Posterior probabilities correspond to the frequency at which a given node is found in the postburn-in trees.

## RESULTS

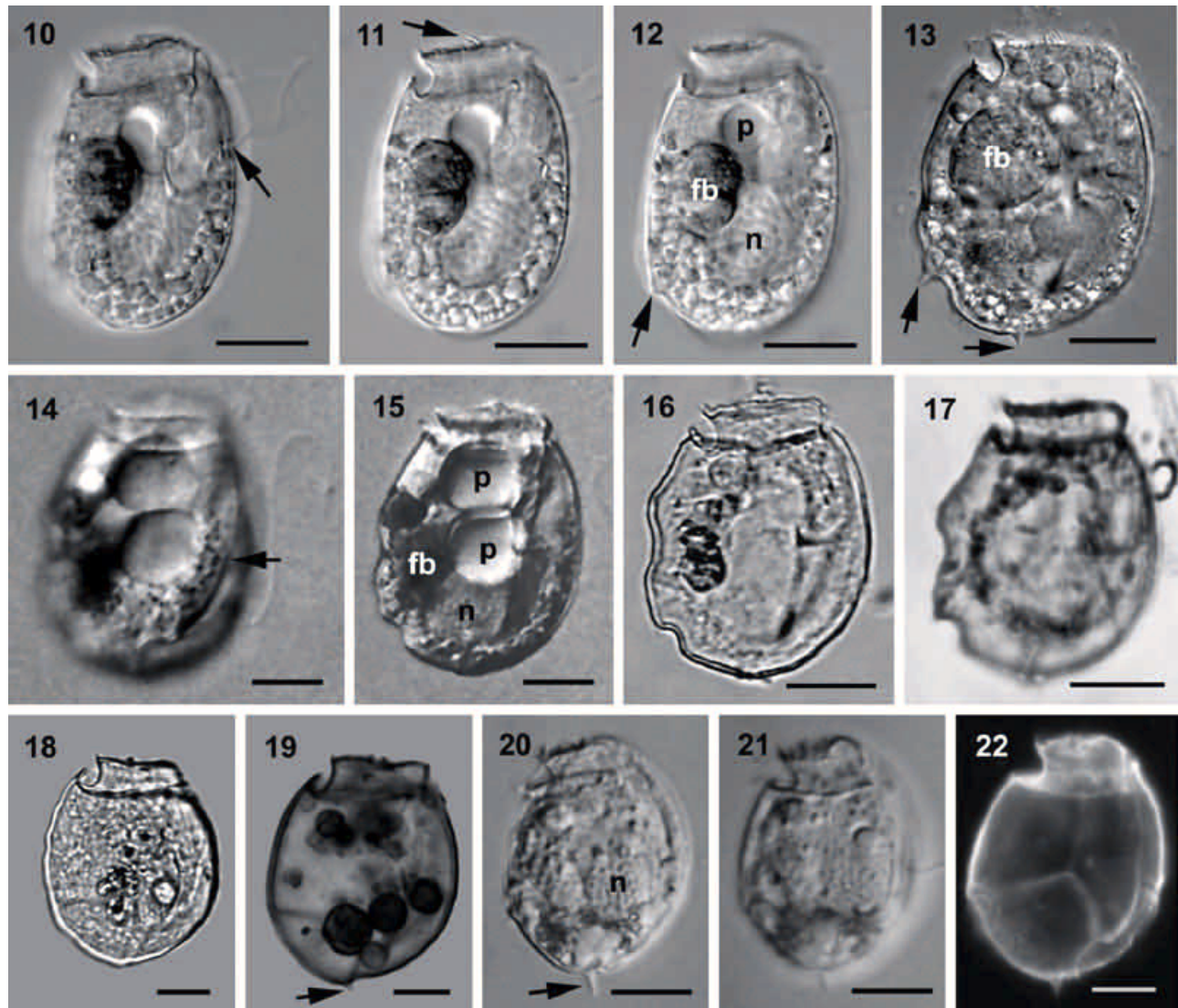
### Morphology

Light micrographs and a drawing of lateral views (Figs 10–23), line drawings of the thecal plate tabulation (Figs 24–26), scanning electron micrographs (Figs 27–49), and light micrographs of the sulcal area and the dissected sulcal plates (Figs 50–56) of *Sabulodinium undulatum* are shown.

Cells were laterally flattened, more or less oval with a truncated apex in lateral view (Figs 10–22) and elongated-elliptical in ventral view (Fig. 50). The dorsal margin of the hyposome was, in most cases, characteristically irregular in shape (i.e. 'undulating'; Figs 13–17), but this feature seemed to be very variable (Figs 10–22). The episome was very small and flat, with a characteristically pointing dorsal side (Figs 10–23). The cingulum was deep and not displaced, and the sulcus was positioned on the right lateral side of the cells (Figs 14,19,22,23). The specimens were generally colorless (lacked chloroplasts) and could contain colored food-bodies that were located in the mid-dorsal half of the hyposome (Figs 10–16). The round to oval nucleus was positioned in the posterior half of the hyposome and one or two pusules were visible in the upper half of the hyposome in the middle of the cell depth (Figs 10–12,14,15). The transverse flagellum ran completely around the cell inside of the cingulum and the longitudinal flagellum seemed to insert about half of the way down the hyposome (Figs 10,14) and was somewhat longer than the cell length.

Cells were 32.0–42.0  $\mu\text{m}$  long and 25.0–36.0  $\mu\text{m}$  deep in Germany; 29.0–35.0  $\mu\text{m}$  long and 20.0–25.0  $\mu\text{m}$  deep in Canada; 27.5–42.5  $\mu\text{m}$  long and 19.8–32.5  $\mu\text{m}$  deep in Russia; and 29.0–32.5  $\mu\text{m}$  long and 18.5–22.5  $\mu\text{m}$  deep in Japan. The total size range for the species worldwide was 27.5–42.5  $\mu\text{m}$  long and 18.5–36.0  $\mu\text{m}$  deep. Depth means the length along the lateral sides (dorsoventral width). Additional details relating to cell sizes are shown in Table 2.

The following descriptions of theca details and morphological variation are for the type variety, *S. undulatum* var. *undulatum* (see *Taxonomy* below), if not otherwise indicated. The plate formula was apical pore complex (APC) 5' 1a 6'' 5c 4s 6''' 1'''' (Figs 24–56). All thecal plates were smooth with scattered, relatively large, rimmed pores (39,40,43–49) that were not visible under the light microscope. The variability of the dorsal theca shape was demonstrated by SEM (Figs 28–38). The lower dorsal margin of the cell was either slightly irregular (undulating) with rounded edges (Figs 28,29,35), or had more pronounced undulations (Fig. 37), containing one spiny dorsal edge (Figs 31,36) or two spiny edges – one dorsal and one posterior (Figs 30,32–34,38). This variation was observed within one geographic population (in Germany). Cells with spiny edges were also observed in Canada (Figs 13,40) along with an additional variant possessing a nearly smooth/straight dorsal margin with one dorsal spiny edge (Figs 10–12,39). A variety with a more rounded and totally smooth/straight dorsal margin, which sometimes had a very tiny posterior spine, was observed in Russia (Figs 18,19,42). These specimens represent the proposed new variety *S. u.* var.



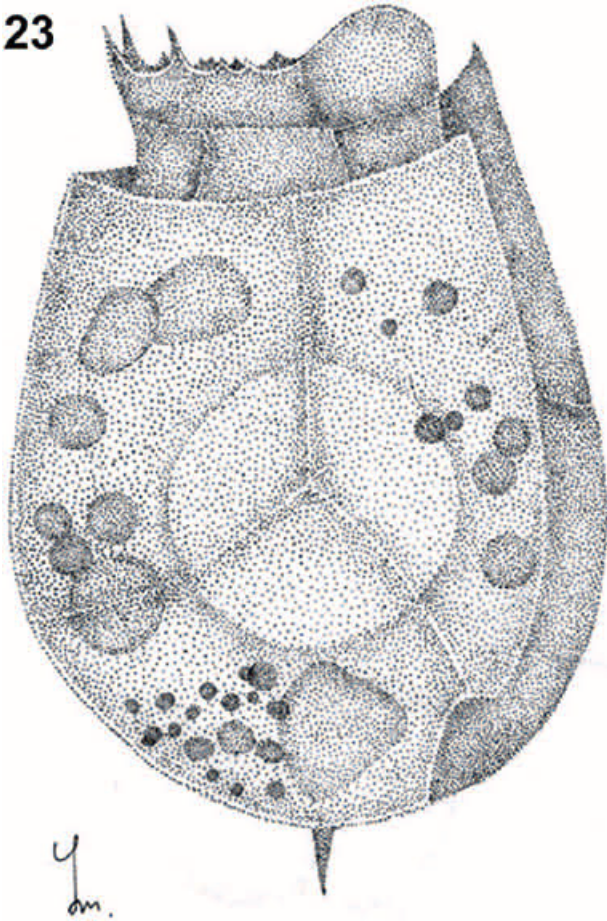
**Fig. 10–22.** Light micrographs of *Sabulodinium undulatum*. 10–12. Same cell (Canada) in different focal planes showing the general morphology. 10. Note the insertion of the longitudinal flagellum (arrow). 11. Focus on the parallel projections on the episome (arrow). 12. The arrow points on the spiny dorsal edge and pusule, food body and nucleus are visible. 13. Cell (Canada) with undulating dorsal hyposome margin with dorsal and posterior spine (arrows). 14,15. Same cell (Germany) in different focal planes showing the general morphology. 14. Focus on the right lateral side; note the insertion of the longitudinal flagellum (arrow). 15. Note the pusule, food body, and nucleus. 16. Cell (Germany) with undulating dorsal hyposome margin without spines. 17. Fixed cell (Russia) with undulating dorsal hyposome margin. 18. Specimen (Russia) with smooth/straight dorsal margin without spines, representing the proposed new variety *S. undulatum* var. *glabromarginatum* (see *Taxonomy* herein). 19. Specimen (Russia) with smooth/straight dorsal margin with one tiny posterior spine (arrow), representing the proposed new variety *S. undulatum* var. *glabromarginatum* (see *Taxonomy* herein). 20,21. Same cell in different focal planes showing the general morphology (Japan). This specimen is representing the proposed new variety *S. undulatum* var. *monospinum* (see *Taxonomy* herein). 20. Specimen with smooth/straight dorsal margin with a prominent posterior spine (arrow), also note the nucleus (n). 22. Epifluorescent micrograph of a FB 28-stained cell showing the tabulation pattern of the right lateral cell side (Japan). This specimen is representing the proposed new variety *S. undulatum* var. *monospinum* (see *Taxonomy* herein). Scale bars = 10  $\mu$ m. fb, food body; n, nucleus; p, pusule.

*glabromarginatum* (see *Taxonomy* below). In addition, we found a more rounded and totally smooth/straight cell shape with a prominent antapical spine in Japan (Figs 20–23). These specimens represent the proposed

new variety *S. u.* var. *monospinum* (see *Taxonomy* below). The hypothecal plate formula seems to be the same in all of these varieties, with some slight size differences associated with the sixth postcingular plate



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**Fig. 23.** Line drawing showing the right lateral view of the *Sabulodinium undulatum* morphotype from Japan with one prominent antapical spine. This specimen is representing the proposed new variety *S. undulatum* var. *monospinum* (see *Taxonomy* herein).

and the first antapical plate (e.g. Figs 31,32,39, compared to the others).

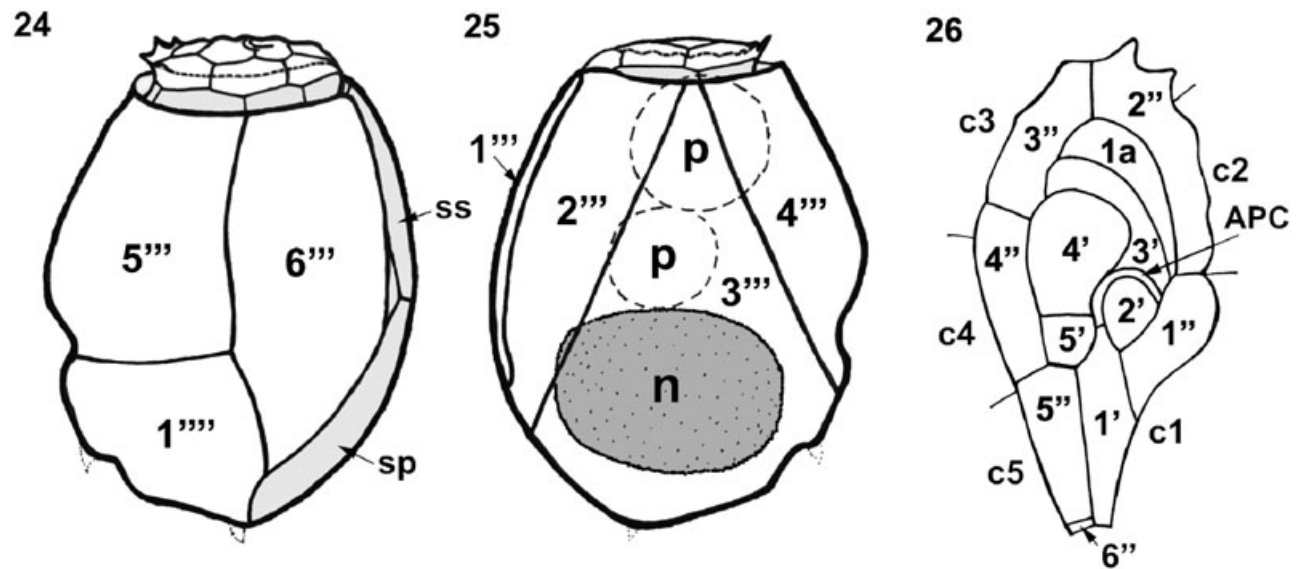
The APC was almost never visible, because it was covered by two protrusions from the apical plates (Figs 45,46) or mucus. These two pore-coverings were named 'upright projections', which is consistent with the terminology used for a very similar structure in other dinoflagellates (see *Discussion*). These upright projections could be visible in lateral views of the cell (Figs 48,49), even under the light microscope (Fig. 11). The outer pore plate (Po) had two parallel 'lips' running straight in the center of the plate (Figs 47,49). The shape of the pore was not visible. There were five apical plates of very different shape (Figs 26,45,46). The first apical plate (1') was elongated; the second (2') was small and rounded and protruded over the Po plate; the third (3') was narrow, running over the dorsal episome part; the fourth (4') varied in size and had a rounded shape; and the fifth (5') was small and pentagonal (Figs 26,45,46). One

anterior intercalary plate (1a) was elongated with a bent shape and was positioned dorsally (Figs 26,45,46). Six precingular plates were observed, with the sixth (6'') being extremely tiny (Figs 26,45,46). The second (2'') and third (3'') precingular plates had a wavy to spiny outer margin (Figs 39,41,45,46,48,49). The dorsal end of both of these plates formed upwards-pointing projections of the margin (Figs 48,49), that were responsible for the characteristic dorsal shape of the episome. The precingular plates had a very thick appearance in the lateral cell view, because the border of the plates was running down into the cingulum (Figs 24,25,48).

The cingulum was deep, not displaced and consisted of five plates (Fig. 26). It ran completely around the cell and made contact with the sulcus. The postcingular plates formed a smooth, narrow cingular list (Figs 39–42,49), running like a collar around the upper hyposome border.

The hyposome was covered mainly by six large plates, five postcingular plates and one antapical plate (Figs 24,25,27–38). In addition, one very narrow first postcingular plate (1''') ran along the left sulcal margin (Figs 25,43,44). The left lateral hyposome consisted of three large postcingular plates (2'''–4'''), with the third postcingular plate (3''') having a nearly triangular appearance with rounded posterior margin (Figs 25,33–38,40). The right lateral hyposome consisted of three large plates, the fifth (5''') and sixth (6''') postcingular plates and the antapical plate (1''') (Figs 24,28–32). The sixth postcingular plate had a (sulcal) list along its ventral margin (Figs 28–30,32,41). A special feature of this species/genus was the position of the antapical plate, which was restricted to the right lateral cell side of the cell. The dorsal and antapical spines, if present, were part of the antapical plate margin (Figs 30–32,39,41). The fourth and fifth postcingular plates could have a dorsal, pointed edge, which looked like an additional spine (Figs 34,37).

The lateral flatness (Figs 27,50) of the cell made it difficult to study the sulcus. Part of the sulcus was visible at the right lateral side, where it ran down the cell from the cingulum to the antapex (Figs 24,28–32,39,41). The posterior sulcal plate (sp.) was visible in all of the SEM images. Sometimes the left sulcal plate (ss) was also partly visible, lying above the sp. plate and making contact with the cingulum (Fig. 24). By dissecting single cells under the light microscope, we were able to discern four sulcal plates (Figs 51–56). The right sulcal plate (sd, Figs 51,52) and the small anterior sulcal plate (sa, Figs 51,52) were hidden behind the sixth postcingular plate in the right lateral cell views. The longitudinal flagellum seemed to emerge from a point where the sp. and ss plates meet.



**Fig. 24–26.** Line drawings of *Sabulodinium undulatum* showing the thecal plate tabulation pattern. 24. Right lateral view, bold line around the hypotheca indicates the position of the 'presagittal' suture. 25. Left lateral view, bold line around the hypotheca indicates the position of the 'presagittal' suture. 26. Apical view of the epitheca with indicated positions of the cingular plates. APC, apical pore complex; c1–c6, cingular plate series; n, nucleus; p, pusule; sp, posterior sulcal plate; ss, left sulcal plate; 1'–5', apical plate series; 1a, first anterior intercalary plate; 1''–6'', precingular plate series; 1'''–6''', postcingular plate series; 1''''', first antapical plate.

## Taxonomy

*Sabulodinium undulatum* var. *undulatum*  
(Figs 10–17, 24–41, 43–56)

Typification: Saunders and Dodge (1984), Figure 31a–c.

Taxonomical syn. *Phalacroma kofoidii* (colorless variety) *sensu* Herdman (1924).

Misidentifications: *Thecadinium petasatum*, partim, *sensu* Baillie (1971).

*Thecadinium kofoidii sensu* Larsen (1985).

Slightly elongated oval specimens with more or less undulating dorsal theca margin. The antapical plate could form one (dorsal) or two (dorsal and posterior) small spines. Cell sizes are 27.5–42.0 µm long and 20.0–36.0 µm deep.

Habitat: Known from eulittoral and sublittoral sand in Germany, the Netherlands, Denmark, UK, France, Canada, and Russia.

*Sabulodinium undulatum* var. *glabromarginatum*  
(Figs 18, 19, 42)

More round specimens with smooth/straight dorsal theca border. The antapical plate could form one very small posterior spine. Cell sizes are 35.0–42.5 µm long and 30.0–32.5 µm deep.

Diagnosis: *Cellula rotundior cum recta/levi thecae margine*. *Scutella antanteriora aliquando habens unam minimam posteriorem spinam*. *Cellulae 35.0–42.5 µm longae et 30.0–32.5 µm altae*.

Holotype: Figure 18.

Habitat: Known from sublittoral sand in Russia.

*Sabulodinium undulatum* var. *monospinum*  
(Figs 20–23)

More round specimens with smooth/straight dorsal theca border. The antapical plate forms one prominent posterior spine. Cell sizes are 29.0–32.5 µm long and 18.5–22.5 µm deep.

Diagnosis: *Cellula rotundior cum recta/levi thecae margine*. *Scutella antanteriora habens unam magnam prominentem posteriorem spinam*. *Cellulae 29.0–32.5 µm longae et 18.5–22.5 µm altae*.

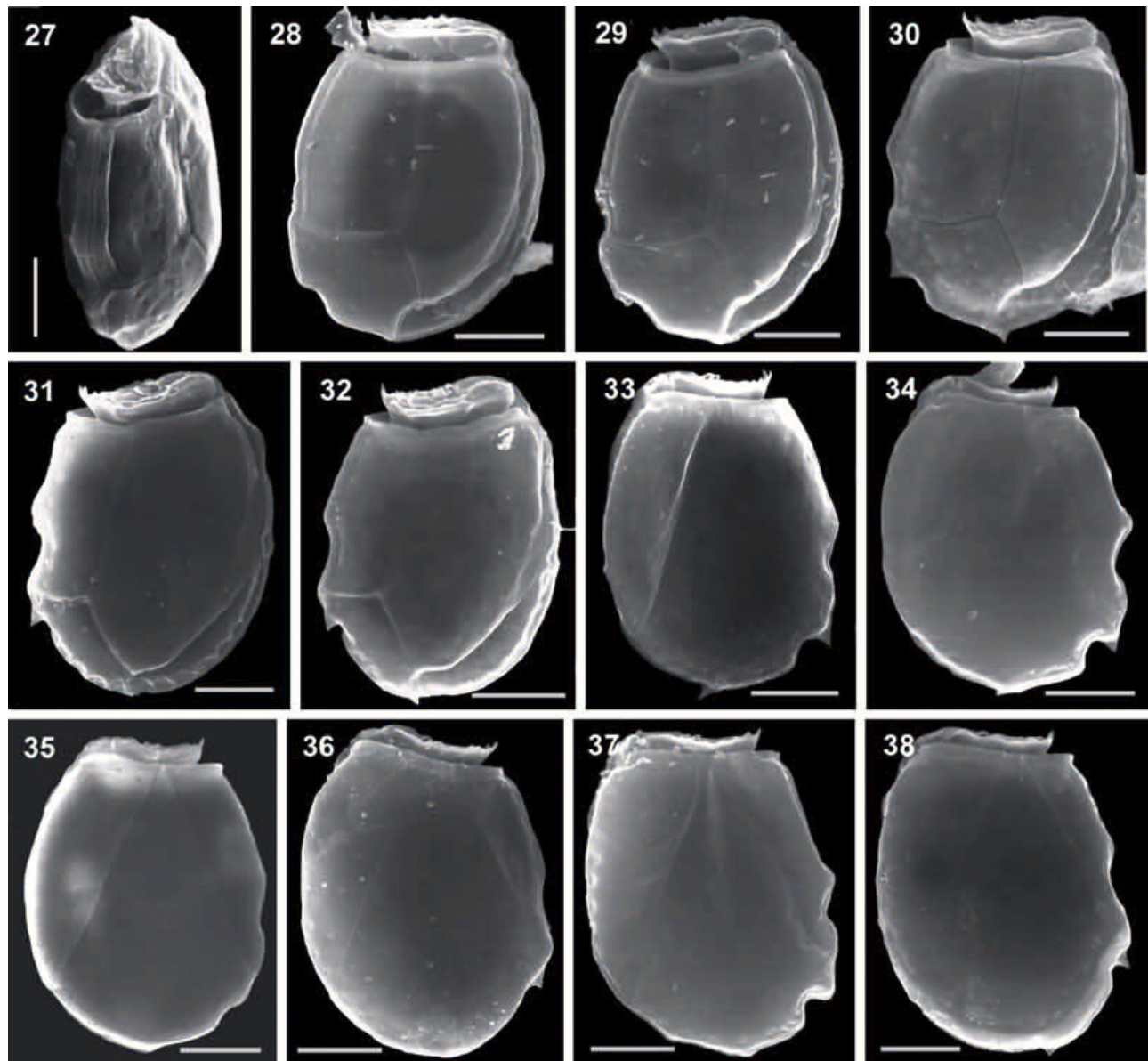
Holotype: Figure 20.

Habitat: Known from beach sand in Japan.

## Molecular phylogeny

We generated two different SSU rDNA sequences from a PCR product derived from genomic DNA extracted from one (wild) multicell sample of *Sabulodinium undulatum* var. *undulatum*. The two sequences differed in six nucleotide positions, which were included in the analyses (1732 included sites). A 24-nucleotide-fragment was missing (deletion) in one sequence (excluded from the analyses). In both the ML and Bayesian analyses, the nearest sister lineage to the *Sabulodinium* clade was *Dinophysis norvegica*, but the relationship was supported by very weak bootstrap





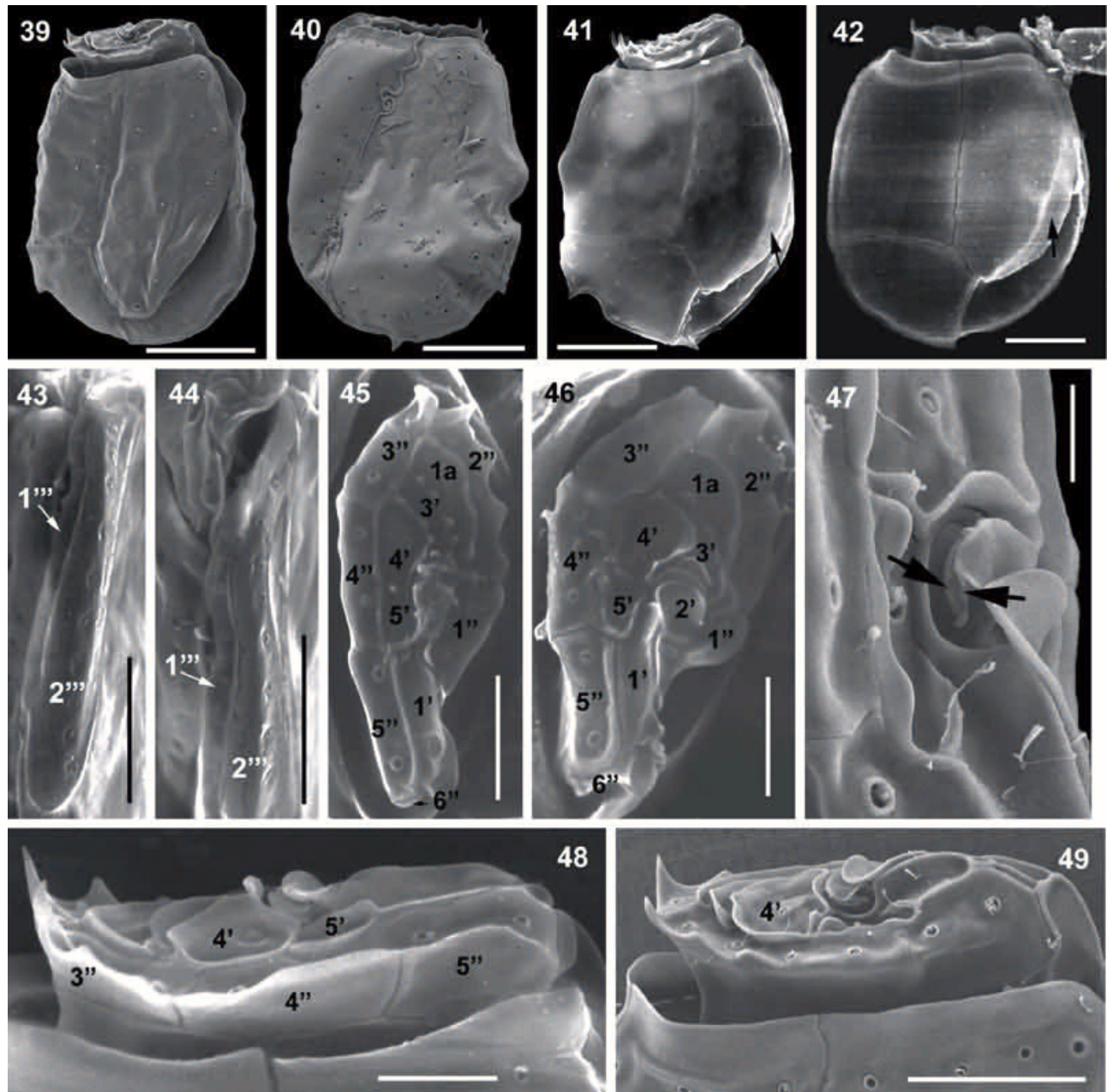
**Fig. 27–38.** Scanning electron micrographs of *Sabulodinium undulatum* showing a dorsal view and the morphological variability of the dorsal hyposome margin in the German population. 27. Dorsal view showing the ‘presagittal’ suture. 28–32. Right lateral views. 33–38. Left lateral views. Scale bars = 10 μm.

values and Bayesian posterior probabilities (Fig. 57). The *Sabulodinium* clade was only distantly related to other sand-dwelling, thecate dinoflagellates (included taxa: *Adenoides eludens* (Herdman) Balech, *Thecadinium kofoidii* (Herdman) Larsen, *Thecadinium yashimaense* Yoshimatsu, Toriumi et Dodge, *Thecadinium dragescoi* Balech, *Pileodinium ciceropse* Tamura et Horiguchi, *Amphidiniella sedentaria* Horiguchi, and *Roscoffia capitata* Balech), which branched as several independent lineages from an unresolved dinoflagellate backbone. All of the sand-dwelling taxa have distinctive and unusual tabulation patterns (Table 3) and their

relationships to other dinoflagellate taxa and to one another were not resolved with the phylogenetic analyses of SSU rDNA.

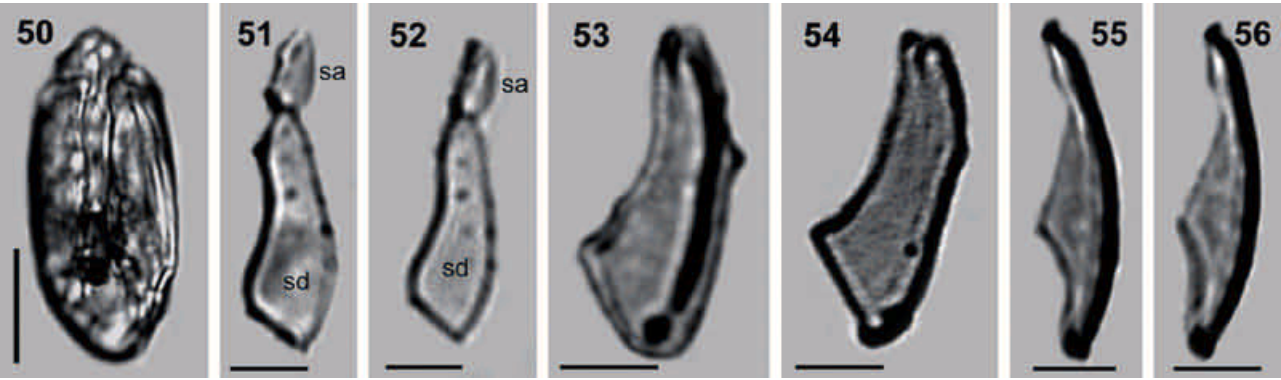
### Biogeography and ecology

**Geographic distribution:** The species has only been recorded in northern temperate regions of the world (Fig. 58). The different varieties of *Sabulodinium undulatum* were found (i) in the North Frisian Wadden Sea, Germany (ii) in the southern part of the west coast of British Columbia, Canada (iii) in Peter the Great Bay,



**Fig. 39–49.** Scanning electron micrographs of *Sabulodinium undulatum*. 39. Right lateral side of a Canadian morphotype with nearly straight dorsal hyposome margin and a dorsal spiny edge. 40. Left lateral side of a Canadian specimen with undulating dorsal hyposome margin. 41. Right lateral side of a Russian specimen with undulating dorsal hyposome margin and sulcal list (arrow). 42. Right lateral side of a Russian specimen with straight/smooth dorsal hyposome margin, tiny posterior spine, and sulcal list (arrow), representing the proposed new variety *S. undulatum* var. *glabromarginatum* (see *Taxonomy* herein). 43,44. Detail of the ventral side of a cell (from Germany) showing the very narrow first postcingular plate (1''') running along a part of the ventral margin of the second postcingular plate (2''). 45,46. Apical views of the epitheca showing the plate morphologies (German specimens). 47. The apical pore complex is visible underneath two parallel 'upright projections'. Note the two 'lips' (arrows) in the center of the pore plate (Canadian specimen). 48,49. Two right lateral views of the epitheca showing general morphology, the two parallel 'upright projections' over the apical pore and the thick border of the precingular plates running down into the cingulum. Note the upwards pointing dorsal margin of the epitheca (Fig. 48. German and Fig. 49 Canadian cell). Scale bars = 10 µm in Figures 39–44; 5 µm in Figures 45,46,48,49 and 1 µm in Figure 47.





**Fig. 50–56.** Light micrographs of a cell in ventral view and of dissected sulcal plates from Russian specimens. 50. Ventral view showing the sulcal area. 51,52. Small anterior sulcal plate (sa) and large right sulcal plate (sd) still connected to each other. 53,54. Posterior sulcal plate. 55,56. Left sulcal plate. Scale bars = 5 µm in Figures 51–56 and 10 µm in Figure 50.

**Table 2.** Known size ranges of *Sabulodinium undulatum* and its varieties

	Length (µm)	Depth (µm)
<i>Sabulodinium undulatum</i> var. <i>undulatum</i>		
Herdman (1924) (UK)	About 30.0	
Baillie (1971) (Canada)	29.0–35.0	20.0–25.0
Present study (Canada)	29.0–32.0	20.0–22.0
Saunders and Dodge (1984) (UK)	35.0	25.0
Present study (Germany)	32.0–42.0	25.0–36.0
Present study (Russia)	27.5–35.0	19.8–30.0
<i>Sabulodinium undulatum</i> var. <i>glabromarginatum</i>		
Present study (Russia)	35.0–42.5	30.0–32.5
<i>Sabulodinium undulatum</i> var. <i>monospinum</i>		
Present study (Japan)	29.0–32.5	18.5–22.5
<i>Sabulodinium undulatum</i> Worldwide		
	27.5–42.5	18.5–36.0

Sea of Japan, Russia (iv) in Aniva Bay and at the eastern coast of Sakhalin Island (v), Okhotsk Sea, Russia, and (vi) at Ishikari Beach, Hokkaido, Japan.

Temporal and spatial distribution at different localities: *Sabulodinium undulatum* was found in all eulittoral areas throughout the year, and it was also present in sublittoral samples in Germany. Maximum cell numbers were registered in spring. These findings were confirmed by the eulittoral samples from Canada. In Russia, Peter the Great Bay, two varieties occurred all year in sublittoral samples, with *S. undulatum* var. *undulatum* only being present from March to September with higher cell numbers in August. The cell concentration of the population of *S. undulatum* var. *glabromarginatum* was always low. In Japan, the variety with a single posterior spine, *S. undulatum* var. *monospinum*, was probably present throughout the year in supralittoral (beach) samples and occurred at all supralittoral stations. The seasonal distribution is sum-

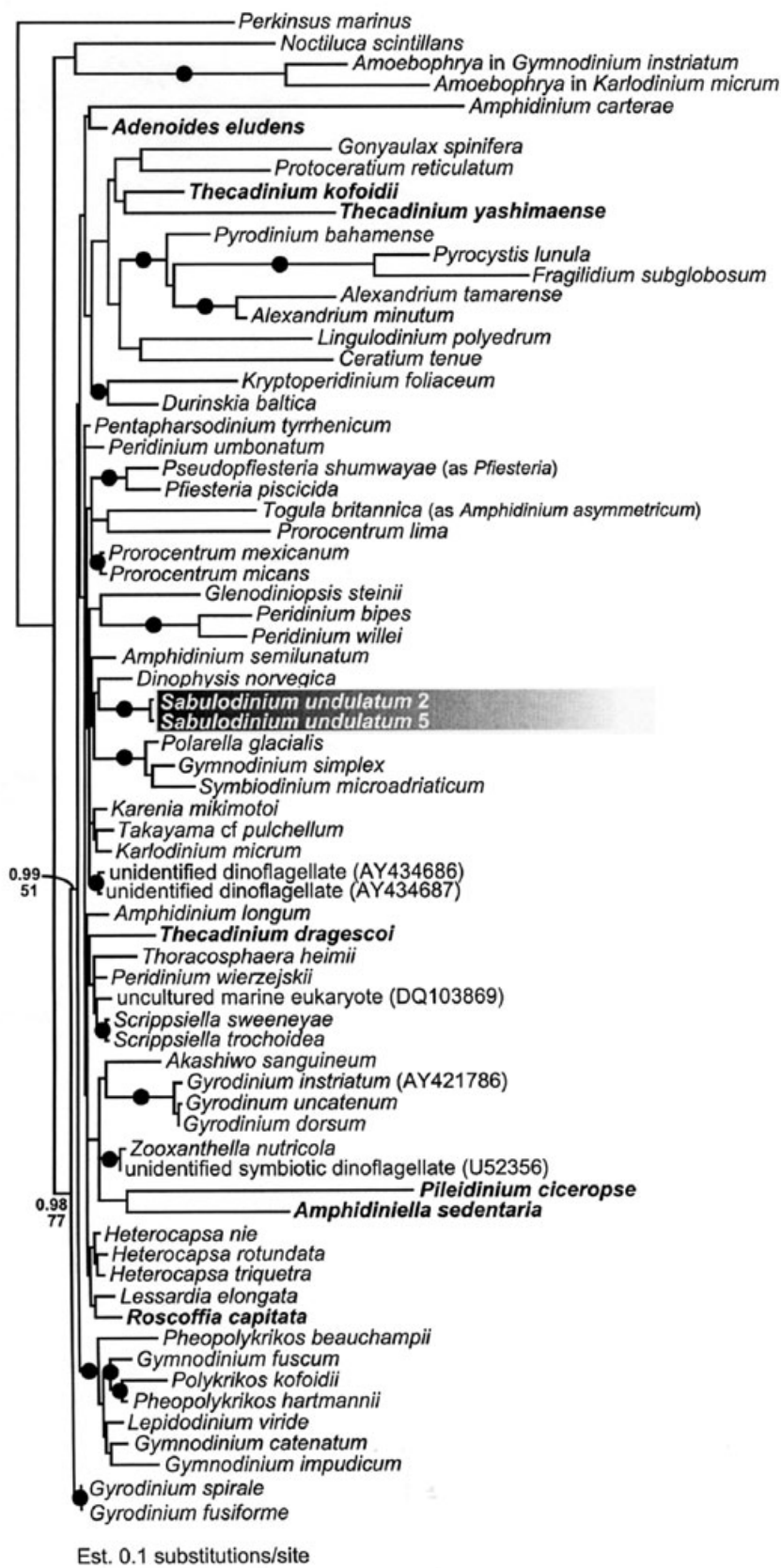
marized in Table 4 and sampling dates for *S. undulatum* records are given in Table 1.

The spatial distribution within profiles through a habitat was investigated for a eulittoral area in Germany and for a beach in Japan (Figs 8,9). Every station in the tidal flat area profile was sampled 20 cm in depth, and the samples were divided into four subsamples of 5 cm each (Fig. 59). *Sabulodinium undulatum* var. *undulatum* occurred in all tidal areas and all investigated depths (except in the 10–15 cm depth at the 80 m station) at the stations (Fig. 59). This does not mean that at every sampling date the species were observed in every subsample, but over the investigation period records accumulated for all subsample sites. Most frequently specimens were found in the upper 5 cm of the sediment at all stations at the same sampling date. It also occurred in sublittoral samples taken sporadically. The Japanese variety *Sabulodinium undulatum* var. *monospinum* was recorded at all stations of the beach profile, but never at all stations at one sampling date. Interestingly, there seems to be a ‘movement’ from the lower beach stations (5 = 5 m and 4 = 10 m) to higher beach stations (2 = 20 m and 1 = 25 m) over the period of a year (Figs 9,60). Specimens were recorded at the stations 5 and 4 from April to July, at station 3 from August to December, at station 2 in November, January, and February, and at station 1 in December (Fig. 60). This could be a first indication that population movement in the beach profile occurs, with the species moving up into higher (more inland) sites from spring to winter and back again.

DISCUSSION

*Sabulodinium undulatum* has some characteristic morphological features: (i) a small episome with the dorsal edge pointing upwards, (ii) two parallel projections on the epitheca covering the apical pore, (iii) insertion of





**Fig. 57.** Gamma-corrected maximum likelihood (ML) tree ( $-\ln L = 17\,541.553$ ,  $\alpha = 0.34$ , 8 rate categories) inferred using the Hasegawa-Kishino-Yano (HKY) model of substitution on an alignment of 71 small subunit rRNA gene (SSU rDNA) sequences and 1571 unambiguously aligned sites. Numbers at the branches denote gamma-corrected bootstrap percentages of 100 replicates using weighted neighbor-joining (top) and Bayesian posterior probabilities – GTR (bottom). Black dots on branches denote bootstrap percentages and posterior probabilities of 95% or higher. Sequences derived from this study are highlighted in shaded boxes. Sequences from sand-dwelling taxa available from GenBank are highlighted with bold letters.

**Table 3.** Plate formulas of 'unusual' thecate, sand-dwelling dinoflagellates

	Po	'	a	''	x	c	s	'''	P	'''
<i>Sabulodinium undulatum</i> <sup>a,p</sup>	Po	5	1	6	—	5	4	6	—	1
<i>Planodinium striatum</i> <sup>b,p</sup>	—	3	—	7	—	6	?	3	—	1
<i>Cabra matta</i> <sup>c,p</sup>	Po	4	—	4	x	3	?	5	—	1
<i>Herdmania littoralis</i> <sup>d,p</sup>	Po	3	2	6	x	7	3	6	1	1
<i>Amphidiniopsis kofoidii</i> <sup>e</sup>	Po	4	3	7	—	3?	3	5	—	2
( <i>Amphidiniopsis korewalensis</i> ) <sup>f</sup>	Po	4	3	6	x	4?	4	6	—	2
( <i>Thecadinium dragescoi</i> ) <sup>g</sup>	Po	4	3	7	x	6	?	5	—	2
<i>Rhinodinium broomeense</i> <sup>h,p</sup>	Po	3	1	5	—	4	?	5	—	1
<i>Roscoffia capitata</i> <sup>i</sup>	Po	3(4)	—	5	—	3	4	5	—	1
<i>Thecadinium kofoidii</i> <sup>j,l</sup>	Po	4	1	3	—	5	5	4	—	1
<i>Plagiodinium belizeanum</i> <sup>k,p</sup>	Po	5	—	—	—	5	5	5	—	1
<i>Pseudothecadinium campbellii</i> <sup>l,p</sup>	Po	4	2	4	—	4	5?	5	—	1
<i>Adenoides eludens</i> <sup>m,p</sup>	Po	4	—	—	—	6	4	5	5	1
<i>Amphidiniella sedentaria</i> <sup>n,p</sup>	Po	4	1	7	—	5	4	6	—	2
<i>Pileodinium ciceropse</i> <sup>o,p</sup>	—	1	—	5	—	4	4	5	—	1

<sup>a</sup>Present study, <sup>b</sup>Saunders and Dodge (1984), <sup>c</sup>Murray and Patterson (2004), <sup>d</sup>Hoppenrath (2000d), <sup>e</sup>Dodge and Lewis (1986), <sup>f</sup>Murray and Patterson (2002), <sup>g</sup>Hoppenrath *et al.* (2004), <sup>h</sup>Murray *et al.* (2006), <sup>i</sup>Hoppenrath and Elbrächter (1998), <sup>j</sup>Hoppenrath (2000b), <sup>k</sup>Faust and Balech (1993), <sup>l</sup>Hoppenrath and Selina (2006), <sup>m</sup>Hoppenrath *et al.* (2003), <sup>n</sup>Horiguchi (1995), <sup>o</sup>Tamura and Horiguchi (2005).  
<sup>p</sup>Monospecific. ( ), no type species.

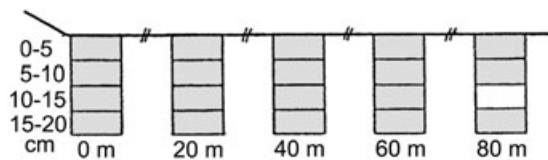
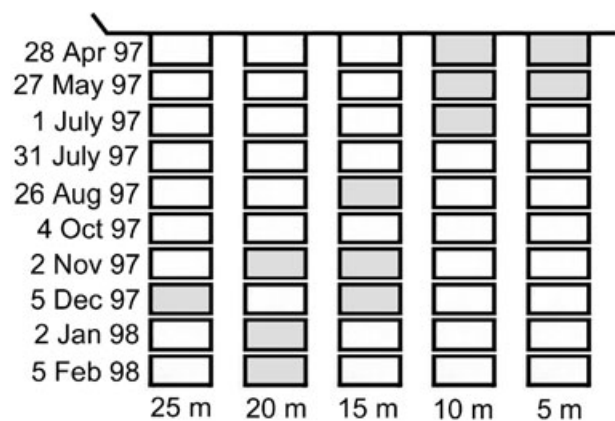


**Fig. 58.** World map showing the geographic distribution of *Sabulodinium undulatum*. Dots, present study; triangle, data from the literature; 1, Northfrisian Wadden Sea, Germany; 2, southern part of the west coast of British Columbia, Canada; 3, Peter the Great Bay, Sea of Japan, Russia; 4, Aniva Bay, Sea of Okhotsk, Russia; 5, eastern coast Sakhalin Island, Sea of Okhotsk, Russia; 6, Ishikari Beach, Hokkaido, Japan; 7, Port Erin, Isle of Man, UK (Herdman 1924); 8, Holy Island, Northumberland, UK (Saunders & Dodge 1984); 9, North Sutherland, Scotland (Dodge 1989); 10, Roscoff, France (Saunders & Dodge 1984); 11, Danish Wadden Sea, Denmark (Larsen 1985); 12, the Dutch Wadden Sea (Koeman, pers. comm.).

**Table 4.** Seasonal distribution of *Sabulodinium undulatum* at different localities

	J	F	M	A	M	J	J	A	S	O	N	D
Germany	+	+	+	+	+	+	+	+	+	+	+	+
Canada	–	+	+	+	+	+	+	+	+	+	?	?
Russia†	–	?	+	–	+	+	+	+	+	–	–	?
Russia‡	+	?	+	+	+	+	+	+	+	+	+	?
Japan§	+	+	?	+	+	?	+	+	?	–	+	+

†*S. undulatum* var. *undulatum*. ‡*S. undulatum* var. *glabromarginatum*. §*S. undulatum* var. *monospinum*. ?, no sample/data.

**59****Fig. 59.** Schematic drawing of the sampling sites of the profile through the eulittoral at the List harbor tidal flat, Germany, showing the distribution of *Sabulodinium undulatum* var. *undulatum* (gray subsample in the profile means that *S. undulatum* was present). Every 20 m a sampling site was sampled 20 cm in depth and the sample divided into four subsamples each of 5 cm depth. The 0 m site corresponded to the border between the steep beach face and the tidal sand flat. The 80 m site was close to the medium low tide line.**60****Fig. 60.** Schematic drawing of the sampling sites of the profile through the beach in Japan, showing the distribution of *Sabulodinium undulatum* var. *monospinum* (gray in the profile means that *S. undulatum* was present).

the longitudinal flagellum nearly half way down the hyposome, (iv) the sulcus lying on the right lateral cell side, and (v) the antapical plate completely positioned at the right lateral cell side. Some of these features were also observed in other thecate dinoflagellate

species, but none showed the complete set. In addition, the thecal plate formula is different from all other taxa with an 'unusual' tabulation pattern (Table 3). For these reasons, maintaining a distinct genus for this species is warranted. Under the light microscope, the species is most difficult to distinguish from *Planodinium striatum* Saunders et Dodge (Saunders & Dodge 1984), and in fact, the description of *Thecadinium petasatum* by Baillie (1971) probably includes observations on both *S. undulatum* and *P. striatum*. The theca morphology of these two species is significantly different (Table 3) and the upwards-pointing dorsal epitheca helps to distinguish them when viewed with the LM. The apical pore of *S. undulatum* (Figs 47,49) resembles the apical pores of *Thecadinium kofoidii* and *Thecadinium neopetasatum* Saunders et Dodge in respect of the two parallel 'lips' (projections) in its center (Hoppenrath 2000b). *Thecadinium kofoidii* has one and *T. neopetasatum* also two (Hoppenrath 2000b). We do not know the shape of the apical pore of *S. undulatum*, which is needed for a more detailed comparison. The flagellar insertion resembles the one in *T. kofoidii* (Hoppenrath 2000b). In both species, *S. undulatum* and *T. kofoidii*, the longitudinal flagellum seems to be displaced in the posterior direction and insert apart from the transverse flagellum. However, the plate formula of the two species is different (Table 3), and *T. kofoidii* is photosynthetic. The position of the antapical plate on the right lateral cell side is shared only with the photosynthetic *Pseudothecadinium campbellii* Hoppenrath et Selina, albeit the hypothecal plate arrangement is entirely different (Table 3) (Hoppenrath & Selina 2006). To our knowledge, this feature is only shared by *S. undulatum* and *P. campbellii*.

Two characteristic features of *S. undulatum* are shared with certain species of the genus *Sinophysis*: (i) the sulcus lying on the right lateral side of the cell and (ii) the micromorphological structure of the epitheca (Figs 47–49), which resembles the parallel, curved, upright projections of *Sinophysis* species (e.g. *Sinophysis microcephala* Nie et Wang, *Sinophysis canaliculata* Quod, Ten-Hage, Turquet, Mascarell et Couté, and *Sinophysis stenosoma* Hoppenrath; Faust 1993; Quod *et al.* 1999; Hoppenrath 2000c; Selina & Hoppenrath 2004). The genus *Sinophysis* belongs to the order Dinophysiales (e.g. Selina & Hoppenrath 2004). Dinophysiales are characterized by a sagittal suture, which has a zigzag course and extends the entire length of the cell (Fensome *et al.* 1993). This sagittal suture completely splits the theca of dinophysialean taxa into two lateral halves. The hypotheca of *Sinophysis*, as for *Dinophysis*, consists mainly of two extremely large plates nearly covering the complete lateral cell sides. The thecal plate arrangement of *S. undulatum* does not really resemble the tabulation pattern of *Sinophysis* or *Dinophysis* species. However, the hypothecal plate arrange-



ment of *Sabulodinium* shows a sagittal suture-like feature, in that the hypothecal plates could be split in two lateral halves along a 'sagittal' suture with a smooth margin (Figs 24,25,27). Interestingly, a fossil taxon is known to have a sagittal suture being present only on the hypotheca, namely *Nannoceratopsis* Deflandre (Fensome *et al.* 1993). This genus is the only one in the order Nannoceratopsiales, which, together with the order Dinophysiales, were classified within the subclass Dinophysiphyidae (Fensome *et al.* 1993). The subclass is circumscribed as 'Dinophyceans which possess a cingulum, sulcus and sagittal suture' (Fensome *et al.* 1993). In addition to the sagittal suture, which has a zigzag course and may either extend the entire length of the cell or only the length of the hyposome, the flagella are inserted ventrally and emerge from a single flagellar pore (Fensome *et al.* 1993). The hyposome of *Nannoceratopsis* also consists of two large lateral plates, but the cingulum and the episome resemble the Peridiniphyidae type of tabulation. Therefore, these features in *Nannoceratopsis* are inferred to be transitional between Dinophysiales and Peridiniphyidae (Fensome *et al.* 1993; Taylor 2004).

The characteristics of *Sabulodinium* seem to provide additional continuity to the transition between Nannoceratopsiales/Dinophysiales and Peridiniphyidae. For instance, *Sabulodinium* possesses a possible precursor to the sagittal suture, which we propose to name the 'presagittal suture', with a smooth border extending only on the hyposome. Moreover, *Sabulodinium* possesses an epitheca and cingulum with the Peridiniphyidae form of tabulation and still has several large plates covering the lateral cell sides and most probably two flagella pores. The sister relationship between *Sabulodinium* and *Dinophysis* in our SSU rDNA phylogenies (Fig. 57) is consistent with this scenario. Unfortunately, the support for this topology was very weak, and no molecular sequence data are available for *Sinophysis* and *Nannoceratopsis*. Nonetheless, this hypothesis of morphological character evolution, which is described and illustrated in Figure 61, can be tested and refined with additional molecular phylogenetic markers from extant taxa.

*Sabulodinium undulatum* showed a relatively wide range of morphological variability in the dorsal hyposome margin: totally smooth without spines and undulations, smooth with an antapical spine, undulated, and undulated with spines. The hypothecal plate pattern seems to be identical in all observed populations. The epithecal tabulation is only known for morphotypes described from Germany. Slight differences were already detected for the sizes of the sixth postcingular, the first antapical plates and the fourth apical plate. Moreover, the most striking differences in the hyposome morphology seem to correlate with distinct habitats. From these observations, we conclude that we are

dealing with varieties, and we propose to distinguish three varieties of *Sabulodinium undulatum*: *S. undulatum* var. *undulatum*, *S. undulatum* var. *glabromarginatum*, and *S. undulatum* var. *monospinum*.

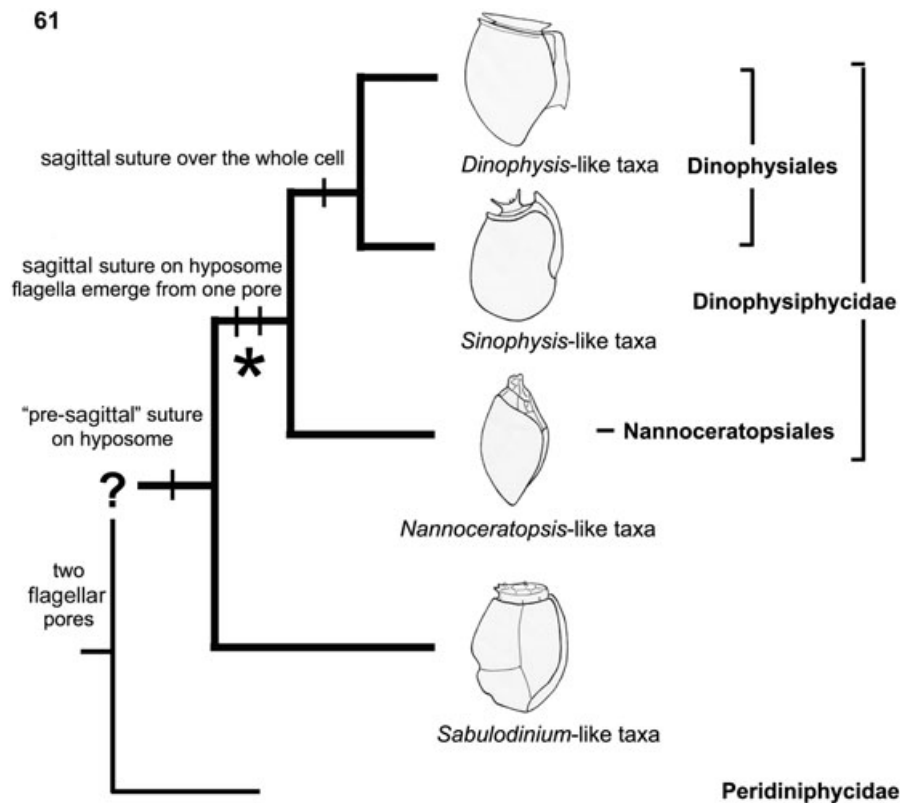
The distribution of *S. undulatum* seems to be restricted to the northern temperate regions of the world (Fig. 58). In the present study, we found the different varieties of *S. undulatum* (1) in the North Frisian Wadden Sea, Germany, (2) in the southern part of the west coast of British Columbia, Canada, (3) in Peter the Great Bay, Sea of Japan, Russia, (4) in Aniva Bay and at the eastern coast of Sakhalin Island, (5), Okhotsk Sea, Russia, and (6) at Ishikari Beach, Hokkaido, Japan. Published findings of *S. undulatum* are (7) Port Erin, Isle of Man, UK (Herdman 1924) (8) Holy Island, Northumberland, UK (Saunders & Dodge 1984) (9) North Sutherland, Scotland (Dodge 1989) (10) Roscoff, France (Saunders & Dodge 1984; note added in proof on p. 283), and (11) Danish Wadden Sea, Denmark (Larsen 1985). It was also found in (12) the Dutch Wadden Sea (53°26.57'N, 06°30.49'E) in 1992 (Koeman, pers. comm. 1992). There are no records from tropical regions or the southern hemisphere.

To the best of our knowledge, no comparable data about temporal and spatial distributions of any other sand-dwelling dinoflagellate species have been published yet.

The beach habitat is extremely variable in its physical conditions, with changes of the water temperature from -2°C to +22°C and of the salinity from 1 to 36. *Sabulodinium* occurred from -2°C to +20°C and 4–35 salinity. In eulittoral areas (in Germany) the temperature range of the interstitial water was -1.6 to +22.5°C but the salinity range was only 30–32 during the morning when sampling took place. In the sublittoral habitat, changes of the water temperature from -1.4 to +24.3°C and of the salinity from 2 to 35 occurred. In general, the marine interstitial habitat in eulittoral areas is comparable, with even higher total temperatures at low tides in summer during midday, high salinities at dry summer days and low tide during midday (high evaporation of interstitial water) and low salinities during raining at low tide times (freshwater input). For the Canadian eulittoral site (Boundary Bay), a temperature range from 0.0 to 32.0°C and a salinity range from 12 to 33 is known (Baillie 1971). Therefore, our data help demonstrate that sand-dwelling dinoflagellates are tolerant of wide-ranging changes in physical parameters.

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**Fig. 61.** A hypothetical framework for understanding character evolution in *Sabulodinium undulatum* and Dinophysiphyceae. A Peridiniphyceae-like ancestor gave rise to a *Sabulodinium*-like lineage possessing a small episome and a large hyposome with a Peridiniphyceae-like plate pattern and a smooth sagittal suture-like structure ('presagittal suture') over the hyposome. This lineage possessed two flagellar pores and a sulcus lying on the right lateral side of the cell. The *Sabulodinium*-like lineage gave rise to the Dinophysiphyceae consisting of two main sister lineages, namely the Dinophysiales and Nannoceratopsiales. In the most recent common ancestor of the Dinophysiphyceae, the sagittal suture became serrated (i.e. zigzag-shaped), the two flagellar pores merged into one, and the lateral plates fused (\*). In the Nannoceratopsiales lineage, the sagittal suture extended only over the hyposome and the sulcus had a real ventral position. In the Dinophysiales lineage, the serrated sagittal suture extended over the whole cell. Within the Dinophysiales lineage, the right lateral position of the sulcus and the two upright parallel projections in *Sinophysis* were retained from the *Sabulodinium*-like ancestor, and the sulcus subsequently migrated to a real ventral position like that found in *Dinophysis*.

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