

# STUDIES ON THE PLANKTONIC CILIATE (PROTOZOA, CILIOPHORA) GENUS *GYMNOZOOM* (MEUNIER, 1910), WITH DESCRIPTION OF A NEW SPECIES *G. SMALLI* N. SP. FROM THE SOUTHERN COAST OF NORWAY AND SUPPLEMENTAL DESCRIPTION OF *G. INTERMEDIUM* FROM THE BARENTS SEA

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## SARSIA



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Two species of the genus *Gymnozoum* have been identified on the basis of protargol stained samples. *G. intermedium* which was found in field and cultivated samples from the Barents Sea, had five more kineties than in the original description. One contractile vacuole pore (sometimes two) was observed. This had pronounced canals situated in the middle of the cell, usually between somatic kineties n-7 and n-8. An area with densely packed kinetosomes was observed near the posterior end of one kinety, kinety 'n'. *G. intermedium* is cannibalistic in culture and may feed on smaller specimens. A new species, *Gymnozoum smalli* n. sp. is described based on a sample from the southern coast of Norway. The cell is small, ellipsoid (42  $\mu\text{m}$  x 30  $\mu\text{m}$ ) and has approximately 36 straight somatic kineties and one short curved kinetofragmon at its posterior end. Four of the somatic kineties extend forward and form semicircles around one half of the oral area. In between the semicircling kineties and the cytostome, eight short kineties, densely packed with kinetosomes, are also located. The macronucleus is ellipsoid (20 x 9  $\mu\text{m}$ ) and the micronucleus (2.3 x 1.8  $\mu\text{m}$ ) is spherical. A contractile vacuole pore may be seen between kineties n-4 and n-5. The prominent cytopharyngeal basket is heavily stained in the anterior half and less stained in the posterior half. Nothing is known of the feeding habit of this species. *G. smalli* n. sp. is the first species in this genus reported from a non-polar region.

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KEYWORDS: ciliates; marine; planktonic; description; *Gymnozoum*.

## INTRODUCTION

The genus *Spiroprorodon* was erected by FENCHEL & LEE (1972) who described *S. glacialis* from interstitial Antarctic sea ice. The next species described in this genus was *S. garrisoni*, also found in samples from Antarctica by CORLISS & SNYDER (1986), who also noted that the samples contained more species in the same genus. DALE (1990) and AUF DEM VENNE (1990) first reported *Spiroprorodon* sp. from the Arctic. The species observed by DALE (1990) was later described as *Spiroprorodon intermedium* by AGAÏHA & al. (1993). AUF DEM VENNE (1994) reported that there were probably several species of *Spiroprorodon* present in his samples from the Greenland Sea. ALEKPEROV & MAMAJEVA (1992) erected the new family Kryoprorodontidae and the genus *Kryoprorodon* when describing *K. arcticum* collected from the Bering Sea and the Chuckchee Sea. This genus is very similar to *Spiroprorodon*. On the basis of similarities between

*Spiroprorodon* sp. and the 'forgotten' genus *Gymnozoum* (MEUNIER, 1910), *Spiroprorodon* was recently synonymized with *Gymnozoum* by PETZ & al. (1995). Due to priority *Gymnozoum* is the valid name of the genus. In their paper PETZ & al. (1995) described a new species *Gymnozoum sympagicum* and synonymized *Spiroprorodon garrisoni* (CORLISS & SNYDER, 1986) with *Gymnozoum viviparum* (MEUNIER, 1910) and transferred the genus *Kryoprorodon* (ALEKPEROV & MAMAJEVA, 1992) to *Gymnozoum*. *Gymnozoum viviparum* (MEUNIER, 1910) is thus the type species and it belongs to the family Kryoprorodontidae (ALEKPEROV & MAMAJEVA, 1992).

The present study is based on samples from the Barents Sea and from the Skagerrak. *Gymnozoum intermedium* from field and cultured samples from the Barents Sea is described in further details. *Gymnozoum smalli* n. sp. from the Skagerrak is a new species.

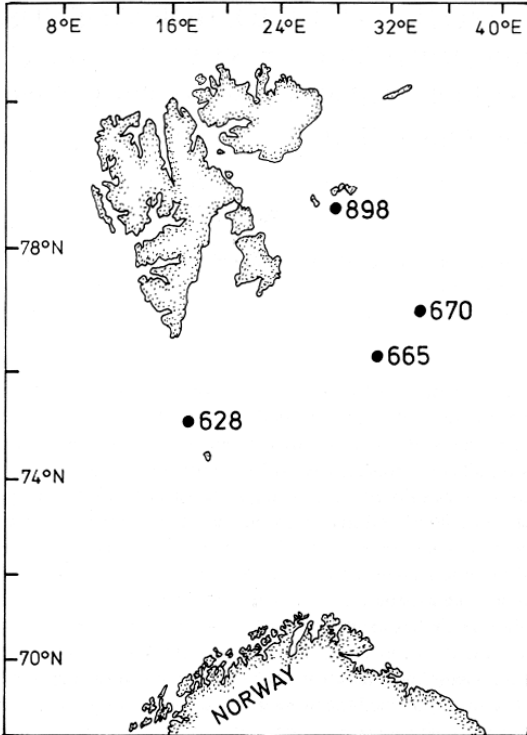


Fig. 1. Map of the Barents Sea where *Gymnozoum intermedium* was found. Wild samples in 1984 from Stns 628, 665, and 670. Cultured samples in 1985 were based on water from Stn 898.

## MATERIAL AND METHODS

The samples containing *Gymnozoum intermedium*, were collected from surface waters of the Barents Sea (Fig. 1) during PRO MARE cruises with R/V *G.O. Sars* 28 May - 18 June 1984 (HASSEL & al. 1984) and 29 July - 19 August 1985 (LOENG & al. 1985). The sampling site for *Gymnozoum smalli* n. sp. was in a bay on the south coast of Norway (Fig. 2). Table 1 shows dates, positions, depths, temperatures, and salinities at the stations.

### *Gymnozoum intermedium*

Two samples were collected by means of a small phytoplankton net (30  $\mu$ m mesh size) from 0-0.5 m at Stn 665 and Stn 670 on 3 June 1984. One sample was collected using a 30-litre Niskin bottle at a depth of 5 m at Stn 628 on 1 June 1984. One litre of this sample was carefully concentrated to about 15 ml using a 20- $\mu$ m plankton mesh. Within a few hours about 15 ml of each of these three samples were preserved with Bouin's fixative (LEE & al. 1985) in ca 25-ml plastic scintillation vials.

The 1985 samples originated from a water sample taken on 12 Aug. with a 30-litre Niskin bottle (Stn 898 from 10 m). Several subsamples of ca 30 ml were incubated in 50-ml Nunc cultivation bottles together with various combinations of the dinoflagellates *Scrippsiella trochoideis* and *Heterocapsa triquetra* and the Prymnesiophyceae *Isochrysis* sp. *G. intermedium* started to grow in three of these samples. These samples were maintained at ca 6-7° C onboard the ship under a 12/12 hour

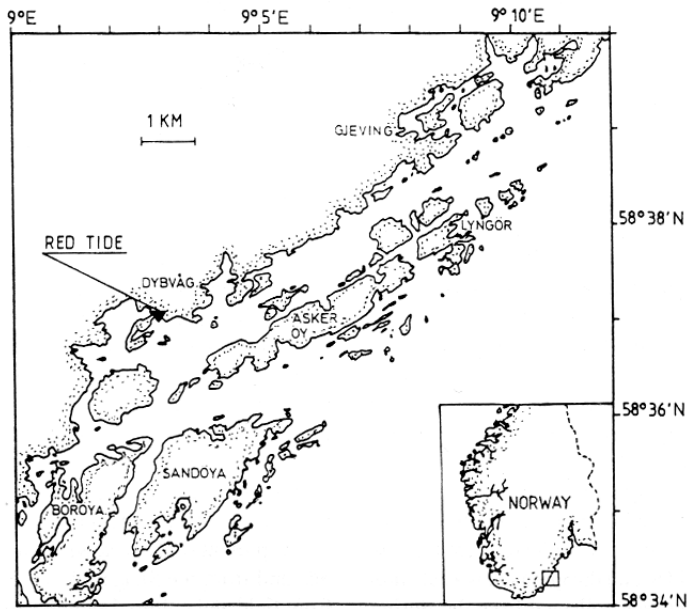


Fig. 2. Map of the Skagerrak area of the south coast of Norway where *Gymnozoum smalli* n. sp. was found in a *Tiarina fusus* red tide sample in the Dybvåg Bay.

light/dark cycle and brought to the Institute of Marine Biology, University of Bergen, where they were kept at 4-5° C under a 12/12 hour light/dark cycle (strong light). When the algal concentrations were low, more algae were added to the cultures. In the period 6 Sept - 3 Oct 1985, four subsamples of cultures with growing strains of *G. intermedium* were preserved with Bouin's fixative as noted above.

The cultures were always contaminated with diatoms (probably *Chaetoceros borealis*, *C. debilis*, and *Nitzschia* sp.) in addition to the algae given as food. Attempts to bring the ciliate into pure cultures with a single algal species as food failed. The ciliate cultures died on 7 Oct 1985, due to a temperature increase resulting from a broken fuse.

#### *Gymnozoum smalli* n. sp.

The sample in which *G. smalli* n. sp. was collected was taken from the surface waters in a small bay on the southern coast of Norway (Fig. 2) on 24 Nov 1986 during a local ciliate bloom of *Tiarina fusus*. The sample was preserved with Bouin's fixative (LEE & al. 1985). Details of the ciliate bloom event are given in DALE & DAHL (1987). The Bouin's fixed samples were stained using Small's protargol technique (see AUF DEM VENNE & al. (1995) which is a modification of LEE & al. (1985)) and studied under oil immersion in Leitz HM-lux or Aristoplan equipped with a 100 x PlanApo (1.32 NA). Drawing of the species were done using a camera lucida, and the microphotographs were taken with a Wild Leitz MPS 46 photoautomat.

## RESULTS

### *Gymnozoum intermedium*

Table 2 gives the sizes of the cell, macronucleus, micronucleus and the number of somatic kineties and oral kinetofragments of *G. intermedium*. The sizes of cultured specimens appeared to be smaller than the few observed wild specimens. *G. intermedium* has approximately 30 somatic kineties, most of them oriented longitudinally from the oral end and extending close to the aboral end (Figs 3A, B; 4A, B). Well stained lines near the kinetosomes were observed but no direct connections were seen. Five kineties are curved; the kinetofragma and somatic kinety number S4, following the terminology in CORLISS & SNYDER (1986) are short, whereas kineties number S1, S2, and S3 are long extending from the aboral end to the oral end where they curve to the left, partly surrounding the oral end with a semicircle (Figs 3B, F; 4C, D). Some of the remaining somatic longitudinal kineties (approximately kineties number n-2, n-3 and n-23 and n-24) are shorter than most of the others. The kinetofragma and the kineties S3 and n-24 partly surround a non-ciliated, striated field on the posterior part of the cell (Figs 3B; 4E). Generally the spacing of kinetosomes in each kinety decreases somewhat toward the aboral

Table 1. Dates, positions, depths, temperatures, and salinities at the stations where *Gymnozoum intermedium* and *Gymnozoum smalli* n. sp. were found. Data from 1984 from LOENG & al. (1984), and H. Loeng (pers. commn), from 1985 from HASSEL & al. (1985), and from 1986 from DALE & DAHL (1987).

Species	Sampling date	Station	Positions	Depth (m)	Temp. (° C)	Salinity (‰)
<i>G. intermedium</i>	1 June 1984	628	75°15' N 18°00' E	5	1.4	34.8
	3 June 1984	665	76°24' N 31°40' E	0	0.5	34.5
	3 June 1984	670	77°10' N 33°29' E	0	-1.2	34.0
<i>G. intermedium</i>	12 Aug 1985	898	78°35' N 28°29' E	10	2	33.3
<i>G. smalli</i> n. sp.	26 Nov 1986	-	58°37' N 9°30' E	0.1	6.5-7	12-14

Table 2. Average sizes of *Gymnozoum intermedium* and *Gymnozoum smalli* n. sp. (Bold: average; italics: range; sample size in brackets).

	Cell		Macronucleus		Mic.-nucleus	Number	
	Length µm	Width µm	Length µm	Width µm	Sizes µm	Somatic kineties	Oral kineto- fragma
<i>G. intermedium</i> field samples	<b>83</b> (3) <i>78-86</i>	<b>51</b> (3) <i>42-62</i>	<b>29</b> (2) <i>28-29</i>	<b>15</b> (4) <i>11-17</i>	-	<b>29</b> (4) <i>29</i>	<b>8</b> (3) <i>8</i>
<i>G. intermedium</i> cultiv. samples	<b>61</b> (14) <i>32-88</i>	<b>37</b> (16) <i>20-62</i>	<b>22</b> (9) <i>12-31</i>	<b>11</b> (16) <i>6-20</i>	<b>3.4x2.7</b> (5)	<b>29.7</b> (15) <i>26-32</i>	<b>8</b> (15) <i>8</i>
<i>G. smalli</i> n. sp.	<b>42</b> (9) <i>35-49</i>	<b>30</b> (11) <i>22-37</i>	<b>20</b> (9) <i>11-27</i>	<b>7</b> (11) <i>5-10</i>	<b>2.3x1.8</b> (2)	<b>37.1</b> (10) <i>35-40</i>	<b>7.5</b> (11) <i>6-8</i>

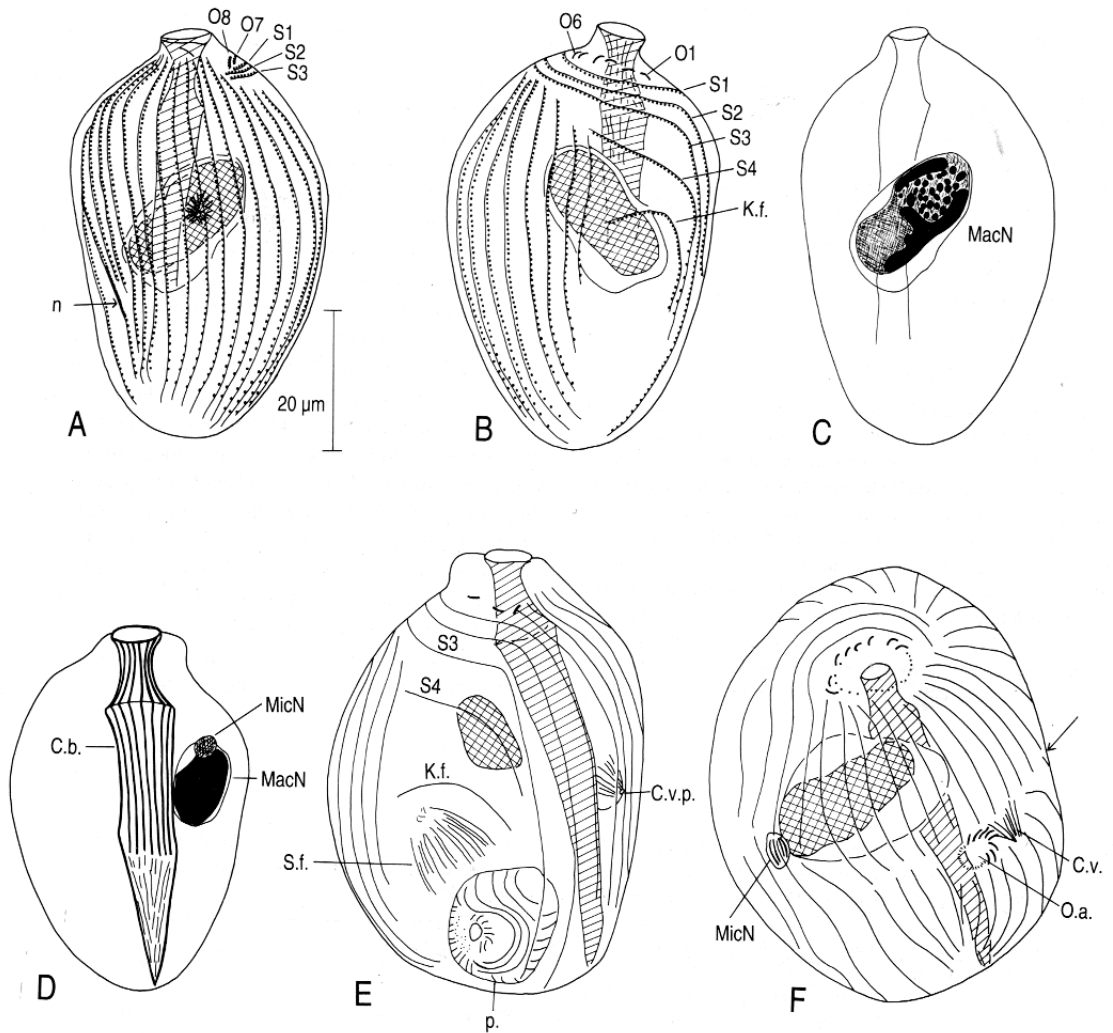


Fig. 3. General outline of *Gymnozoum intermedium*: A. Somatic ciliation, note kinety 'n' with the densely spaced kinetosomes in the posterior part and contractile vacuole pore between kineties n-7 and n-8. B. Somatic ciliation on the other side, note kineties S1, S2, and S3 (spiralling kineties) and the reduced spiralling kinety S4 and the short kinetofragmon. Note also six of the eight oral kinetofragma. C. Macronucleus. D. Macronucleus and micronucleus, cytopharyngeal basket. E. Specimen with smaller individual inside; note striated field. F. Early divider; note the oral anlage of the opisthe near the contractile vacuole pore and the fission furrow (broken lines of the somatic kineties, arrow). Scale bar: 20  $\mu$ m. Legends: S1-S4 = spiralling somatic kineties. n = kinety n. O1-O8 = oral kinetofragments. S.f. = striated field. C.v.p. = contractile vacuole pore. K.f. = kinetofragmon. MacN = macronucleus. MicN = micronucleus. C.b. = cytopharyngeal basket. p = prey. O.a. = oral anlage.

end of the cell, but kinety 'n' has a very dense row of kinetosomes near its aboral end (Figs 3A; 4B). The somatic cilia are around 3-4  $\mu$ m long with the exception of those attached to the kinetofragmon, which are longer. At the anterior end between the cytostome and kinety number

S1, eight oral membranelles (kinetofragma) are found, each of them usually with an adjacent pair of single kinetosomes (not shown in the figure) between the membranelles and the cytostome (Figs 3F; 4C). Membranelle O1 often has a markedly different angle to

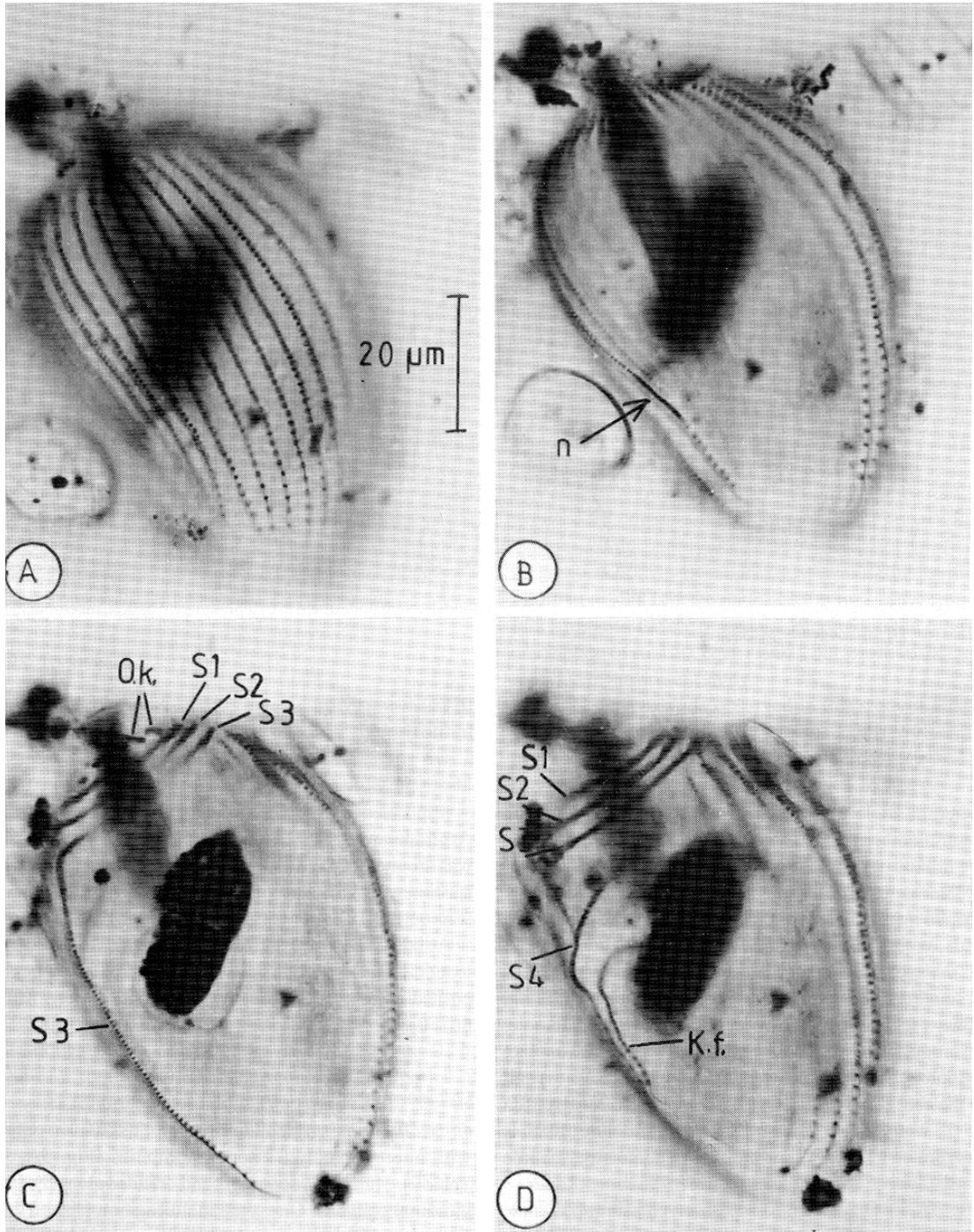


Fig. 4. Micrographs of *G. intermedium* from Stn 665. A and B seen from outside the cell, C, D and E seen from inside the cell. A. Somatic ciliation. B. Somatic ciliation, note kinety 'n', with the closely spaced kinetosomes near its posterior end. C. Spiralling kineties S1, S2, and S3, oral kinetofragments, macronucleus with nuclear membrane. D. Spiralling kineties S1, S2, S3 and reduced kinety S4 plus the kinetofragmon. E and F see next page. Scale bar: 20 µm. Legends: see Fig. 3.

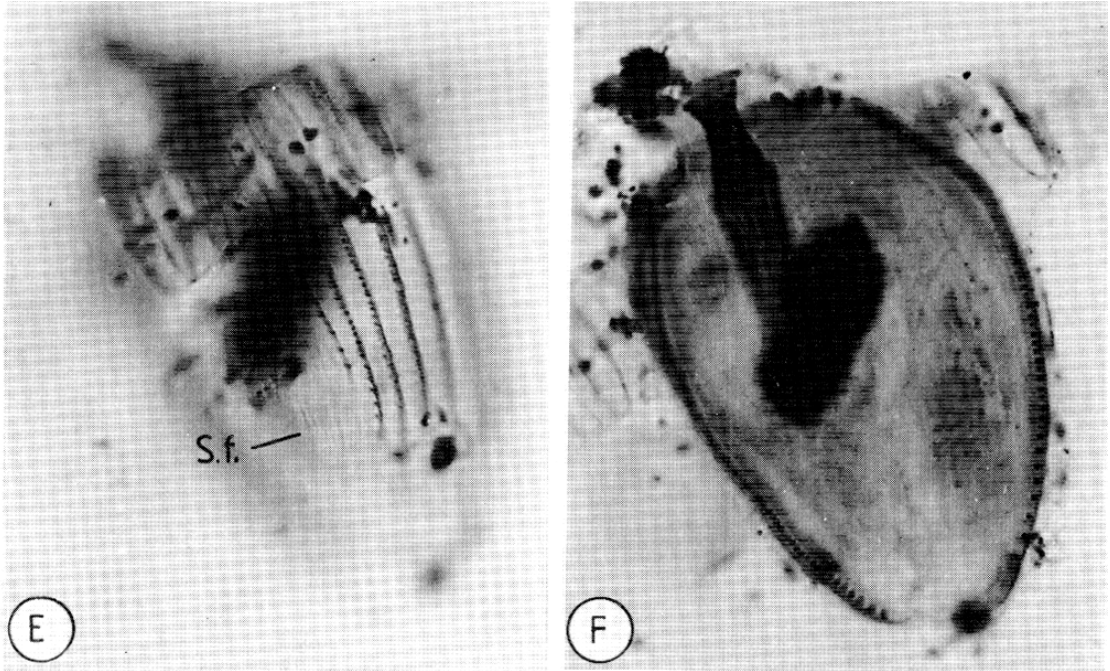


Fig. 4E, F. Micrographs of *G. intermedium* from Stn 665. E. Striated field. F. Nematodesmata in cytopharyngeal basket, macronucleus. Scale bar: 20  $\mu\text{m}$ . Legends: see Fig. 3.

the axis of the cell compared to the other membranelles. The cilia of the oral membranelles are around 10  $\mu\text{m}$  long.

The nuclear apparatus consists of a fairly large kidney shaped macronucleus, which stains differently in its two ends, giving the appearance of being bipartite (Figs 3C; 4C). A nuclear membrane is also evident. The micronucleus is small and located close to the macronucleus. It is not always seen and is usually less densely stained than the macronucleus (Fig. 3D). From the cytostome, a sword like cytopharyngeal basket extends obliquely through the cell to the aboral end (Figs 3D; 4B). It appears to consist of ca 15-18 well-stained nematodesmata. A structure which is interpreted as the pore and canals of a contractile vacuole is usually seen approximately between kineties n-7 and n-8 (Fig. 3A, F). One specimen was observed with two vacuole pores, the second was situated close to the first between the neighbouring kineties. A few cells in the same division stage with only one macro- and micronucleus were also observed. In the micronucleus some parallel lines were observed (Fig. 3F). The oral anlage appeared between kineties n-5 and n-7, close to the contractile vacuole pore.

It appeared to grow when fed diatoms and dinoflagellates. At temperatures 3-4° C, the generation time of the species was around 3-4 days (T. Dale, own obs.). In several large specimens, smaller specimens could be seen inside the cell (Fig. 3E).

A slide with the redescribed species resides the slide collection of T. Dale (slide no. 415/78c) and will later be deposited at Zoological Museum at University of Bergen (no. 66750).

#### *Gymnozoum smalli* n. sp.

*G. smalli* n. sp. is a small, ellipsoid (42 x 30  $\mu\text{m}$ ) ciliate with approximately 37 somatic kineties (Fig. 5A, B; Table 2). Thirty-six are straight and oriented along the longitudinal cell axis. One, the kinetofragmon is short and curved, and is situated posteriorly (Figs 5A; 6E, F). The kinety 'n' may show somewhat increased density of kinetosomes at the posterior end. The four somatic kineties S1-4 curve to the left in the anterior end so that each forms a semicircle at one side of the oral area (Figs 5A, B, C; 6B, F). It is unclear whether S4 is continuous or discon-

tinuous with the longitudinal somatic kinety. Between the cytostome and the spiralling kineties, eight oral membranelles (kinetofragma), densely packed with kinetosomes and usually with two single kinetosomes adjacent to each, are also found (Fig. 5C). The cell has a large (20 x 9 µm) ellipsoid macronucleus (Figs 5D; 6C) and a small micronucleus (2.3 x 1.8 µm). Following the numbering system of CORLISS & SNYDER (1986), a contractile vacuole pore may be found between kineties n-4 and n-5 (Fig. 5A). The cytopharyngeal basket of the cell is prominent (Fig. 4D), and may extend to the posterior end of the cell. The first half is usually more darkly stained than the posterior half. The number of nematodesmata is approximately 15-17. None of the 11 inspected specimens contained any larger items which could suggest anything of the food habits of *G. smalli* n. sp. *Gymnozoum smalli* n. sp. however, may be preyed upon by *Tiarina fusus* as one specimen of *Gymnozoum* sp., probably *G. smalli* n. sp. was seen in one *T. fusus* in the same sample. The name *G. smalli* n. sp. is given in honour to Eugene B. Small who opened the world of ciliate taxonomy to me. A slide with the type specimen resides in the slide collection of T. Dale (slide no. 437/84 a) but will later be deposited at Zoological Museum, University of Bergen (no. 66749).

#### DISCUSSION

The genus *Gymnozoum* was erected by MEUNIER (1910) who described *G. viviparum* based on fixed (formol) planktonic samples collected from the Barents Sea in 1907. In many respects, however, *Gymnozoum*, resembles the genus *Spiroprorodon* (FENCHEL & LEE, 1972). First, many drawings of *Gymnozoum viviparum* show a prominent cytopharyngeal basket similar to that of *Gymnozoum (Spiroprorodon) glacialis*, *G. intermedium*, and *G. smalli* n. sp. Second, MEUNIER (1910) frequently refers to the nucleus as being bipartite, and this is also seen in many of his drawings. The bipartite nucleus is also seen in *G. garrisoni* and *G. intermedium*. Third, MEUNIER (1910) notes similarly spaced lines ('stries élastiques') from pole to pole. In fig. XXI, no. 11, he shows a total of 24 lines that could be interpreted as kineties. Fourth, in two drawings (fig. XX, no. 18, fig. XXI, no. 9) of a total of ca 30 drawings, he shows four lines circling the oral end. These could be interpreted as kineties S1-4. Fifth, the divider shown in fig. XXI, no. 8, is similar to the pattern seen in *G. intermedium*. Sixth, MEUNIER (1910) shows several specimens with smaller individuals of the same species inside which he interpreted as embryos. These may well represent preys consumed cannibalistically as suggested by KAHL (1930) similar to those seen in the cultures of *G. intermedium*. However, it is problematic to synonymize *Gymnozoum* with the ciliate genus *Spiroprorodon*, as

MEUNIER (1910:180) stated that *Gymnozoum* was naked, an observation reflected in the name. One might assume that MEUNIER simply overlooked the cilia, but that would be curious, as he was able to see the cilia of many other ciliates. He does not indicate any special field without lines (kineties) similar to the prominent unciliated field seen *G. intermedium* and *G. garrisoni*, but this might be inconspicuous as in *G. smalli* n. sp. Despite the apparent absence of cilia in *G. viviparum* (MEUNIER, 1910), the other characteristics are so similar that it is concluded that *Spiroprorodon* is a junior synonym of *Gymnozoum*. This view is also supported by the recent paper of PETZ & al. (1995) who synonymized the genera. In order to verify this, it would be interesting to reexamine the samples of Meunier, if they still exist.

If the genera are synonymized, *G. intermedium* or *G. smalli* n. sp. are probably not the same species as *G. viviparum*. It is difficult to determine the sizes of *Gymnozoum viviparum* from MEUNIER (1910), but according to KAHL (1930) it is between 130 and 140 µm and is thus much larger than *G. intermedium* and *G. smalli* n. sp. The numbers of lines (or kineties) which is approximately 2 x (12-13) = 24-26 in *G. viviparum*, is markedly less than the approximately 37 kineties in *G. smalli* n. sp., but is in the same order as in *G. intermedium*. It is similar to the ca 25 kineties reported in the original description of AGATHA & al. (1993), but less than the ca 30 reported in the present study.

The *G. intermedium* observed in this study is similar to the original description by AGATHA & al. (1993) in the major respects. The most notable differences are that the present specimens have more somatic kineties (29.7 vs 24.9), fewer fibers in the cytopharynx (15-18 vs 36) and shorter cilia (3-4 µm vs 11 µm). The cultured specimens are smaller (61 x 37 µm vs 76 x 59 µm) but the few wild specimens (83 x 51 µm) are in the same size range as that described by AGATHA & al. (1993). The present specimens also have a contractile vacuole pore, not reported in the original description, but this may be easily overlooked. It is therefore assumed that these differences do not justify erection of a new species.

*G. intermedium* is usually not a common species within the assemblages of planktonic marine ciliates in the Barents Sea (T. Dale, own obs.). The actual food of the species is not known. It appears to be a cannibal given the opportunity, as several specimens in the cultured samples contained smaller individuals of the same species.

*G. smalli* n. sp. is a new species because the somatic ciliation is different from that of previously described species. The most pronounced difference lies in the short kinetofragmon and the small unciliated field. In addition, the cell is smaller (except for *G. sympagicum*) and has more kineties than the other described species except for *G. glacialis* which, however, is much larger and have five

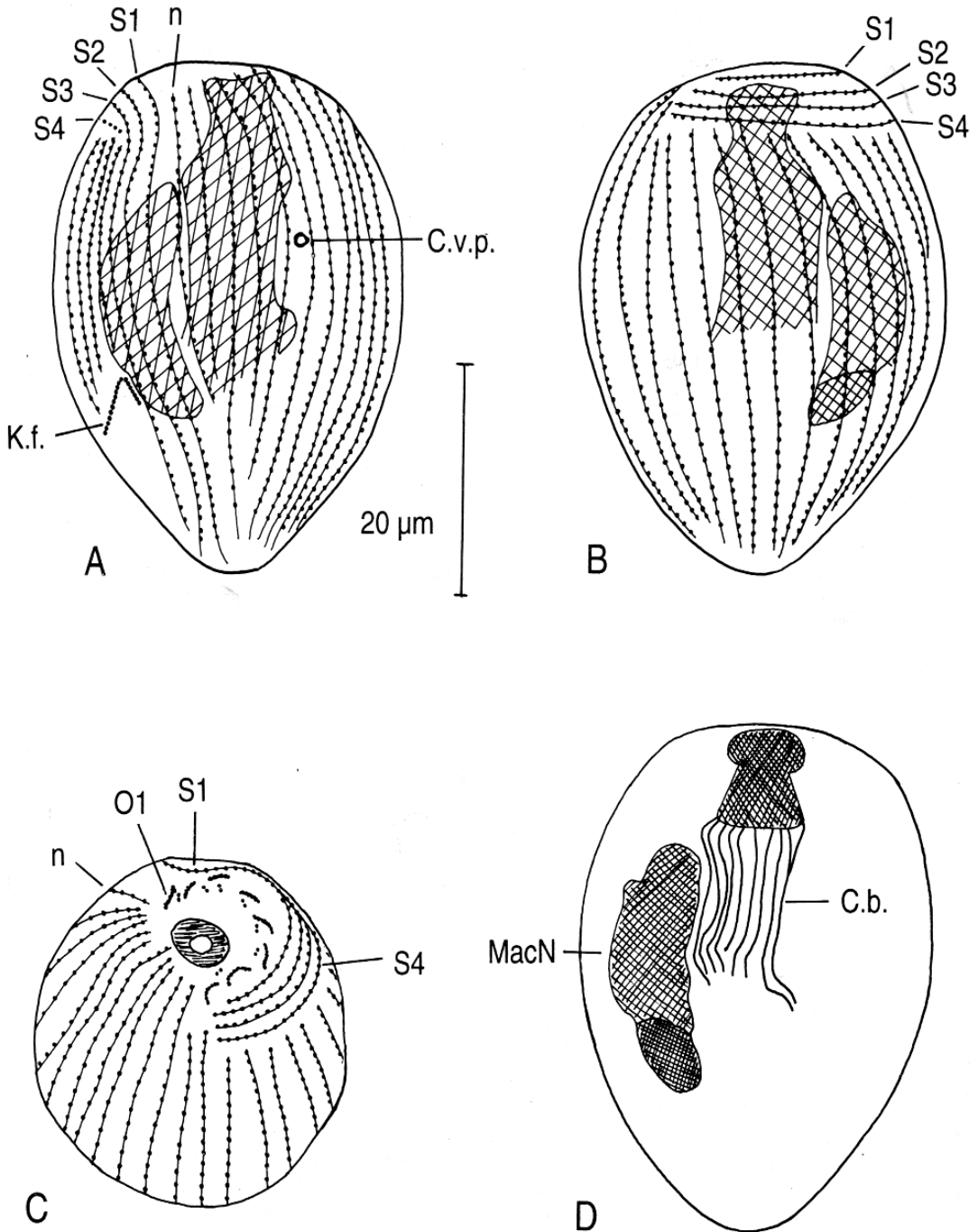


Fig. 5. General outline of *Gymnozoum smalli* n. sp.: A. somatic ciliation, contractile vacuole pore between kineties n-3 and n-4, spiralling kineties S1, S2 and S3 continuous with the longitudinal somatic kineties and S4 kinety disconnected (?) from its somatic longitudinal kinety. Short kinetofragmon with densely spaced kinetosomes. B. somatic ciliation on the other side showing four spiralling kineties. C. ciliation at the oral end of the cell, four spiralling kineties, eight oral membranelles with adjacent double kinetosomes. D. macronucleus and cytopharyngeal basket. Scale bar: 20 μm. Legends: see Fig. 3.



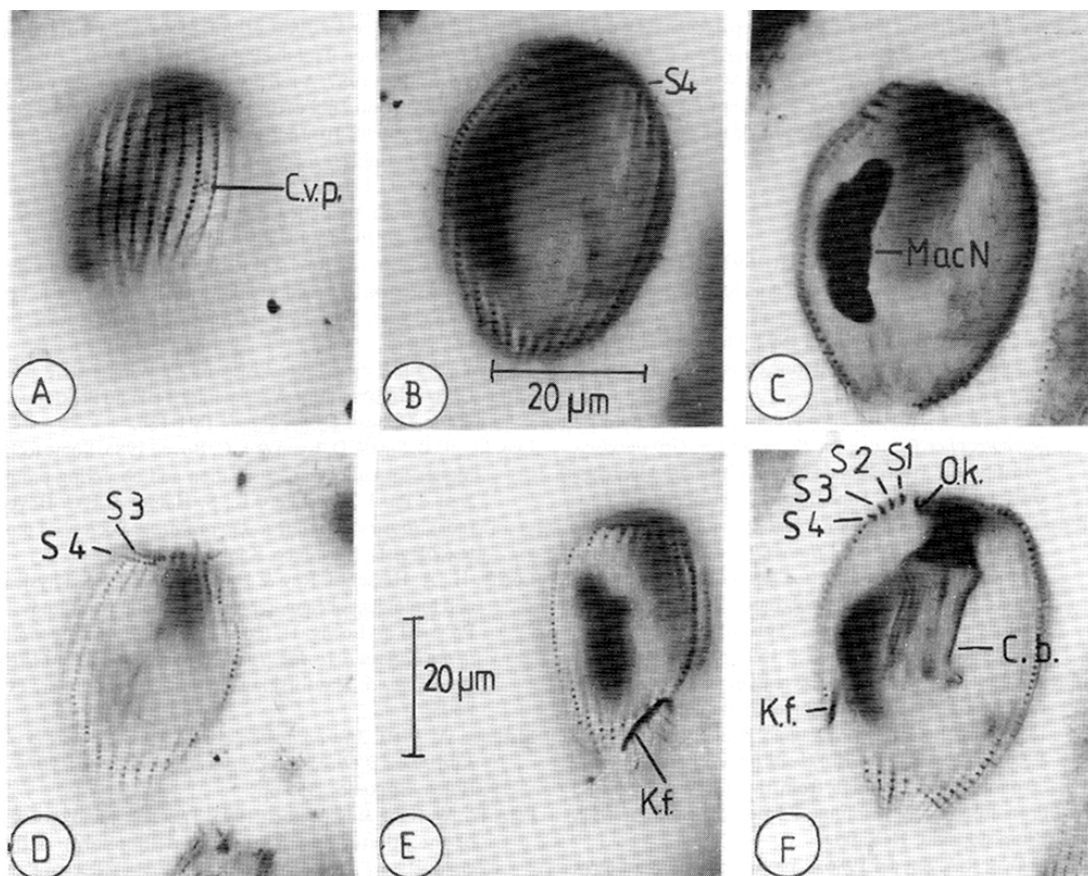


Fig. 6. Micrographs of *Gymnozoum smalli* n. sp.: A. Somatic ciliation, contractile vacuole pore. B. Somatic ciliation, spiralling kineties. C. Four spiralling kineties and macronucleus. D. Somatic ciliation, anterior terminal end of spiralling kineties three and four (seen through the cell). E. Kinetofragmon with long cilia. F. Cytopharyngeal basket, kinetofragmon, four spiralling kineties, one oral membranelle. Scale bars: 20 µm, scale bar in picture B valid for picture A, B, C and F; scale bar in picture E valid for picture D and E. Legends: see Fig. 3.

spiralling kineties. The kinty 'n' is comparatively shorter than in *G. intermedium* and does not have the same area with densely packed kinetosomes. The contractile vacuole pore observed in this species is situated in the middle of the cell between kineties n-3 and n-4. To date, all other species in *Gymnozoum* have been observed in polar regions. *G. smalli* n. sp. is thus the first species of this genus found in a boreal region.

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