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On Papulifères, putative ciliate cysts of diverse morphologies, with new observations from the plankton of the Chukchi Sea (Arctic Ocean)

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Abstract. In the early 20th century, Alphonse Meunier described "Papulifères" as a group of enigmatic forms of unknown taxonomic affinity characterized by possessing a hyaline pimple, a "*papula*". In the early 1980's Papulifères were equated with cysts of tintinnid ciliates. The most conspicuous forms, the large *Fusopsis,* have been widely found, and are now known to resemble the cyst of a certain species of oligotrich ciliate (i.e., *Cyrtostrombidium boreale*). Thus today, Papulifère forms are often assumed to be cysts of oligotrich ciliates. Here we report on 26 Papulifère forms, of more or less distinct morphologies, found in the plankton of the Chukchi Sea. We found forms resembling some of those described by Meunier, and recorded here for the first time since Meunier's reports, and others that do not resemble any of Meunier's Papulifères. Here, we first review the literature on Papulifères, then we present the surprising variety of forms we found in Chukchi Sea, and for some, we provide for the first time data on morphological variability. With this report we have expanded the catalogue of observed Papulifère forms and documented variability in the dimensions of some morphotypes. However, we urge caution in assigning a ciliate species name to any given Papulifère form in the absence of corroborating data. There is a need for observational and/or sequence-based data to elucidate the identity of Papulifère forms.

Keywords: *Fusopsis*, *Piropsis*, *Sphaeropsis*, microzooplankton, tintinnids, oligotrichs

INTRODUCTION

Since 2012 plankton samples have been collected during cruises in the Chukchi Sea each August and analyzed to provide data on the tintinnid ciliates of the Chukchi Sea. Occasionally, forms resembling more or less, the enigmatic forms known as Papulifères were encountered, their occurrences noted, and the forms were imaged. However, in a sample collected in 2022 from one station, a large variety of apparently new forms were found motivating the present report. Here, by way of an introduction, we first present a historical review of the enigmatic forms known as Papulifères as many protistologists are likely unfamiliar with them. We then present images of the forms we found, and data on the size variability of many of them, to our knowledge, data previously not available.

In 1910 Alphonse Meunier published a monographic study of the microplankton of the Kara and Barents Seas of the Arctic Ocean (Meunier 1910). In it he described a new group of admittedly unknown taxonomic affinity and uncertain coherence: "Papulifères". The forms, of a surprising variety of shapes and sizes, shared a single morphological character, a '*papule*', a button or pimple on one end. Meunier divided Papulifères into three genera, grouping forms with roughly similar overall shape. *Fusopsis* contained six spindle-shaped 'species', *Piropsis* grouped four pyriform 'species', and *Sphaeropsis* grouped 9 roughly spherical 'species'. A few years later, Meunier published a second study of the near-shore plankton of Belgium (N.W. Europe) that included depictions of more forms of *Fusopsis* and *Sphaeropsus*, without full binomials, noted simply as 'sp.' (Meunier 1919). These are all shown in Fig. 1. Unfortunately, Meunier's descriptions were deficient in key aspects. He provided no direct information on the sizes of the forms depicted nor the ranges of sizes encountered. The illustrations in the monographs were described as all of the same relative size, 500 times actual size, and to determine actual size knowledge of the dimensions of the form on the original printed page is needed. He also did not note the exact locations where given specimens were found.

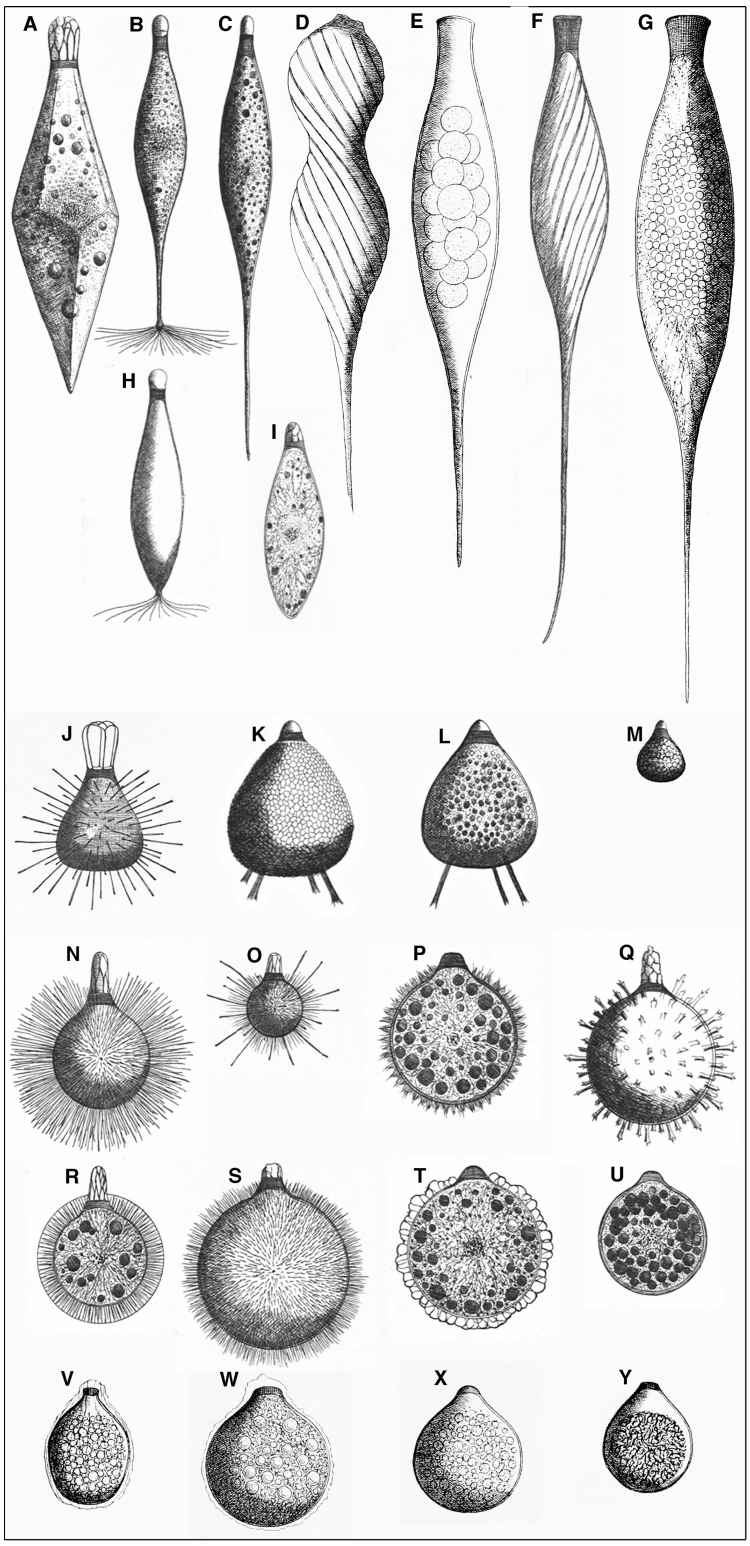

Fig. 1. Meunier's Papulifères from Meunier 1910, 1919. A-I: *Fusopsis* forms, J-M: *Piropsis* forms; N-Y: *Sphaeropsis* forms. Meunier's names for each, approximate sizes as he depicted them (figures above were re-sized to fit into a single plate), and locations in his reports are given in Table 1.

Table 1. Papilulifères of Meunier shown in Fig. 1: names, approximate actual sizes (largest dimension, LD, µm) based on the size of the image printed, and the location of the figures and text descriptions in Meunier 1910 and Meunier 1919.

Fig. 1	Meunier name	Meunier figure	LD (µm)	Meunier text
A	*Fusopsis polyedra*	1910 Plate 6 fig. 22, 23	200	1910 p 101
B	*Fusopsis umbricula*	1910 Plate 6 fig. 19, 20	150	1910 p 99-100
C	*Fusopsis elongata*	1910 Plate 6 fig. 21	220	1910 p 98-99
D	*Fusopsis* sp	1919 Plate 23 fig. 10	220	1919 p 43
E	*Fusopsis* sp.	1919 Plate 23 fig. 9	230	1919 p 43
F	*Fusopsis spiralis*	1910 Plate 7 fig. 6	270	1910 p 99
G	Fusopsis sp.	1919 Plate 23 fig. 8	290	1919 p 43
H	*Fusopsis flagifera*	1910 Plate 7 fig. 7, 8	115	1910 p 100
I	*Fusopsis pauperata*	1910 Plate 6 fig. 24, 25	60	1910 p 100
J	*Piropsis acineta*	1910 Plate 7 fig 9	100	1910 p 102
K	*Piropsis reticulata*	1910 Plate 6 fig 26, 27	75	1910 p 102
L	*Piropsis polita*	1910 Plate 7 fig. 12, 13	65	1910 p 103
M	*Piropsis minuta*	1910 Plate 7 fig. 29	25	1910 p 103
N	*Sphaeropsis longisetosa*	1910 Plate 7 fig. 14, 15	80	1910 p 105
O	*Sphaeropsis heterosetosa*	1910 Plate 7 fig. 10, 11	40	1910 p 103
P	*Sphaeropsis nivais*	1910 Plate 7 fig. 13	75	1910 p 104-105
Q	*Sphaeropsis echinata*	1910 Plate 7 fig. 16	90	1910 p 105
R	*Sphaeropsis nimbata*	1910 plate 7 fig. 21	75	1910 p 104
S	*Sphaeropsis brevisetosa*	1910 Plate 7 fig. 12	95	1910 p 104
T	*Sphaeropsis spumosa*	1910 plate 7 fig. 17-20	75	1910 p. 105
U	*Sphaeropsis laevigata*	1910 Plate 7 fig. 24, 25	50	1910 p. 106
V	*Sphaeropsis* sp.	1919 Plate 23 fig. 6	62	1919 p 43
W	*Sphaeropsis* sp.	1919 Plate 23 fig. 4	62	1919 p 43
X	*Sphaeropsis* sp.	1919 Plate 23 fig 5	50	1919 p 43
Y	*Sphaeropsis* sp.	1919 Plate 23 fig 7	50	1919 p 43

The first illustrations (Fig. 2) and speculations as to the nature of the forms Meunier named the Papulifères were made by others, before his studies, from observations of forms from various locations in the North Atlantic. The first record appears to be that of Canu who found odd forms (Fig. $2A_1$, $2A_2$), with one resembling one of Meunier's *Fusopsis* sp. (Fig. 1 G), in plankton net material in the coastal waters of Boulogne-sur-Mer (N.W. France). He speculated that the two might be egg cases of a trematode parasite of planktivorous fish, but also noted some resemblance to a tintinnid ciliate (Canu 1893). The second record appears to be that of Vanhöffen who described a similar form as a small-tailed cyst of unknown origin (Fig. 2 B) from a sample taken in the Karajak fjord in (S.W. Greenland) in September 1893 (Vanhöffen 1897). The third record, from material collected in near shore waters of Canso (E. Nova Scotia, Canada) in 1901 and 1902 was reported by Wright. His illustration (Fig. 2C) showed a form greatly resembling Meunier's *Fusopsis spiralis* (Fig. 1 F), quoting Canu, as "what has been supposed to be the pelagic egg of a trematode" (Wright 1907). Meunier, in his first study (Meunier 1910), mentioned only the reports of Canu and Wright; Meunier himself did not speculate as to the nature of the Papulifères. However, in his second study (Meunier 1919), he reported finding a tintinnid lorica (a

Favella sp., noted as *Cyttarocylis ehrenbergii*) containing a cyst with a *papula*. This led him to conclude that Papulifères were likely cysts, but of infusoria other than tintinnids, since none resembled the cyst in the tintinnid he had found.

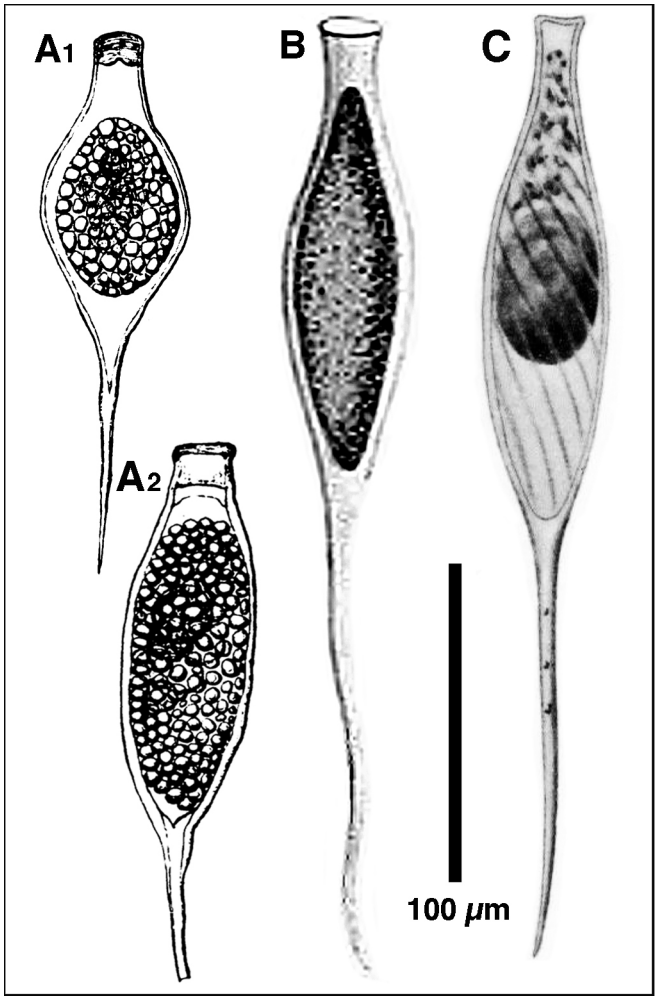

Fig. 2. The early illustrations of forms, of distinct morphologies, which would later come to be known as *Fusopsis*, from reports pre-dating Meunier's studies naming them as such. Canu (1893) depicted two forms, A_1, and A_2 (figs. 8 and 9, respectively, in Canu 1893), which he found in plankton net samples from coastal waters of Boulogne-sur-Mer (NW France). Vanhöffen reported finding the form B (Plate 6, fig. 5 in Vanhöffen 1897) in a plankton net samples from a fjord in western Greenland. Wright (1907) illustrated a form (Plate 5, fig. 4 in Wright 1907) that he found in a plankton net sample from the coastal waters on New Brunswick (E. Canada).

A Papulifère was possibly listed in an anonymous catalogue of animal plankton as "*Sphaeropsis*" in the category "*Incertae sedis et Ova varia*", found in the plankton of Baltic near Finland in May 1911 (Anon. 1916). A full 30 years passed after Meunier's 1919 monograph before another detailed report of the occurrence of Papulifères was published, that of Kufferath in 1950. In a study on plankton samples collected in the southern North Sea and across the English Channel, he reported finding several varieties of *Fusopsis* (Fig. 3). Kufferath described them as 'appearing to be the cysts of tintinnids or other ciliates' (Kufferath 1950). Thus, by 1950, quite a large variety of

Fusopsis-like forms had been recorded. If each of the depictions published from 1893 to 1950 were to be taken to be a distinct 'species', the Papulifère species of *Fusopsis* alone would number 19. After Kufferath's study, there were but two reports mentioning any of Meunier's Papulifères. A *Fusoposis* form was listed in a report concerning plankton of the port of Ostend on the coast of Belgium (De Pauw 1969) and *Piropsis polita* (shown here as Fig. 1 L) was reported by Horner (1978) as among the plankton of the Chukchi and Beaufort Seas (Arctic Ocean).

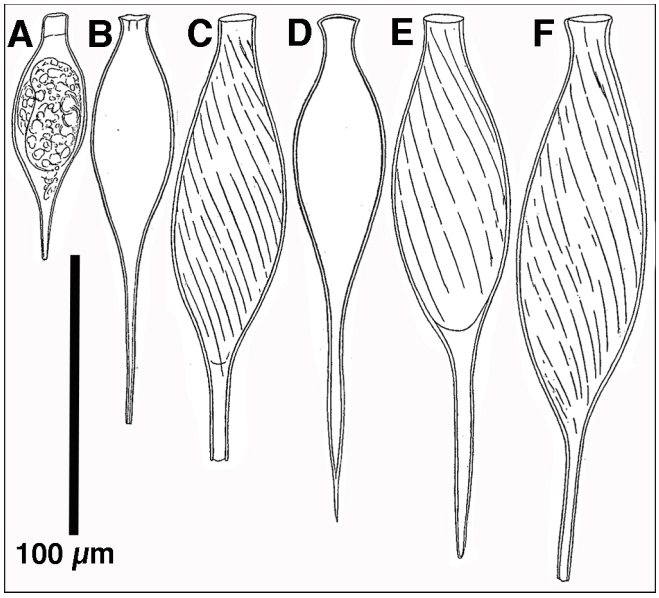

Fig. 3. The illustrations of the variety of forms, all described as *Fusopsis*, from Kufferath (1950) that he found in plankton samples from the southern North Sea and the English Channel (NW Europe). The original illustrations shown here as A to F appear in Kufferath 's report, respectively, as 16, 17, 20, 18, 19, and 21.

It is fair to say that overall, there was little interest in Papulifères until 1978 when Reid and John published a paper on the cysts of tintinnids found in the samples collected by the Continuous Plankton Recorder survey program (Reid & John 1978). They argued that Paulifères are cysts of tintinnids based on observations, like those of Meunier, that a *Favella* lorica contained an apparent cyst with a *papula*. They further argued that the extinct flask-shaped Chitonozoa, known from micropaleontology studies, may be tintinnid cysts. Interestingly, at the time, only one study had been published based on actual observations of encystment in a tintinnid, that of Biernacka (1952), on *Tintinnopsis subacuta*, and the cyst produced was not flask-shaped, nor did it have a *papula.* In a subsequent paper, Reid and John described several more Papulifère forms as tintinnid cysts (Reid and John 1981). All of the supposed tintinnid cysts of Reid and John are shown in Fig. 4. Notably, none of the Papulifère forms described by them as tintinnid cysts has ever been observed inside a tintinnid lorica which is a strong argument against an identity as a tintinnid cyst, an observation first made by Davis (1986). Despite a distinct lack of evidence that any of the forms shown in Fig. 4 are cysts of tintinnids, many reports do describe them as tintinnid cysts

(Della Tommasa et al. 2000; Rubino et al. 2000; Adamonis et al. 2007; Pieńkowski et al. 2011; Matsouka et al. 2018a; Allan et al. 2020; Pieńkowski et al. 2020; Mudie et al. 2021a).

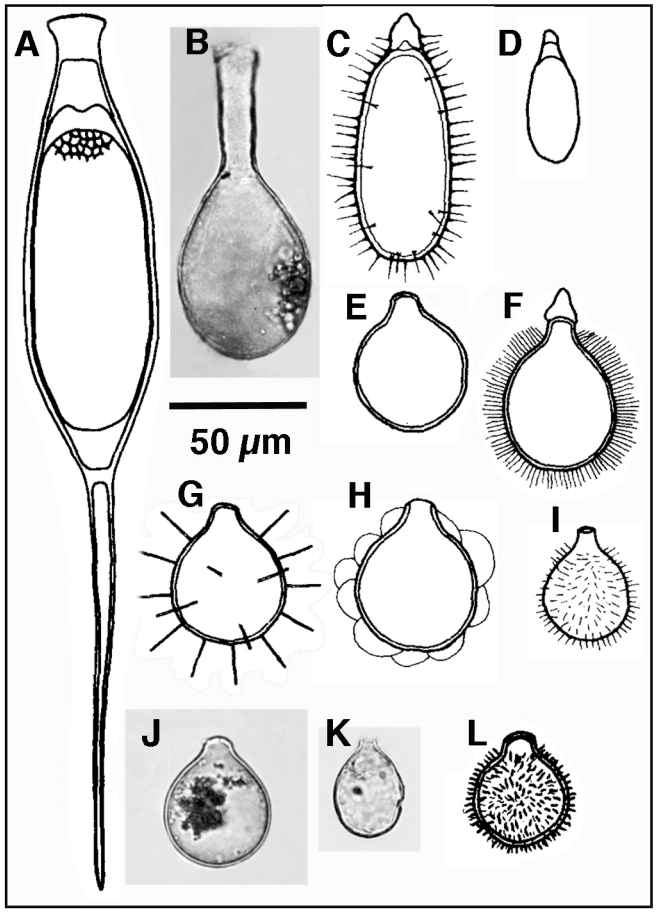

Fig. 4. Illustrations of Papulifère forms said to be tintinnid cysts by Reid and John. From Reid and John 1978: A, B, J, K, & L. From Reid and John 1981: C, D, E, F, G, H, & I. Some were given specific designations: B: "cyst type P"; C: "cyst type S"; D: "cyst type T"; E: "cyst type M"; F: "cyst type F"; G: "cyst type Q"; H: "cyst type K; I: "cyst type N"; L: "cyst type O". Some of these specific designations are still in use in the micropaleontology literature (e.g. Mudie et al. 2021a,b)

The first and only unambiguous direct link between any of Meunier's Papulifère forms and a living organism was the study by Kim et al. (2002) showing that the planktonic oligotrich *Cyrtostrombidium boreale* emerged from a cyst greatly resembling one of Meunier's *Fusopsis* species, similar to the ones shown here as Fig. 1E or Fig. 1G. Subsequently, in the literature, all Papulifère-like forms were considered to be the cysts of oligotrich ciliates (*e.g.*, Moscatello et al. 2004; Ichinomiya et al. 2008; Price & Pospelova 2011; Heikkilä et al. 2016; Rubino et al. 2017; Matsuoka et al. 2018b). Recently, six long-tailed *Fusopsis* specimens, similar in gross morphology to those shown in Fig. 1F and 2C, from the Chukchi and Labrador Sea were sequenced and all found to be most closely related to *Cyrtostrombidium* species (Gurdebeke et al. 2023). Still, it is important to note that, with the exception

of the large *Fusopsis* forms mentioned above, there is no evidence linking a Papulifère form to a particular living organism. Nonetheless, we believe it is worthwhile to provide documentation of these forms from the plankton of the Chukchi Sea as future studies may uncover evidence of their nature and/or occurrence in other localities. Furthermore, as will be shown here, there appear to be many more forms of Papulifères than those described by Meunier.

Here we document a very wide variety of the Papulifère forms, most of which appear to be previously unknown, we found in plankton net samples from the Chukchi Sea. We also provide rudimentary data on the size ranges of the some of the forms. While the forms we found may be ciliate cysts, in the absence of formation or germination evidence, or nucleic acid sequence data, they should be described as possible or putative ciliate cysts.

MATERIALS AND METHODS

We have previously reported in detail the material and methods used in our studies of Chukchi Sea and North Pacific Ocean microplankton (Dolan and Yang 2017, Dolan et al. 2014, 2016, 2021). Briefly, over the past 12 years, survey cruises of the Chukchi Sea have been conducted by Korea Polar Research Institute, usually in July and August, and include sampling microplankton using a 20 μm mesh-size plankton net at 20-50 locations each year. The samples were examined primarily for enumerating tintinnid ciliates. Aliquots of lugol's-preserved net samples, 1 - 3 ml volumes, were examined in settling chambers using an inverted microscope equipped with DIC optics (Olympus IX71) and a Canon Eos 5D Mark II digital camera. Very occasionally, forms resembling ciliate cysts were encountered, the longest dimension measured using a calibrated ocular micrometer, and imaged using the digital camera. Longest dimensions were recorded using a 20x objective. Hence, the dimensions recorded were relatively rough, to the nearest 5 microns. The forms were assumed to be ciliate cysts as the first found resembled the distinctive cyst of *Cyrtostrombidium boreale*, formally known only as one of Meunier's 'Papulifères', *Fusopsis* (Kim et al. 2002). A few such forms were encountered in samples from 2015 and 2021. However, in a sample collected in 2022 from one station, a large variety of forms were found prompting a thorough examination of the sample (65 ml of net material, representing material from 2 m^3), and motivating the present report.

RESULTS

Overall, Papulifère forms were rarely, and only sporadically, observed in plankton net samples from the Chukchi Sea collected during annual cruises over the past 12 years. They were encountered in only seven of the grand total of 308 Chukchi Sea samples examined, and the seven samples were from only 3 of the 12 cruises. Pooling all of our observations, we found a total of 108 Papulifère specimens. Samples containing Papulifère forms were from sites scattered across the Chukchi Sea without any

apparent relationship with the total depth of the site, latitude, or distance from shore (Fig. 5). The samples in which the Papulifère forms were found were collected at different times of day (Table 2). We found Papulifère forms only in samples collected in 2015, 2021, and 2022. The majority of Papulifère forms were found in a single sample from the 2022 cruise with a few forms encountered in several samples collected in 2021, and one sample collected in 2015 (Table 2).

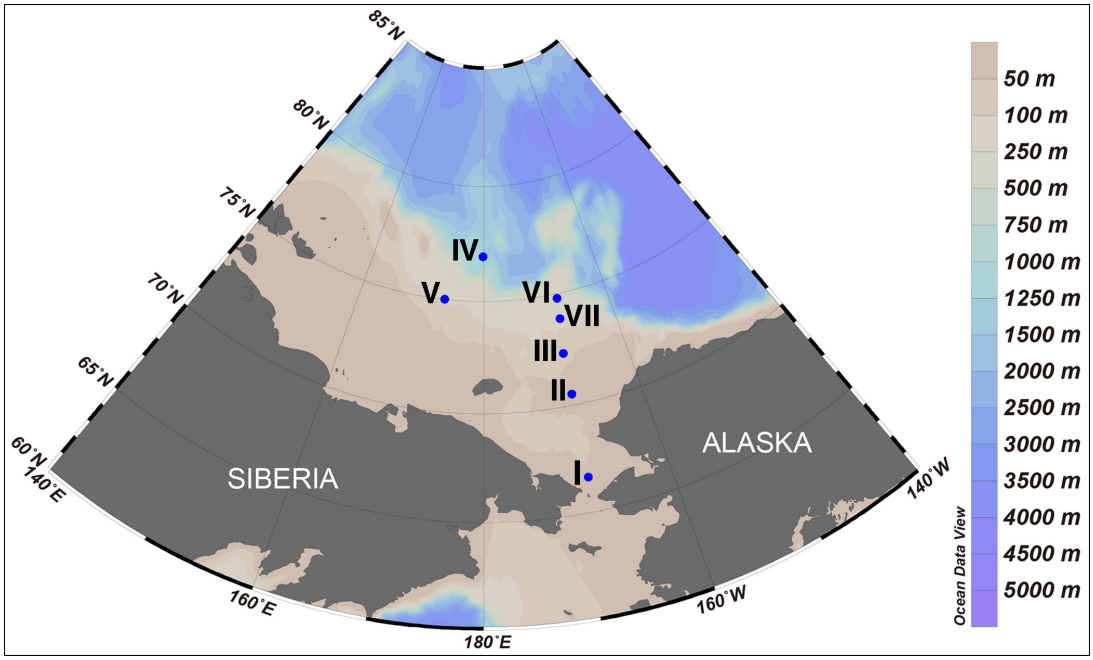

Fig. 5. Locations of the sampling sites in the Chukchi Sea where Papulifère forms were found in plankton net tow material gathered during survey cruises in 2015, 2021, and 2022. The sites are numbered I to VII in chronological order of sampling. Details of the sites and sampling are given in Table 2.

Table 2. Details of the plankton net samples in which Papulifère forms were found. Sample code refers to location labels shown in Fig. 5. Sample Designation is composed of the cruise code and station number.

Sample Code (Fig. 2)	Sample Designation	Date m/d/yr time	Lat/Long	depth sampled	site depth	*Fusopsis* forms Fig. 4	Sphaeropsis forms Fig. 6
I	ARA06B St 2	8/20/2015 16:35	66.3°N/-168.7°E	0-35	200	2F	
II	ARA12B St 14	7/23/2021 21:00	70.5°N/-168.7°E	0-30	200	2I	
III	ARA12B St 18	7/23/2021 17:15	72.3°N/-168.7°E	0-40	200		3F
IV	ARA12B St 36	7/29/2021 17:30	76.9°N/179.8°E	0-100	300	2H	
V	ARA12B St 52	8/42021 5:25	75°N/173.5°E	0-100	300	2G	
VI	ARA12B St 72	8/10/2021 10:15	74.8°N/-168°E	0-100	300	2C	
VII	ARA13B St 16	7/272022 22:00	73.8°N/-168°E	0-100	310	2A, 2B, 2D, 2E, 2I-2L	3A-3E, 3G-3N

Shown in Figure 6 are the 12 forms we found which could be placed among the spindle-shaped and oblong-shaped Papulifère in Meunier's genus *Fusopsis*. Three, (Fig. 6A, B and C) could be described as long-tailed, and resemble most closely Meunier's *Fusopsis spiralis* shown in Fig. 1F, in terms of maximum width to length. However, they none have the spiral surface stripes, and they differ among themselves in the shape of the *papula* or terminal cap. The form shown in Fig. 6D corresponds closely with Meunier's *Fusopsis flagrifera*, shown in Fig. 1H and the form shown in Fig. 6E corresponds closely with Meunier's *Fusopsis umbricula* shown in Fig. 1B. The form shown in Fig. 6F resembles that shown in Fig. 3D, as it also resembles *Fusopsis flagifera*, but it differs from form shown in Fig. 6D being shorter in length and having terminal filaments relatively thicker and fewer in number. The fact that the terminal filaments are bent is perhaps a plankton net treatment artifact as we have seen specimens of the forms shown in Fig. 6D and Fig. 6E with some terminal filaments bent. The forms shown in Figs. 6G, 6H, 6I, 6J, 6K, and 6L appear to be distinct from any of Meunier's forms. The forms shown in Figs. 6H, 6I, 6k, and 6L have distinctive pointed *papula* caps similar to that of the form in Fig. 1C. Other distinctions of the *Fusopsis* forms we found are given in Table 3.

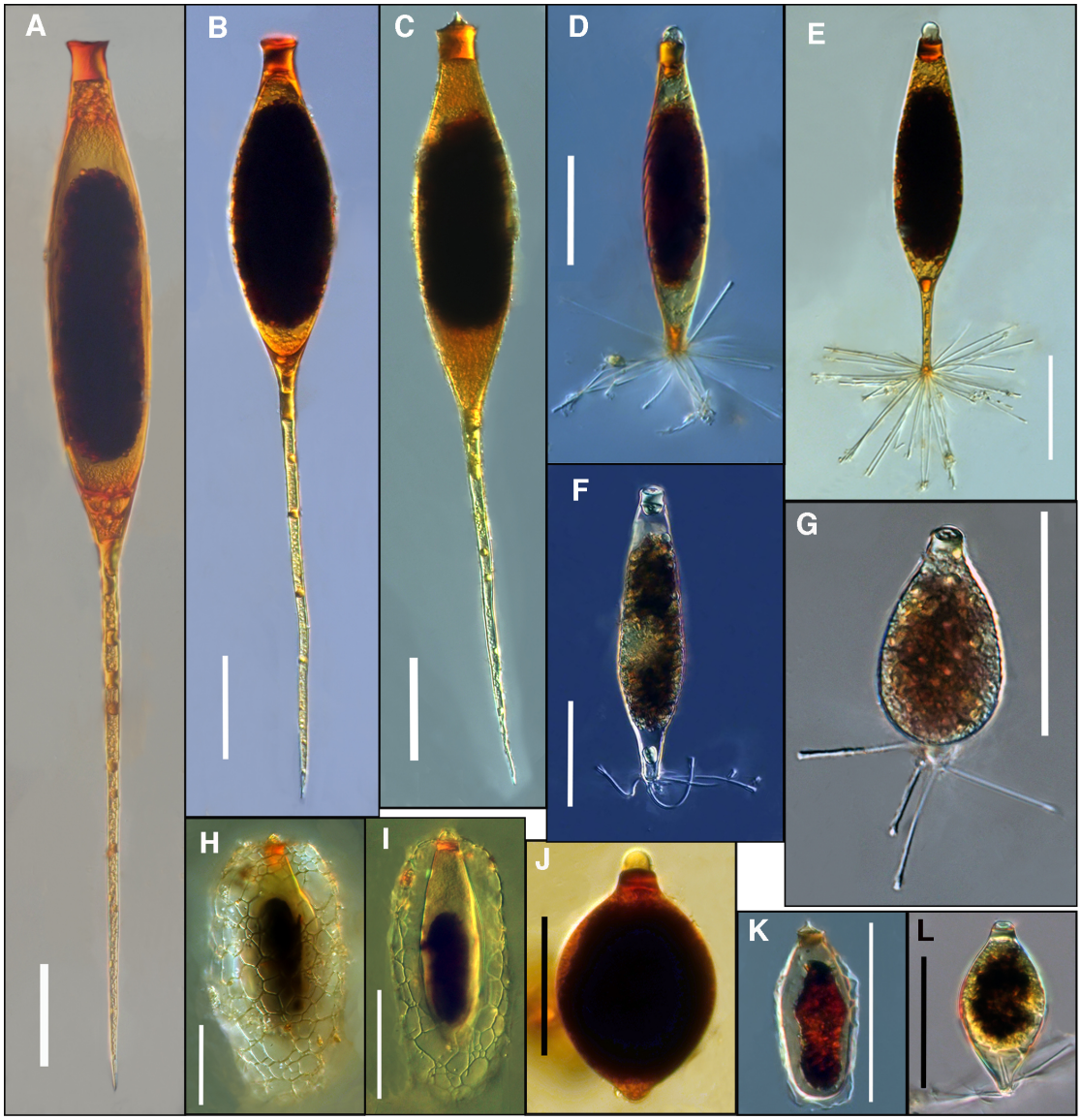

Fig. 6. The 12 fusiform (spindle-shaped, and oblong) *Fusuposis* papuliferid cyst forms found in Chukchi Sea plankton net tow material. The specimens shown in A, B, D, E, I, K, and L are all from the 2022 station 16. Scale bars all represent 50 µm.

Table 3. Dimensions, notes on morphology, and possible affinities of the 12 *Fusoposis* forms found in the Chukchi Sea samples shown in Fig. 6 with Meunier's *Fusopsis* forms shown in Fig. 1. Longest dimension (LD) and maximum breadth [MB] are given in µm, excluding terminal filaments. When two specimens were found the range is given. When multiple specimens were found, the means are given ± standard deviation and number of specimens found (n).

Chukchi Sea Figure 6 (LD, µm) [MB, µm]	**Notes on Morphology**
Fig. 6A (385-510) [38-50]	Resembles *Fusopsis spiralis* (Fig. 1F) in overall shape but no surface markings were visible, papule differs from 6B, 6C
Fig. 6B (365) [53]	Resembles *Fusopsis spiralis* (Fig. 1F) in overall shape but no surface markings were visible, papule differs from 6A, 6C
Fig. 6C (375) [50]	Resembles *Fusopsis spiralis* (Fig. 1F) in overall shape but no surface markings were visible, papule differs from 6A, 6B
Fig. 6D (122±27.7, n=9) [31±6.9, n= 9]	Resembles *Fusopsis flagrifera*, terminal filaments fewer, slightly thicker than in form 6E
Fig. 6E (147±19.5, n=53) [32±4.3, n=53]	Resembles *Fusopsis umbracula*, large size range found, terminal filaments more numerous and thinner than in form 6D
Fig. 6F (125) [30]	5 thick terminal filaments
Fig. 6G (55) [25]	3-4 thick terminal filaments
Fig. 6H (160) [90]	Unlike any Meunier form, interior capsule 120 x 40 µm
Fig. 6I (135) [55]	Unlike any Meunier form, interior capsule 90 x 25 µm
Fig. 6J (90) [55]	Unlike any Meunier form
Fig. 6K (50) [20]	Unlike any Meunier form, interior capsule 45 x 15 µm
Fig. 6L (65-65) [40-40]	Unlike any Meunier form, 8-9 terminal filaments

The 14 spherical or oblong forms of Papulifère we found in Chukchi Sea samples, corresponding to Meunier's *Sphaeropsis*, are shown in Fig. 7 and the dimensions of the forms are given in Table 4. Seven of the *Sphaeropsis* forms resembled one of Meunier's *Sphaeroposis* species (Table 4). Two of the four, shown in Fig. 8A and 8E, resembled Meunier's *S. heterosetosa* (shown in Fig. 1O), and were nearly identical except in size. Three forms, Fig. 7D, 7L, and 7M had an outer layer resembling a corrugated membrane like that of Meunier 's *Sphaeropsis spumosa*, but differing in

other aspects as indicated in Table 4. We found seven spherical forms of Papulifère that do not resemble any that Meunier described as *Sphaeropsis*. Other distinctions of the *Sphaeropsis* forms we found are given in Table 4.

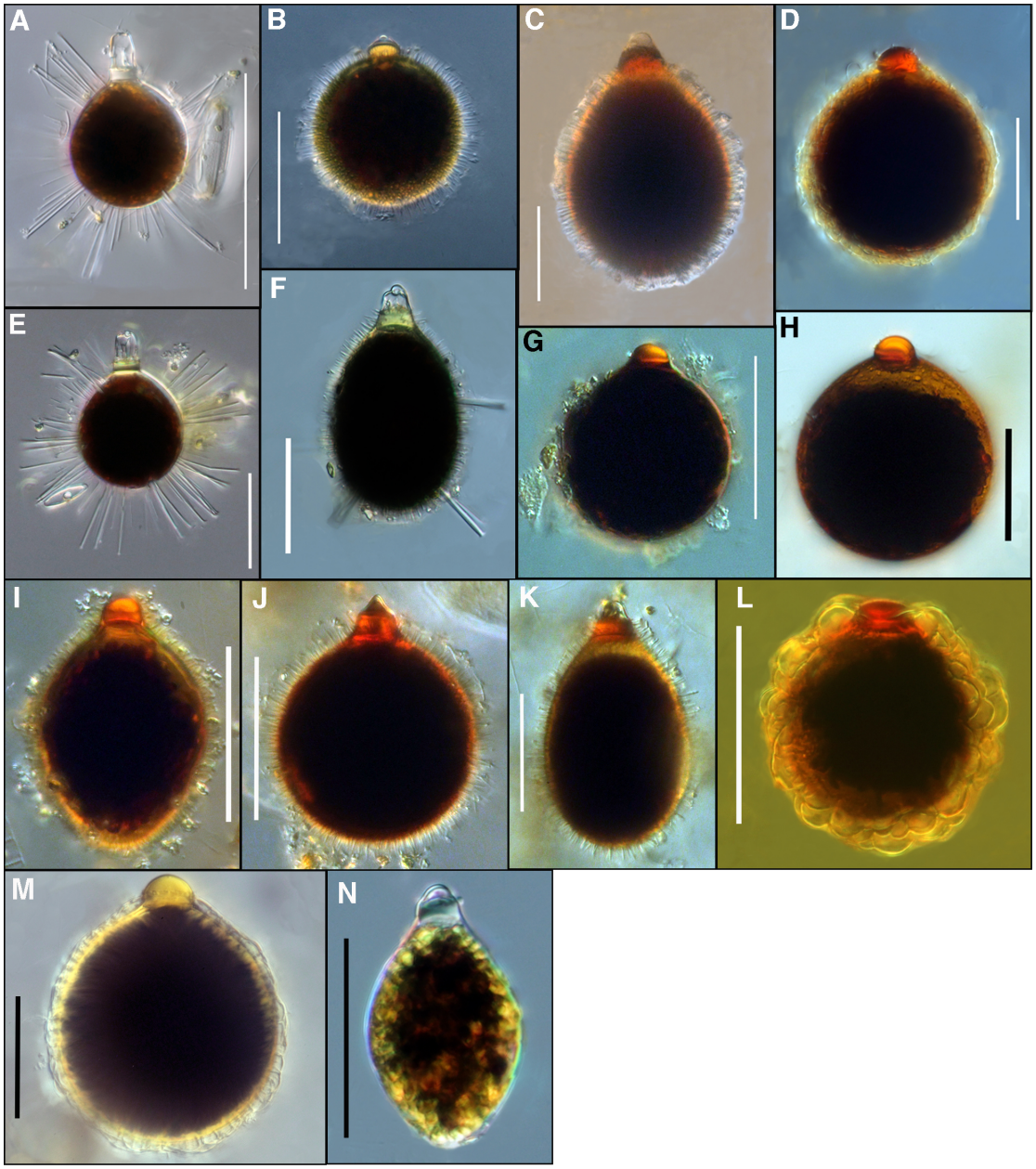

Fig. 7. The 14 spherical/ovoid *'Sphaeropsis*' papuliferid cyst forms found in Chukchi Sea plankton net tow material. All the specimens shown are from the 2022 sample station 16 (sample VII in Table 2), except the one shown in Fig. F. Scale bars all represent 50 µm.

Table 4. Dimension, morphological notes and affinities of the 14 *Sphaeropsis* forms found in the Chukchi Sea samples shown in Fig. 7 with Meunier's *Sphaeropsis* forms shown in Fig. 1. Longest dimension (LD) and maximum breadth [MB] are given in µm, excluding spines or outer membrane as in Fig. 7L. When two specimens were found the range is given. When multiple specimens were found, the means are given ± standard deviation and number of specimens found (n).

Chukchi Sea forms in Fig. 7 (LD, µm)	**Notes on Morphology**
Fig. 7A (45±8.7, n=3) [28±5.4, n= 3]	Resembles *Sphaeropsis heterosetosa* (Fig. 1.O), opaque portion of papule about 1/4 of papule length, longest spines about 2/3 MB
Fig. 7B (74±7.2, n=20) [62±6.1, n=20]	Resembles *Sphaeropsis brevisetosa* (Fig. 1.S), spherical without papule, opaque portion of papule 1/2 papule length
Fig. 7C (130)[75]	Oblong, unlike any Meunier form, opaque portion of papule about 3/4 of papule length
Fig. 7D (85) [65]	Surface appears corrugated, resembles *Sphaeropsis spumosa* (Fig. 1.T), papule without hyaline portion
Fig. 7E (70-80) [48-55]	Resembles *Sphaeropsis heterosetosa* (Fig. 1.O), opaque portion of papule about 1/4 of papule length, longest spines about 2/3 MB, larger than 7A
Fig. 7F (100) [55]	Oblong, unlike any Meunier form, opaque portion of papule about 3/4 of papule length, long spines length about 1/2 MB
Fig. 7G (59±4.2, n=5) [45±3.9, n=5]	Smooth surface, unlike any Meunier form, papule without hyaline portion, resembles Fig. 7.H but smaller
Fig. 7H (80-100) [72-90]	Smooth surface, unlike any Meunier form, papule without hyaline portion, resembles Fig. 7.G but larger
Fig. 7I (66±7.4, n=6) [42±4.7, n=6]	Oblong, unlike any Meunier form, papule without hyaline portion, resembles Fig.7.K but smaller
Fig. 7J (75) [55]	Resembles *Sphaeropsis brevisetosa* (Fig. 1.S), and Fig.7B but larger and papule without hyaline portion
Fig. 7K (100-105) [52-55]	Oblong, unlike any Meunier form, resembles Fig.7C but elongate and shorter, opaque portion of papule about 3/4 of papule length
Fig. 7L (64±4.8, n=4) [43±3.2, n=4]	Surface appears corrugated, resembles *Sphaeropsis spumosa* (Fig. 1.T) and Fig. 7.D, but with thicker membranous coat, papule without hyaline portion
Fig. 7M (110) [90]	Surface appears corrugated, resembles *Sphaeropsis spumosa* (Fig. 1.T), Fig. 7.D, and but larger than Fig. 7.D, papule without hyaline portion
Fig. 7N (65) [35]	Smooth surface, oblong, unlike any Meunier form, opaque portion of papule about 3/4 of papule length

DISCUSSION

We found a surprising diversity of Papulifère forms, most of which appear to be previously unknown. The sample that provided an extraordinary variety of forms was sample VII, from 2022 cruise in the Chukchi Sea. It yielded 16 different forms, 8 *Fusopsis* forms, and 8 *Sphaeropsis* forms. There was no obvious difference between the Papilifère-rich sample and those from the preceding and succeeding stations sampled. The sample had the same ciliate fauna in which no Papulifère were found in terms of the species present and their abundances. The ciliate fauna in sample VII and nearby stations was dominated by moderate-sized oligotrichs (30 µm length), a euplotid species about 100 µm long, and the tintinnids *Pytchocylis obtusa*, *Leprotintinnus pellucides*, *Salpingella faurie*. No specimens of encysted tintinnids were observed. With regard to the observed ciliate fauna, it should be noted that all of the samples were from a 20 µm net pore-size plankton net which likely under-sampled small species. Any differences in quantities or composition of small taxa in the Papulifère-rich sample compared to other samples would not have been detectable. Furthermore, it appears that taxa may form Papulifères much larger than the vegetative cell. In regard to the size of the known Papulifère cyst formed by the oligotrich *Cyrtostrombidium boreal*, the *Fusopsis*-type cyst it forms was reported to range in maximim length from 200 to 245 µm and to be formed when the vegetative cells are 50 to 60 µm in length (Kim et al. 2002). Thus a *Fusopsis*-form cyst may be much larger in longest dimension than the vegetative cell forming the cyst. The Papulifère specimens we found all contained dark matter stained by the Lugol's solution used to preserve the samples. Thus, we did not encounter any "empty" Papulifère forms such as those in Fig. 4 from Continuous Plankton Recorder samples.

Some of the *Fusopsis* Papulifère forms we found in Chukchi Sea are quite likely ciliate cysts. Kim et al. (2002) showed that the oligotrich ciliate *Cytrostrombidium boreale* emerges from cysts like those shown in Fig. 4 as A, B. and C. These same *Fusopsis* forms, greatly resemble forms Gurdebeke et al. (2023) showed (except for a lack of surface stripes), based sequence-based evidence, to be closely related to *Cytrostrombidium*. However, even with regard to such known cysts, we lack knowledge of both morphological variability and developmental stages. Thus, it is possible that some of the forms we distinguished as distinct, for example, based on the criterion of size (e.g., Fig. 7.A and 7.E), are actually from a single population. Others we distinguish might be simply developmental stages of a single type, for example, the forms in Fig. 6D being an early developmental stage of the form shown in 6E.

For some of the forms we found, morphological variability could not be estimated as only one specimen was encountered. However, for many forms we provide, for the first time some estimates of variability in overall dimensions (see Tables 3 and 4). Two forms were found in numbers sufficient for a preliminary examination of morphological variability, in particular, the frequency distribution of longest dimension. We hypothesized that the frequency distribution of a total length, if bi-

modal, suggests distinct populations may have been sampled whereas a more or less normal distribution supports a view that a single population was sampled. Two forms were found in sufficient numbers to examine frequency distributions of longest dimension. We found 53 specimens of the form resembling *Fusopsis umbracula* (shown in Figs. 1E and 6E), and 20 specimens of the form resembling *Sphaeropsis brevisetosa* (shown in Figs. 1S and 7B). The frequency distributions for both forms is right-shifted normal and thus provides no evidence that distinct populations were sampled (Fig. 8) but rather suggests that single populations with a wide ranges of largest dimension were sampled. The fact that the two forms found in numbers sufficient to examine morphological variability, albeit crudely (recall that measurements were made to the nearest 5 µm), were found to have wide ranges of longest dimensions indicates that it may be unwise to distinguish forms based on size differences alone, as we have with regard to the 3 pairs of Figs. 7A and 7E, Figs. 7G and 7H, and Figs. 7D and 7M. However, we retained the distinctions for now as later 'lumping' is possible but not 'splitting'.

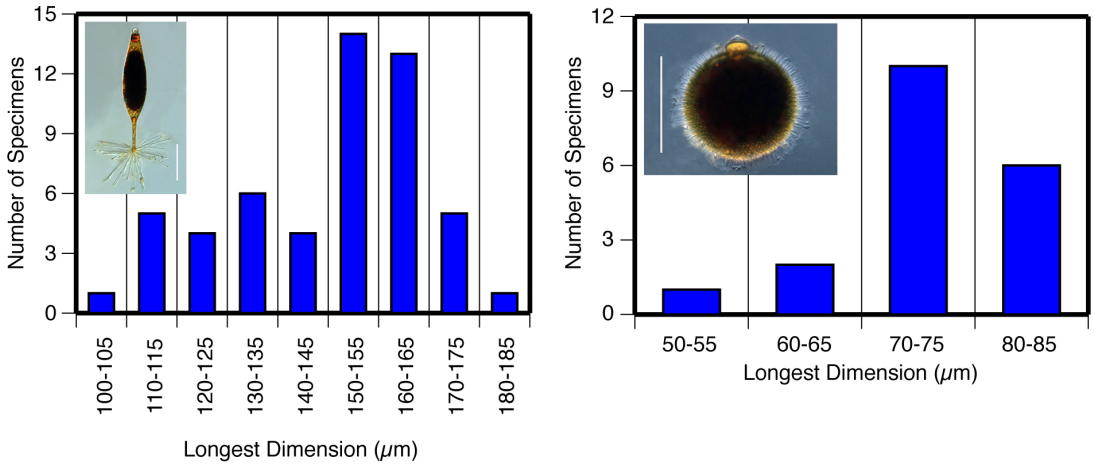

Fig. 8. Frequency distributions of the largest dimensions of the two forms found in the greatest abundance. The left panel shows the distribution of 53 specimens of the form shown in Fig. 6E, resembling Meunier's *Fusopsis umbracula* (Fig. 1B), parsed into size-classes of longest dimension. The right panel shows the distribution of 20 specimens of the form shown in Fig. 7B, resembling Meunier's *Sphaeropsis brevisetosa* (Fig. 1S), parsed into size-classes of longest dimension. The distributions of the size-classes appears more 'normal' than bi-modal' suggesting that single populations were sampled with wide size-ranges.

An interesting observation is that Papulifères have so far only been found in the marine plankton of temperate or polar systems. For example, the Continuous Plankton Recorder charts of the North Atlantic show many *Fusopsis* forms have been found in samples from North of about 45°N up into Arctic waters, but not south of about 45°N (Fig. 9). Thus, Papulifères appear to be restricted to, or markedly more common in, systems with large seasonal changes in the plankton where one might expect resting cyst formation to be more common than in systems with less seasonality such as in the tropics. However it is also a fact that temperate systems of the Northern

Hemisphere have been much better sampled than the tropics with regard to planktonic protists (e.g., Fig. 10.1 in Dolan and Pierce 2013 showing global tintinnid species records). Consequently the lack of records of Papulifères from subtropical or tropical systems may be due, at least in part, to under-sampling of warm water regions.

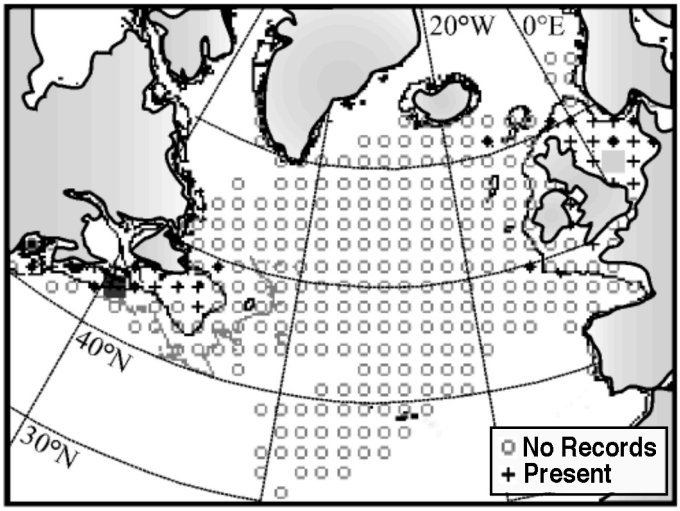

Fig. 9. Geographical distribution of the records of the occurrences of *Fusopsis* in the 155,000 samples collected with the Continuous Plankton Recorder (CPR) across the North Atlantic from 1958 to 1998, adapted from CPR (2004). Note the occurrence records from north of approximately 45°N and into the Arctic waters, in contrast to the absence of records from localities south of about 45°N. The CPR is a plankton sampling device towed by ships of opportunity which provides samples of the plankton of near surface waters captured on a filter gauze of approximately 270 µm mesh. For details see Beaugrand (2004) and CPR (2004). For the history of the device see Dolan (2022).

Ideally identification of putative ciliate cysts should be derived from germination experiments *(e.g.*, Paranjape 1980; Kamiyama 1996) and/or sequencing of the nucleic acids of the cyst (*e.g.*, Gurdebeke et al. 2023). Obviously, germination experiments are not possible with fixed material and sequencing can be difficult or impossible depending upon the fixative. Nonetheless, tempting as it may be to assign a presumptive ciliate cyst to a specific taxon based solely on morphology, we caution against such a practice. Here we have attempted only to show that some of the large variety of Papulifère forms we encountered in Chukchi Sea samples resembling forms depicted in previous reports on Papulifère as well as apparently novel forms. We also provide some data on the size variability of some the forms we encountered. We hope to encourage further studies of these forms with interesting morphologies that are still today enigmatic!

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REFERENCES

Adamonis, S., Concheyro, A., & Alder, V. 2007. Protistas autótrofos y heterótrofos: silicoflagelados, ebridianos y tintinídos. In: H.H. Camacho & M.I. Longobucco (eds.) Los Invertebrados Fósiles Tomo I, Fundación de Historia Natural Félix de Azara, Vazquez Mazzini Editores, Buenos Aires, p. 133–145.

Allan, E., de Vernal, A., Krawczyk, D., Moros, M., Radi, T., Rochon, A., Seidenkrantz, M.S. and Zaragosi, S., (2020) Distribution of dinocyst assemblages in surface sediment samples from the West Greenland margin. *Mar. Micropaleo*., **159**: p.101818.

Anon. 1916. B. Plankton animal, *ICES J. Mar. Sc*i., Volume s1, Issue 70: 35–83, https://doi.org/10.1093/icesjms/s1.70.35

Beaugrand, G. (2004). Continuous plankton records: plankton atlas of the North Atlantic Ocean (1958–1999). I. Introduction and methodology. Mar. Ecol. Prog. Ser., Suppl., 3-10.

Biernacka, I. (1952) Studies on the reproduction of some species of the genus *Tintinnopsis* Stein. *Ann. Univ. Mariae Curie-Sklodowska Sect. C*, **6**:211-247.

Canu, E. (1893) Notes de biologie marine, fauniques ou éthologiques. *Annal. Stat. Aquicole Boulogne-sur-Mer*, **1**: 99-116.

Continuous Plankton Recorder Survey Team. (2004). Continuous plankton records: plankton atlas of the North Atlantic Ocean (1958–1999). II. Biogeographical charts. Mar. Ecol. Prog. Ser., Suppl., 11-75.

Davis, C. C. (1986) A comparison of the zooplankton in two Newfoundland bays with differing influences from major currents. Internat. Rev ges. Hydrobiol. Hydrogr., **71**:11-47.

Della Tommasa L, Belmonte G, Palanques A, Puig P, Boero F (2000) Resting stages in a submarine canyon: a component of shallow-deep-sea coupling? *Hydrobiologia* **440**:249 –260

De Pauw, N. (1969). Contribution à l'étude du plankton dans le port d'Ostende. *Biologisch Jaarboek*, **37**:186-261.

Dolan, J. R. (2022) Pioneers of plankton research: Alister Hardy (1896-1985). J. Plankton Res., **44**:477-485.

Dolan, J.R., Pierce, R.W. 2013. Diversity and distributions of tintinnids. in Dolan, J.R., Montagnes, D.J.S., Agatha, S., Coats, D.W., Stoecker, D.K. (eds) The Biology and Ecology of Tintinnid Ciliates: Models for Marine Plankton. Oxford, John Wiley & Sons, LTD., 214-243.

Dolan, J. R., Yang, E. J. (2017) Observations of apparent lorica variability in *Salpingacantha* (Ciliophora: Tintinnida) in the Northern Pacific and Arctic Oceans. *Acta Protozool*., **56**:217-220.

Dolan, J. R., Yang, E. J., Kim, T. W., Kang, S. H. (2014). Microzooplankton in a warming Arctic: A comparison of tintinnids and radiolarians from summer 2011 and 2012 in the Chukchi Sea. *Acta Protozool*., **53**:101-113.

Dolan, J. R., Yang, E. J., Kang, S. H., Rhee, T. S. (2016) Declines in both redundant and trace species characterize the latitudinal diversity gradient in tintinnid ciliates. *ISME J.*, **10**:2174-2183.

Dolan, J. R., Moon, J. K., Yang, E. J. (2021) Notes on the occurrence of tintinnid ciliates, and the nasselarian radiolarian *Amphimelissa setosa* of the marine microzooplankton, in the Chukchi Sea (Arctic Ocean) sampled each August from 2011 to 2020. *Acta Protozool.*, **60**:1-11.

Ganser, M. H., Bartel, H., Weißenbacher, B., Andosch, A., Lütz-Meindl, U., Radacher, P., Agatha, S. (2022) A light and electron microscopical study on the resting cyst of the tintinnid *Schmidingerella* (Alveolata, Ciliophora) including a phylogeny-aware comparison. *Eur. J. Protistol.*, **86**:125922.

Gurdebeke, P.R., Mertens, K.N., Rajter, L., Meyvisch, P., Potvin, E., Yang, E.J., André, C., Pospelova, V., Louwye, S., (2023) The ciliophoran affinity of *Radiosperma textum*, and its relation to other marine ciliate cysts. *Mar. Micropaleo.*, **178**: p.102185.

Heikkilä, M., Pospelova, V., Forest, A., Stern, G. A., Fortier, L., Macdonald, R. W. (2016) Dinoflagellate cyst production over an annual cycle in seasonally ice-covered Hudson Bay. *Mar. Micropaleo.*, **125**:1-24.
Horner, R.A. (1978) Beaufort Sea plankton Studies. *Environmental assessment of the Alaskan Continental Shelf: Annual reports of principal investigators*, **5:**85-142.

Ichinomiya, M., Nakamachi, M., Fukuchi, M., Taniguchi, A. (2008) Resting cells of microorganisms in the 20-100 μm fraction of marine sediments in an Antarctic coastal area. Pol. Sci., **2**:27-32.

Kamiyama, T. (1996) Determination of the abundance of viable tintinnid cysts in marine sediments in Hiroshima Bay, the Seto Inland Sea of Japan, using a modified MPN method. *J. Plankton Res*, **18:**1253-1259.

Kamiyama, T. (2013). Comparative biology of tintinnid cysts. in: *The Biology and Ecology of Tintinnid Ciliates: Models for Marine Plankton*, edited by: Dolan, J. R., Montagnes, D. J. S., Agatha, S., Coats, D.W., and Stoecker, D. K., 171–185,Wiley-Blackwell, Hoboken, New Jersey, USA, 171-185.

Kim, Y. O., Suzuki, T., Taniguchi, A. (2002) Systematics and Evolution-A New Species in the Genus *Cyrtostrombidium* (Ciliphora, Oligotrichia, Oligotrichida): Its Morphology, Seasonal Cycle and Resting Stage. *J. Euk. Microbiol.*, **49**:338-343.

Kufferath, H. (1950) Recherches sur le plancton de la mer flamande (mer du Nord méridionale): 1. Quelques flagellés, protistes et" cætera". *Institut Royal des Sciences Naturelles de Belgique*, **26**:1-43.

Meunier, A., 1910. *Microplankton des Mers de Barents et de Kara*. Duc d'Orléans. Campagne arctique de 1907. Imprimerie scientifique Charles Bulens, Bruxelles. 355 p.

Meunier, A., 1919. *Microplancton de la Mer flamande*. 4me partie. Les Tintinnides et cetera. Mém. Mus. Roy. Hist. Nat. Belg. 8, 59 p.

Montagnes, D. J., Lowe, C. D., Poulton, A., Jonsson, P. R. (2002) Redescription of *Strombidium oculatum* Gruber 1884 (Ciliophora, Oligotrichia). *J. Euk. Microbiol*., **49**:329-337.

Moscatello, S., Rubino, F., Saracino, O. D., Fanelli, G., Belmonte, G., Boero, F. (2004) Plankton biodiversity around the Salento Peninsula (South East Italy): an integrated water/sediment approach. *Sci. Mar*., **68**(S1):85-102.

Mudie, P. J., Marret, F., Gurdebeke, P. R., Hartman, J. D., Reid, P. C. (2021a). Marine dinocysts, acritarchs and less well-known NPP: tintinnids, ostracod and foraminiferal linings, copepod and worm remains. *Geological Society, London, Special Publications,* **511**:159-232.

Mudie, P.J., Leroy, S.A.G., Marret, F., Gerasimenko, N.P., Kholeif, S.E.A., Sapelko, T., and Filipova-Marinova, M., (2021b) Nonpollen palynomorphs: Indicators of salinity and environmental change in the Caspian–Black Sea–Mediterranean corridor, In Buynevich, I.V., Yanko-Hombach, V., Gilbert, A.S., and Martin, R.E., (eds)., *Geology and Geoarchaeology of the Black Sea Region: Beyond the Flood Hypothesis*, Geological Society of America Special Paper 473, p. 89–115.

Paranjape, M. A. (1980) Occurrence and significance of resting cysts in a hyaline tintinnid, *Helicostomella subulata* (Ehre.) Jorgensen. J. Exp. Mar. Biol. Ecol., **48:**23-33.

Pieńkowski, A. J., Mudie, P. J., England, J. H., Smith, J. N., Furze, M. F. (2011). Late Holocene environmental conditions in Coronation Gulf, southwestern Canadian Arctic Archipelago: evidence from dinoflagellate cysts, other non‐pollen palynomorphs, and pollen. *J. Quatern. Sci*., **26**:839-853.

Pieńkowski, A. J., Kennaway, S., Lang, S. I. (2020) Aquatic palynomorphs from modern marine sediments in a reconnaissance transect across the Northwest Passage–Baffin Bay region. *Mar. Micropaleo*., **156**:101825.

Price, A. M., Pospelova, V. (2011) High-resolution sediment trap study of organic-walled dinoflagellate cyst production and biogenic silica flux in Saanich Inlet (BC, Canada). *Mar. Micropaleo*., **80**:18-43.

Reid, P. C., & John, A. W. (1978). Tintinnid cysts. J. Mar. Biol. Assoc. United Kingdom, 58:551-557.

Reid, P. C., John, A. W. (1981). A possible relationship between chitinozoa and tintinnids. *Rev. Palaeobot. Palynol*., **34**:251-262.

Rubino, F., Belmonte, M., Galil, B.S. (2017) Plankton resting stages in recent sediments of Haifa port, Israel (Eastern Mediterranean)- distribution, viability and potential environmental consequences. Mar. Poll. Bull., **116**: 258-269.

Vanhöffen, E. 1897. Grönland-Expedition der Gesellschaft für Erdkunde zu Berlin 1891-1893. 2: 1-384.

Wright, R. R. (1907). The plankton of eastern Nova Scotia waters. Cont. Canad. Biology, 39th Ann. Rept. Dept. of Marine and Fisheries, Fisheries Branch. Ottawa, **31**:1-21.