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**Short Communication**

# New observations of Papulifères, putative ciliate cysts, from the plankton of the Chukchi Sea (Western Arctic Ocean) in August of 2023

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**Abstract**. We recently documented the existence of 26 different forms of Papulifères, presumptive ciliate cysts, from plankton net tow material of the Chukchi Sea gathered in 2015, 2021, and 2022. The forms appeared to be rare, found in only 7 of the 308 samples taken in annual August surveys from 2010 to 2022. Thus, we were surprised to find them relatively common and widespread in samples gathered in August of 2023. We found 18 different Papulifére forms, 8 of which appear to be new forms, in samples from 19 of the 36 stations sampled. They were found in localities ranging from 70.5°N to 80°N. Here we report on these Papulifére forms found in 2023, providing information on morphologies and geographical distributions. With this report we have expanded the catalogue of observed Papulifére, and photographed, forms of the Chukchi Sea to a grand total of 34 of morphologically distinct forms. However, we continue to urge caution in assigning a ciliate identity to any given Papulifére form in the absence of corroborating data.

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

## INTRODUCTION

In a recent publication in this journal (Dolan et al. 2023), we provided the first review of Alphonse Meunier's enigmatic "Papulifères", from their first descriptions until recent years. Briefly, they were first illustrated as 'eggs' in a report by Meunier on microorganisms of sea ice (Meunier 1909), and then described, with species names, as possible cysts of unknown forms in his report on the microplankton of the Kara and Barents Seas (Meunier 1910), and augmented in his study of the plankton of the North Sea (Meunier 1919). For many years, little attention was given to Papulifères until they were considered to be the cysts of tintinnid ciliates by Reid and John (1978, 1981), and are still described, as such, by some authors (e.g., Allan et al. 2020; Pieńkowski et al. 2011; Mudie et al. 2021). However, over 20 years ago, one of the largest and most conspicuous of Meunier's Papulifères, a spindle-shaped *Fusopsis*, was found to be the cyst of the marine planktonic oligotrich *Cyrtostrombidium boreale* in the North

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Pacific Ocean (Kim et al. 2002). Subsequently, many studies have considered various Papulifère forms to be the cysts of oligotrich ciliates (Moscatello et al. 2004; Ichinomiya et al. 2008; Price and Pospelova 2011; Heikkilä et al. 2016; Rubino et al. 2017; Matsuoka and Ishii 2018). Recently, Gurdebeke et al. (2023) sequenced large spindle-shaped *Fusopsis* forms from the Chukchi and Labrador Seas, and found them to be most closely related to *Cyrtostrombidium* species, confirming Kim et al.'s findings concerning *Fusopsis* forms found in the North Pacific Ocean.

In our Papulifère review paper (Dolan et al. 2023), we reported on finding Papulifère forms not reported upon since their descriptions by Meunier, and documented the existence of 13 apparently new forms (i.e., unlike any reported on by Meunier), all from samples from the Chukchi Sea (Western Arctic Ocean) taken in 2015, 2021, and 2022. Nonetheless, we found Papulifères to be quite rare as we found them in only seven of the over 300 samples taken in annual survey campaigns since 2010. Consequently, we were surprised to find Papulifère forms in many samples from the 2023 survey campaign conducted in August of 2023. Here, in this brief report, we provide images of the new Papulifère forms found, and details of the locations in which they were found as well the new observations of Papulifère forms that we recently described (Dolan et al. 2023).

## METHODS AND MATERIALS

We have previously reported in detail the material and methods used in our studies of Chukchi Sea microplankton (Dolan and Yang 2017, Dolan et al. 2014, 2021, 2023). Sample collection methods, and analysis have been consistent with all microscopical examinations done by the first author, for this and the earlier, reports. For this report, data and samples were collected in the Chukchi Sea from onboard the Korean Research Icebreaker *Araon* in August of 2023. Station locations are shown in Fig. 2, with the exact locations and sampling dates given in Table 1. Samples for chlorophyll determinations at discrete depths (4–8 depths per station, depending on water column depth) were obtained using a Niskin bottle rosette. Plankton net tows were used to sample the microplankton community in which Papulifère forms were found. For chlorophyll a determinations, water samples of 0.3–1 l were filtered through a 0.7 μm Whatman glass fiber filter (GF/F). Chlorophyll *a* concentrations were determined onboard using a Turner Designs Trilogy model fluorometer calibrated using commercial chlorophyll *a* standards. For details of the protocols see Lee et al. (2007). Net tows were made with a 20-μm mesh plankton net of 0.45 m diameter towed from 100 m depth to the surface, except in shallow water stations. Net tow material was fixed with Lugol's (2 % final concentration). Aliquots of net tow material (1–3 ml) were examined in settling chambers using an inverted microscope equipped with DIC optics. Nominal concentrations of the forms reported on here were calculated based on the theoretical volume of water filtered in the net hauls, calculated from the net diameter and the depth of the net tow, and the final volume in the net cod end. Considering that some net clogging likely occurred, the nominal volumes of water filtered are likely underestimates. Furthermore, the nominal volumes of water sampled and the depth strata sampled varied among stations. Consequently, the concentrations reported here serve only to indicate very rough relative abundances among stations where Papulifère forms were found.

## RESULTS AND DISCUSSION

In the net tow material from the 2023 samples, we found Papulifère forms in 19 of the 36 stations sampled. They were far more common than in all the previous 13 years of sampling in which they were found in only a few samples in 2021 and one sample each in the years 2015 and 2022 (see Dolan et al. 2023) and it was thus unclear if station locations might be linked to the presence of Papulifères. Our 2023 samples containing Papulifére forms were widely distributed in the latitudinal zone sampled in 2023 (Fig. 1), and the locations varied widely in depth, sea surface temperature, and concentrations of chlorophyll a (Table 1). Thus, our 2023 samples indicate that station locations appear to be unrelated to the presence of Papulifères. There were no distinct characteristics of the oligotrich or tintinnid ciliate assemblages in the samples with Papulifères (unpublished observations). Overall, there were no obvious similarities among samples in which Papulifères were found compared to those in which none were found. Eight new forms of Papulifères were found. These are shown in Figure 2 with notes on the morphologies, locations found and concentrations in the samples given Table 2. The new forms consisted of four spindle-shaped

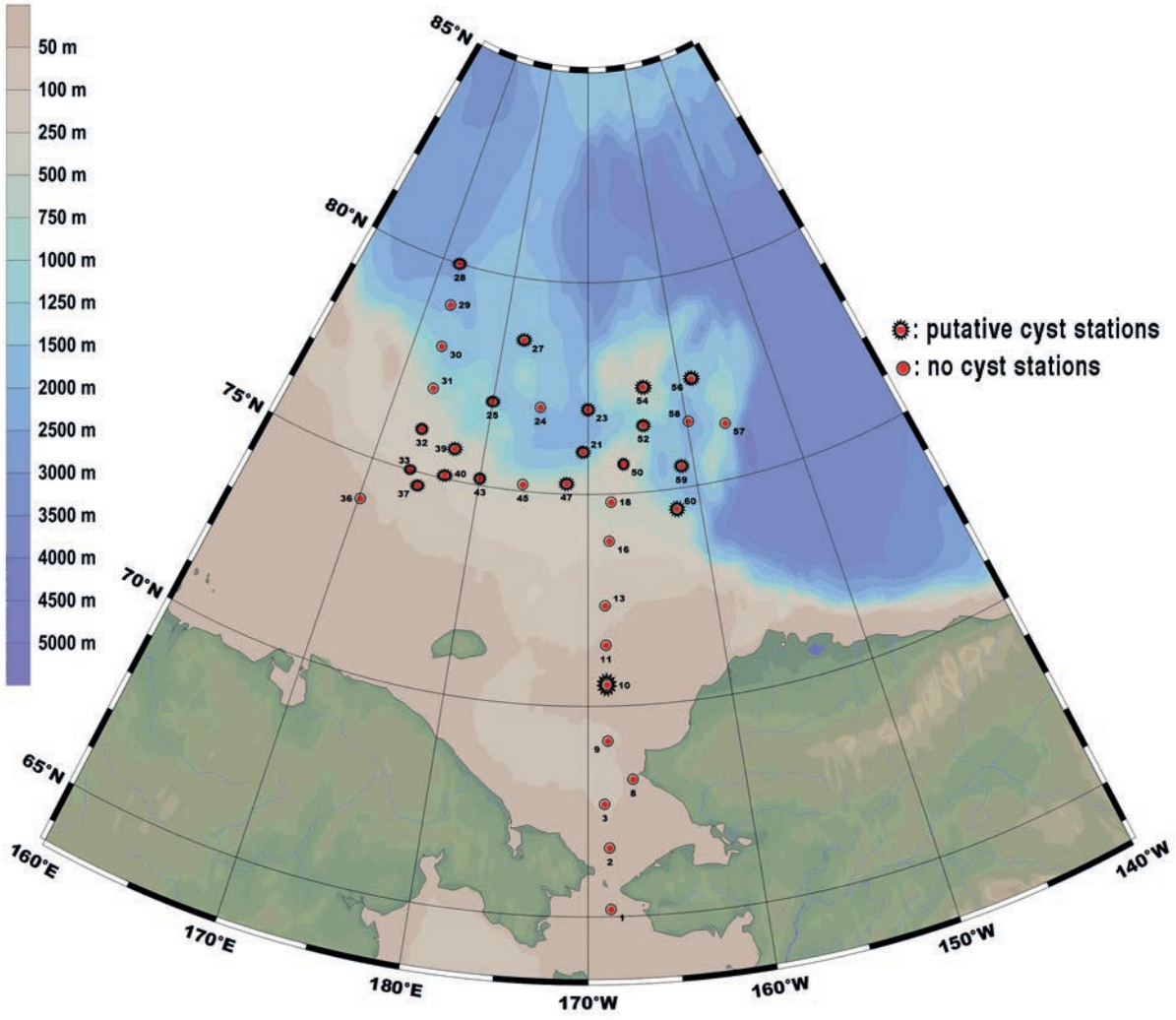


**Fig. 1.** Map of the Western Arctic Ocean showing the 36 station locations sampled using a 20 µm mesh plankton net in the Chukchi Sea region during August of 2023. Note that station locations yielding samples in which Papulifère, putative ciliate cysts forms, were found (putative cyst stations) were located throughout the region sampled. Detailed station characteristics are given in Table 1.

*Fusopsis* forms and four spherical or oblong shaped *Sphaeropsis* forms. In addition, we encountered three *Fusopsis* forms and six *Sphaeropsis* forms from among those described in our previous review (Dolan et al. 2023). Thus, the 2023 samples contained 18 Papulifère forms out of the total of 34 forms now known from the Chukchi Sea region.

We are at loss to explain why more Papulifère forms were found in 2023 compared to previous years. For example, Arctic sea ice extent in August of 2023 was 5.6 million km$^2$, about the average over the past ten years of 5.5 ±0.33 million km$^2$ (data from the National Snow and Ice Data Center, University of Colorado, Boulder, CO, U.S.A). Average chlorophyll *a* concentration among the 2023 stations (1.3 ±2,37 µg l$^{-1}$) was well within the wide range encountered in previous years of 0.2–1.9 µg l$^{-1}$ (Dolan et al. 2021). Thus, conditions in August of 2023 do not appear to have been clearly different from those of August in previous years. One might speculate that once an item is found, and identified as of interest, in further examinations, it is less likely to be overlooked and hence more often will be 'found'. However, re-examination of samples which yielded no cysts, from those sites near 'cyst stations' (see Fig. 1) failed to uncover cysts not previously encountered. Thus, we are inclined to believe that the

**Table 1**. Summary data for the 36 stations sampled in 2023. Depth given is the vertical extent of the plankton net tow from the depth indicated to the surface. Chlorophyll *a* concentration (Chl a) is average integrated concentration (µg L-1) throughout the water column from the surface to approximately the depth of the plankton net tow. Cyst types found refer to the new forms shown in here in Figure 2 (2A-2H), and in the Figures 6 (6A, 6E, 6D) and 7 (7A, 7G, 7H, 7I, 7J, 7K, 7N) in Dolan et al. (2023). For convenience, the supplementary file contains images of all now known Chukchi Sea Papulifère forms in two plates, one showing the 16 spindle-shaped *Fusopsis* forms, another showing the 18 the spherical and oblong *Sphaeropsi*s forms.

| St # | Date Aug 2023 | lat (N°) | long (W°) | Tow Depth (m) | Station Depth | Chl a | SST (C°) | cyst types found |
|---|---|---|---|---|---|---|---|---|
| 1 | 3 | 65,17 | –168,69 | 45 | 55 | 1,45 | 9,9 | Ø |
| 2 | 3 | 66,63 | –168,69 | 35 | 45 | 7,47 | 7,8 | Ø |
| 3 | 3 | 67,67 | –168,96 | 45 | 55 | 9,92 | 6 | Ø |
| 8 | 4 | 68,24 | –167,12 | 38 | 48 | 0,74 | 12,6 | Ø |
| 9 | 4 | 69,17 | –168,67 | 43 | 53 | 0,58 | 9,9 | Ø |
| **10** | **5** | **70,50** | **–168,67** | **35** | **45** | **6,48** | **6** | **2E, 2F** |
| 11 | 5 | 71,43 | –168,67 | 40 | 50 | 0,75 | 7,9 | Ø |
| **13** | **5** | **72,36** | **–168,66** | **50** | **60** | **6,16** | **1** | **6A** |
| 16 | 6 | 73,89 | –168,19 | 100 | 183 | 1,5 | 1 | Ø |
| 18 | 6 | 74,80 | –167,90 | 100 | 195 | 0,413 | 1 | Ø |
| **21** | **7** | **76,00** | **–170,49** | **100** | **1315** | **1,22** | **–0,5** | **6A, 7H, 7K, 7N** |
| **23** | **8** | **77,00** | **–170,00** | **100** | **2214** | **0,53** | **–1,1** | **6A** |
| 24 | 8 | 77,00 | –174,99 | 100 | 2012 | 0,34 | –1,3 | Ø |
| 25 | 9 | 77,00 | 179,95 | 100 | 1081 | 0,09 | –1,2 | Œ |
| **27** | **12** | **78,54** | **–177,58** | **100** | **1009** | **0,07** | **–1,2** | **7K** |
| **28** | **13** | **80,00** | **172,40** | **100** | **2702** | **0,14** | **–1** | **6A** |
| 29 | 14 | 79,00 | 172,80 | 100 | 2561 | 0,15 | –0,9 | Ø |
| 30 | 14 | 78,00 | 173,20 | 100 | 1133 | 0,14 | 0,3 | Ø |
| 31 | 15 | 77,00 | 173,60 | 100 | 740 | 0,4 | 0,2 | Ø |
| **32** | **15** | **76,00** | **173,61** | **100** | **265** | **0,2** | **–1,1** | **7H** |
| **33** | **16** | **75,00** | **173,60** | **100** | **147** | **0,07** | **–0,06** | **6E** |
| 36 | 16 | 74,00 | 170,16 | 40 | 52 | 0,1 | –0,8 | Ø |
| **37** | **17** | **74,69** | **174,62** | **60** | **72** | **0,2** | **–1** | **2A** |
| **39** | **18** | **75,73** | **177,18** | **100** | **499** | **0,15** | **–1,3** | **2G** |
| **40** | **20** | **75,07** | **176,80** | **100** | **196** | **0,31** | **–1,2** | **2B, 2H, 7I, 7N** |
| **43** | **21** | **75,16** | **–179,97** | **100** | **539** | **0,35** | **–1,4** | **6D, 6E, 7J** |
| 45 | 21 | 75,15 | –176,00 | 100 | 327 | 1,36 | –1 | Ø |
| **47** | **22** | **75,24** | **–171,97** | **100** | **505** | **2,73** | **–0,7** | **6A, 7J** |
| **50** | **23** | **75,69** | **–166,64** | **100** | **392** | **3,1** | **–0,9** | **6A, 7H, 7N** |
| **52** | **23** | **76,57** | **–164,36** | **100** | **550** | **0,019** | **–1,1** | **7G** |
| **54** | **24** | **77,47** | **–164,10** | **100** | **280** | **0,32** | **–1,3** | **2C, 7J** |
| **56** | **25** | **77,49** | **–158,73** | **100** | **1323** | **0,02** | **–1,3** | **7H, 7J** |
| 57 | 26 | 76,30 | –156,22 | 100 | 725 | 0,23 | –0,1 | Ø |
| 58 | 26 | 76,52 | –159,78 | 100 | 2113 | 0,21 | –1 | Ø |
| **59** | **27** | **75,50** | **–161,15** | **100** | **2098** | **0,2** | **–0,5** | **2D, 7A** |
| **60** | **27** | **74,52** | **–162,15** | **100** | **1596** | **0,27** | **1,8** | **2G, 6A, 7A, 7H** |

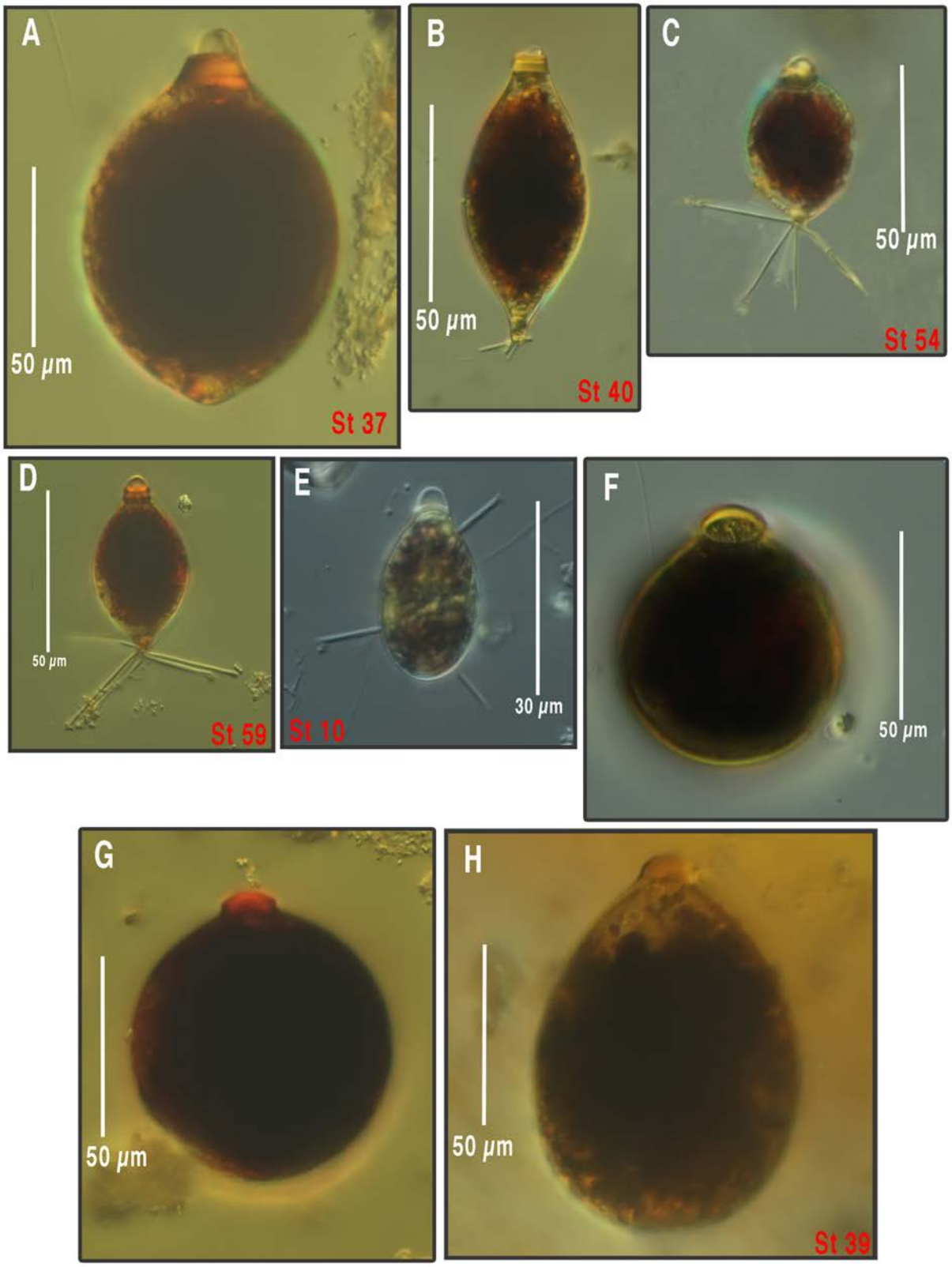


**Fig. 2.** New forms of Papulifères found in samples from the Chukchi Sea region taken in August 2023. Forms A–D are new spindle-shaped *Fusopsis* forms, and Forms E–H are new spherical or oblong-shaped *Sphaeropsis* forms. Morphology notes and stations in which each were found along with the nominal concentrations found in the samples are given in Table 2.

**Table 2**. The morphological characteristics and sample data (stations and nominal concentrations) of the apparently new Papulifère forms found in the samples taken in 2023. LD refers to longest dimension of the main body (excluding filaments if present). In Morphology Notes, 'Differs from fig. x.x' refer to the figures in Dolan et al. 2023; figures are also given in the Supplementary File.

| Cyst type | Morphology Notes | **Station** (s) found [conc. #$L^{-1}$] *# specimens* |
|---|---|---|
| 2A | *Fusopsis sp.,* spindle smooth surface/LD 115 µm<br>Differs from fig 6j in larger size and absence of a posterior knob | **37** [0.01] *2* |
| 2B | *Fusopsis sp*., spindle smooth surface/LD 75–85/µm short (3–5µm) filaments Differs from figs 6 f,g,l in larger size and spine lengths & numbers | **40** [.007] *1* |
| 2C | *Fusopsis sp*., spindle smooth surface/LD 50µm/5 long, 25 µm filaments<br>Differs from fig 6c in overall shape small size | **54** [0.016] *1* |
| 2D | *Fusopsis sp*., spindle smooth surface/LD 50 µm/ 3 pairs 30 µm filaments<br>Differs from fig 6g,l in larger size and filaments in pairs | **59** [0.007] *1* |
| 2E | *Sphaeropsis sp*., oblong smooth surface/ LD 35µm/ 3 10 µm filaments<br>Differs from fig 7f in smaller size and smooth surface | **10** [0.04] *1* |
| 2F | *Sphaeropsis sp.,* oblong smooth surface/LD 70 µm<br>Differs from fig 7h in smaller size and ovoid shape | **10** [0.01] *4* |
| 2G | *Sphaeropsis sp.,* spherical smooth surface/LD 80 µm<br>Differs from fig 7h in smaller size | **39** [0.08] *2*, **60** [0.08] *1* |
| 2H | *Sphaeropsis sp.,* oblong smooth surface/LD 105 µm<br>Differs in overall size and shape from other Sphaeropsis in fig7 | **40** [0.004] *1* |

samples from 2023, for reasons unknown to date, contained more putative cysts than the samples from previous years.

As we noted in our earlier review of Papulifère forms (Dolan et al. 2023), the actual identities of the forms are unknown with the singular exception of the one large spindle-shaped *Fusopsis* form shown to be formed by the oligotrich *Cyrtostrombidium boreale* by Kim et al. 2002, and sequence data obtained by Gurdebeke et al. (2023) from specimens found in the field relating such forms to *Cyrtostrombidium* species. Nonetheless, one cannot ignore the possibility that species other than *Cyrtostrombidium* may form similar cysts. In addition, no developmental data is available on any Papulifère forms. Thus, although the forms we described in our previous study, and in this report, appear to be distinct morphologically, we can not exclude the possibility that some of our 'distinct forms' are simply developmental stages of other 'distinct forms'. We reiterate the contention that as Papulifère forms, with the singular exception of the *Cyrtostrombidium*- related large *Fusopsis*, should probably not be taken to be ciliate cysts, but are rather denoted as putative ciliate cysts in the absence of corroborating observations. With the singular exception of the study by Gurdebeke et al. (2023), we lack molecular sequencing data and fine structure analysis from electron microscope examinations. We hope with this report to stimulate research into the nature of these intriguing forms!

**Supplementary material**. The supplementary file is a compendium of illustrations Papulifère, provided here as a convenient visual guide to the forms observed to date. It consists of three plates. Plate 1 shows photomicrographs of all of the *Fusopsis* forms of the Chukchi Sea region. Plate 2 shows photomicrographs of all of the *Sphaeropsis* forms, of the Chukchi Sea region. Plate 3 shows all of Meunier's illustrations of Papulifère forms found in samples from the Kara Sea (Meunier 1909, 1910) and the coastal waters of Belgium (Meunier 1919).

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