Kentrophoros Field Manual

Resources for working with meiofaunal marine ciliates

Brandon Seah
MPI for Marine Microbiology
compiled 2012–2018

Introduction

This manual compiles field and laboratory resources for working with *Kentrophoros* and similarly soft-bodied, marine meiofaunal ciliates. These were tested and adapted in the course of my thesis research over several field seasons between 2012 and 2017 at the Hydra Institute field station on Elba, the Wattenmeerstation Sylt on the North Sea coast, Carrie Bow Caye field station in Belize, and the Marine Biological Laboratory in Helsingør.

Additional appendices of taxonomic descriptions and on the geographical distribution of *Kentrophoros* are also provided as the literature is frequently difficult to access and scattered across numerous journals, many of which are not yet available online.

Further information on meiofaunal field techniques can be found in the book by Giere (2009), and in the doctoral dissertation of Fenchel. Methods for working with ciliates, particularly for microscopy and taxonomy, are available from the compilation by Foissner (2014) and in *Protocols in Protozoology*, published by the International Society of Protistologists.

1. Equipment

1.1. Typical equipment for field work

Standard equipment

- Beakers and spoons (for decanting)
- Metal knives or spatulas (for slicing cores)
- Pipetters
- Uhlig apparatus (see “Extraction methods”)
- Glass finger bowls, glass Petri dishes
- Black rubber matting (cut to size, as a dark background for glass bowls to see ciliates)
- Shallow black plastic trays (for sorting)
- Binocular stereo microscope (for handling, dissection)
- Light source (flexible-necked LED lamps, e.g. from IKEA, are cheap and convenient)
- Hot plate, portable weighing scale, …
- Nylon mesh, 150 micron
- Wooden clothes pegs, rubber bands (cut up old bicycle tubes)
- Measuring cylinders, pitchers, plastic funnels
- Forceps, scalpels
- Permanent markers, pens, labels
- Vacuum pump (if performing filtering)
- Refractometer, thermometer

Consumables

- Heat-sterilized glass Pasteur pipettes (wrap glass Pasteur pipettes in aluminium foil and sterilize in a muffle oven
- Rubber bulbs for Pasteur pipettes, or rubber tubing if mouth pipetting is preferred
- Exetainers, 5.9 or 12 mL (for keeping ciliates alive in short term with local sediment and seawater, or for transport)
- Microtubes (2 mL, 1.5 mL flip-caps, and 2 mL, 0.5 mL screw-caps)
- Kautex plastic bottles
- Plastic Petri dishes (5 cm and 10 cm diameter are most useful)
- Plastic pipette tips (1 mL and 100 microL are the most useful)
- Plastic bulb Pasteur pipettes
- Filters for preparing sterile seawater (syringe and bottle-top)
- Syringes (50 mL, 20 mL), needles
- Centrifuge tubes (50 mL, 15 mL)
- Ice cube bags or trays if using Uhlig method

- Glass slides and cover slips
- Paper filters (for pre-filtering seawater)

1.2. Preparing hydrophobic glassware by silanization

Hydrophobic glassware may be useful for handling extremely sticky species. However, with practice, uncoated glass may be used successfully. Stickiness may indicate problems with salinity.

Materials

SERVA siliconizing solution in isopropanol (sold for coating UV cuvettes)

Method

1. Dip glassware (e.g. glass Pasteur pipettes) into siliconizing solution, under fumehood.
2. Drip dry
3. Wrap with Al foil, bake in oven 100-160 C for > 2 h

Notes

- Siliconized layer is heat-resistant. Sterilize with dry heat. Remove with NaOH solution or saturated methanol solution. Coating can be corroded by strong acids and bases.
- See the SERVA manufacturer's manual for more information (catalog number 35130)
- Silanized pipettes are not suitable for handling samples for metabolomics

2. Extraction methods

As a member of the soft-bodied mieofauna, Kentrophoros has been regularly overlooked because it is usually not recovered by typical decanting-and-sieving methods, which tend to break up fragile organisms. The Uhlig seawater ice method made it possible to routinely extract karyorelict ciliates from sediment with almost quantitative efficiencies, but because of the osmotic and temperature shock that the ciliates are exposed to, they may not survive for very long after extraction. Meiofauna extraction methods have been reviewed by Giere (2009).

Table. Pros and cons of different extraction methods

Method	**Advantages**	**Disadvantages**
Digging a hole	Relatively gentle	Sediment has to be exposed (e.g. low tide)
Decantation	Can process large quantity of sediment Organisms recovered alive	Can be harsh – fragile species broken
Uhlig method	Organisms recovered alive Minimal contamination from dead biomatter – easier to sort Supposedly quantitative	Small quantities of sediment only (~10 to 100 mL) Osmotic shock – organisms eventually disintegrate Requires access to freezer, special apparatus
Magnesium chloride		Small quantities of sediment only Not all species anesthetized
Bulk fixation + Percoll	Described in (Wickham et al. 2000)	Poor preservation of morphology No live organisms recovered

2.1. Digging a hole

Simply digging a hole in the beach and letting water drain into the hole from surrounding sediment, bringing in ciliates and other meiofauna, e.g. (Sauerbrey 1928; Xu et al. 2011). This requires sediment of the correct grain size and porosity, that remains intact enough, but is not so porous that the meiofauna is poor. I have not successfully used this method myself.

2.2. Decantation

This refers to stirring up sediment in clean seawater, letting the heavier sediment grains settle, and then quickly decanting off the supernatant (including meiofauna and organic debris) into a tray or Petri dish for sorting.

Standard method (Elba) – Sediment from Sant' Andrea and Cavoli is relatively coarse and with little organic debris. The sediment is usually collected in large (ca. 10 L) closed buckets by Scuba divers and kept under local seawater at all times. Because the ciliates are relatively rare, a large amount of sediment has to be sorted. Pour some clean seawater into a small open bucket. Using a glass bowl, gently scoop out half a bowl of sediment from the collection bucket, keeping it as much as possible under seawater at all times. Transfer into the open bucket, which should have enough seawater to cover the sediment. Pour clean seawater from a large pitcher (2 L) into the open bucket, held at an angle, aiming for the bottom of the sediment such that it swirls it around and agitates it maximally. Immediately decant supernatant into a shallow black plastic tray for sorting. Repeat once or twice if necessary.

Nivå Bay – Ciliate meiofauna in Nivå is dense enough that one can do the following: Slice about 1 to 1.5 inches of sediment from a push core into a beaker filled with seawater. Stir up the sediment vigorously, but not so much that bubbles form, let the sand briefly settle, and decant into Petri dishes for sorting under the microscope. A “rest” period is usually required for the organic debris to settle down, and the ciliates to resume their usual movement, as they tend to freeze up immediately after being agitated.

2.3. Uhlig seawater-ice method

Originally described by (Uhlig 1964). The apparatus relies on cold, saline seawater to drive ciliates out of a sediment sample, through a nylon mesh (to keep the sediment out) into a collecting dish filled with seawater. Only live organisms will be recovered, and mostly soft-bodied organisms. One rarely finds nematodes and other species there.

Materials

Plexiglass or PVC piping, 5 cm internal diameter and about 15 cm long
Nylon mesh, 150 µm spacing
Silicone glue gun
Wooden clothes pegs
Rubber band (e.g. cut from a used bicycle inner tube)
Cotton wool
Seawater ice (made from filtered local seawater)
Petri dishes (5 cm diameter)
Petri dishes (10 cm diameter)
Filtered local seawater

Method

1. Building the apparatus: Cut out pieces of nylon mesh to fit the mouth of the plastic piping. Hot-glue the mesh to one opening of the pipes (use rubber bands to help hold it taut). Trim away the excess mesh. Disassemble the clothes pegs. For each tube, use rubber band to hold three half-pegs in place as “legs”.
2. Set small petri dish in larger one (larger one contains spillover seawater). Fill small dish with filtered seawater until it is almost brimming over.
3. Position the Uhlig tube with mesh side down over the small petri dish. Adjust height of legs until the mesh is in contact with the seawater surface. Adjust and top up with extra seawater if necessary to remove any bubbles at the interface.
4. Take a sediment core, or scoop of sediment with a spoon, enough to fill the lower 1/3 or ½ of the Uhlig tube. Gently drop this sediment into the tube.
5. Put a small piece of cotton wool on top of the sediment as padding. Dampen with some seawater.
6. Put 4 to 6 cubes of seawater ice on top of the cotton wool padding.
7. Allow the ice to melt. Overflow seawater can be removed from the outer petri dish with a pipette and discarded.
8. After ice has completely melted, remove the Uhlig tube from above and examine the inner dish for ciliates.

Versions of the Uhlig apparatus

Figure. Uhlig apparatus Mark II (left) and Mark III (right).

Mark I
The apparatus as originally described by Uhlig – Tubes are held in place by clamps and retort stands above the collecting dish.
Mark II
Design that I saw at the AWI station on Sylt. Tubes are held in place on a plastic rack by thick rubber bands. Fabricated at the MPI by Georg Herz.
Mark III
Improvised design built at Carrie Bow Caye in Belize after suggestions from the station manager Craig Sherwood. Each tube has three legs (disassembled wooden clothes pegs) held in place by a rubber band. The height above the collecting dish can be easily adjusted by pressing downwards. The legs slide and adjust their position, but have enough friction that they do not bounce back.

Notes

- Plastic petri dishes are more convenient than glass. It is much easier to maintain a convex meniscus ("brimming full") with a plastic dish, they lie flatter, and it is easier to replace them although they can always be reused several times.
- Seawater ice should be put into the freezer at least 2 days in advance. With a conventional household -20 degree C freezer, it usually takes > 24 h for the brine to freeze up properly. Disposable plastic ice cube bags are the most convenient way to make the cubes.
- In a hot climate, put a bowl in the freezer too. When you take the ice cubes out, keep them in the cold bowl so that they don't melt completely before you can bring the cubes to the apparatus.
- Overflow seawater can be discarded, but when the sediment is unusually dense with ciliates, it can frequently also contain ciliates. It might be worth to have a look at the first few pipette-fuls of overflow water.
- I often prepare two petri dishes for each Uhlig tube. When the ice is halfway melted, I swap out the collecting dishes below and can already get started with examining the first one.
- Kentrophoros is quite fragile and sensitive to osmolarity changes, so the ciliates may not last very long after extraction. It is best to work quickly and not to leave dishes standing about for too long, especially if the laboratory temperature is very different from the water temperature.
- Paracatenula and soft-bodied flatworms can also be successfully recovered by the Uhlig method, though their population densities are usually too low for this to be an efficient method.
- The sediment must be fine enough to hold together in the tube. Coarse sediment is not suitable for this method because all the water simply drains out at once.

2.4. Magnesium chloride relaxation

After (Fauré-Fremiet 1950). The use of $MgCl_2$ as a relaxant is common in zoology.

Materials

12% (w/v) magnesium chloride solution
Filtered seawater

Method

1. Prepare magnesium chloride solution. Caution: Do not use if the magnesium chloride fizzes and sputters when dissolving. Technical-grade magnesium chloride will leave a powdery residue that does not dissolve. Filter before use.
2. Mix 5 mL of 12% $MgCl_2$ with 20 mL of local seawater, or equivalent (e.g. 10 mL of 7.1% $MgCl_2$ with 15 mL seawater).

Notes

- This has been tested with Kentrophoros from Fetovaia, Elba, where local seawater salinity is 38-39 PSU.
- This doesn't work with all Kentrophoros species. Some are not affected by the magnesium chloride solution, or start to disintegrate.

3. Fixation methods

Fixing specimens in the field requires some patience and luck. Not all methods will work with every species, and preservation of morphology for microscopy is the most challenging because it depends to some extent on temperature, salinity of local seawater, and handling.

3.1. Fixation for DNA/RNA extraction

I have tested a few methods: direct freezing, 70% ethanol, and RNAlater. For DNA extraction, I have tested the Chelex protocol and the DNeasy Blood and Tissue kit (Qiagen). For RNA extraction, I used RNeasy Plus Micro Kit (Qiagen) The best results have been with RNALater and the DNeasy kit, following manufacturer's instructions.

Notes

4. For specimens preserved in 70% ethanol, evaporate ethanol before proceeding with DNA extraction, using the Concentrator Plus (or Speedvac) at V-AL setting for at least 30 min (ca. 0.5 mL sample volume). Proceed immediately with DNA extraction with DNeasy kit.
5. Individual Kentrophoros cells are small. Avoid contamination by using microcentrifuge tubes that have been UV-treated and handled with clean forceps for collection, heat-sterilized glass pipettes, and 1-2 transfers through dishes of filtered seawater (more than 2 transfers usually results in disintegration of fragile cells). Alternatively use pre-sterilized screw-cap microcentrifuge tubes (2 mL volume), which are less likely to pop open during transport.
6. If using "homebrew" RNAlater, filter through 0.22 µm membrane, and UV-sterilize aliquots before bringing to the field. About 200-500 µL of RNAlater per specimen to fix (a Kentrophoros cell in a small droplet of seawater will constitute about 20-50 µL volume). Add the RNAlater to the specimen, and not the other way around, for ease of handling. Otherwise the cell may get fixed while inside the Pasteur pipette and stick to it.
7. Store specimens at 4 ºC, though RNAlater should not freeze even at -20 ºC. Storage at room temperature does not appear to have any effect for a few days, though systematic experiments have not been done to determine the exact effects. Both DNA and RNA have been successfully extracted from single Kentrophoros cells of medium-sized morphotypes after > 6 months storage in RNAlater at 4 ºC.
8. For DNA/RNA extraction, remove excess RNA later from specimen. For Kentrophoros, it is not always possible to see the fixed specimen, or it may be stuck to the tube, or the host cell may have disintegrated. Simply centrifuge the sample (for example, 8000 rcf for 1 min) and gently pipette away the overlying RNAlater. Perform digestion and extraction directly in the collection tube. Check DNA/RNA concentration after extraction with Bioanalyzer or Qubit.

9. Recommendations from Bruno Huettel: Store extracted DNA/RNA in DNA Lo-Bind tubes (Eppendorf) to minimize adsorption to the plastic walls. Store at 4 ºC instead of freezing, to avoid freeze-thaw cycles which degrade DNA.

3.2. Fixation for FISH (on slides)

Materials

Filtered seawater
20% formaldehyde (Electron Microscopy Sciences, Hatfield, Penn. USA)
Deionized water
70% ethanol
Absolute ethanol
Poly-lysine-coated glass slides
Cool-packs, frozen
Dissecting microscope
Diamond pen

Method

1. Evaporate filtered seawater on a hotplate to ½ original volume.
2. Mix: 1 part concentrated seawater, 1 part 20% formaldehyde, 3 parts filtered seawater, to obtain 4% formaldehyde in seawater (FA/sw). [NB: This is important only for the very small Kentrophoros species. For the larger morphotype, simply mix 1 part 20% formaldehyde with 4 parts filtered seawater.] Cool the 4% FA/sw in ice bath. Warm FA will cause cells to disintegrate quickly.
3. Pick *Kentrophoros* cells into filtered seawater in Petri dish, to isolate them from other meiofauna. Place dish on cool pack to cool the cells.
4. Place poly-lys slide on cool pack to cool the glass. Cooling prevents cells from contracting too vigorously when fixed, and reduces the chances of disintegration.
5. Pick 3 to 5 *Kentrophoros* cells onto the slide surface (normally about 2 to 3 survive the entire process, in various states of intactness). Eventual droplet of seawater should have about 50 microL volume.
6. Allow cells to settle and uncoil themselves. Ideally, they should start gliding around on the surface of the glass.
7. Slowly pipette 50 microL of 4% FA/sw onto the slide, such that the droplet merges slowly with the seawater droplet, from the side of the droplet that is furthest away from the cells. It is important to do this slowly. Too quick, and the cells will begin to swirl around, or contract too strongly, and disintegrate. The final concentration of the FA should be about 2%.
8. Place the slide carefully into a Petri dish and allow it to fix in a fridge at 4 deg C for about 2 h.
9. Remove the seawater and FA carefully by pipetting from the side of the droplet. The cells should have settled to the glass and should remain stuck to it. The edges of the droplet will eventually sweep over the cells, leaving them behind and dry. **If cells have become detached from the glass**, do this instead: Carefully remove as much of the FA and seawater as possible by pipetting without removing the cells too. Dilute with cold deionized water, and again remove as much of the droplet as possible. Repeat this a few times, then place the slide on a warm surface (e.g. the top of the

light source power box) and allow the water to evaporate.
10. With a diamond scribe, scratch a mark on the reverse side of the slide to indicate where the cells are. If you don't have a diamond scribe, memorize the location of the cells on the slide.
11. Dip the slide three times in deionized water to wash. The length of time immersed is immaterial. The action of drawing the slide through the water surface is responsible for most of the washing. Blot the back of the slide to remove excess water.
12. Dip the slide three times in 70% ethanol to dehydrate. Blot the back of the slide.
13. Dip the slide three times in 96% ethanol. Blot the back of the slide and allow it to air-dry. Check that the cells are still attached. The cells should have become much more transparent due to the extraction of sulfur globules from the bacteria, and will be harder to locate. If you haven't done so already, mark the position of the cells with a diamond scribe on the back of the slide.
14. Place slide in a slide box and store frozen.

Ref: Sylt field trip 2012 and Elba summer 2013

Notes

Not ideal for larger species, but may be practical with species that are too small to be handled or even seen once fixed in fluid medium.

3.3. Fixation of *Kentrophoros* cells for FISH with agarose-lined dish

Large Kentrophoros fixed in bowls or dishes tend to stick to the substrates. A solution is to fix them in agarose-lined dishes, and then cut them out with the adhering agarose block.

Materials

Filtered seawater
Deionized water (MQ)
4% formaldehyde / seawater solution (as above)
Agarose powder
Ethanol solutions: 25%, 50%, 75%

Method

1. Prepare 1% or 1.5% (w/v) agarose in seawater. May not dissolve completely. Pour a shallow layer into small Petri dishes and allow them to set. Store in fridge for up to a few days.
2. Flood dish with 4% formaldehyde / seawater.
3. Pick large Kentrophoros individuals into the formaldehyde solution. Quick immersion is essential or they will tend to stick to pipette. Be sure to rinse pipette thoroughly in water and seawater afterwards. Traces of formaldehyde will make the individuals in the sorting bowl very sticky.
4. Fix at r.t. for 1 to 3 h (or in fridge for 6+ h).
5. Remove cells from dish (if necessary, cut out the surrounding agarose), and rinse in MQ water for ca. 1 h.
6. Dehydrate through ethanol series: 25%, 50%, 75% for ca. 1 h each step.
7. Storage in 75% ethanol in glass vials.

Ref: Field book Elba 2013 vol. 3 pg. 82

3.4. Fixation for metabolomics

Samples can be fixed for metabolomics (e.g. GC-MS, LC-MS) in cold, HPLC-grade methanol, and stored at -20 °C until use.

Notes

- "Handling controls" of the same methanol fixative processed through the same labware should be produced in parallel. Plastic microtubes leach compounds such as plasticizers that should be subtracted from the total metabolite count.
- Silanized glass pipettes should not be used to handle samples for metabolomics. The silane coating will leach a large diversity of silane compounds that will be detected by e.g. GC-MS.
- Methanol-fixed samples can also be used for DNA/RNA extraction, however the cells are more challenging to locate in the tube than RNAlater-fixed material.

3.5. Fixation for TEM/SEM

SEM protocol is adapted from Niko Leisch. PHEM buffer protocol has been published (Montanaro et al. 2016). Quality of fixation has not yet been verified!

EMBed 812 protocol for TEM was adapted from these websites:

- http://www.ihcworld.com/_protocols/em/em_en_bloc_method.htm
- http://bioimaging.dbi.udel.edu/sites/bioimaging.dbi.udel.edu/files/manuals/General%20TEM%20Processing%20Protocol%20for%20Ultrastructure.pdf
- http://www.pharma.uzh.ch/research/neuromorphology/researchareas/neuromorphology/Protocols/EM_protocols.pdf

Materials

For PHEM buffer
PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)) – pH buffer
HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) – pH buffer
EGTA (ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid) – chelating agent
Magnesium chloride hexahydrate
5M KOH

Fixatives and waste disposal
25% glutaraldehyde (aq.) (in glass vials, from EMS)
20% formaldehyde (aq.) (in glass vials, from EMS)
2% osmium tetroxide (aq.) (in glass vials, from EMS)
Sodium hydroxide solution (for deactivating glutaraldehyde for disposal)
Vegetable oil or ascorbic acid (for quenching osmium tetroxide for disposal)

For sample mounting
Silicon wafers (for SEM sample mounting)
Poly-lysine solution, 1 mg/mL (for SEM sample mounting)

Method

PHEM Buffer 10x stock

1. Weigh the following amounts of solid reagents: 18.14 g PIPES, 5.96 g HEPES, 3.805 g EGTA, and 0.41 g $MgCl_2 \cdot 6H_2O$; makes 100 mL of 10x stock.
2. Add PIPES to about 30 mL of water in a measuring cylinder with stir bar. Add 5M KOH dropwise until completely dissolved.
3. Add HEPES and EGTA. EGTA will cause precipitation of PIPES. Add 5M KOH dropwise again until solution clears.
4. Add magnesium chloride.
5. Adjust pH to 7.4 with 5M KOH. Final volume should be close to 100 mL. Add water until final volume is 100 mL. If the volume mark is exceeded during pH adjustment, add water to 125 mL to get 8x stock instead.
6. Aliquot and freeze.

Primary fixation

1. Fixative 1 – 2.5% glutaraldehyde in 3x PHEM buffer.
2. Fixative 2 – 2% glutaraldehyde, 2% formaldehyde, 2x PHEM buffer.
3. Washing solution – 4.5x PHEM buffer.
4. In block glass dish (embryo dish), transfer Kentrophoros in a small drop of seawater. Add Fixative 1 (pre-cooled, at 4 ºC) to the dish, using gentle pipetting to prevent the cells from sticking to the dish or each other. Fix for ca. 12 h at 4 ºC.
5. Transfer to fixative 2 for ca. 12 h at 4 ºC.
6. Wash in washing buffer 3x, and store refrigerated until use.

Post-fixation and dehydration for SEM

1. Prepare silicon wafers by pipetting poly-Lys solution on surface, leaving for a few seconds, and then removing solution by pipetting and air-drying. Poly-Lys solution can be re-used; store frozen.
2. Prepare 1% osmium tetroxide by dilution with water from 2% stock. Take necessary precautions as outlined in MSDS!
3. Transfer cells from PHEM buffer to osmium tetroxide solution. Fix for ca. 2 h at room temperature in block glass bowl.
4. Wash 3 x with water.
5. Adhere cells to silicon wafers by pipetting them onto the coated surface, and allowing them to settle.
6. Bring the specimen through ethanol series by immersing the entire wafer: 30, 40, 50, 60, 70, 80, 90, 96, 100, 100%.
7. [Bring through 1:1 ethanol:acetone mixture, and 100% acetone.]
8. Dry specimen on critical-point dryer.

Post-fixation, dehydration, and embedding for TEM

1. Prepare 1% osmium tetroxide by dilution with water from 2% stock (stock can be stored frozen in microtubes sealed in a secondary container).
2. Transfer cells from PHEM buffer to osmium tetroxide solution. Fix for ca. 2 h at room temperature.
3. Wash 3 x with water.
4. Bring through ethanol series: 30, 40, 50, 60, 70, 80, 90, 96, 96, 100, 100, 100%
5. Exchange 3 x with 100% acetone.

6. Prepare EMBed 812 resin mixture according to manufacturer's instructions (use "hard" formulation): prepare mixture without hardener, then add hardener to aliquots just before using. Avoid making bubbles when mixing.
7. Exchange acetone for 1:1 mixture of EMBed 812 mixture / acetone. Infiltrate at room temperature with gentle shaking for 1 h.
8. Exchange mixture for 2:1 mixture of EMBed 812 mixture / acetone. Infiltrate at room temperature with gentle shaking overnight.
9. Prepare new aliquot of EMBed 812 with hardener. Exchange mixture with acetone for 100% embedding mixture. Infiltrate at room temperature with gentle shaking for 2 h.
10. Place specimens in silicone rubber mold. Top up mold with embedding mixture. Polymerize in 60 ºC oven for 48 h.

Waste disposal

- Glutaraldehyde (up to 2%) can be chemically deactivated by adding sodium hydroxide for over 8 h. The solution is then neutralized with acid or bicarbonate and discarded.
- Osmium tetroxide can be inactivated with vegetable oil or ascorbic acid solution (less messy), both of which should turn black on exposure to the osmium tetroxide. The inactivated osmium can be disposed with other heavy metal waste.

4. Staining methods

4.1. Fernandez-Galiano silver staining

Also known as the “silver carbonate” method. This is probaby a misnomer, because although silver carbonate is used in preparing the silver solution, the silver ions are complexed in solution and are not directly associated with carbonate.

This protocol has been modified from (Fernández-Galiano 1976; Ma et al. 2003; Foissner 2014) to minimize reagent use, and optimized for the staining of few specimens. Compared to the other silver staining methods, this is the most convenient and portable method for field work, requiring as special equipment only a hot plate and microscopes.

Materials

Rio-Hortega ammoniacal silver
Prepare 10% (w/v) silver nitrate, 5% (w/v) sodium carbonate, and 25% (w/v) ammonia.
To 10 mL silver nitrate, add 30 mL sodium carbonate slowly while stirring. White precipitate forms, becoming gradually yellowish. Then add ammonia dropwise while stirring, until all the precipitate disappears (about 5 mL of ammonia is necessary). Dilute with water to final volume of 250 mL. A solution that was inadvertently ca. 10% more dilute was used by me without problems.

Peptone/Tryptone solution
Substituting Bacto Tryptone digest from casein instead of Peptone in the original recipes.
To 96 mL water, sprinkled 4 g of tryptone on the surface, allowed to dissolve without stirring. Added 925 µL of 20% formaldehyde as preservative (original recipe: 0.5 mL of 37% formaldehyde, i.e. formalin).

Other materials
Pyridine (l.)
2.5% (w/v) of sodium thiosulfate (aq.)
4% formaldehyde in seawater
4% formaldehyde in distilled water
Glass slide (Superfrost Plus)
Hot plate (best option is the BioShake IQ with flat plate instead of tube holder)
Piece of beeswax
Deionized water
Filtered seawater

Fernandez-Galiano fluid (prepare fresh each day)
Add in the following order:
Pyridine, 15 µL

Rio-Hortega solution, 175 µL
Tryptone solution, 40 µL
Water (deionized), 800 µL

Method

1. Mark out big circle on glass slide (about 1/3 of area) with beeswax (alternative: use a histological pap pen).
2. Pipette live Kentrophoros into the middle of the circle, remove excess seawater until only about a drop (ca. 50-75 µL) remains.
3. Add 50 µL of 4% formaldehyde/SW to the drop to fix Kentrophoros, while monitoring under dissecting microscope. Swirl gently to mix, and when ciliate has stopped moving, pipette away some of the drop and replace with more 4% formaldehyde to bring up to full strength. Fix for about 1-3 minutes.
4. Remove formaldehyde/SW while monitoring under dissection microscope, until only small drop of water remains enclosing Kentrophoros. Rinse with 100 µL of water three times, to remove as much seawater salts as possible. Kentrophoros H will often detach from slide, so be careful not to inadvertently remove the ciliate as well.
5. Post-fix with 50 µL of 4% formaldehyde in distilled water, for about 1-3 min.
6. Add 50 µL of Fernandez-Galiano fluid to the formaldehyde drop. Incubate at room temperature for 1 min. Drop should remain more or less colorless and clear.
7. Place on pre-warmed hotplate (55-60 ºC; I have used 58 ºC), with shaking (250 rpm works best, but manually is ok too). Watch for color change – drop should start turning golden-brown around the edges, and shiny film of silver may form on the surface. Blackish-grey color and powdery appearance is not desirable, and was observed when too much pyridine and Rio-Hortega silver solution was used in the Fernandez-Galiano fluid.
8. Wait until the whole drop is more or less golden-brown (shaking helps to distribute color evenly). The ciliate itself should appear as a dark brown or black spot. Remove from hot plate.
9. Check that ciliate is still attached to the slide; if not, the following steps have to be done carefully to avoid losing the cell!
10. Pipette away most of the drop of fluid. Dilute with about 50 µL of water.
11. Put on coverslip and observe the water-mounted specimen under brightfield illumination. Coverslip may be supported by beeswax at the corners, or with other supports (e.g. Vaseline), to prevent it from completely flattening the specimen.
12. Document as completely as possible with micrographs. Infraciliature should be stained black. Nuclei in Kentrophoros H were not stained.
13. To prepare permanent slide:
    a. Remove coverslip carefully, to avoid destroying specimen. Check under dissecting microscope.
    b. Rinse again with distilled water. Blot away most of the water, leaving only a small drop surrounding the specimen.
    c. Add a drop of 50 µL of 2.5 % sodium thiosulfate to fix the impregnation. Monitor under dissecting microscope. Incubate for about 5 min.
    d. Rinse off thiosulfate well with distilled water. Traces of thiosulfate will destroy the preparation, according to Ma et al.
    e. Air-dry (Foissner recommends mixing with albumen but I did not find this necessary, though it may improve the quality).
    f. Bring through ethanol series (70%, 80%, 96%), ethanol:Roti-Histol mixture

(1:1), and 100% Roti-Histol.

g. Blot off excess Roti-Histol, and coverslip with a drop of Permount. Leave to set overnight in fume hood.

Notes

- Infraciliature and nuclei don't always stain well, especially in midbody region and in freshly-collected specimens. Perhaps it depends on the physiological state of the cells, and maybe the sulfur content of the symbionts?
- Deionized water used to make the solutions has to be clean – traces of salt or seawater will cause the mixture of formaldehyde and Fernandez-Galiano fluid at step 6 to become cloudy. Then the reaction will not proceed properly and staining will fail.
- I recommend using disposable plastic pipette tips instead of glass pipettes, to avoid carry-over of contaminating traces of salt and other substances.
- Aqueous pyridine solution can also be used in place of pure pyridine for preparing the Fernandez-Galiano fluid. It may be easier to transport dilute pyridine solutions because pure pyridine is highly flammable (although the pyridine vapor is still flammable).
- Perform staining in a well-ventilated place!

4.2. DAPI-staining of fixed *Kentrophoros* cells

DAPI staining will reveal cell nuclei of Kentrophoros, however the symbiont cells will fluoresce only poorly (this has also been observed for other sulfur-oxidizing bacteria). With a long-pass epifluorescence cube, polyphosphate granules in the symbiont cells can be visualized as small yellow inclusions.

Materials

Fixed *Kentrophoros* cells on glass slides
DAPI working solution (1 microg/mL)
Deionized water
VectaShield mounting medium
Coverslips

Method

1. Thaw the slide of fixed cells.
2. Stain cells with 100 microL of DAPI solution for 5 min.
3. Rinse with 100 microL of water.
4. Rinse again with 100 microL of water for 1 min.
5. Remove water and air-dry (ca. 30 min).
6. Pipette VectaShield (about 25 microL for a 18 mm square coverslip), and carefully lay down coverslip. A larger coverslip is preferable to a smaller one, because oil from an immersion lens can easily leak over the side if the coverslip is too small.

4.3. Acridine orange staining of fixed cells

Materials

Acridine orange stock solution

PBS solution, 10x
Tween-20, 20% aq.
Glycerol, 100%
Vectashield mounting medium

Method

1. Dilute AO stock 50x with PBS 1x
2. Prepare glycerol:PBS (1x) concentration series (1:3, 1:1, 3:1)
3. Prepare PTw solution: 1x PBS with 0.8% Tween-20 (100 mL PBS 10x, 900 mL water, 4 mL Tween-20 20%)
4. Rinse or rehydrate fixed material (e.g. stored in ethanol, or from fixative) with PTw while shaking (4 changes, 5 min each)
5. Stain sample with AO solution (30 min, room temperature, shaking)
6. Bring through glycerol concentration series (in Petri dishes), 30 min per step
7. Mount on slide with Vectashield mounting medium
8. Use Scotch tape to support the cover slip on sides, and seal with nail polish
9. Imaging with Fluos/A488 filter on epifluorescence microscope

Notes

- Acridine orange gives much stronger signal than DAPI but also has much non-specific fluorescence.
- In practice there is also fluorescence of intracellular inclusions and cilia, though this might be desirable for a general image of cellular features.

4.4. Synthesis of protargol

Protargol is used for silver staining of ciliates. However, it is no longer commercially available in the grade or purity that is necessary for this application. This protocol is lightly modified from (Pan et al. 2013).

Reagents

Reagent	Quantity (1 ×)	Quantity (0.5 ×)
Water, MQ	350 mL	175 mL
Ethanol, 100% anhydrous	300 mL	150 mL
Silver nitrate	20 g	10 g
Acetone	500 mL	250 mL
Ammonium hydroxide, 29%	5 mL	2.5 mL
Peptone gelysate (BD no. 211870)	50 g	25 g

Expected yield: 7 to 12 g of protargol (for 1 × quantity)

Procedure

(All quantities 0.5 × of original recipe)

Day 1. Purify peptone and precipitate silver proteinate

- Add 25 g peptone to 500 mL beaker containing 150 mL ethanol 100%.
- Add 25 mL water while shaking mixture.
- Allow brownish, gummy precipitate to settle, pour off cloudy supernatant, **keep precipitate**. Stand for 5 min to evaporate residual fluid from precipitate.
- → **Set heating plate to 60°C max.**

- Dissolve precipitate in 20 mL water, warm on heating plate to max 60 °C while stirring constantly.
- When completely dissolved (10-20 min), add 60 mL ethanol to solution while stirring constantly. Solution will become milky.

- Cover beaker and cool it with running water for 30 min to ensure complete precipitation.
- Carefully pour off supernatant, **retaining gummy precipitate** and allow to stand for 10 min.
- Dissolve precipitate in 20 mL distilled water while gently swirling beaker. Resulting solution is “purified peptone”

Figure. “Purified peptone” before being redissolved in water (left), and final protargol powder product (right).

- Measure purified peptone into (glass) measuring cylinder. Pour half of the solution back into 500 mL beaker and the other half into 100 mL beaker.

- Add 1 mL ammonium hydroxide to purified peptone in large beaker.

- Dissolve 10 g silver nitrate in 30 mL distilled water. Add silver nitrate solution to peptone.
- Light brown precipitate forms and slowly settles to bottom of beaker.

- Seal both beakers with parafilm and store for 12-24 h in cool (13-18 °C) dark place.

Day 2. Dry protargol

- Pour off and discard supernatant from large beaker, **retaining precipitate**
- Add 50 mL distilled water and let it stand on precipitate for 10 min at room temperature **without stirring**
- Pour off water, add another 50 mL distilled water and stand for 10 min
- Pour off water, let precipitate stand for 5 min

- Add reserved purified peptone from small beaker to large beaker, dissolve by gentle swirling
- Dissolution may be hastened by heating to no more than 60°C while stirring constantly
- Cool to room temperature
- Measure pH with pH meter. If pH < 8.0, add concentrated ammonia dropwise until pH between 8.0 and 9.0

- Add 50 mL acetone, while stirring slowly with glass rod ca. 5 min
- Solution becomes cloudy. Stand for 5 min, discard milky acetone supernatant and **retain precipitate**
- Stand for 10 min, add 50 mL acetone, stir slowly, stand for 5 min, and pour off milky supernatant, **retaining precipitate**
- Repeat above step until acetone remains clear and is not milky.
- Precipitate will gradaully achieve consistentcy of stiff paste that adheres to beaker walls. Scrape off from sides of beaker with glass rod and transfer to ceramic mortar, covered under 15 mL of acetone.

- Pulverize paste to powder under acetone with pestle (30 min to 2 h)
- Paste becomes brittle and flaky, acetone begins to get fine particles. Pour acetone with fine particles into glass funnel to retain fine particles
- Add another 15 mL aliquot (or more) of acetone to repeat pulverization. Requires about 10 iterations before paste is fully pulverized
- Place acetone-moist filter paper for 30 min at room temperature then transfer to completely dry Petri dish
- Spread product in dish with the end of a clean dry glass slide, scraping constantly to evaporate acetone (20-30 min).
- Completely dry, very fine, light brown powder should result. Store in brown glass or opaque polyethylene bottle in cool, dark, dry place.

5. Field characters

The taxonomy of *Kentrophoros* is poorly developed and has relied mostly on characters from fixed specimens, especially the number and arrangement of nuclei. Live specimens can be challenging to describe, especially when they are very small. However, some distinguishing characteristics can already be observed in the field, and can help in pre-sorting specimens into provisional morphotypes.

Overall size

Length tends to be more variable than width for a given species, because most species appear to reproduce asexually by simple fragmentation, but despite this fact, overall size can already be useful in sorting the most obvious cases.

Involution

Flat (symbiont-bearing surface exposed), **tubular** (symbiont-bearing surface enclosed like a tube, e.g. *K. fistulosus*), ***canalis*-like** (enclosure is not complete, leaving an exposed groove down the middle, *K. canalis*), or **pseudotrophosomal** (enclosed and the inner surface is thrown into folds that appear like regular series of pouches, *K.* sp. H). Tubular and pseudotrophosomal cases usually have a **midline seam** visible, where the cell margins meet.

Shape of cell margin

Flat or **wavy** ("ruffled"); though this can be variable depending on the movement and contractile state of the cell.

Overall shape

Especially the angle at the "shoulder" where the symbiont-free "head" and "tail" meet the symbiont-bearing body region. This can be **straight/narrow**, or with pronounced **cuspidation,** or simply **acute** (cf. terminology for leaf shapes in botany). Symbiont-free head and tail can be prominent and long, or only barely visible. However, because they are the most fragile parts of the body, it is common to see "headless" specimens running about. It is worth noting down anyway if a specimen has an obvious head/tail or not just in case.

Features along midline

Clear midline points – The nuclei of most *Kentrophoros* species are arranged along the median axis. In many species, the symbiont coat is interrupted directly above the nuclei, which has the appearance of clear points or "holes" in the symbiont coat along the midline. These can be **round** or appear more like **transverse bars**. They can be **single** (e.g. *K. uninucleatus*) or **many** (e.g. *K. fistulosus*).
In some species, the cytoplasm is thickened along the median axis. This **median thickening**

can be pronounced enough to be visible in live specimens, and can be **straight** or **wavy**.

Contractility

Strongly contractile (considerable foreshortening of body length), **moderately** (slight foreshortening of body length, rapidly reversed), or **not apparently** contractile. This is a character that can only be observed in live specimens. However, it can confound the fixation of specimens if relaxation is not used beforehand.

A. *Kentrophoros* species descriptions in the literature

Many published descriptions of *Kentrophoros* are in publications that are difficult to find, and also in languages other than English. For convenience, the original diagnoses and redescriptions of *Kentrophoros* species are reproduced here, and translated into English from the original languages (shown in smaller typeface), with citations to the original sources. Directly quoted material (including translations) are in blue.

The type species of the genus is *K. fasciolatum* Sauerbrey 1928. Nomenclature was reviewed by Foissner (1995), where he determined Centrophorella Kahl 1935 to be illegitimate, and amended names to reflect the masculine grammatical gender of *Kentrophoros*.

Genus *Centrophorella*

Alfred Kahl, 1934, Ciliata ectocommensalia et parasitica. Die Tierwelt der Nord- und Ostsee, Band II c. 65 (Fig. 5.32) (under fam. Loxodidae)

Provisorisch angeschlossen wird hier die Gattung *Centrophorella* nom. nov. (für *Kentrophoros* Sauerbrey 1928) *Centrophorella fasciolata* (Sauerbrey 1928) (Fig. 5.32) – Größe sehr variabel, 100 bis 300 µ; die nackte Seite mit Bazillen besetzt (nach KAHLS Beobachtung); wohl mundlos. – Kiel: Sandgrund, verbreitet (SAURBREY, KAHL); auf Sand von Helgoland und Sylt je ein Stück (KAHL).

Genus *Centrophorella* nom. nov. (for *Kentrophoros* Saurbrey 1928)

Centrophorella fasciolata (Saurbrey 1928) (Fig. 5.32) – Very variable in size, 100 to 300 µm; the bare side is covered in Bacilli (according to Kahl's observations); possibly mouthless. – Kiel: Sands, widespread (Saurbrey, Kahl), 1 specimen each from the sands of Helgoland and Sylt (Kahl).

Family Kentrophoridae and genus *Kentrophoros*

Improved diagnoses from: W Foissner, 1995, "*Kentrophoros* (Ciliophora, Karyorelictea) has oral vestiges: a reinvestigation of *K. fistulosus* (Fauré-Fremiet, 1950) using protargol impregnation" *Archiv für Protistenkunde*, 146: 167-176.

Kentrophoridae JANKOWSKI, 1980: Loxodida JANKOWSKI, 1978 with very likely functionless oral structures reduced to nematodesmata-bearing, condensed dikinetids in anterior body region. Left side covered with mucous material inhabitated [sic] by symbiotic sulphur bacteria phagocytised through cell surface and used as food source. Monotypic: *Kentrophoros* SAUERBREY, 1928.

Remarks: Jankowski mentioned a new family Kentrophoridae already in 1975 without, however, providing any characterization or type genus. Thus, the name is illegitimate, i.e. not in accordance with the rules of nomenclature. I thus date the Kentrophoridae with 1980, when JANKOWSKI characterized them in more detail, albeit vaguely and incompletely. The thoughless [sic] actions of JANKOWSKI (1975, 1978) might explain why SMALL & LYNN (1985) gave very ambiguous authorships, viz. "order Loxodida Jankowski, n. ord.", respectively, "Kentrophoridae Jankowski, n. fam."

Kentrophoros SAUERBREY, 1928: With diagnosis of family. Probably diphyletic or

polyphyletic as indicated by the different nuclear configurations (two macronuclei with micronucleus in between, many nuclear groups) and body shapes (with or without hyaline ends). Infraciliature of type species, *K. fasciolatus*, not yet known.

List of species

7. *Kentrophoros canalis* Wright 1982
8. *Kentrophoros fasciolatus* Sauerbrey 1928
9. *Kentrophoros faurei* (Dragesco 1954a)
    j. Basionym: *Centrophorella faurei*
11. *Kentrophoros fistulosus* (Fauré-Fremiet 1950)
    l. Basionym: *Centrophorella fistulosa*
13. *Kentrophoros flavus* Raikov and Kovaleva 1968
14. *Kentrophoros gracilis* Raikov 1963
15. *Kentrophoros grandis* (Dragesco 1954a)
    p. Basionym: *Centrophorella grandis*
17. *Kentrophoros lanceolatus* (Fauré-Fremiet 1951)
    r. Basionym: *Centrophorella lanceolata*
19. *Kentrophoros latus* Raikov 1962
20. *Kentrophoros longissimus* (Dragesco 1954b)
    u. Basionym: *Centrophorella longissima*
22. Kentrophoros minutus (Dragesco 1960)
    w. Basionym: *Centrophorella minuta*, species inquirenda**
24. *Kentrophoros ponticus* Kovaleva 1966
25. *Kentrophoros trichocystus* (Dragesco 1954b)
    z. Basionym: *Centrophorella trichocystus*
27. *Kentrophoros tubiformis* Raikov & Kovaleva in Kovaleva 1966
28. *Kentrophoros uninucleatus* Raikov 1962

Kentrophoros canalis

JM Wright, 1982, “Some sand-dwelling ciliates of South Wales” *Cahiers de Biologie Marine*, 23: 282-284 (Fig. 6)

This is a slow-moving thigmotactic ciliate. The anterior end is blunter than the posterior. In incident light, it is opaque with a lighter central region extending throughout its length.

Length between 300 and 600 µm, average 400 µm.

The apparent lightness of the central region is an effect produced by folds of the longitudinal edge extending towards, but not reaching the central longitudinal axis. The bacteria that cover the surface of the ciliate are absent from the ‘inner’ surface, producing the lighter region. The bacterial covering is so dense that it has not been possible to determine the number of kineties. These bacteria measure 2.5 by 0.5 µm, smaller than those recorded by Fauré-Fremiet (1950) on the surface of *K. fistulosa* (Fauré-Fremiet, 1950) and also smaller than those recorded from *K. latum* Raikov, 1962 by Raikov (1974).

The nuclear material is made up of four or more usually five macronuclei and two micronuclei. The macronuclei vary in diameter between 4 and 6 µm. The arrangement of the nuclei follows a typical pattern with two macronuclei close together in the longitudinal axis, then two micronuclei, followed by two macronuclei that are closely associated followed by the most posteriorly located macronucleus. The two micronuclei are always closely associated and may be in juxtaposition or, less commonly, in tandem. They measure 2 µm

across. The distance from first to last macronucleus accounts for 15 percent of the ciliates length.

Individuals of this species occur infrequently and in Swansea Bay only; a dozen preparations are available.

This species is characterized by the lighter longitudinal region extending from anterior to posterior; no other species of *Kentrophoros* shows this folding.

Kentrophoros fasciolatum

E Sauerbrey, 1928, “Beobachtungen über einige neue oder wenig bekannte marine Ciliaten” *Archiv für Protistenkunde*, 62: 381-384

II. Allgemeine Morphologie

9. Größe. Die durchschnittliche Länge des Infusors beträgt bei mittlerer Kontraktion, bei welcher das Tier ungefähr die Form von Fig. 44 hat, 270 µ, die größte Breite 38 µ.
10. Farbe. Die von mir gefundenen Exemplare hatten auf der Oberseite eine gelbliche Farbe, die Unterseite war dagegen farblos.
11. Form. Die äußere Form hängt von dem jeweiligen Kontraktionszustand ab. Bei mittlerer Kontraktion sieht das Infusor aus, wie es Fig. 44 darstellt. Es hat eine sehr langgestreckte Form, die die größte Breite in der Mitte hat und sich nach vorn und hinten verschmälert. Sowohl das vordere wie das hintere Ende ist abgerundet. Der Körper ist bandförmig. Die stärkste Streckung zeigt Fig. 45d. In Fig. 46 sehen wir die größte Kontraktion, die ich beobachtet habe. Fig. 45a—c geben Übergangsformen zwischen den Extremalformen an. Vorderes und hinteres Körperende sind gewöhnlich nicht voneinander zu unterscheiden. Ich bezeichne als vorderes Ende deshalb dasjenige, mit dem das Infusor meistens vorwärts schwimmt. An diesem fand ich bei dem in Fig. 44 dargestellten Exemplar im ersten Sechstel des Körpers einen schmalen von links vorn nach rechts hinten zeigenden Spalt, der ungefähr ein Drittel der Körperbreite an dieser Stelle einnimmt. Vielleicht war das der Mund, vielleicht war es aber auch nur eine Verletzung. Bei allen anderen Exemplaren war diese Spalte nicht zu beobachten. Eine kontraktile Vakuole habe ich bei keinem Tier gesehen. Sie scheint zu fehlen. Auch über die Afteröffnung vermag ich keine Angaben zu machen.

III. Differenzierungen des Ectoplasmas.

1. Cilien. Die Unterseite des Tieres, d.h. diejenige, die beim ruhigen Gleiten nach unten gerichtet ist, ist dicht mit Cilien besetzt. Diese stehen in Furchen, deren Zahl nicht festgestellt werden konnte. Die Cilien sind lang und zart. An den beiden Enden überragen sie den Körperrand. Sie bilden hier Wimperbüschel, die meist deutlich sichtbar sind, während man die Cilien an den Rändern sonst nie sieht.
2. Stacheln. Das Auffallendste und Interessanteste an dem Tier sind die Stacheln, die die Oberseite bedecken. Sie sitzen dicht nebeneinander, ohne aber eine Regelmäßigkeit der Anordnung erkennen zu lassen. Meistens überragen sie die Ränder (Fig. 44), doch kommt es auch vor, daß sie nicht ganz bis an den Rand reichen. Dann bleibt, wie es in Fig. 47 dargestellt ist, ein unregelmäßiger, hyaliner Saum rundherum. Die Stacheln scheinen unbeweglich und stark vom Körper abgespreizt. Sie bestehen aus einer sehr stark lichtbrechenden Substanz und bedingen die charakteristische Färbung des Tieres. Zerfließt dieses, so bleiben die Stacheln erhalten und man kann sie leicht untersuchen. Zunächst sieht man, daß sie sehr verschiedene Form haben (Fig. 48). Es gibt dreieckige, solche, die von elliptischer Gestalt sind, andere sind konkav-konvex und wieder andere rechteckig. In ihrem Innern finden sich kleine Körnchen, die ein noch stärkeres Lichtbrechungsvermögen haben, als die Stacheln selbst. Die Körnchen liegen in verschiedenen Ebenen und wechseln sehr in der Zahl. Es gibt Stacheln mit nur einem Körnchen, solche die zwei oder drei haben. Die Höchstzahl beträgt fünf.
   Fig. 49 zeigt einen Teil des Tieres von der Kante gesehen. Man sieht auf der einen Seite die cilien, auf der anderen die Stacheln. Dazwischen liegt das Plasma, dessen Breite noch nicht einmal die Länge der Stacheln erreicht.

IV. Entoplasmatische Einschlüsse

11. Kern. Das Infusor hat 1—3 Kerne, die ungefähr in der Körpermitte liegen. Sie haben eine rundliche Form und sind meistens ungleich in der Größe. Im Innern findet sich das Chromatin in großen Ballen (Fig. 46).
12. Vakuolen. Schon bei schwacher Vergrößerung fällt an dem Tier eine Reihe heller Bläschen in der

Längsachse des Körpers auf (Fig. 44). Sie liegen nicht immer in eine Reihe, zum Teil sind sie auch zweireihig, wie es Fig. 46 angibt. Ich hielt sie zuerst für Kerne. Da sie sich mit Kernfarbstoffen jedoch nicht färben ließen, war diese Annahme hinfällig. Bei einer Behandlung mit Neutralrot nahmen sie eine rote Farbe an und ließen alle Übergänge von karminrot zu rosa erkennen. Es handelt sich also um Vakuolen, deren Inhalt langsam von saurer Natur zu basischer übergeht. Auf Grund dieser Reaktion nehme ich an, daß es sich hier um Nahrungsvakuolen handelt. Die Nahrungsaufnahme selbst habe ich nie beobachtet. Wegen der geringen Plasmamasse kann das Tier sicher nur sehr kleine Nahrung aufnehmen und es ist höchstwahrscheinlich Bakterienfresser. Neben diesen Vakuolen beobachtete ich an einem Exemplar rings um den Rand in ungefähr gleichem Abstand sehr kleine helle Flecke (Fig. 46). Ein Verschwinden und eine darauffolgende Neubildung habe ich nicht gesehen.

V. Bewegung

Die häufigste Bewegungsform ist ein langsames Gleiten unter schwacher Rotation. Meistens tritt dabei eine mehrfache Überscheidung auf (Fig. 45a—d). Zuweilen ist auch eine wellenförmige Bewegung zu beobachten. Das Tier kann sowohl vorwärts wie rückwärts schwimmen. Oft liegt es bewegungslos, spiralig aufgerollt wie eine Uhrfeder oder zum Knäuel zusammengeballt am Boden der Kulturschale und ist dann nur sehr schwer zu erkennen. Das Herausfangen mit der Pipette ist nicht ganz leicht. Das Infusor klebt nämlich sehr am Glase und muß oft erst losgespritzt werden.

VI. Artdiagnose

Das Infusor ist ungefähr 270 µ lang. Es hat eine langgestreckte Form und fällt sofort durch seine bandförmige Gestalt auf. Die eine Seite ist mit Cilien bedeckt, die andere dagegen mit stark lichtbrechenden stachelartigen Gebilden besetzt. Diese geben dem Tier eine gelbliche Farbe. Die bewimperte Seite dagegen ist farblos. In der Mitte sieht man ein oder zwei Reihen heller Bläschen. Der Kern ist rundlich und besteht aus 1—3 Gliedern.

VII. Systematische Stellung

Wegen der geringen Anzahl von Exemplaren ist die Beschreibung dieses interessanten Infusors äußerst lückenhaft. Daher ist es vorerst unmöglich, ihm eine bestimmte Stelle im System anzuweisen.

II. General morphology

1. Size. The average length of the infusorian in moderate contraction, in which the animal has approximately the appearance of fig. 44, is 270 µ, with maximum width 38 µ.
2. Color. The specimens found by me had a yellowish color on the upper surface, whereas the lower surface was colorless.
3. Shape. The external appearance is dependent on the state of contraction. At moderate contraction the infusorian appears as in fig. 44. It has a very elongate shape, that the greatest width is in the middle and narrows towards the front and rear. Both the front and rear ends are rounded. The body is ribbon-shaped. The strongest aspect is shown in Fig. 45d. In Fig. 46 we see the strongest contraction that I have observed. Figs. 45a-c give the transitional forms between the extreme forms. The front and rear ends of the body are usually not distinguishable. I therefore call the front end that which the infusorian uses to swim forwards with. I found, as shown in Fig 44, an instance where the first sixth of the body a narrow gap facing from the left-front to the right-rear, which takes up about a third of the body width at this point. Probably it was the mouth, or probably only an injury. In all other cases, this gap was not observed. I have not observed a contractile vacuole in any animal. It seems to be missing. I am also unable to make any report on the anus.

III. Differentiation of the ectoplasm

8. Cilia. The lower surface of the animal, i.e. the one that is directed downwards when it is calmly gliding, is densely covered with cilia. These stand in furrows, the number of which could not be determined exactly. The cilia are long and delicate. At both ends they extend beyond the edge of the body. They form there ciliary clumps that are

usually clearly visible, whereas one would never see the cilia at the edges.

9. Spines. The most striking and interesting aspect of the animal are the spines, that cover the upper surface. They sit close to each other, but without a discernible regularity to their arrangement. Mostly they extend beyond the edges (fig 44), but it also happens that they do not completely reach beyond the edge. Then remains, as is shown in Fig. 47, an irregular hyaline hem all around. The spines appear motionless and strongly attached to the body. They consist of a highly refractive substance and are responsible for the characteristic color of the animal. [When the animal] disintegrates, the spines remain and one can easily investigate them. First one sees that they have very different shapes (Fig. 48). There are triangles, those that are elliptical in shape, others are concave-convex and some others rectangular. In their interior are found small granules, which have an even stronger refractive index than the spines themselves. The granules lie in different planes and change much in number between the spines. There are spines with only one granule, and those with two or three. The greatest number is five. Fig. 49 shows a part of the animal seen from the edge. One sees on one side the cilia, and on the other the spines. Between lies the plasma, whose width does not even reach the length of one of the spines.

IV. Endoplasmic inclusions

3. Nucleus. The infusorian has 1 to 3 nuclei, which are approximately in the middle of the body. It has a round shape and is mostly uneven in size. Within is found the chromatin in a large ball (Fig. 46).

4. Vacuoles. Even a low magnification shows on the animal a row of bright bubbles on the long axis of the body (Fig. 44). They do not always lie in a single row, some are also in two rows, as shown in Fig. 46. I thought at first that they were nuclei. As they did not stain with nuclear dyes, this guess was invalid. Upon treatment with neutral red they took on a red color, all in the range from carmine to pink could be recognized. It acts on vacuoles, whose contents slowly change from acidic to basic in nature. On the basis of this reaction I believe that these stained here are food vacuoles. The food ingestion itself I have not observed. Because of the small plasma mass can the animal safely only a very small quantity of food ingest and it is most likely feeding on bacteria. Near these vacuoles I observed in one instance ringing around the edge at about the same distance very small light spots. I have not seen their disappearance and subsequent new formation.

V. Movement

The most common form of movement is a slow glide with gentle rotation. Mostly it occurs with many overlaps (Fig. 45a-d). Sometimes a wave-like motion is also observed. The animal can swim both forwards and backwards. It often lies without movement, spirally rolled up like a clock spring or rolled up in a knot at the bottom of the culture dish and is then very difficult to recognize. The extraction with the pipette is not easy. That is to say, the infusorian sticks to the glass and must first be squirted off.

VI. Species diagnosis

The infusorian is about 270 µ long. It has an elongated shape and is immediately noticeable through its ribbon-like shape. One side is covered with cilia, the other with strongly refractive spine-shaped structures. These give the animal a yellowish color. The ciliated side however is colorless. In the middle one sees one or two rows of light bubbles. The nucleus is round and consists of 1 to 3 members.

VII. Systematic position

Because of the small number of exemplars is the description of this interesting infusorian extremely patchy. Therefore it is initially impossible to assign it a specific place in the system.

Redescription of *Centrophorus fasciolatus*

Alfred Kahl, 1931, Urtiere oder Protozoa, I: Wimpertiere oder Ciliata (Infusoria), 2. Holotricha außer den im 1. Teil behandelten Prostomata. Die Tierwelt Deutschlands Teil 21. pg. 203.

Centrophorus fasciolatus SAUERBREY, 1928 (Fig. S. 196, 15). Gr. 270 . 38 µ. Schlank lanzettlich, vorn und hinten kurz gerundet, bandartig flach, sehr dehnbar. Eine Seite platt, dicht mit langen Wp. besetzt, die andere Seite flach gewölbt, gelblich, dicht mit stabartigen Bildungen besetzt. Die Autorin nennt sie wohl etwas irreführend Stacheln. Es scheint eher eine stabförmig gegliederte Gallerthülle (Tektin) zu sein, die ähnlich wie bei verschiedenen Oldesloer Salzinfusorien, aber auch bei mehreren Süßwasserformen einen Schutz bei plötzlicher Konzentrationsänderung des Mediums gewähren wird. Das Tier wird sich dabei einrollen (Aussüßung durch Regen oder Austrocknung) und durch die einseitige Hülle geschützt in den Ruhestand übergehen. 1-3 rundliche ungleiche Kernteile. c.V. nicht beobachtet, doch dürften die in einer Reihe liegenden kleinen Vakuolen die Exkretion besorgen (Verf.) Über die Md.einrichtung und Ernährung fehlen obachtet (siehe Abbildung), der aber wohl kaum von Bedeutung ist. Bewegung gleitend unter schwacher Verdrehung, manchmal wellig bewegt; oft spiralig aufgerollt. Aus kleinen Ostseewassertümpeln bei Kiel, in vier Exemplaren beobachtet. Über die systematische Stellung läßt sich vorläufig nicht viel sagen; vielleicht steht es Lionotus nahe, doch sind kleinerlei Trc. nachgewiesen.

Centrophorus fasciolatus Saurebrey, 1928 (Fig. pg. 196, 15). Size 270 × 38 µm. Thinly lanceolate, abruptly rounded on the anterior and posterior ends, flat and ribbon-like, very fleixble. One side is flat and thickly covered with long cilia, the other side is slightly elevated, yellowish, and thickly covered with rod-shaped structures. The authoress misleadingly called them “spines”. It is rather appears to be a gelatinous coat (tectin) shaped into rods, that is similar to the diverse saline infusorians of Oldesloe [Oldesloe salt marshes], but which also in many freshwater affords a protection against sudden changes in the concentration of the medium. The animal would therefore roll itself up (freshwater influx because of rain or drying-out) and through the shell on one side survive while protected in a dormant state. 1-3 round, dissimilar nuclei, contractile vacuole not observed, but it may take care of excretion through small vacuoles that are arranged in one row. […] Movement is gliding under gentle pressure, sometimes moving in waves; often rolled up in a spiral. From small Baltic water pools at Kiel, four specimens were observed. On the systematic position there is not much to say at present; probably it is close to Lionotus, …

Notes:

“Centrophorus” is an illegitimate homonym of Centrophoros *Müller & Henle, 1837 (Pisces), according to Foissner (1995).*

In the appendix to this volume (pg. 826, volume 30 of the Tierwelt Deutschlands series) published in 1935, Kahl corrects his interpretation of the “Stacheln” to say that they are probably symbiotic sulfur bacteria.

Redescription of *Centrophorella fasciolata*

Jean Dragesco, 1960, “Les ciliés mésopsammiques littoraux (Systématique, morphologie, écologie)”. *Travaux de la Station biologique de Roscoff* (N.S.) 12 : 177. (NB: Publication of the author's doctoral thesis at the University of Paris, dated 18 June 1956)

La plus commune de toutes, mais aussi la plus variable, Centrophorella fasciolata a été longuement décrite par E. Faure-Fremiet (1950). Découvert par Sauerbrey (1928) et revu par Kahl (1933-1935) et Noland (1937), mais toujours en petite quantité, ce curieux cilié s'est toujours montré très abondant, aussi bien à Concarneau qu'à Banyuls et à Roscoff. Contrairement done à l'opinion des auteurs anciens, il semble aujourd'hui évident que C. fasciolata est un cilié très commun, car nous l'avons vu souvent en quantité prodigieuse. La structure fine de cette espèce, ainsi que celle des bactéroïdes symbiontes qui recouvrent la face non ciliée, ont été admirablement décrites par Faure-Fremiet (1950-1951) et nous n'y reviendrons pas. Nous ajouterons seulement que nous l'avons rencontrée en énormes quantités dans le sable fin, mais saprobe, de l'Ile Verte (il nous a, d'ailleurs, toujours semblé que les Centrophorella étaient des ciliés plutôt saprobes). Dans la plupart de cas, la taille est extrêment variable, la longueur pouvant atteindre 800 µ ou descendre à 200 µ seulement. Un individu, particulièrement filiforme, atteignait 1 600 µ et possédait une extrémité caudale étalée et arrondie. Il s'agissait peut-être d'une espèce nouvelle que nous n'avons pas eu la possibilité d'étudier plus en détail.

The most common of all, but also the most variable, *Centrophorella fasciolata* was extensively described by E. Faure-Fremiet (1950). Discovered by Sauerbrey (1928) and reviewed by Kahl (1933-1935) and Noland (1937) but always in small quantities, this curious ciliate was always very abundant, both in Concarneau at Banyuls and Roscoff. Unlike the opinions of the previous authors, it seems obvious today that C. fasciolata is a very common ciliate, as we have often seen it in prodigious quantities. The fine structure of this species, as well as the symbiont bacteroids that cover the non-ciliated surface, have been admirably described by Faure-Fremiet (1950-1951) and we do not return to this again. We only add that we have met with huge quantities in fine, but saprobic, sand of Ile Verte (it always seemed to us that the *Centrophorella* ciliates were saprobes). In most cases, the size is extremely variable, with length up to 800 µm or down to only 200 µm. An individual, that was especially narrow, reached 1600 µm and had a spread and rounded caudal extremity. This was possibly a new species that we did not have the opportunity to study in detail.

Centrophorella faurei

Jean Dragesco, 1954, “Diagnoses préliminaries de quelques ciliés psammophiles nouveaux” *Bulletin de la Societe zoologique de France* 79: 61 (fig. 2 f)

Trouvée dans le sable fin et vaseux de l’Aber de Roscoff. Espèce de grande taille (750 µ) se rapprochant de la *C. lanceolata* de Fauré-Fremiet mais bien plus volumineuse et présentant trois groupes de noyaux (23 macronuclei et une demi-douzaine de micronuclei). Longue tête transparente et partie terminale effilée. Bactéries symbiotes très réfrigentes.

Found in fine sand and mud of l’Aber de Roscoff. Large species (750 µm) similar to C. lanceolata of Fauré-Fremiet but larger and with three groups of nuclei (23 macronuclei and half-dozen micronuclei). Long transparent head and tapered end portion. Symbiotic bacteria very refractile.

Redescription of *Centrophorella faurei*

Jean Dragesco, 1960, “Les ciliés mésopsammiques littoraux (Systématique, morphologie, écologie)”. *Travaux de la Station biologique de Roscoff* (N.S.) 12 : 181. (NB: Publication of the author's doctoral thesis at the University of Paris, dated 18 June 1956)

Cette nouvelle espèce a été trouvéé dans le sable fin, mais vaseux, de l'Aber de Roscoff (en face du Laboratoire) ainsi que sur la plage de Banyuls-sur-Mer. Cilié de grande taille (env. 1 000 µ), ressemblant quelque peu à la C. lanceolata Fauré-Frémiet, mais s'en différenciant par sa taille considérable et son appareil nucl'eaire plus complexe (constitué par trois groupes de noyaux totalisant de 20 à 25 macronuclei et plusieurs micronuclei). Les cinéties n'ont pas pu être comptées mais sont assez nombreuses. La partie antérieure, en forme de bec, et l'extrémité caudale pointue, sont allongées et très transparentes. Les bactéroïdes phorétiques sont incolores, mais de grande taille et bourrés de granules très réfringentes (d'o`u teinte générale presque noire dans toute la zone

centrale du cilié). L'animal est fortement aplati et très thigmotactique et sa nage est lente car il tourne en hélice et s'enroule autour de son axe comme un ruban. Cette espèce est très commune, surtout dans les sables méditerranéens.

This new species was found in the sandy, but muddy, Aber de Roscoff (facing the Laboratory) and on the beach of Banyuls-sur-Mer. Large ciliate (approx. 1000 µm), somewhat resembling *C. lanceolata* Fauré-Frémiet, but differentiated by its considerable size and more complex nuclear apparatus (consisting of three nuclear groups totaling 20 to 25 macronuclei and many micronuclei). The kineties could not be counted but are quite numerous. The anterior part is shaped like a beak, while the caudal end is elongated and very transparent. The phoretic bacteroids are colorless, but large and full of highly refractive granules (where appearing mostly back in the central part of the ciliate). The animal is highly flattened and very thigmotactic and its swimming is slow because it helically wraps itself around its axis like a ribbon. This species is very common, especially in Mediterranean sands.

Centrophorella fistulosa

E. Fauré-Fremiet, 1950, “Écologie des Ciliés psammophiles littoraux” *Bulletin biologique de la France et de la Belgique*, 84: 56—61

Moins répandue et moins fréquente que C. fasciolata, cette grande espèce n’est cependant pas rare dans la couche superficielle des sables de Mousterlin et de Pouldohan.

C. fistulosa mesure de 700 µ à 2,8 mm. de long, pour 45 µ de diameter et compte ainsi parmi les plus grandes espèces de la faunule infusorienne microporale. Au premier abord, les grands individus nématomorphes, cylindriques, flexueux et d’aspect sombre peuvent être confondus avec Trachelocerca phaenicopterus ou quelque autre forme voisine (fig. 12); un examen plus attentive de la région antérieure, aplatie en forme de spatule, transparente, longue de près de 60 µ, large de 16 à 20 µ, ciliée, légèrement recourbée vers la gauche à son extrémité apicale, ne montre aucune trace de bouche, et rappelle, à la dimension près, la région antérieure de C. fasciolata.

En arrière de ce lobe, le corps s’épaissit brusquement en un long cylinder sombre terminé par un prolongement caudal transparent, mince et plus ou moins long. Toute la surface du corps cylindrique est ciliée; elle montre l’apparence d’une couche ectoplasmique transparente entourant un endoplasme rendu très sombre, ou Presque noir par la présenece d’un grand nombre de corps réfringents allongés, serrés perpendiculairement à la zone ectoplasmique superficielle, sauf le long d’une ligne apparemment dorsale, près de laquelle ils s’inclinent parallèlement au grand axe du corps.

L’écrasement, ou la cytolyse de l’Infusoire montre aussitôt que ces bâtonnets sont des elements bactérioïdes identiques à ceux de C. fasciolata, bien que l”egèrement plus grands, et dépourvus de coloration proper. Cette nouvelle espèce montrerait donc l’aspect paradoxal d’une localization interne des bactérioïdes qui forment, chez la précédente, un revêtement superficiel? Il ne s’agit, en fait, que d’une fallacieuse appraence.

L’observation attentive des individus isolés dans l’eau de mer, ou circulant entre quelques grains de sable, montre l’étonnante souplesse avec laquelle C. fistulosa s’enroule et se déroule en multiples boucles et replies; mais de place en place de corps s’élargit en lobes foliacés don’t l’une des faces est couverte par le tapis des bactérioïdes. Il apparait alors que cet Infusoire est en forme de ruban comme C. fasciolata, don’t il diffère par les dimensions plus grandes, et surtout par le fait que le corps extrêmement plat et large de plus de 70 µ est ordinairement replié sur lui-même, face dorsale en dedans, les deux bords, droit et gauche, se rejoignant sur une ligne médiane; il en résulte l’aspect paradoxal d’un corps cylindrique ou plus exactement tubulaire, don’t la surface externe est constituée par la face ventrale ciliée, tandis eque le tapis des bactérioïdes dorsaux se trouve à l’intérieur, Les réactifs fixateurs, qui contractent la mince lame protoplasmique, provoquent presque toujours l’ouverture du tube, et font apparaître l’aspect rubané réel (fig. 13).

Comme pour C. fasciolata, l’examen de C. fistulosa, soit à l’état vivant, soit après fixation et coloration des cils, ne révèle aucune trace apparente d’un dispositif buccal; mais celui-ci existe certainement, car on peut observer dans le cytoplasma, à côté d’inclusions indéterminées, la présence d’ingestats authentiques, tels que de petites Diatomées.

L’appareil nucléaire de C. fistulosa est constitué par une série médiane d’éléments irréguliers, dont le nombre varie avec la longueur du corps et peut dépasser 40; chacun de ceux-ci apparait comme un amas de 5 à 8 granules de 1 à 1,5 µ, donnant très fortement la réaction de Feulgen, enveloppés par une membrane irrégulière et

parfois indistincte (fig. 14). Les apsects de division n'ayant pas été observés, l'interprétation de cette curieuse structure, correspondant probablement au macronucléus, ne peut être discutée.

Les bactérioïdes couvrant la face dorsale de C. fistulosa se distinguent de ceux portés par C. fasciolata par leur taille un peu plus grande et l'absence de couleur propre. Les autres caractères structuraux de ces bâtonnets, identiques chez les deux espèces de Centrophorella, seront examinés maintenant.

Le revêtement dorsal des Centrophorella, examiné soit en coupe optique, sur les replis de l'Infusoire vivant ou fixé, soit en coupe microtomique normale à la surface ectoplasmique, apparait formé de bâtonnets réfringents, dressés perpendiculairement à la surface du corps, à laquelle ils adhèrent par leur extrémité proximale (fig. 15). Pour une même région, ces bâtonnets sout tous exactement de même hauteur, ce qui donne l'aspect en tapis brosse déjà cité, avec un niveau externe parfaitement uniforme. La répartition de ces éléments sur la surface ectoplasmique est remarquablement homogène, mais elle ne montre aucun ordre défini, aucun alignement régulier; les bâtonnets sont dépourvus de toute espèce de mouvement; les espaces qui les séparent sont inférieurs à leur propre diamètre.

La cytolyse de l'Infusoire provoque immédiatement la séparation des bâtonnets qui, libérés, présentent l'aspect de Bactéries immobiles (fig. 16), ou seulement agitées de mouvements browniens.

Sous l'action de quelques réactifs, la solution iodo-iodurée par exemple, le recouvrement dorsal se sépare en bloc, comme une croûte rigide, du corps protoplasmique lamellaire; ce fait suggère l'existence, entre les bâtonnets, d'une substance interstitielle coagulable.

Les corps bactérioïdes isolés appraissent comme des bâtonnets légèrement courbes, un peu plus épais à leur extrémité distale libre qu'à leur extrémité proximale fixée.

Ces corps sont réfringents et non biréfringents.

Ils ne sont altérés ni par les solvents, ni par les réactifs fixateurs, ni par la desiccation; l'eau distilée paraît affaiblir leur réfringence, sans les gonfier visiblement. Leur aspect est hétérogène, et l'on peut observer la presence de granules plus réfringents que le bâtonnet lui-même. L'éclairage sur fond noir avec un condensateur cardioïde, dessine nettement les contours brilliants des bâtonnets et montre dans leur masse obscure la presence de quelques granules plus ou moins brilliants (fig. 16).

L'examen des corps bactérioïdes disperses révèle nombre d'aspects suggérant la possibilité de leur division par fissuration longitudinale. Sur fond noir, le premier aspect de cette fissuration est un trait brilliant coupant l'extrémité distale élargie et pouvant s'accroitre sur toute la longeur du bâtonnet; d'autres images correspondraient à la separation divergente de deux bâtonnets produits par cette division. La succession *réelle* de ces différents stades n'a pas été suivie sur un même élément.

Les colorations couramment utilisées en bactériologie ne teignent pas fortement les bâtonnets; ces corps ne prennent pas la coloration de Gram; ils se colorent faiblement par les bleus de tolouidine ou de méthylène, la thionine, la fuchsine de Ziel utilisée comme colorant de fond.

L'examen attentif des frottis traités par les mélanges de Giemsa, ou de Romanowski, comme par le bleu de toluidine ou la thionine phéniquée, montre, sur un fond pâle, la présence de petits granules basophiles, bleu-violacé, dont la coloration n'est jamais intense. Une hydrolyse préalable par HCl N à 60°, selon Robinow, ne modifie pas l'aspect de ces teintures; la technique de Feulgen, après des durées d'hydrolyse chlorhydrique comprises entre 2 et 12 minutes, ne donne aucun effet positif.

A côté de ces résultats peu significatifs, on notera que l'iode en solution iodurée colore 'electivement le revêtement des bâtonnets en gris bleu violacé, deux réactions sont négatives aprèes action de la salive à 37°, et l'on en doit conclure à la présence de composés polysaccharidiques. L'examen à fort grossissement des corps bactérioïdes isolés montre que de tels composés sont localisés sur de gros granules; que ceux-ci disparaissent sous l'action de la salive tandis que le corps des bâtonnets persiste; et que les petits granules légèrement basophiles déjà décrits, se trouvent distribués entre les inclusions polysaccharidiques.

L'ensemble de ces résultats ne permet pas encore de définir avec certitude la nature des corps bactérioïdes; il paraît cependant très probable, d'après A. Lowff qui a bien voulu les examiner, que ces corps soient des microorganismes phorétiques (conformément à l'interprétation de Kahl), et plus spécialement des Caulobactéries au sens de Henrici; leur division longitudinale rappelle étroitement, en effet, la bipartition des espèces du genre *Pasteuria* observée par Metchnikoff et par Henrici.

Less widespread and less common than *C. fasciolata*, this large species is, however, not uncommon in the surface layer of sands at Mousterlin and Pouldohan.

C. fistulosa measures 700 µ to 2.8 mm long, to 45 µ in diameter, and is thus one of the largest species of the microporal infusorian fauna. At first, the nematomorphic, cylindrical, sinuous and dark appearance of large individuals may be confused with *Trachelocerca*

phaenicopterus or some other similar form (Fig. 12). A more careful examination of the anterior region – flattened spatula-shaped, transparent, about 60 µ long, 16 to 20 µ wide, ciliated, slightly curved to the left at its apical end, showing no trace of mouth – recalls the anterior region of *C. fasciolata*.

Behind this lobe, the body thickens abruptly to a long dark cylinder that is terminated with a transparent caudal extension, thinner and more or less long. The entire surface of the cylindrical body is ciliated; it shows the appearance of an ectoplasmic transparent layer surrounding an endoplasm made very dark, almost black by the presence of a large number of elongated refractile bodies, arranged at right angles to the surface ectoplasmic zone, apparently except along a dorsal line, near which they are inclined parallel to the long axis of the body.

Crushing or cytolysis of the infusorian immediately shows that these rods are bacteria of the type identical to those of *C. fasciolata*, though slightly larger and lacking the proper color. The new species thus shows the paradox that its bacteria are internally localized, which in the previous [species] appears to be a surface coating? It is in fact a false appearance.

Careful observation of individuals isolated in seawater, or moving between a few grains of sand, showed the amazing flexibility with which *C. fistulosa* wraps and runs multiple loops and waves, but from place to place its body widens into leafy lobes where one side is covered with a carpet of bacteria. It appears that the infusorian is ribbon-shaped as is *C. fasciolata*, from which it differs by its larger size, and especially by the fact that the extremely flat and wide body of 70 µ is usually folded over itself, the dorsal surface within the two sides, right and left, joining at a center line, resulting in the paradoxical aspect of a cylindrical tubular body, or more precisely, that the outer surface is formed by the ventral ciliated surface, while the carpet-like bacteria are within. Fixatives, which cause a contraction of the thin protoplasmic blade, almost always cause an opening of the tube, and show the real ribbon-like appearance (fig. 13).

As with *C. fasciolata*, examination of *C. fistulosa*, either in a living state or after fixation and staining of its cilia, exhibited no trace of an oral apparatus, but one certainly exists, as in the cytoplasm can be observed the presence of authentic ingested objects such as small diatoms, adjacent to unknown inclusions.

The nuclear apparatus of *C. fistulosa* consists of a median series of irregular elements, whose number varies with the length of the body and can exceed 40; each thereof appears as a cluster of 5 to 8 granules of 1 to 1.5 µ, giving a very strong Feulgen reaction, enveloped by an irregular and sometimes indistinct membrane (Fig. 14). Aspects of division have not been observed, the interpretation of this curious structure, probably corresponding to the macronucleus, can be discussed.

The bacteria covering the dorsal surface of *C. fistulosa* differ from those carried by *C. fasciolata* by their slightly larger size and lack of color. Other structural features of these rods are identical in both species of *Centrophorella*, and will be examined now.

The dorsal coating of *Centrophorella*, examined in optical section, on the folds of the living infusorian or fixed in microtome sections normal to the ectoplasmic surface appears as refractile rods, erect perpendicular to the body surface to which they adhere by their proximal end (Fig. 15). For the same region, these rods all reach exactly the same height, which gives the appearance of a carpet brush mentioned above, perfectly uniform externally. The distribution of these elements on the ectoplasmic surface is remarkably homogeneous, but it shows no defined order, no regular alignment, the rods are without any kind of movement,

and the spaces between them are smaller than their own diameter.

Cytolysis of the infurosian immediately causes the separation of the rods, which when released still have the appearance of immobile bacteria, or are stirred by Brownian motion.

Under the action of some reagents, such as iodine solution, the dorsal covering is separated en bloc as a rigid crust from the lamellar protoplasmic body, thus suggesting the existence between the rods of a coagulable interstitial substance.

The isolated bacteria appear as slightly curved rods, slightly thicker at the distal free ends to their proximal attached ends.

These bodies are refractive but non birefringent.

They are not altered by either solvents or reagents by fixing or desiccation. Distilled water seems to weaken their refractive power, without visible swelling. Their appearance is heterogeneous, and one can observe the presence of granules more refractive than the rods themselves. Dark-field illumination with a cardioid condenser clearly shows the bright outlines of the rods and shows in their dark mass the presence of some more or less bright granules (Fig. 16).

Examination of scattered bacterial bodies reveals many aspects suggesting the possibility of their division by longitudinal fission. On a black background, the first appearance of this fission is a bright line cutting the enlarged distal end, and may be increasing over the entire length of the rod. Other images correspond to the separation of two divergent rods produced by this division. The actual sequence of these stages has not been followed in the same element.

The dyes commonly used in bacteriology do not strongly bind to these rods. These bodies do not take the Gram stain, they are colored slightly by toluidine blue or methylene thionine, with Ziel fuchsin dye used as counterstain.

Careful examination of smears treated with the solutions of Giemsa or Romanowski for toluidine blue or thionine carbolic shows on a pale background the presence of small basophilic granules, blue-violent, the color is never intense. Prior hydrolysis with HCl at 60°C, according to Robinow, does not alter the appearance of these dyes. The technique of Felgen, after hydrochloric hydrolysis times between 2 and 12 minutes, gives no positive effect.

Besides these poorly significant results, we note that iodine in iodide solution colors selectively the rods purple-blue, the two reactions are negative after the action of saliva at 37°, and we must conclude the presence of polysaccharide compounds. Examination of isolated bacteria at high magnification shows that such compounds are located on large granules, that they disappear under the action of saliva while the body of the rods still remains; and the small basophilic granules already described are distributed between the polysaccharide inclusions.

Taken together, these results do not yet allow us to define with certainty the nature of the bacterial bodies, but it seems very likely, according to A. Lwoff who kindly examined them, that these bodies are phoretic microorganisms (according to the interpretation of Kahl), especially within the Caulobacteria sensu Henrici; the longitudinal division reminds closely, in fact, the bipartition of the genus *Pasteuria* observed by Metchnikoff and Henrici.

Redescription of *Centrophorella fistulosa*

Jean Dragesco, 1960, “Les ciliés mésopsammiques littoraux (Systématique, morphologie, écologie)”. *Travaux de la Station biologique de Roscoff* (N.S.) 12 : 177. (NB: Publication of the author's doctoral thesis at the University of Paris, dated 18 June 1956)

Admirablement étudiée par Faure-Frémiet (1950), qui l'a découverte dans les sables fins du Mousterlin, cette superbe espèce est assez fréquente dans la région de Roscoff, et nous avons pu la filmer et l'observer assez fréquemment. En tout point semblable à la description donnée par notre maître, G. [sic] fistulosa mesure, en moyenne, 1 mm de long. et sa masse totale est assez considérable. Nous rappelons ici que les curieux bactéroïdes phorétiques, qui sont régulièrement implantés sur toute la face non ciliée, semblent pouvoir être définis comme étant des caulobactéries (au sens de Henrici).

Admirably described by Faure-Fremiet (1950), who discovered this in the sands of Mousterlin, this beautiful species is fairly common in the Roscoff area, and we were able to film and observe it quite frequently. Similar in all respects to the description given by our teacher, *C. fistulosa* measures on average 1 mm long, and its total mass is quite considerable. We recall here that the curious phoretic bacteroids, which are regularly attached across the non-ciliated face, do not seem to be matching the definition of caulobacteria (in the sense of Henrici).

Redescription of *Kentrophoros fistulosus*

W Foissner, 1995, “*Kentrophoros* (Ciliophora, Karyorelictea) has oral vestiges: a reinvestigation of *K. fistulosus* (Fauré-Fremiet, 1950) using protargol impregnation” *Archiv für Protistenkunde*, 146: 167-176.

Morphometric data shown in Table 1 are repeated in this section only as needed for clarity. The interpretation of cortical fine structures is partially based on the transmission electron microscope study by Raikov (1972a). All observations are from field material. Thus, it cannot be excluded that different species were seen. However, the uniform nuclear configuration and the normal coefficient of variation (10.6%, Tab. 1) of the somatic kinety number indicate that all specimens studied were from the same species despite their high size variability.

Size in vivo about 500-2000 x 20-30 µm, difficult to measure because specimens were restless and largest individuals, probably up to 3 mm long (cp. Raikov 1972b), were always convoluted and only partially preserved in protargol preparations (Figs. 1-3, 33, 37). Very slender, filiform, length/width ratio highly variable, viz. 30:1-70:1 (n=10). Mid-body region in protargol slides and SEM preparations usually distinctly broader than in live specimens, because tube-shaped portion evolutes and flattens more or less completely due to preparation procedures (Figs. 4-8, 38, 39, 52, Tab. 1).

Body only about 5 µm thick, appears black, except for hyaline and narrowed ends, and flattened ribbon-like at low magnification (< x50) although tubularly involuted in central region (Figs. 1, 3, 14, 33, 35-37). Blacking caused by dense lawn of sulphur bacteria having refractile granules inside (Figs. 10, 11, 53, 54). Black body portion tube-shaped with epibiontic bacteria lawn inside and ciliated right surface outside (Figs. 13, 19, 43, 52). Disturbed and dying specimens often lose tubular shape, i.e. became 50-100 µm wide, frequently showing bright median ribbon due to regional loss of bacteria lawn (Figs. 4, 34, 38, 39). Anterior body region more or less distinctly narrowed and with short rostrum, flattened up to 2:1, very hyaline and fragile, anterior and lateral margins slightly thickened (Figs. 15, 16, 40). Posterior body region (tail) very similar to anterior region, but narrower, less distinctly thickened at margin, and evenly rounded at end, which, however, becomes

slightly club-shaped in disturbed and prepared specimens (Figs. 19, 24, 50).

Morphology and morphometry of nuclear apparatus as described by Raikov (1972b), who corrected Fauré-Fremiet's (1954) misinterpretations. 10-30 roundish to ellipsoid nuclear groups, each comprising an average of 4 macronuclei and 2 micronuclei, are most common (Raikov 1972b, Tab. 1, Figs. 1, 14, 18). Macronuclear nodules usually contain single, large chromocentre (Fig. 30a), easily mistaken as micronucleus if nuclear envelope is weakly stained (Fauré-Fremiet 1954; Raikov 1972b).

Cortex colourless, gelatinous and very flexible, distinctly striated by refractile granules extending between ciliary rows. Granules – very likely secretory ampullae as in K. latus (Raikov 1972a, 1974a) – 1-1.5x0.6 µm in size, yellowish, especially conspicuous in hyaline body regions (Figs. 9, 40-42, 44), impregnate occasionally with protargol and explode to 2-3 µm sized blisters when cells are pressed between slide and cover glass.

Cytoplasm rather transparent, contains many 1x0.5µm sized granules, but lacks food and contractile vacuoles. Movement sluggish, winds worm-like between sand grains and glides slowly on petri dish bottom with rostrate end ahead; acontractile but often spiral and/or convoluted (Figs. 1, 2, 35, 37). Conspicuous and beautiful body undulations performed by flat specimens which lost tubular involution (Figs. 4, 38, 39).

Infraciliature composed of dikinetids throughout. Cilia 8-10 µm long, rather stiff, on right surface arranged in equidistantly spaced, longitudinal rows most of which became shortened toward body ends, i.e. abut on left side kinety (Figs. 19, 31, 32, 45). Both basal bodies ciliated in anterior and posterior region of cell, posterior basal bodies barren in tubular body portion (Figs. 15-18, 22, 23, 25, 26, 30-32, 47, 51). Dikinetidal axes parallel to main body axis, except for obliquely oriented and more closely spaced dikinetids at anterior end. Oblique and anterior dorsal dikinetids with conspicuous fibres originating from anterior basal bodies, form basket-like structure right of cell median (Figs. 20, 23, 28, 29, 45-49). Dikinetids also condensed at posterior end of cell, give rise to distinct tuft of slightly elongated caudal cilia and conspicuous bundles of fibres extending about 20 µm into tail (Figs. 17, 24-26, 50). At left side of ciliary rows a distinct, ribbon-like myoneme, at right a faintly-stained postciliary microtubule bundle (Figs. 30, 51).

Left side with 2 kineties at margins of cell. Dikinetids of right kinety have the posterior basal body ciliated and an *anteriorly* extending (postciliary?) fibre associated with the anterior basal body; dikinetids of left kinety have the anterior basal body ciliated and a *posteriorly* extending (postciliary?) fibre associated with the posterior basal body (Figs. 21, 22, 24, 25, 45-48). This curious pattern can be reasonably explained if it is assumed that these kineties are in reality a single kinety curving around cell margins.

Epibiontic bacteria

My observations on the epibiontic bacteria and spirilla agree with those of Fauré-Fremiet (1950), Raikov (1971), and Hedin (1977). I thus restrict the description to a few new observations. The spirilla are 5-15x0.5 µm in size and found only on the surface of the ciliate, i.e. between the proximal end of the rod-shaped bacteria (Figs. 11, 12, 58). Both, bacteria and spirilla, are embedded in a 4-5 µm thick layer of mucous material almost equalling the length of the bacteria (Fig. 59). In many specimens patches of bacteria detached due to the preparation procedure, leaving a honeycombed mucus layer (Figs. 56, 57). The mucus was not preserved in all specimens observed (Figs. 55, 58). The contact between bacteria and host is weak and no attachment structures were observed on the ciliate surface (Fig. 58). However, the narrowed proximal extremity of the bacteria was sometimes slightly broadened (Figs. 55,

58), i.e. formed an inconspicuous capitulum as described by Raikov (1971).

Kentrophoros flavum

IB Raikov & VG Kovaleva, 1968, “Complements to the fauna of psammobiotic ciliates of the Japan Sea (Posjet Gulf)” *Acta Protozoologica* 6: 325-327

Body flat, ribbon-like, incontractile, with a median cytoplasmic thickening running along the dorsal body surface (Fig. 11 A, B, D). Living animals yellow. Anterior end forming a poorly expressed rostrum. Posterior end rounded. Trichocysts absent. Body length 300—600 µ.

Ciliature present only on the ventral body surface (as in all *Kentrophoros* species), consisting of 12 longitudinal rows. Dorsal surface completely covered with yellowish elongate symbiotic sulphur bacteria, about 4 µ long (Fig. 11 C). The bacteria attach to the pellicle with one of their ends (Fig. 11 A, D). The ventral surface of living ciliates is usually concave, the dorsal, convex, so that the bacteria protrude not only upward but also sideward (Fig. 11 D). Mouth absent.

Nuclear apparatus consisting of 9—28 macronuclei arranged in a single row along the body (in the median cytoplasmic thickening). The nuclei often (but not always) form groups of 2 macronuclei and 1 micronucleus (Fig. 11 E). The macronuclei, only 2 µ in diameter, contain a single nucleolus and a single Feulgen-positive chromocenter. The micronuclei are only 1-1.2 µ in diameter (Fig. 11 F).

Biotope: Fine sand of the Churkhado spit.

According to the body form, number of kineties and nuclear morphology, this species resembles *Kentrophoros gracile* Raikov, 1963. But *K. flavum* differs from *K. gracile* by its larger size (300—600 µ versus 300—350 µ), yellow color, and presence of the longitudinal cytoplasmic thickening (the latter is well seen in vivo but worse in fixed animals). The bacteria cover in *K. flavum* the whole body including the rostrum (in *K. gracile* the rostrum is free of bacteria). The bacteria themselves are shorter (4 µ) and thicker in *K. flavum* than in *K. gracile* (6 µ). The number of nuclei in *K. flavum* (9—28 macronuclei, 3—14 micronuclei) is greater than in *K. gracile*, which has 7—10 macronuclei, 4—6 micronuclei. Finally the nuclei themselves are smaller in *K. flavum* (the diameter of macronuclei is in *K. flavum* 2 µ, in *K. gracile* – 3—4 µ; the diameter of micronuclei, 1 µ and 2 µ respectively).

Redescription of *Kentrophoros flavus*

Y Xu, J Huang, A Warren, KAS Al-Rasheid, SA Al-Farraj, W Song, 2011, “Morphological and molecular information of a new species of *Geleia* (Ciliophora, Karyorelictea) with redescriptions of two *Kentrophoros* species from China” *European Journal of Protistology* 47: 179-181

Improved diagnosis : Highly flattened *Kentrophoros* about 250—600 µm x 30—60 µm in vivo; 9-49 macronuclei and 3-17 micronuclei arranged in a long irregular row; about 12—19 somatic kineties on right side of cell; densely packed epibiontic bacteria covering left side of cell.

Description of Qingdao population: Cell size in vivo mostly about 300—500 µm x 40—50 µm , cell moderately contractile and flexible, highly flattened and ribbon-like; body length:width ratio about 8—14:1 when fully extended although only about 5:1 in contracted specimens (Figs 5A—C, 6A, B, D). Anterior and posterior portions slightly narrowed (Figs 5A, 6A, B, H, I). Body opaque due to the dense lawn of epibiontic bacteria (about 4 µm x 1

µm), completely covering left side of cell and extending beyond body margins (Figs 5F, G, H, 6C, E—I). Cytoplasm colorless, rather transparent. Elongated, vacuole-like, longitudinal strand extending almost entire length of body along cell meridian marking the position of the nuclear apparatus (Figs 5A, 6A, B). Neither food vacuoles nor contractile vacuoles observed. Locomotion by gliding on bottom of Petri dish, usually attaching to substrate when disturbed.

Fourteen to 19 longitudinal somatic kineties on right side composed of dikinetids throughout (Figs 5I, 6J). Dikinetidal axes parallel to main body axis, except for obliquely oriented and more closely spaced dikinetids at anterior end (Figs 5K, M, 6K, L). Marginal dikinetids at posterior end of cell densely arranged (Figs 5L, N, 6M, N). Single kinety on left side, curving around cell margin almost forming a circle (Figs 5J, M,. N, 6L, N). Somatic cilia about 7 µm long in vivo.

Nuclear apparatus forming a longitudinally oriented strand along cell meridian, composed of 21—49 macronuclei, each about 2—4 µm in diameter, and 9—17 micronuclei (Figs 5D, E, J, 6J, O, P). Nuclei mostly globular or ellipsoidal but unusual nuclear shapes were found in one out of 18 specimens, which were probably micronuclei undergoing mitosis (Figs 5D, 6P) and macronuclear primordia (Figs 5D, 6P; Raikov 1994).

Kentrophoros graciles

IB Raikov, 1963, “Ciliates of the mesopsammon of the Ussuri Gulf (Japan Sea)” *Zoologiceskij Zurnal*, 42: 1765

Translated by Brenda Seah

The body is flat and tape-like (fig. 7, *1*). Living infusoria are colourless. The front end of the body is bent to the right, but does not form the typical “rostrum”; the back end is rounded. As with other species of *Kentrophoros*, an oral groove could not be observed. Trichocysts are absent.

The ciliated coat is present only on the physiologically ventral side of the body and consists of 12 longitudinal rows. The whole dorsal side is entirely covered with colourless symbiotic rodlike sulphur bacteria (Caulobacteriales), about 6 µ long (fig. 7, *2*), which attach to the pellicle by one of their own ends and position themselves perpendicular to the surface of the infusoria’s body, like brushes. Only the very front end of the body is free of these symbionts.

The nuclear apparatus is represented by 7-10 macronuclei (fig. 7, *3*, *Ma*) and 4-6 Feulgen-positive micronuclei (*Mu*), arranged in one row along the body. The macronuclei, coloured by the Feulgen stain, are very small (3-4 µ), spherical, and contain 1-2 nucleoli (*H*) and 1-3 chromocentres (*X*).

The length of the body is 300-350 µ; the infusoria are not shortened, and they move by slowly sliding on the substrate. Its biotope is the fine and middle-sized sand of the Sea of Japan.

This species is superficially reminiscent of *K. fasciolatum* Sauerbrey (Fauré-Fremiet, 1950), but differs from the latter in the large number of rows of cilia and numerous nuclei.

Redescription of *Kentrophoros gracilis*

Y Xu, J Huang, A Warren, KAS Al-Rasheid, SA Al-Farraj, W Song, 2011, “Morphological and molecular information of a new species of *Geleia* (Ciliophora, Karyorelictea) with redescriptions of two *Kentrophoros* species from China” *European Journal of Protistology* 47: 179-181

Improved diagnosis: Size rather variable, about 150-600 µm × 25-70 µm in vivo; body flattened and ribbon-like; 7-25 macronuclei and 4-21 micronuclei arranged in a line along the cell meridian; 10-13 ciliary rows on right side of cell; densely arranged epibiontic bacteria covering left side of cell except for anterior end.

Redescription: Cell size mostly about 150-600 µm × 25-70 µm in vivo; body flattened and ribbon-like (Figs 7A, D, G, 8A-D, M). Cell flexible but not contractile (Figs 7B, C, 8D-F). Anterior part slightly curved and forming a short rostrum with both ends broadly rounded (Figs 7A, E, 8A, B, D, G).

Cell often slightly yellow-brownish at low magnifications, colorless or grayish at high magnifications. Left side of cell except anterior end covered by rod-like epibiontic bacteria, 4-5 µm × 1 µm in size that are arranged perpendicularly to cell surface (Figs 7A, E, F, 8A, B, D, G, Q). Bright longitudinal strand along cell meridian caused by nuclear apparatus which comprises 10-25 macronuclei and 9-21 micronuclei arranged in a longitudinal row; macronuclei globular, about 2-7 µm across, micronuclei about 1.0-2.5 µm in diameter (Figs 7A, I, J, 8A, B, D, J, K, L, N). No food vacuoles or contractile vacuoles observed. Locomotion by sluggish gliding on bottom of Petri dish.

Infraciliature as shown in Figs 7H, I, 8K, L. About 10-13 ciliary rows on right side (Figs 7H, 8K-M, P). Somatic kineties composed of dikinetids most of which are loosely arranged with their axes parallel to main body axis, although those at the anterior end are more closely spaced wth axes oriented obliquely to the main body axis (Figs 7H, K, 8P). On left side, one circle kinety surrounds body margin as seen in *K. flavus* (Figs 7I, L, 8O, R).

Centrophorella grandis

Jean Dragesco, 1954, “Diagnoses préliminaries de quelques ciliés psammophiles nouveaux” *Bulletin de la Societe zoologique de France* 79: 60 (fig. 2 e)

Trouvée dans le sable fin de l’Aber de Roscoff. Grande espèce different de *C. fistulosa* par le fait qu’elle ne présente jamais d’enroulement en gouttière mais, au contraire, un grand applatissement du corps. Appareil nucléaire constitué par 27 à 30 macronuclei et une dizaine de micronuclei, répartis en 4 groupes. Bactéries symbiotes incolores et de grande taille.

Found in the sand of l’Aber de Roscoff. Large species differing from *C. fistulosa* by the fact that it does not have a channel from involution but a largely flattened body. Nuclear apparatus comprises 27 to 30 macronuclei and 10 micronuclei, divided into 4 groups. Bacterial symbionts colorless and large.

Redescription of *Centrophorella grandis*

Jean Dragesco, 1960, “Les ciliés mésopsammiques littoraux (Systématique, morphologie, écologie)”. *Travaux de la Station biologique de Roscoff* (N.S.) 12 : 180-181. (NB: Publication of the author's doctoral thesis at the University of Paris, dated 18 June 1956)

Trouvée dans le sable fin de l'Aber de Roscoff, cette grande espèce est assez proche de Centrophorella fistulosa Fauré-Frémiet, mais ne s'enroule jamais en gouttière comme cette dernière. Elle présente, tout au contraire, un grand aplatissement du corps. Sa taille dépasse 1000 µ de long pour une largeur de 110 µ, son volume est done considérable. L'appareil nucléaire est constitué par seulement 4 à 6 groupes de plusieurs macronuclei (5-8), peu chromatiques, ainsi que 1 à 2 micronuclei du type normal. Done, en tout, 27-35 macronuclei et 7 à 9 micronuclei (réaction nucléale de Feulgen). Ainsi qu'il arrive chez les autres espèces du genre, l'ouverture buccale n'est pas perceptible, mais la disposition des cils laisserait penser qu'elle est antérieure et sur le côté convexe du bec, ainsi qu'il advient chez les Lionotus. Le côté cilié montre de très nombreuses cinéties très difficiles à compter, à cause de l'aspect noirâtre de l'animal, aspect dû à la forte refringence des bactéroïdes phorétiques qui garnissent le côté

glabre (et qui sont, en fait, incolores). Les ingesta semblent être constitués par des diatomées.

Found in the sands of Aber de Roscoff, this large species is large like *Centrophorella fistulosa* but not rolled up in a pipe like it is. It has, on the contrary, a greatly flattened body. Its size exceeds 1000 µm with a width of 110 µm, its volume is therefore considerable. The nuclear apparatus consists of only 4 to 6 groups of several macronuclei (5-8), colorless, and 1 to 2 micronuclei of the normal type. Thus in all there are 27-35 macronuclei and 7-9 micronuclei (nuclear Feulgen reaction). As happens in other species of this genus, the mouth opening is not noticeable, but the arrangement of the cilia would suggest that it is anterior and on the convex side of the "beak", as is the case in *Lionotus*. The ciliated side shows many kineties that are very difficult to count, because of the dark appearance of the animal, due to the strong refractive power of the phoretic bacteroids that furnish the smooth side (and which are actually colorless). Ingesta [food vacuoles?] appear to be filled with diatoms. [!?]

Centrophorella lanceolata

E. Fauré-Fremiet, 1951, "The marine sand-dwelling ciliates of Cape Cod" *Biological Bulletin* 100: 65-67 (Fig. 2)

The enigmatic genus *Centrophorella* (see Kahl, 1933, 1935 and Fauré-Fremiet, 1950), first described by Sauerbrey under the name *Kentrophoros*, probably belongs to the family Amphileptidae, according to Kahl.

The different species are strongly thigmotactic, very fragile and difficult to examine in vitro. The use of $MgCl_2$ solution shows that they are much more frequently represented in the microporal interstitial fauna than it was supposed at first. I did not observe in the fine sand of Barnstable Harbor the very large species *C. fistulosa* Fauré-Fremiet of the Brittany coast, but I found, besides *C. fasciolata* Sauerbrey, a new one which I call *C. lanceolata* n. sp. It seems highly probably that it can be found elsewhere, and a form recently observed at Roscoff, in Brittany, by Dragesco (personal communication) is perhaps the same.

Centrophorella lanceolata is a very flattened and ribbon-like ciliate, mneasuring about 460 to 520 micra in length, 40 micra wide and 5 to 6 micra thick. It tapers at the ends; the anterior part is slender, with a sort of neck preceding the buccal edge, curved on the right side and bearing a row of long cilia. The posterior end is pointed.

The ventral surface is ciliated, and the dorsal one is covered with an accumulation of dark sulfur bacteria, except at a narrow lateral margin at the neck and at the tail.

The associated sulfur bacteria, rod-shaped and all of the same length, measure about 10 micra; they are attached to the dorsal surface of the ciliate infusorian body by one end, and arranged in a parallel manner like the bristles of a brush. That is a characteristic of the so-called Caulobacteria—the genus *Pasteuria* (Metschnikoff), for example. As is the case among others of this group, they divide longitudinally.

The dark appearance of the bacteria of *C. lanceolata* is due to the presence in their cytoplasm of highly refractile sulfur granules; in fact, these bacteria are really colorless, as are those of *C. fistulosa*, whereas those of *C. fasciolata* show a pink color.

It is known that the nuclei of the *Centrophorella* (Kahl, 1933, 1935; Fauré-Fremiet, 1950) are very small; *C. lanceolata* shows five to six globular macronuclei about 4 micra in diameter, and some small micronuclei.

The association of the *Centrophorella* with the Caulobacteria is a perfectly constant

character; in each case these bacteria are sulfur bacteria, but they differ in size and color with each species of Centrophorella. It would be interesting to define the nature, specific or adaptively induced, of these differences. On the other hand, it is noteworthy that the bacteria constantly associated with a heterotrichous ciliate, *Parablepharisma pellitum* Kahl, are quite different and do not contain sulfur.

Redescription of *Centrophorella lanceolata*

Jean Dragesco, 1960, “Les ciliés mésopsammiques littoraux (Systématique, morphologie, écologie)”. *Travaux de la Station biologique de Roscoff* (N.S.) 12 : 179. (NB: Publication of the author's doctoral thesis at the University of Paris, dated 18 June 1956)

Découverte par Fauré-Frémiet (1951), dans les sables du Cape Cod (U.S.A.), cette espèce a été revue par nous-même à Roscoff, vers la même époque et par Bock (1952) dans la région de Kiel. L'espèce observée par nous était, en tous points, semblable à celle décrite par notre maître, et il nous semble inutile d'y ajouter quelque chose.

Discovered by Fauré-Frémiet (1951) in the sands of Cape Cod (USA), this species was reviewed by us in Roscoff at about the same time as Bock (1952) in the Kiel area. The species observed by us was in all respects similar to what was described by our teacher, and it seems unnecessary to add anything.

Kentrophoros latum

IB Raikov, 1962, “Les ciliés mésopsammiques du littoral de la Mer Blanche (U.R.S.S.) avec une description de quelques espèces nouvelles ou peu connues” *Cahiers de Biologie Marine* 3: 354-356 (Fig. 12)

Synonyme: Centrophorella grandis Dragesco – seulement dans l’article de Raikov (1960), pas dans les travaux de Dragesco (1954, 1960).

Dans l’article de l’auteur sur les Ciliés mésopsammiques de la Mer de Barentz, cette nouvelle espèce a été identifiée, par erreur, avec Centrophorella grandis (Dragesco. 1954). Après la parution de la monographie de Dragesco (1960) comportant une description plus détaillée de C. grandis, il est devenu clair que nous avions eu alors une espèce nouvelle. Pendant l’été 1961, elle a été trouvée aussi dans la Mer Blanche.

Le corps a une forme de large ruban, aux bords ondulés (fig. 12a). Les Ciliés sont incolores; en lumière incidente, ils paraissent blancs laiteux. Le bout antérieur est asymétrique – un peu recourbé vers la droite, mais sans former un “bec” typique. La region postérieure ne forme qu’une pointe assez vague (fig. 12a) ; elle n’est jamais effilée comme une queue.

Un renflement median passé le long du corps sur la face non ciliée. Sur le vivant, il appraît comme une bande plus sombre (fig. 12a). Les autres parties du corps sont très plates (fig. 12b). Il n’y a pas de trichocystes.

Comme dans les autres espèces du genre Kentrophoros, la bouche est apparemment absente. La ciliature se restraint à la face physiologiquement ventrale; elle est compose de 30 à 45 cinéties. La face dorsale est entièrement couverte de Caulobactéries symbionts en forme de bâtonnets qui s’implantent par une de leurs extrémités (fig. 12c). Ces bactéries sont incolores, de 5 à 6 µ de long et 0,5 µ de large.

L’appareil nucléaire est constitué par 1 à 4 noyaux composes, qui se trouvent l’un derrière l’autre dans le renflement median du cytoplasme. Ils apparaissent, sur le vivant, comme des vésicules claires (fig. 12a, NC). Les noyaux composes ressemblent à ceux de certaines Trachelocercidae. Entourés d’une membrane commune (fig. 12d), ils contiennent des nucléoles (N) et des petits grains chromatiques (Chr), appartenant évidemment à plusieurs macronuclei conflués. Au centre de chaque noyau compose, se trouvent les micronuclei Feulgen-positifs, habituellement au nombre de 2 (fig. 12d, Mi); plus rarement il y en a 1,3 ou 4.

Longeur: 600 à 1.200 µ. Les Ciliés ne sont pas contractiles; irrités, ils s’enroulent sur eux-mêmes en pelote.

Distribution: Mer de Barentz et Mer Blanche.

Biotype: sables oligosaprobes fins et moyens.

Comme il a déjà été note plus haut, cette espèce est voisine de Kentrophoros grandis (Dragesco, 1954, 1960). Mais K. grandis a 4 à 6 groupes nucléaires, qui sont constitu’es par des macronuclei et des micronuclei libres, tandis que, chez K. latum, ces groupes fusionnent toujours en noyaux composes. D’ailleurs, le renflement

longitudinal caractéristique de notre espèce n'est pas décrit pour K. grandis.

Synonym: *Centrophorella grandis* Dragesco – only in the article of Raikov (1960), not in the work of Dragesco (1954, 1960).

In the article by the author on the mesopsammic ciliates of the Barents Sea, this new species was identified by mistake with *Centrophorella* grandis (Drageseco, 1954). After the publication of the monograph by Dragesco (1960) with a more detailed description of *C. grandis*, it became clear that we had then a new species. During the summer of 1961, it was also found in the White Sea.

The body has the shape of a wide ribbon, the edges corrugated (fig 12a). The ciliates are colorless, in incident light they appear milky white. The anterior end is asymmetrical – a little curved to the right, but does not form a typical "rostrum". The posterior region only forms a vague tip (fig 12a); it is never as a tapered tail.

A median bulge passes along the body at the non-ciliated surface. When alive, it appears as a dark band (fig 12a). Other parts of the body are very flat (fig 12b). There are no trichocysts.

As in other species of the genus *Kentrophoros*, the mouth is apparently absent. The ciliature is restricted to the physiologically ventral surface, and is composed of 30-45 kineties. The dorsal surface is completely covered with rod-shaped Caulobacterial symbionts, attached by one of their ends (fig 12c). The bacteria are colorless, 5 to 6 µ long and 0.5 µ wide.

The nuclear apparatus consists of 1 to 4 compound nuclei, which lie one behind the other in the median bulge of the cytoplasm. They appear in life as clear vesicles (fig 12a, NC). The compound nuclei resemble those of some Trachelocercidae. Surrounded by a common membrane (fig 12d), they contain nucleoli (N) and small chromatic grains (Chr), obviously belonging to several confluent macronuclei. In the center of each compound nucleus are the Feulgen-positive micronuclei, usually numbering 2 (fig 12d, Mi), more rarely are there 1, 3, or 4.

Length: 600 to 1200 µ. Ciliates are not contractile; when irritated they roll themselves up into a ball.

Distribution: Barents Sea and White Sea

Biotype: Fine and medium oligosaprobic sand.

As has already been noted above, this species is close to *Kentrophoros grandis* (Dragesco 1954, 1960). But *K. grandis* has 4-6 nuclear groups, which are composed of free macronuclei and micronuclei, while in *K. latum*, these groups always merge into compound nuclei. Moreover, the characteristic longitudinal bulge of our species is not reported for *K. grandis*.

Centrophorella longissima

Jean Dragesco, 1954, "Diagnoses preliminaries de quelques ciliés nouveaux des sables de Banyuls-sur-Mer (I)" *Vie et Milieu* 4: 636 (fig. 1 m)

Splendide espèce de très grande taille (L: 1.600 µ) rencontrée seulement à deux reprises. Caractérisée par la presence de gros trichocystes (ou protrichocystes) sphériques réfrigents régulièrement disposés sur les deux bords du corps aplati. Huit groupes de noyaux. Bactéroïdes peu réfrigents, aspect general transparent.

Beautiful species of a large size (length: 1600 µm) encountered only twice. Characterized by the presence of large trichocysts (or protrichocysts); refractile spheres regularly arranged on both sides of the flattened body. Eight groups of nuclei. Bacteria weakly-refractile, general

appearance transparent.

Redescription of *Centrophorella longissima*

Jean Dragesco, 1960, “Les ciliés mésopsammiques littoraux (Systématique, morphologie, écologie)”. *Travaux de la Station biologique de Roscoff* (N.S.) 12 : 182-184.

Note: This paper is the published version of the author's doctoral thesis at the University of Paris, dated 18 June 1956

Cette très belle espèce a été trouvée par nous dans le sable fin de la plage de Banyuls-sur-Mer (1). Peu fréquente (rencontrée seulement à deux reprises), cette splendide Centrophorella atteint 1 600 µ de longueur et sa forme est celle d'un long ruban aplati, dont la région antérieure est recourbée, pour constituter un bec assez prononcé, tandis que la région caudale s'effile en une longue pointe. Le cytoplasme est incolore et transparent, tandis que les bactéroïdes phorétiques sont peu réfringents et de grande taille (du type C. trichocystus). De la sorte, la région médiane de l'animal reste assez transparente pour permettre l'observation des organites cellulaires. Un détail de structure très caractéristique permet de reconnaître aisément cette curieuse espèce: des corpuscules sphériques réfringents sont régulièrement répartis sur tout son pourtour et s'entremêlent avec des petits protrichocystes transparents ovalaires. (Il s'agit probablement de trichocystes, mais nous n'avons pas observé leur explosion). L'appareil nucléaire est constitué par sept à dix groupes de plusieurs macronuclei et micronuclei (le peu de matériel dont nous avons pu disposer ne nous a pas permis de réaliser des colorations nucléaires). La nage de ce cilié est lente et spiralée, l'animal s'enroulant en hélice ou adhérant fortement au support.

This beautiful species was found by us on the fine sandy beach of Banyuls-sur-Mer (1). Uncommon (only encountered twice), this splendid *Centrophorella* reaches 1600 µm in length, and its form is that of a long flat ribbon. The anterior region is curved to form a quite pronounced beak, while the caudal region tapers to a long point. The cytoplasm is colorless and transparent, while the phoretic bacteroids are weakly refractile and large (of the type of *C. trichocystus*). In this way, the middle region of the animal remains sufficiently transparent to permit observation of the cell organelles. A detail of a very characteristic structure can easily recognize this curious species: refractile spherical particles are uniformly distributed over its entire periphery and intermingle wth small transparent oval protrichocysts (they are probably trichocysts, but we have not observed their explosion). The nuclear apparatus consists of seven to ten groups of several macronuclei and micronuclei (the little material that we could have did not allow us to achieve nuclear staining). The ciliary swimming is slow and spiral, the animal winds into a helix and adheres strongly to the support.

Centrophorella minuta

Jean Dragesco, 1960, “Les ciliés mésopsammiques littoraux (Systématique, morphologie, écologie)”. *Travaux de la Station biologique de Roscoff* (N.S.) 12 : 184. (NB: Publication of the author's doctoral thesis at the University of Paris, dated 18 June 1956)

Note: Dragesco marked this as “n. sp. ?”, i.e. a doubtful species.

Une curieuse espèce de petite taille (170 µ) a été vue par nous, une seule fois, dans le sable fin de la “Station 3” (Swedmark) de l'Aber de Roscoff. De forme ovalaire (pyriforme) cette espèce contractile montrait un bec antérieur transparent tandis que la région terminale était tout à fait arrondie. Le nombre de cinéties atteint dix environ et de fins protrichocystes garnissaient les espaces intercinétiques. Le côté glabre était abondamment garni de bactéroïdes phorétiques très réfringents. L'appareil nucléaire n'a pas pu être observé, à cause de la densité extraordinaire des bactéroïdes.

A curious species of small size (170 µm) was seen by us only once in the fine sand of “Station 3” (Swedmark) at Aber de Roscoff. With an oval (pyriform) shape, this contractile species shows an anterior transparent beak, while the terminal region was quite rounded. The

number of kineties reached about ten, and fine protrichocysts filled the interkinety spaces. The smooth side was heavily covered with very refringent phoretic bacteroids. The nuclear apparatus could not be observed, because of the extraordinary density of the bacteroids.

Kentrophoros ponticum

VG Kovaleva, 1966, “Infusoria of the mesopsammon in sand bays of the Black Sea” *Zoologiceskij Zurnal* 45 (11) : 1608—1609

Translated by Brenda Seah

The body of the infusorium is flat and tape-like (fig. 7, *1*). Its colour is brown in transmitted light and white in incident light. The front end of its body is bent to the right and forms a typical “rostrum”; the back end is pointed, but does not extend into a tail.

Rows of cilia are present only on the physiologically ventral side of the body: the number of rows varies from 17 to 20. The dorsal side is covered with a layer of symbiotic rodlike bacteria (fig. 7, *3*). The rostrum and back end of the body of the infusoria is not covered with bacteria.

The nuclear apparatus is represented by only one nuclear group (fig. 7, *2*), arranged in the form of a rosette in the centre of the body. The nuclear group consists of 6 small *Mu* and 6-12 *Ma*. The macronuclei contain one large nucleolus and one chromocentre in the form of a not very large Feulgen-positive granule.

The length of the body is 300-400 µ. Movement is by slow gliding on the surface of the substrate. Upon mechanical irritation the infusoria does not contract, but curls up into a ball. It is found mainly in fine, moderately dirty sand.

This species, by its external appearance, somewhat recalls *Kentrophoros fasciolatum* Sauerbrey and *K. uninucleatum* Raikov, but differs from them in the structure of its nuclear apparatus and large number of rows of cilia.

Centrophorella trichocystus

Jean Dragesco, 1954, “Diagnoses préliminaries de quelques ciliés psammophiles nouveaux” *Bulletin de la Societe zoologique de France* 79: 62 (fig. 2 g)

Petite espèce (longueur 250 µ) trouvée dans le sable fin mais vaseux de l’Aber de Roscoff. De forme lancéolée cette espèce montre des trichocystes marginaux expossifs et un appareil nucléaire constitué par seulement 3 macronuclei. Face droite recouverte de bactéries symbiotes très peu réfrigentes.

Small species (length 250 µm) found in the muddy sand of l’Aber de Roscoff. The lanceolate form of this species shows marginal expossifs(?) trichocysts, and one nuclear apparatus that contains only 3 macronuclei. The right surface is covered in weakly-refractile symbiotic bacteria.

Redescription of *Centrophorella trichocystus*

Jean Dragesco, 1960, “Les ciliés mésopsammiques littoraux (Systématique, morphologie, écologie)”. *Travaux de la Station biologique de Roscoff* (N.S.) 12 : 181-182. (NB: Publication of the author's doctoral thesis at the University of Paris, dated 18 June 1956)

Cette curieuse espèce, bien différente de toutes celles que nous venons de voir, a été trouvée dans le sable fin, mais vaseux et saprobe, de certains échantillons récoltés dans l'Aber de Roscoff. De petite taille (L = 250 µ) cette espèce se caractérise par sa forme lancéolée, sa grande transparence, sa teinte jaune clair, sa couverture de

bactéroïdes, peu refringents et peu abondants, sa ciliature assez lâche, la présence d'ingestas (vacuole assez considérable remplie de granules très fins refringents). Deux caractéristiques assez inattendues viennent compléter cette description succincte: Centrophorella trichocystus est pourvue de trois macronuclei vésiculeux, finement granuleux (d'un type différent de tous ceux que nous avons pu voir jusqu'a présent) ainsi que d'assez gros trichocystes explosifs, en forme de navettes et régulièrement implantés sur tout le pourtour.

This curious species, very different from any that we have seen, was found in the fine sand, although muddy and saprobic, of certain samples collected in Aber de Roscoff. This small (L= 250 µm) species is characterized by its lanceolate shape, high transparency, light yellow hue, weakly-refractile and scanty coverage of bacteroids, loose cilia, and the presence of ingestas (large vacuoles full of very fine refractile granules). Two rather unexpected features complete this brief description: *Centrophorella trichocystus* is provided with three vesicular macronuclei, finely granular (of a different type from what we have seen until now) and rather large explosive trichocysts in the shape of spindles that are regularly placed around the entire circumference.

Kentrophoros tubiformis

Raikov & Kovaleva in VG Kovaleva, 1966, “Infusoria of the mesopsammon in sand bays of the Black Sea” *Zoologiceskij Zurnal* 45 (11) : 1607-1608 (fig. 4-5)

Translated by Brenda Seah

In I. B. Raikov’s article (1963) on the infusoria of the mesopsammon of the Sea of Japan, this species was wrongly identified as *Kentrophoros fistulosum* Fauré-Fremiet, 1950, from which it differed only by details of the structure of its nuclear apparatus. After we discovered in the sands of the Black Sea two species of infusoria of the genus *Kentrophoros* – one of which by all appearances, including the structure of the nuclei, turned out to be identical to the species of Fauré-Fremiet, and the other identical to the Far Eastern form [i.e., the one found in the Sea of Japan – transl.] – it emerged that I. B. Raikov had found a new species. Having obtained material for comparison, we were able to produce a description of this new species.

The body of *Kentrophoros tubiformis* is tubiform, the borders of which touch only in the middle of the body (fig. 4, *3*). This tube is not stable and upon fixation or mechanical irritation often unfurls, taking on the appearance of a wide ribbon (fig. 5, *1*), unlike *K. fistulosum*, which upon even complete preparation very often distinctly looks like a tube. The coloration of the infusoria is dark in transmitted light and milky white in incident light. The front end of the body has the appearance of a rostrum, slightly bent to the right. A mouth could not be observed. The back end of the body is pointed, but does not extend into a tail.

There is a ciliated covering only on the physiologically ventral side of the body and consists of 25-35 longitudinal rows. The dorsal side is covered by a continuous layer of symbiotic bacteria (4-5), attached perpendicular to the body of the infusoria (fig. 4, *3*). The rostrum and back end of the body are free of bacteria. Upon the formation of the tube, the edges of the body bend towards the dorsal side; in this way, the dorsal side with the bacteria forms the inside of the tube.

The nuclear apparatus presents itself as nuclear groups, the number of which varies from 7 to 50. In the nuclear groups are free macronuclei (4-8) and micronuclei (2-6). The macronuclei contain 1-3 nucleoli and scattered chromatin granules (fig. 5 and 4, *2*).

The length of the body is from 400 to 1500 µ, and the specimens from the Far East are thinner/smaller than those from the Black Sea. Movement is by crawling along the substrate. Their distribution: fine and middle-sized moderately dirty sand on the Black Sea (rare) or Sea

of Japan.

This species differs from *K. fistulosum* Fauré-Fremiet (fig. 6) in the basic structure of the nuclear apparatus. The species of Fauré-Fremiet has complex nuclei, the number of which varies from 3 to 45. The complex nuclei are compact groups, surrounded by a common envelope and consisting of 2-3 *Mu* and 4-6 merged *Ma*, to which likely belong the large/coarse chromocenters which can be distinguished with difficulty from the micronuclei, and also 1-2 nucleoli (fig. 6, *2*).

Kentrophoros uninucleatum

IB Raikov, 1962, “Les ciliés mésopsammiques du littoral de la Mer Blanche (U.R.S.S.) avec une description de quelques espèces nouvelles ou peu connues” *Cahiers de Biologie Marine* 3: 356-357 (Fig. 13)

Ciliés lancéolés, don’t l’extrémité antérieure du corps a la forme d’un bec fin, pointu et légèrement recourbé à droite (fig. 13a). La terminaison caudale est effilée en une queue transparente. Les bords latéraux du corps sont droits. Il n’y a pas de renflement median plasmatique. Les Ciliés sont transparents, légèrement jaunâtres. La bouche n’a pas été découverte.

Le côté cilié du corps porte 16 à 17 cinéties. La face dorsale non ciliée est couverte par une “brose” de bactéries sulfuriques, sauf les region du bec et de la queue (fig. 13a). Le corps est souvent recourbé en gouttière, de sorte que la face ciliée s’en trouve à l’extérieur, et la face à bactéries phorétiques, à l’intérieur (fig. 13b). Pourtant, les bords de cette gouttière ne se referment pas en tuyau, comme chez K. fistuloa (Fauré-Fremiet, 1950). Il n’y a pas de trichocystes.

L’appareil nucléaire est représenté par un seul noyau compose (fig. 13c), du même type que dans l’espèce précédente. Deux micronuclei (Mi), ainsi que quelques nucléoles (N) et des grains chromatiques (Chr), se trouvent à l’intérieur de ce noyau.

Longeur du corps: 550 à 650 µ.

Biotope: sables fins assez saprobes de la Mer Blanche (rarement).

Par sa structure extérieure, cette espèce ressemble beaucoup à K. lanceolata (Fauré-Fremiet, 1951). Elle s’en distingue cependant par la structure de son appareil nucléaire: K. lanceolata a 5 à 6 macronuclei disposes le long du corps et quelques micronuclei libres.

Lanceolate ciliate; the anterior end of the body has the form of a rostrum, pointed and slightly curved to the right (Fig 13a). The caudal termination is a tapered and transparent tail. The sides of the body are straight. There is no median plasmatic bulge. The ciliates are transparent and slightly yellowish. The mouth has not been discovered.

The ciliated side of the body carries 16 to 17 kineties. The non-ciliated dorsal side is covered in a “brose” of sulfur bacteria, except the region of the rostrum and tail (Fig 13a). The body is often folded into a canal, so that the ciliated face is outside and the bacteria-carrying face is inside (Fig 13b). However, the edges of the canal do not close it into a pipe, as in *K. fistulosa* (Fauré-Fremiet, 1950). There are no trichocysts.

The nuclear apparatus is represented by a single nuclear complex (Fig 13c), the same type as in the previous case. Two micronuclei (Mi) and some nucleoli (N) and chromatic grains (Chr) are located inside the nucleus.

Body length: 550 to 650 µ.

Biotope: Saprobic fine sands of the White Sea (rare)

In its outer appearance, this species closely resembles *K. lanceolata* (Fauré-Fremiet, 1951). It is however distinguished by the structure of its nuclear apparatus. *K. lanceolata* has 5 to 6 macronuclei which run the length of the body, and some free micronuclei.

B. Geographical distribution of *Kentrophoros*

Locality	Lat	Long	Depth	Reference	Coordinates source
Fetovaia, Elba, Italy	42.7313	10.1534	1.5	(Seah et al. 2017)	Map
Cavoli, Elba, Italy	42.734192	10.185868	12.8	(Seah et al. 2017)	Publication
Sant' Andrea, Elba, Italy	42.808561	10.142275	7.3	(Seah et al. 2017)	Publication
Twin Cayes, Belize	16.82356	-88.10615	1.5	(Seah et al. 2017)	Map
Sagami Bay, Japan	35.003033	139.22475	1178	(Takishita et al. 2010)	Publication
Sagami Bay, Japan	35.003111	139.22505	1177	(Takishita et al. 2010)	Publication
Helgoland, Germany	54.1825	7.885278		(Kahl 1935)	Map
Sylt, Germany	55.0197905	8.4390279	0	KBS	Map
Kiel, Germany	54.3265244	10.1731414		(Sauerbrey 1928)	Map
No. 1 Beach, Qingdao, China	36.1	120.533333	0	(Gao et al. 2010; Xu et al. 2011)	Publication
Roscoff, France	48.7274645	-3.9876599		(Fauré-Fremiet 1950; Foissner 1995)	Map
Barnstable, MA, USA	41.705331	-70.3100708		(Fauré-Fremiet 1951)	Map
Swansea Bay, UK	51.6169324	-3.8857433		(Wright 1982)	Map
Niva Bay, Denmark	55.928927	12.5244175		(Fenchel and Finlay 1989)	Map
Gullmaren, Sweden	58.3370092	11.557029		(Hedin 1977)	Map
Lemon Bay, FL, USA	26.9525108	-82.369216		(Noland 1937)	Map
Dal'niye Zelentsy, Barents Sea	69.1135036	36.0657108		(Raikov 1974)	Map
Wattenmeer, Germany	53.733333	8.505556		(Wickham et al. 2000)	Publication
Gryaznaya Bay, White Sea	53.535	142.57667		(Burkovsky and Mazei 2010)	Map
Baie Omega, Sevastopol	44.601972	33.4430618		(Raikov 1971)	Map
Jubail, Saudi Arabia	27.3720766	49.3879465		(Al-Rasheid 1998)	Map
Banyuls-sur-Mer, France	42.4804865	3.1364028		(Dragesco 1954b)	Map
Ussuri Bay, Russia	43.2828429	132.3137113		(Raikov 1963)	Map
Posyet, Russia	42.6261741	130.7926773		(Raikov and Kovaleva 1968)	Map
Astafyev Bay, Russia	42.6149237	131.2087574		(Raikov and Kovaleva 1996)	Map

References

Al-Rasheid K. A. S. 1998. Records of marine interstitial karyorelictid ciliates from Jubail Marine Wildlife Sanctuary in the Gulf-shore of Saudi Arabia. *Arab Gulf J. Sci. Res.*, **16**:595–610.

Burkovsky I. V. & Mazei Y. A. 2010. Long-term dynamics of marine interstitial ciliate community. *Protistology*, **6**:147–172.

Dragesco J. 1954a. Diagnoses préliminiaires de quelques ciliés psammophiles nouveaux. *Bull. Société Zool. Fr.*, **79**:57–62.

Dragesco J. 1954b. Diagnoses préliminaires de quelques ciliés nouveaux des sables de Banyuls-Sur-Mer (I). *Vie Milieu*, **4**:633–637.

Dragesco J. 1960. Les Ciliés mésopsammiques littoraux (Systématique, morphologie, écologie). *Trav. Stn. Biol. Roscoff N S*, **12**:1–356.

Fauré-Fremiet E. 1950. Écologie des ciliés psammophiles littoraux. *Bull. Biol. Fr. Belg.*, **84**:35–75.

Fauré-Fremiet E. 1951. The marine sand-dwelling ciliates of Cape Cod. *Biol. Bull.*, **100**:59–70.

Fenchel T. & Finlay B. J. 1989. *Kentrophoros*: A mouthless ciliate with a symbiotic kitchen garden. *Ophelia*, **30**:75–93.

Fernández-Galiano D. 1976. Silver impregnation of ciliated protozoa: procedure yielding good results with the pyridinated silver carbonate method. *Trans. Am. Microsc. Soc.*, **95**:557.

Foissner W. 1995. *Kentrophoros* (Ciliophora, Karyorelictea) has oral vestiges: a reinvestigation of *K. fistulosus* (Fauré-Fremiet, 1950) using protargol impregnation. *Arch. Für Protistenkd.*, **146**:165–179.

Foissner W. 2014. An update of “basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa.” *Int. J. Syst. Evol. Microbiol.*, **64**:271–292.

Gao S., Strüder-Kypke M. C., Al-Rasheid K. A. S., Lin X. & Song W. 2010. Molecular phylogeny of three ambiguous ciliate genera: *Kentrophoros* , *Trachelolophos* and *Trachelotractus* (Alveolata, Ciliophora). *Zool. Scr.*, **39**:305–313.

Giere O. 2009. Meiobenthology : the microscopic motile fauna of aquatic sediments. 2nd ed. Berlin, Springer-Verlag.

Hedin H. 1977. *Kentrophoros fasciolatum*: a psammobiontic ciliate living symbiotically with sulfur bacteria. *Zool. Revy*, **39**:37–40.

Kahl A. 1935. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 4. Peritricha und Chonotricha; Nachtrag I. *In*: Die Tierwelt Deutschlands. Vol. 30. Jena, G. Fischer. p. 651–886.

Kovaleva V. G. 1966. [Infusoria of the mesopsammon in sand bays of the Black Sea]. *Zool. Zurnal*, **45**:1600–1611.

Ma H., Choi J. K. & Song W. 2003. An improved silver carbonate impregnation for marine ciliated protozoa. *Acta Protozool.*, **42**:161–164.

Montanaro J., Gruber D. & Leisch N. 2016. Improved ultrastructure of marine invertebrates using non-toxic buffers. *PeerJ*, **4**:e1860.

Noland L. E. 1937. Observations on marine ciliates of the Gulf Coast of Florida. *Trans. Am. Microsc. Soc.*, **56**:160.

Pan X., Bourland W. A. & Song W. 2013. Protargol Synthesis: An In-house Protocol. *J. Eukaryot. Microbiol.*, **60**:609–614.

Raikov I. B. 1962. Les ciliés mésopsammiques du littoral de la Mer Blanche (U.R.S.S.) avec une description de quelques espèces nouvelles ou peu connues. *Cah. Biol. Mar.*, **3**:325–361.

Raikov I. B. 1963. [Ciliates of the mesopsammon of the Ussuri gulf (Japan Sea)]. *Zool. Zurnal*, **42**:1753–1767.

Raikov I. B. 1971. Bactéries épizoiques et mode de nutrition du cilié psammophile *Kentrophoros fistulosum* Fauré-Fremiet (étude au microscope électronique). *Protistologica*, **7**:365–378.

Raikov I. B. 1974. Étude ultrastructurale des bactéries épizoiques et endozoiques de *Kentrophoros latum* Raikov, cilié holotriche mésopsammique. *Cah. Biol. Mar.*, **15**:379–393.

Raikov I. B. & Kovaleva V. G. 1968. Complements to the fauna of psammobiotic ciliates of the Japan Sea (Posjet Gulf). *Acta Protozool.*, **6**:309–333.

Raikov I. B. & Kovaleva V. G. 1996. Nuclear groups and diversity of macronuclei of *Kentrophoros tubiformis* (Ciliophora, Karyorelictida) from the Japan Sea. *Eur. J. Protistol.*, **32**:327–335.

Sauerbrey E. 1928. Beobachtungen über einige neue oder wenig bekannte marine Ciliaten. *Arch. Für Protistenkd.*, **62**:355–407.

Seah B. K. B., Schwaha T., Volland J.-M., Huettel B., Dubilier N. & Gruber-Vodicka H. R. 2017. Specificity in diversity: single origin of a widespread ciliate-bacteria symbiosis. *Proc. R. Soc. B Biol. Sci.*, **284**:20170764.

Takishita K., Kakizoe N., Yoshida T. & Maruyama T. 2010. Molecular evidence that phylogenetically diverged ciliates are active in microbial mats of deep-sea cold-seep

sediment. *J. Eukaryot. Microbiol.*, **57**:76–86.

Uhlig G. 1964. Eine einfache Methode zur Extraktion der vagilen, mesopsammalen Mikrofauna. *Helgoländer Wiss. Meeresunters.*, **11**:178–185.

Wickham S., Gieseke A. & Berninger U.-G. 2000. Benthic ciliate identification and enumeration: an improved methodology and its application. *Aquat. Microb. Ecol.*, **22**:79–91.

Wright J. M. 1982. Some sand-dwelling ciliates of South Wales. *Cah. Biol. Mar.*, **23**:275–285.

Xu Y., Huang J., Warren A., Al-Rasheid K. A. S., Al-Farraj S. A. & Song W. 2011. Morphological and molecular information of a new species of *Geleia* (Ciliophora, Karyorelictea), with redescriptions of two *Kentrophoros* species from China. *Eur. J. Protistol.*, **47**:172–185.