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Nanoplankton protists from the western Mediterranean Sea. I. Occurrence, ultrastructure, taxonomy and ecological role of the mixotrophic flagellate *Ollicola vangoorii* (Chrysomonadidae = Chrysophyceae p.p.)*

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SUMMARY: We give an account of the loricate mixotrophic nanoflagellate *Ollicola vangoorii* (basionym *Codonomonas Van Goorii*, synonym *Calycomonas Van Goorii)* (Chrysomonadidae = Chrysophyceae p.p.) in the plankton of the western Mediterranean Sea. Using digital imaging and field-emission scanning electron microscopy (SEM), we describe the lorica morphology and ultrastructure based on some 150 specimens occurring either as dispersed (unattached) cells or cells attached to suspended particulate matter in coastal waters off Barcelona (Catalonia, Spain) in July 1998. The spectrum of morphological variation includes that of *Calycomonas gracilis* sensu Wulff non Lohmann, *Calycomonas gracilis* sensu Espeland & Throndsen non Lohmann, *Calycomonas wulffii* Conrad et Kufferath, *Ollicola cylindrica* (Conrad et Kufferath) Vørs (= *Calycomonas cylindrica* (Conrad et Kufferath) Lund, *Codonomonas cylindrica* Conrad et Kufferath), *Ollicola dilatata* (Conrad et Kufferath) Vørs (= *Calycomonas dilatata* (Conrad et Kufferath) Lund, *Codonomonas dilatata* Conrad et Kufferath), suggesting that those taxa are conspecific with *Ollicola vangoorii*. By contrast, *Ollicola pascheri* (van Goor) Vørs (basionym *Codonomonas pascheri* van Goor) is best maintained as a separate species and retained in the genus *Codonomonas* van Goor. From an ultrastructural viewpoint, the basic architecture of the lorica is that of a fibrillar ribbon coiled around the cell and eventually hardened as a result of inorganic mineralization from the environment. The appearance of the fibrillar component and the degree of mineralization may vary considerably. The cell apex is modified in a newly documented apical cytostome, substantiating an existing observation that cells are capable of ingesting bacteria. Although the statistical correlation between SEM-based nanoflagellate cell counts and epifluorescence-based bacterial cell counts is at best only weakly significant, it is possible that in the Barcelona populations *O. vangoorii* relies partly on bacterivory and therefore its ecological role is partly that of a carbon consumer. We also hypothesize that the presence of an external lorica increases the probability of intercepting bacterial prey, ready to be ingested by the cell in the apical cytostome.

Key words: mixotrophic flagellates, nanoplankton, *Ollicola*, chrysomonads, ultrastructure.

RESUMEN: PROTISTAS NANOPLANCTÓNICOS DEL MEDITERRÁNEO OCCIDENTAL. I. INCIDENCIA, ULTRAESTRUCTURA, TAXONOMÍA Y PAPEL ECOLÓGICO DEL FLAGELADO MIXOTRÓFICO *OLLICOLA VANGOORII* (CHRYSOMONADIDAE = CHRYSOPHYCEAE P.P.) – Aportamos una descripción del nanoflagelado mixotrófico provisto de lorica *Ollicola vangoorii* (basiónimo *Codonomonas Van Goorii*, sinónimo *Calycomonas Van Goorii*) (Chrysomonadidae = Chrysophyceae p.p.) en el plancton del Mar Mediterráneo Noroccidental. Mediante la utilización de imágenes digitalizadas y un microscopio electrónico de barrido (SEM), describimos la morfología de la lórica y la ultraestructura basándonos en 150 especímenes que aparecían ya fueran en células dispersas (no adheridas) ó células adheridas a material particulado suspendido en aguas costeras a poca distancia de Barcelona (Catalunya, España) en Julio de 1998. El espectro morfológico incluye a *Calycomonas gracilis* sensu Wulff non Lohmann, *Calycomonas gracilis* sensu Espeland & Throndsen non Lohmann, *Calycomonas wulffii* Conrad et Kufferath, *Olli-*

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cola cylindrica (Conrad et Kufferath) Vørs (= *Calycomonas cylindrica* (Conrad et Kufferath) Lund, *Codonomonas cylindrica* Conrad et Kufferath), *Ollicola dilatata* (Conrad et Kufferath) Vørs (= *Calycomonas dilatata* (Conrad et Kufferath) Lund, *Codonomonas dilatata* Conrad et Kufferath), sugiriendo que aquellos taxones eran conespecíficos con *Ollicola vangoorii*. En cambio *Ollicola pascheri* (van Goor) Vørs (basionym *Codonomonas pascheri* van Goor) es mejor considerarla como especie separada e incluirla en el genero *Codonomonas* van Goor. Desde un punto de vista ultraestructural la arquitectura básica de la lorica es la de una banda fibrilar que envuelve a la célula en espiral y que acaba endureciéndose debido a la mineralización inorgánica del medio. La aparición del componente fibrilar y el grado de mineralización puede variar considerablemente. El vértice celular se modifica en un citostoma apical lo que confirma una observación previa de que dichas células son capaces de ingerir bacterias. Aunque la correlación entre los recuentos de nanoflagelados determinados por microscopía de barrido (SEM) y los de bacterias por microscopía de epifluorescencia en el mejor de los casos son marginalmente significativos, es posible que las poblaciones de *O. vangoorii* de Barcelona subsistan parcialmente gracias a la bacterivoria y por tanto su papel ecológico es el de ser un consumidor eventual de carbono. Nosotros también hipotetizamos que la presencia de una lórica incrementa la posibilidad de interceptar las presas bacterianas, dispuestas para ser ingeridas por el citostoma apical de la célula.

Palabras clave: flagelados mixotróficos, nanoplancton, *Ollicola*, crisomónidos, ultraestructura.

INTRODUCTION

General introduction to the series

An examination of water samples collected in the spring-summer of 1998 in the Alboran Sea and, to a lesser extent, in coastal waters off Barcelona (Catalonia, Spain), has provided insight into the species diversity and ecological significance of non-coccolithophorid nanoplankton protists in the western Mediterranean Sea. The results form the object of this series of papers. Each paper will deal with one or more taxa within the following groups: cryptomonads, dinoflagellates, haptophytes, prasinophytes, chrysomonads, choanoflagellates, miscellaneous heterotrophic flagellates and *incertae sedis* protists, and naked ciliates. The final paper will give a general retrospective overview.

Modern studies on the diversity of marine nanoplankton protists are at least as necessary as they are scarce, particularly in the case of the Mediterranean Sea. Owing to their abundance and wide distribution, protists within or below the "nano" size range (2-20 μm) play essential rôles within carbon fluxes in the pelagic food web, both in its classical model and in the "microbial loop" model (Azam *et al.*, 1983). Most of these protists are flagellates belonging to a variety of taxonomic groups (Booth *et al.*, 1982; Hannah and Boney, 1983; Nielsen *et al.*, 1993; Fenchel, 1986; Berninger *et al.*, 1991; Booth *et al.*, 1993; Kuuppo, 1994; Mills *et al.*, 1994; Novarino *et al.*, 1997), but other kinds of protists may also be numerically abundant and functionally important. In particular, naked ciliates within the "nano" range (Montagnes *et al.,* 1988b; Pèrez *et al.,* 2000) may be abundant and important as bacterial grazers in the pelagic food web (Sherr *et al.*, 1986; Sherr and Sherr, 1987; Sherr *et al.*, 1989; Rassoulzadegan, 1993).

Apart from the coccolithophorids and few others, the nanoplankton protists have not yet attracted the full attention they deserve from an ecological point of view, and many available ecological studies on the smaller plankton concentrate on larger forms such as diatoms and thecate dinoflagellates within the phytoplankton, and the larger ciliates (tintinnids or naked ciliates) within the protozooplankton. This could be due in part to a long-lasting scarcity of baseline investigations on nanoplankton diversity, not only in the case of flagellates but also within the ciliates, for which taxonomic information was unavailable until relatively recently (Montagnes *et al.*, 1988a; Martin and Montagnes, 1993; Montagnes and Taylor, 1994; Agatha and Riedel-Lorje, 1997). Most likely this lack of published studies is a result of practical difficulties with working with these organisms –their small size, their fragility when brought into contact with chemical fixatives, and the laboriousness of taxonomic identification. These difficulties may well represent some of the reasons leading to the development of so-called "ataxonomic" approaches in nanoplankton ecology, which consist in quantifying populations by functional criteria rather than species. In this context nanoflagellate cells, for instance, may be enumerated *in toto* by size class (e.g. < 5, 5-10, 10-15, 15-20 μm), nutrition mode (e.g. photosynthetic nanoflagellates, PNAN, versus heterotrophic nanoflagellates, HNAN, based on the presence or absence of chlorophyll autofluorescence under epifluorescence microscopy), or a combination of these. In the case of PNAN, biomass estimates other than cell numbers are often preferred, most notably chlorophyll *a* concentrations. Within the functional group approach, ciliates are considered either as heterotrophs or mixotrophs and quantified using recently established methods, although most quantitative studies have dealt with "micro" rather than "nano" forms.

Ataxonomic approaches are entirely appropriate for providing insight into the functioning of nanoplankton communities, but it may also be argued that they are too reductive because they ignore interspecific differences in the rates of carbon primary production by photosyntethic forms or carbon consumption by heterotrophs. Furthermore, owing to the fact that they lack taxonomic resolution by definition, these approaches do not provide information on such topics as the occurrence of toxic species and nanoplankton biogeography, both of which continue to deserve detailed attention. Several nanoplankton flagellates are able or suspected to release toxins in the water column, often with serious adverse effects on the ecosystem (e.g. Kaartvedt *et al.*, 1991; Kaas *et al.*, 1991; Aune *et al.,* 1992; Lindholm and Virtanen, 1992), so it is essential to monitor their distribution and population dynamics. From a biogeographical viewpoint, the hypothesis that many heterotrophic or mixotrophic nanoplankton flagellates and ciliates have a cosmopolitan distribution (Vørs, 1993; Finlay *et al.*, 1998) will undoubtedly benefit from an increasing amount of baseline data. Comparisons with species lists for the Mediterranean Sea would be particularly interesting because this an area of higher species diversity as far as the phytoplankton is concerned (Margalef, 1994), which begs the question of whether or not this is also the case with the heterotrophic and mixotrophic nanoplankton.

In summary, the ataxonomic philosophy is valid within its own remit but we argue that there are also strong grounds for a greater presence of taxonomy in nanoplankton ecology. This requires in turn more background information on the diversity of natural nanoplankton communities, so that as large a dataset as possible can be gathered for identification and comparative purposes. The main factors working against nanoplankton diversity studies are probably the perceived difficulties and uncertainties of taxonomic identification, and –perhaps even more importantly– the consequent prejudices against taxonomic science as a whole. It is reassuring to see that these widespread but unjustified prejudices are deplored also by taxonomically-versed ecologists (Margalef, 1994) in addition to the taxonomists themselves.

Introduction to *Ollicola vangoorii*

Ollicola vangoorii (Conrad) Vørs (Chrysomonadidae = Chrysophyceae p.p.) is a flagellate reported from the plankton of temperate, subpolar, and arctic waters (see references in Vørs, 1992b). In the genus *Ollicola,* cells are characteristically surrounded by a mineralized lorica incorporating Fe, Mn and other elements present in smaller quantities (Espeland and Throndsen, 1986). The function of the lorica remains unknown.

Ever since its original description (Conrad, 1938, as *Codonomonas Van Goorii*) and other early observations (e.g. Wulff, 1919, erroneously identified as *Calycomonas gracilis* Lohmann), *Ollicola vangoorii* has been thought to lack chloroplasts and therefore its nutrition has been regarded as heterotrophic rather than photosynthetic. However, transmission electron microscopy has shown that it contains an apparently typical chrysophyte chloroplast whilst also being able to ingest bacteria (Vørs, 1992b), suggesting mixotrophic nutrition. This kind of nutrition seems to occur in protists more often than usually believed, particularly in the case of flagellates (Boraas *et al.*, 1988; Sanders and Porter, 1988; Jones *et al.*, 1993); it may cause some conceptual inconvenience to the ecologist, challenging as it does the long-established distinction between the "phyto" and "zoo" components of the plankton, i.e. carbon primary producers versus carbon consumers.

We are aware of only two published, verifiable reports of *Ollicola vangoorii* from the Mediterranean Sea (Leadbeater, 1974, as *Kephyrion* spp.; Delgado and Fortuño, 1991, as *Calycomonas wulffii* Conrad et Kufferath). Both are based on electron microscopy, which is indispensable for correct identification because at the light microscopical level the testate amoeba *Paulinella* Lauterborn may be easily mistaken for *Ollicola* (see Johnson *et al.*, 1988). *Ollicola* was also mentioned without illustrations by Margalef (1994), and possibly by Sarno *et al.* (1993) as *Calycomonas* cf. *wulffii*. We found *Ollicola vangoorii* in the western Mediterranean Sea and this has provided us with an opportunity for a detailed report, a study of lorica morphology and ultrastructure, and some considerations on its taxonomy and ecological rôle.

MATERIALS AND METHODS

Sampling

Water samples were collected using Niskin bottles in the spring-summer 1998 in three geographical areas: the Alboran Sea (south-western Mediterranean) and two areas off the coast of Barcelona

(Catalunya, Spain). In the Alboran Sea, between 1 and 16 May 1998 a total of 39 samples were collected at various depths (including the chlorophyll fluorescence maximum depth) from 4 stations on three occasions from RV "Hesperides" during the second leg of the MATER I cruise funded by the Commission of the European Communities MAST III programme. On 28 July 1998, additional samples were collected along a horizontal transect located some 2 km off the coast of Barcelona and running parallel to the shore between the mouth of the Besós river and the Punta de Llobregat. This transect included 8 equidistant stations where samples were collected near the surface from a small boat during a workshop held at CSIC-Instituto de Ciencias del Mar, Barcelona. Between 29 and 30 July 1998, samples were also collected from one station in the port of Barcelona close to the CSIC-Instituto de Ciencias del Mar, during a time-series plankton sampling experiment carried out within the workshop. Samples were collected near the surface and near the bottom (ca. 5 m depth) at 2 hour intervals between 10.00 on 29 July and 12.00 on 30 July inclusive.

Electron microscopy

For nanoplankton analyses, subsamples (50-100 ml) were fixed immediately with a few drops of concentrated non-acidified Lugol's iodine or electron-microscopical grade glutaraldehyde (2.5% final concentration). For each nanoplankton subsample, following agitation 20 ml aliquots were allowed to settle for 24 h on polylysine-coated coverslips, rinsed for 15 min in filtered seawater, osmicated (2% OsO_4 for 30 min), rinsed 3 times in distilled water (5 min each time), dehydrated in a graded isopropanol series, and dried in hexamethyldisylazane (HMDS). Coverslips were mounted with Araldite glue on aluminium stubs, sputter-coated with a 20 nm layer of gold-palladium and observed at 5 kV with a Philips XL 30 field-emission scanning electron microscope (SEM). Additionally, 20 ml aliquots of some subsamples were gently filtered onto polycarbonate filters (pore size 0.8-3.0 µm), which were subsequently processed in the same way as the coverslips. Micrographs were taken on Kodak T-Max 400 roll film.

Morphometry and image analysis

A total of 151 loricas of *Ollicola vangoorii* (mostly from the Barcelona time series samples) were measured on SEM photographic prints using a caliper. No corrections were applied to compensate for SEM tilt angle since all samples were observed untilted. Descriptive statistics were calculated using Microsoft Excel. Photographic prints 7 x 10 cm in size were converted into digital format using a flatbed scanner at a resolution of 600 dpi. Digital images were processed with Adobe Photoshop 3.0 as follows: the "Find Edges" filter was applied to trace lorica contours, after which the contours were re-sized to the same scale and rotated so as to align them in the same direction. A total of 120 such contours were superimposed to give a composite image showing the overall variability of lorica shape.

Bacterial abundance analyses

A few hours after fixation, bacterial sample aliquots (5 ml) were filtered onto 0.2 µm black polycarbonate filters and stained for 5 min with DAPI (4',6-diamidino-2-phenylindole), at a final concentration of 5 µg ml^{-1}. Filters were mounted with low-fluorescence oil on microscope slides and stored frozen until they were examined using a Nikon Diaphot epifluorescence microscope. About 300 bacteria were counted per sample.

Nomenclature

Owing to their possessing photosynthetic, heterotrophic or mixotrophic nutrition we regard chrysomonads/chrysophytes as ambiregnal protists, and therefore consider *Ollicola,* under the jurisdiction of both the botanical (ICBN) and the zoological (ICZN) Codes of Nomenclature, analogous to other groups of protists e.g. cryptomonads (Novarino and Lucas, 1993, 1995).

RESULTS

Occurrence and abundance

Ollicola vangoorii occurred in all of the sample series examined, with SEM-based counts of up to 1000 cells l^{-1} (Table 1). Cells were either dispersed (unattached) or attached to suspended particulate matter (SPM) (Figs. 2A-F). In the Alboran Sea *O. vangoorii* was only present in very low numbers in 1 sample (station A) out of 39 examined. By contrast, in Barcelona it occurred in 5 out of 8 transect samples and 17 out of 28 time series samples. In the time series samples, which were characterised by higher epifluo-

TABLE 1. – Cell counts of *Ollicola vangoorii*. Based on SEM examination of 20 ml sedimented aliquots per sample. ALB = Alboran Sea sample series, T = Barcelona transect sample series, TS = Barcelona time series sample series.

Sample	Cells l^{-1}	Sample	Cells l^{-1}
ALB A	50	TS06S	50
T01	0	TS06B	0
T02	850	TS07S	50
T03	0	TS07B	250
T04	0	TS08S	0
T05	450	TS08B	650
T06	350	TS09S	450
T07	150	TS09B	50
T08	500	TS10S	50
TS01S	0	TS10B	50
TS01B	0	TS11S	1000
TS02S	0	TS11B	400
TS02B	0	TS12S	50
TS03S	0	TS12B	50
TS03B	0	TS13S	100
TS04S	350	TS13B	500
TS04B	100	TS14S	1000
TS05S	100	TS14B	50
TS05B	200		

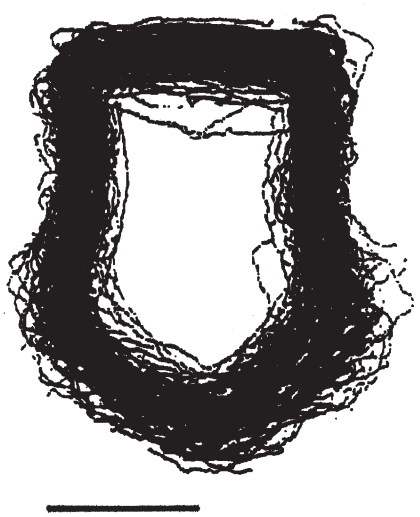

FIG. 1. – Variability of lorica shape of *O. vangoorii* based on 151 specimens from the Mediterranean Sea. Scale bar = 1.7 μm.

TABLE 2. – Lorica morphometry of *Ollicola vangoorii* in the examined samples. Based on SEM examination. L = lorica length, W = lorica width, B = number of bands, SD = standard deviation, CV = coefficient of variation, N = number of observations.

Variable	Min	Max	Mean	Median	Mode	SD	CV %	N
L	2.22	5.09	4.12	4.16	4.07	0.49	11.98	151
W	2.57	4.16	3.19	3.19	3.40	0.37	11.50	151
L/W	0.62	1.67	1.30	1.31	1.27	0.18	13.72	151
B	2	10	6.05	6	6	1.25	20.70	109

rescence bacterial counts (range 8.38 x 10^6 - 1.49 x 10^7 cells ml^{-1}, mean 1.16 x 10^7 cells ml^{-1}, n=28), cell numbers of *O. vangoorii* were higher than the Alboran and transect samples, the latter being characterised by lower bacterial counts (range 8.65 x 10^5 - 2.16 x 10^6 cells ml^{-1}, mean 1.45 x 10^6 cells ml^{-1}, n=8). Values of Pearson's product-moment correlation coefficient between bacterial and *O. vangoorii* counts were weakly significant (p=0.1) in the transect samples, and not significant in the time series samples.

Morphology and ultrastructure

Cells had two strongly unequal flagella, the longer one usually lacking the characteristic hairs as a result of variable chemical fixation (Fig. 2A). For the first time we were able to observe the apical region of the cell, which was modified into a circular, shallow cytostome (Fig. 5F).

Cells were enclosed by a pot-like lorica, although many loricas were empty (possibly as a consequence of chemical fixation). In agreement with existing observations (Vørs, 1992b), loricas were very variable in shape, ranging from narrow forms with a more or less acute posterior end to much broader forms with a rounded or truncated posterior end. (Figs. 2, 3, 4 and 5A). Table 2 summarises the measured morphometric features, and Figure 1 summarises the morphological variability spectrum in our samples.

The lorica ultrastructure was also very variable (Figs. 2C-F, 3, 4). Loricas were usually circular in cross-section (Figs. 2B-C, 5F) but occasionally they were laterally flattened (Figs. 4A-D). They usually had a striated appearance, resulting from the presence of transverse bands with a thickened upper margin (Figs. 2E-F, 3A-C). There were significant negative correlations between the number of bands and the

TABLE 3. – Values of Pearson's product-moment correlation coefficient (r) between lorica length and number of bands (L - B) and lorica width and number of bands (W - B). DF = degrees of freedom, p = significance limits.

Variables	r	DF	p
L - B	- 0.170	149	$0.01 < p < 0.05$
W - B	- 0.297	149	$p < 0.001$

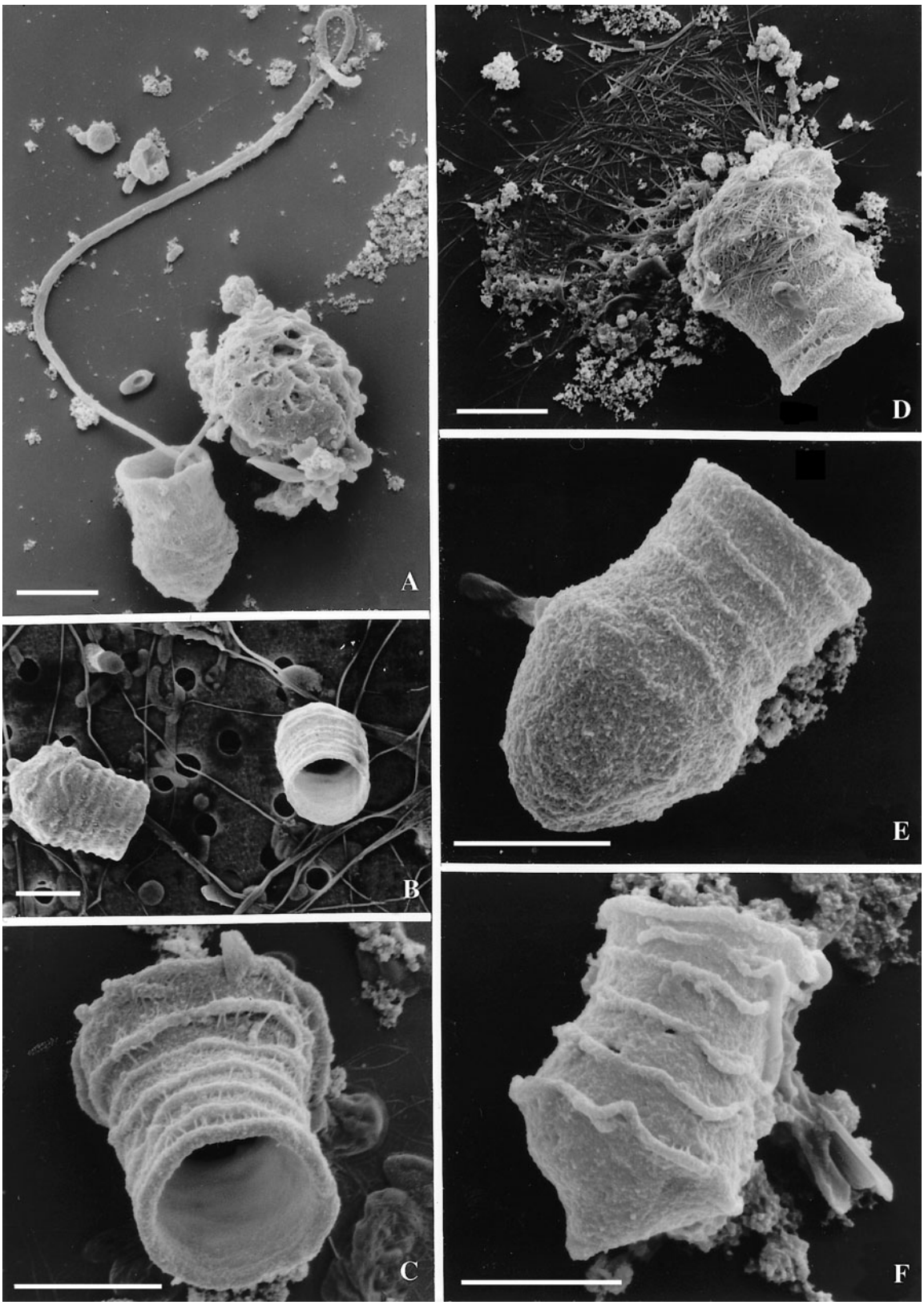

FIG. 2. – Cells and loricas of *O. vangoorii* from the Mediterranean Sea, SEM, HMDS-dried material., showing the variability of the mode of attachment to suspended matter. **A**, whole cell, showing flagella protuding from the lorica opening. **B**, **C**, loricas associated with suspended filamentous material and bacteria (visible in the background). **D**, lorica with abundant fibrils at the posterior end, providing adhesion to suspended particulate matter (visible in the background). **E**, **F**, loricas adhering to suspended particulate matter directly, i.e. without interposed fibrils. Scale bars = 2 μm.

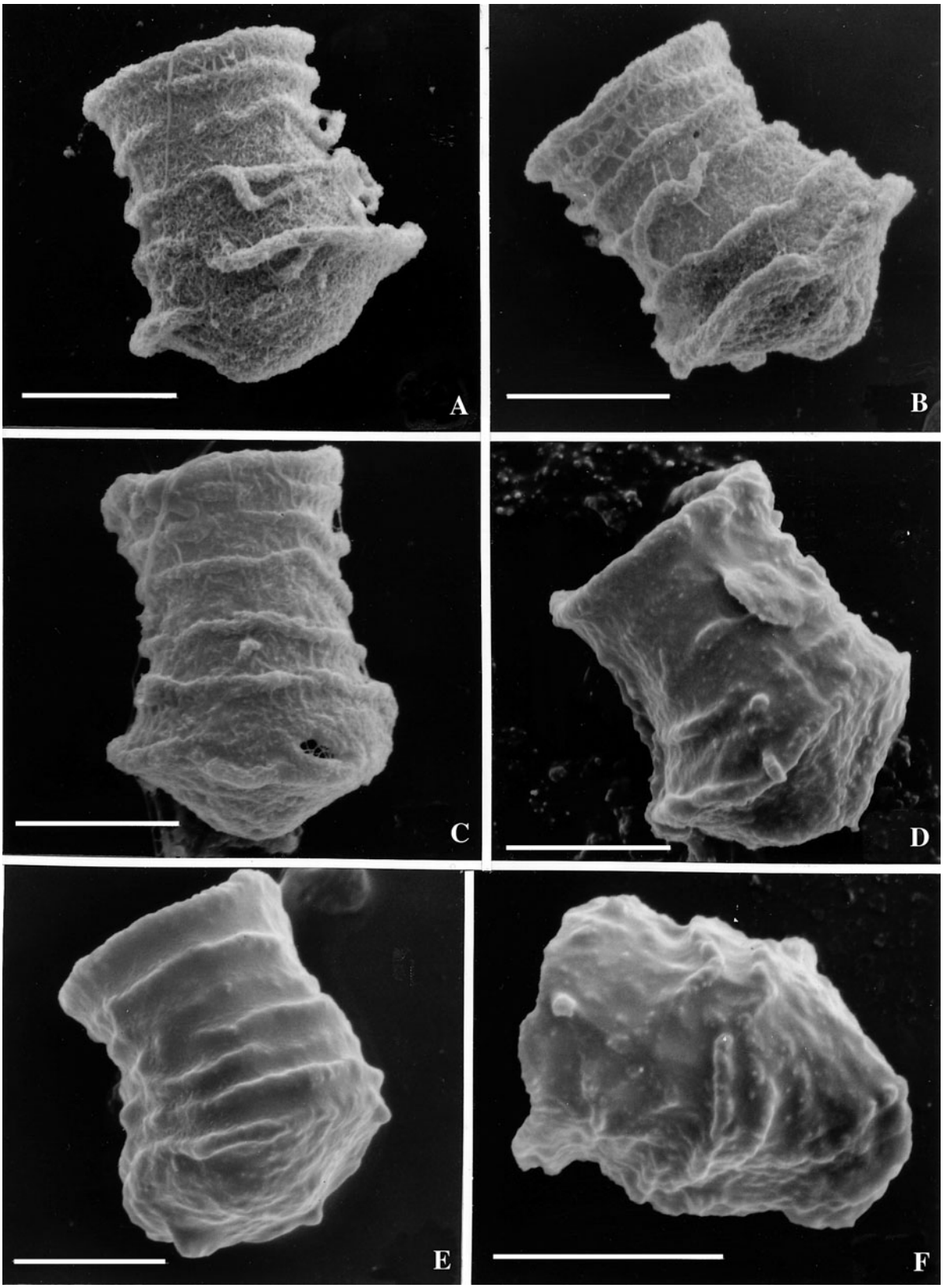

FIG. 3. – Loricas of *O. vangoorii* from the Mediterranean Sea, SEM, HMDS-dried material, showing the structural rôle of fibrils and mineralization in the formation of a mature lorica. In **A**, a scarcely mineralized lorica shows the fibrillar component of the coiled ribbon. In **B** and **C** an increasing number of longitudinal fibrils running between the thickened upper margins of the ribbon provide structural cohesion. Rigidity is provided by mineral deposition from the environment, ranging from moderate (**C**) to very pronounced (**D**-**F**), which may hide the banding of the lorica (e.g. **F**). Scale bars = 2 µm.

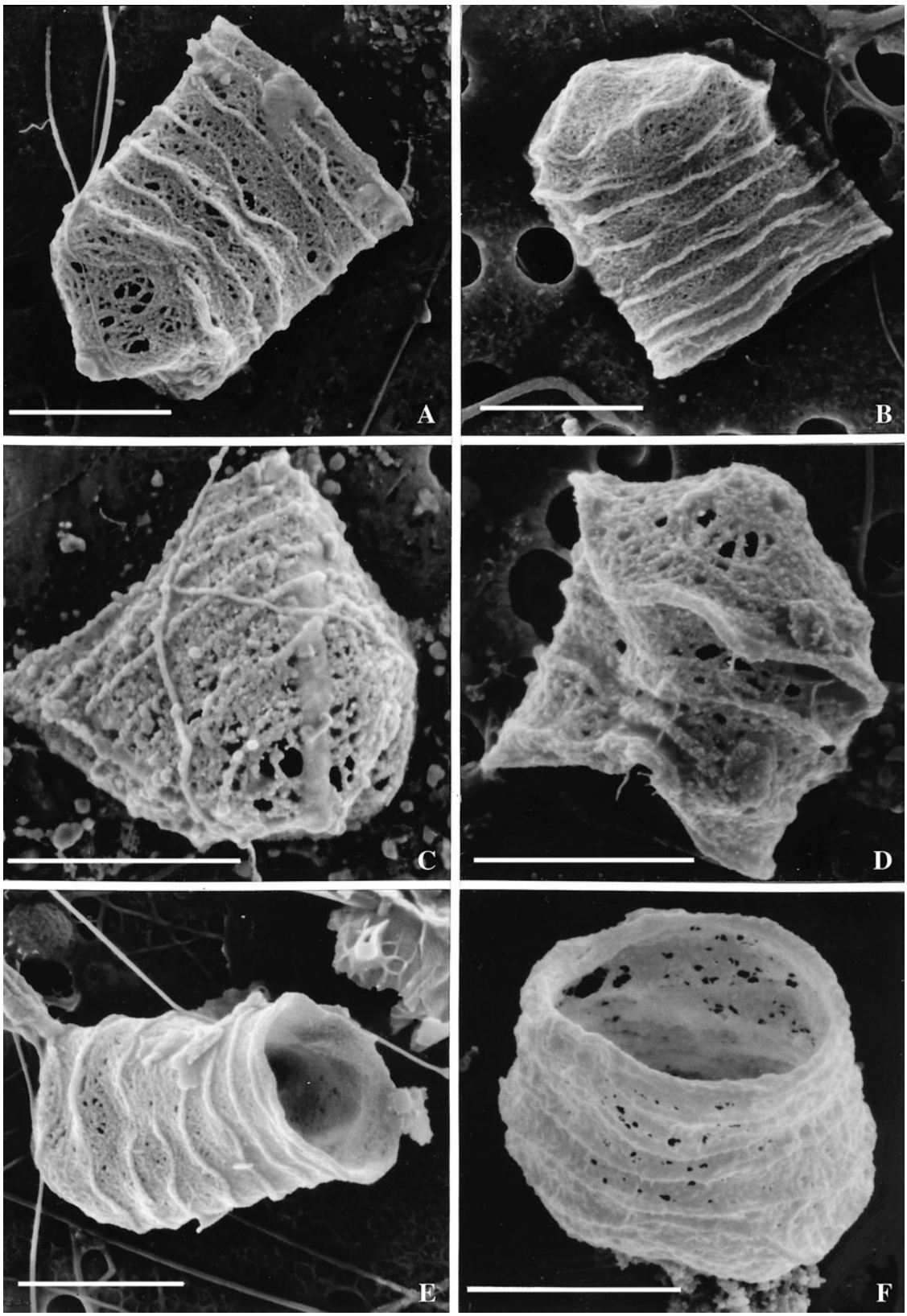

FIG. 4. – Loricas of *O. vangoorii* from the Mediterranean Sea, SEM, HMDS-dried material, showing the relationship between scarce or absent mineralization and lorica rigidity. **A** and **B** show flattened and therefore flexible loricas as a consequence of the absence of mineralization; they differ in the appearance of the fibrillar component (coarse, **A**, versus finer, **B**). **C** and **D** show loricas similar to **A** and **B**, except that the fibrillar component is overall much coarser. **E** and **F** illustrate scarcely mineralized loricas, as shown by the presence of irregular perforations and the slight degree of flattening. Scale bars = 2 μm.

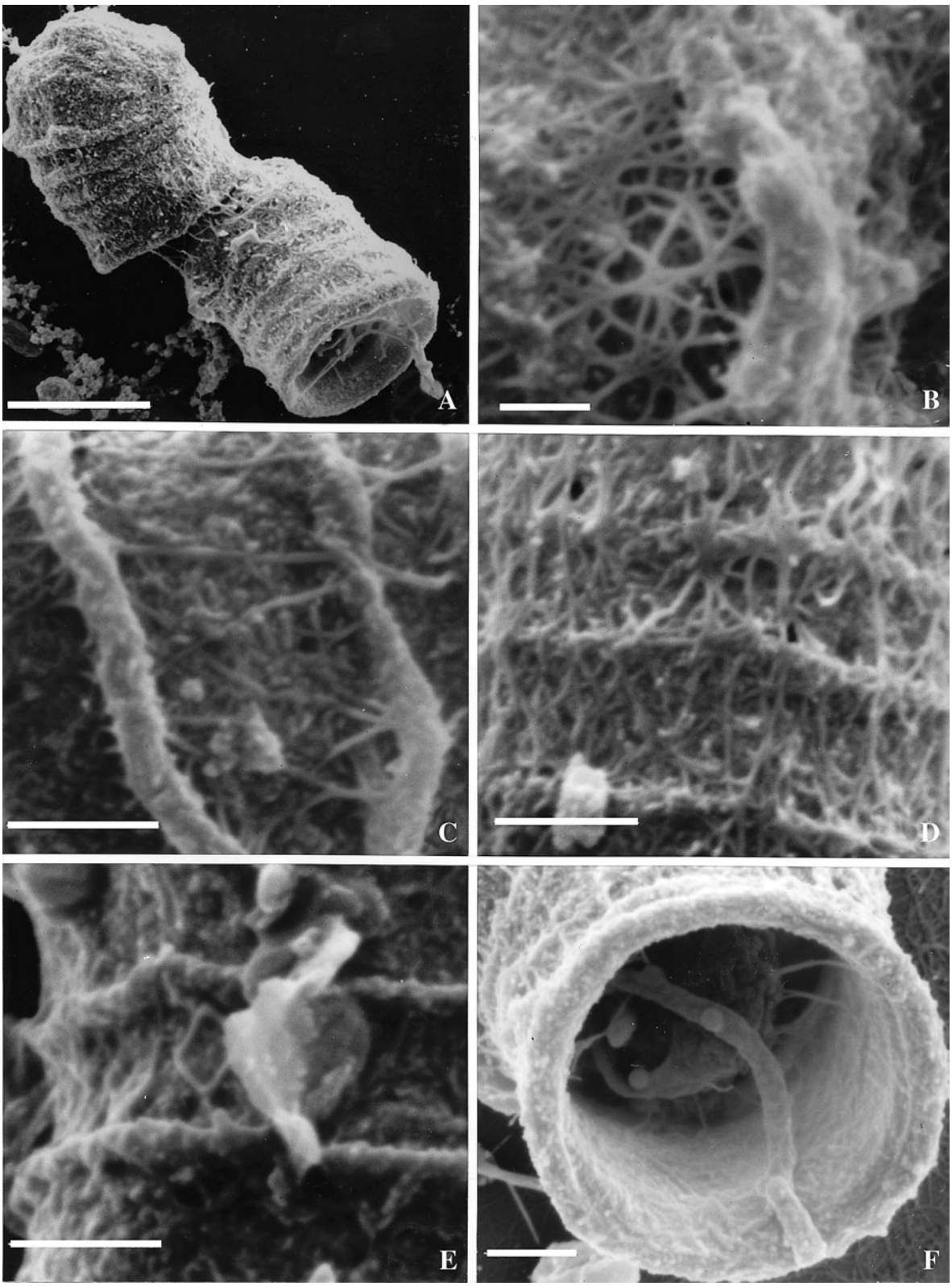

FIG. 5. – Cells and loricas of *O. vangoorii* from the Mediterranean Sea, SEM, HMDS-dried material, showing further characteristics of the fibrillar component and cell morphology. **A**, paired cells adhering by means of fibrils. **B**, basic fibrillar network of the lorica ribbon, showing a characteristic stellate pattern. **C**, longitudinal fibrils running between the thickened margins of adjacent spires of the lorica ribbon. **D**, detail of a lorica with numerous longitudinal fibrils deposited over the basic fibrillar network. **E**, a lorica incorporating a prasinophyte scale (centre of micrograph). **F**, cell inside its lorica, to which it adheres by means of short fibrils. A circular cytostome region is also visible at the base of the flagella. Scale bars: 2 µm (A), 500 nm (C-F), or 250 nm (B).

size of the lorica (Table 3). In heavily mineralized loricas the bands were scarcely visible (Figs. 3E-F). That the degree of mineralization was very variable was also shown by the presence of loricas with irregular perforations (Fig. 4), representing unmineralized areas, and also flattened (and therefore flexible) unmineralized loricas. Unmineralized or moderately mineralized loricas clearly revealed the fibrillar substructure of the lorica wall (Figs. 3A-B, 4A-B, 5B-D), in agreement with an existing observation (Espeland and Throndsen, 1986). Fibrils were also present between the cell surface and the internal face of the lorica (Fig. 5F), anchoring the cell to the lorica itself. Individual fibrils composing the lorica wall were usually about 200-600 nm in length and of variable diameter, usually about 20-40 nm but occasionally much coarser (e.g. Fig. 4C). They formed an intricate network (Figs. 4A-B, 5B-D) in which they often anastomosed to form stellate structures (Fig. 5B). Very long fibrils were also seen which extended beyond the confines of the lorica, providing adhesion to SPM (Fig. 2D) or between paired loricas (Fig. 5A). Alternatively, loricas adhered directly to SPM without interposed fibrils (Figs. 2E-F). The sticky properties of the lorica fibrils were also apparent from the fact that the lorica often incorporated foreign particles (e.g. Fig. 5E, a prasinophyte scale).

DISCUSSION

Our SEM observations show for the first time that cells of *Ollicola vangoorii* have an apical cytostome, substantiating a brief comment by Vørs (1992b) that the cells are capable of ingesting bacteria. Unfortunately the unavailability of suitable live material has prevented us from observing the uptake of bacteria directly. In the populations examined here the correlation between *O. vangoorii* and bacterial counts was at best only weakly significant, which makes it difficult to estimate the significance of bacterivory as a feeding mode for *O. vangoorii* from an ecological perspective. However, in this respect it is interesting to note that the lack of a significant relationship between heterotrophic flagellates and bacteria is a general rather than an exceptional case (Gasol and Vaqué, 1993). If the Barcelona populations relied partly on bacterivory, as we are inclined to believe, then the absence of a significant correlation between *O. vangoorii* and bacteria in the Barcelona time series samples could be explained, perhaps, in terms of prey preference, i.e. relying on only few kinds of bacteria as a food source. In this case, the high bacterial counts in the samples are not necessarily an indication that there was abundant food available for *O. vangoorii* because such counts do not necessarily imply that high numbers of the right kind of bacterial preys were present. Furthermore, it is likely that any grazing activity of SPM-associated *O. vangoorii* cells was mainly directed towards SPM-associated bacteria, in which case the ratio between unattached and SPM-associated bacteria could have been more important than total bacterial numbers in influencing the abundance of *O. vangoorii.*

Owing to the fact that the morphological variability spectrum of *O. vangoorii* in our samples clearly included that of other described species (see Appendix), we regard those species as simple morphological variants (morphotypes) of *O. vangoorii*, confirming previous remarks (Vørs, 1992b). The specimens which we examined morphometrically were found mostly in a sample series from a single geographical location (the Barcelona time series samples). Therefore the simplest assumption is that they all belong to a single species, implying that the other described species in question are conspecific with *O. vangoorii* (see Appendix for details). This assumption, however, is based exclusively on a morphological species concept, and it remains unknown whether or not the morphological continuum of *O. vangoorii* in our samples conceals a number of separate biological species. This sort of question can only be addressed by using genetic and molecular techniques, but unfortunately these were not available during our study.

From an architectural point of view our observations expand a brief comment that the lorica of *Ollicola* is constructed of a "spirally curved fibrous ribbon" (Vørs, 1992b). Each band visible with the SEM represents a spire of the coiled ribbon, with longitudinally directed fibrils providing cohesion between adjacent spires (Figs. 5C-D, 6A-C). The strong negative correlations between the number of bands/spires and cell size could be explained by hypothesizing that as the cell grows, additional material (fibrillar material produced by the cell and/or minerals from the environment) is deposited on the lorica, eventually causing some spires to coalesce. As a result, mature loricas are also likely to be more rigid than immature ones, especially as a consequence of increased mineralization (compare for instance Fig. 3F, a heavily mineralized lorica, and Fig. 4B, an unmineralized lorica).

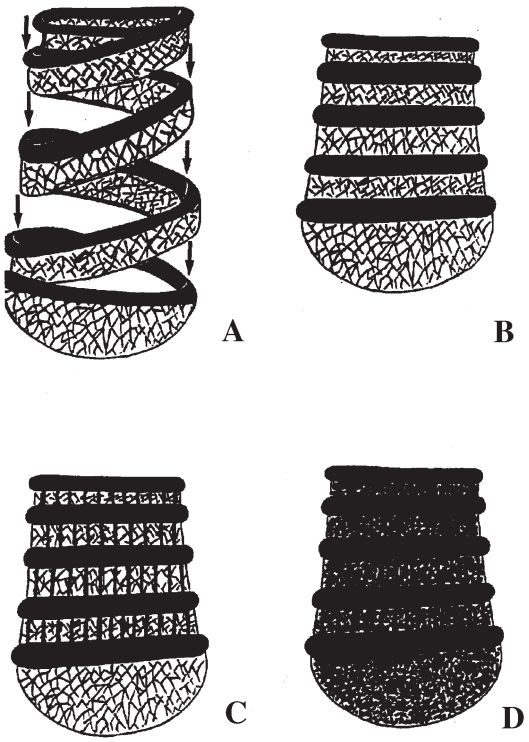

FIG. 6. – Diagrammatic model of lorica architecture of *O. vangoorii* based on the present observations. A fibrous ribbon with a thickened upper margin (**A**) forms the basic architecture of the lorica (**B**) by coiling around the cell (not shown), with each spire underlapping the next most distal one with respect to the lorica opening (**A**, arrows). Longitudinal fibrils running between the thickened margins of adjacent spires (**C**) provide structural cohesion, and mineral deposition from the environment (**D**) increases lorica rigidity.

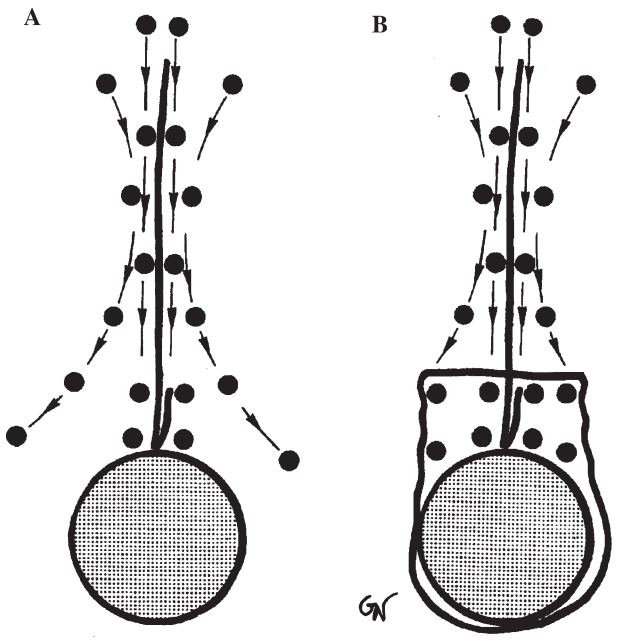

FIG. 7. – Hypothetical feeding behaviour of *O. vangoorii*. In the absence of a lorica, the beating of the long flagellum of a chrysomonad cell produces feeding currents similar to those in **A** (based on data provided for *Paraphysomonas* by Fenchel, 1986), with bacteria transported along the outermost flow lines escaping interception from the cell. The presence of a lorica in *Ollicola* (**B**) might increase the probability of intercepting bacteria transported along the outermost flow lines, effectively trapping them close to the cell surface ready to be ingested in the apical cytostome (not shown). This model does not take into account the slight asymmetry of *Paraphysomonas* feeding currents.

The chemical composition of the lorica fibrils is unknown and we hypothesise that it includes polysaccharides, which are widespread in the cell coverings and other kinds of external secretions produced by protists. For instance, analogous to other loricate chrysomonad flagellates (Herth *et al*., 1977), the lorica fibrils of *Ollicola* could be of a chitinous nature. Alternatively they could contain sulphated mucopolysaccharides, consistently with the demonstrated presence of sulphur (Espeland and Throndsen, 1986), and the fact that the lorica is mineralized. In many other protists and algae, externally secreted mucopolysaccharides –especially sulphated ones– may provide nucleation sites for the inorganic deposition of minerals from the environment (e.g. Novarino, 1993, and references therein). It is interesting to note that, analogous to *O. vangoorii* (Espeland and Throndsen, 1986), the mucilaginous lorica of the euglenid flagellate *Trachelomonas* also incorporates Fe and Mn (Dunlap and Walne, 1985).

The function of the lorica is also unknown and we hypothesize that it increases the probability of intercepting food bacteria. We assume that *Ollicola vangoorii* is similar to other chrysomonads (e.g. *Paraphysomonas* De Saedeleer, *Ochromonas* Vysotskii, *Spumella* Cienkowsky) in terms of the feeding currents produced by the flagellar beat and the mechanism of prey ingestion. (See Fenchel, 1986, for information on the feeding currents of *Paraphysomonas*, Fenchel, 1987, for bacterial ingestion through the cytostome of *Ochromonas*, and Zwart and Darbyshire, 1992, for direct interception feeding of *Spumella*). In the case of non-loricate chrysomonad cells (Fig. 7A), bacteria transported along the flow lines closest to the long flagellum are more likely to come into contact with the apical region of the cell and be ingested than bacteria transported along the outermost flow lines (Fenchel, 1986). Overall, the probability of bacteria eventually being ingested in such a way is likely to be small when the bacterial concentration in the immediate surroundings of the cell is low. Such a condition is probably the rule for pelagic, non-particle associated cells of *O. vangoorii*, whereas SPM-associated cells usually benefit from higher bacterial concentrations on or close to SPM itself.

In *O. vangoorii*, we hypothesize that the lorica wall extending well beyond the cell apex intercepts bacteria transported along the outermost flow lines which would otherwise be missed, effectively trapping them close to the cell surface ready to be ingested in the apical cytostome (Fig. 7B). We do not know whether or not the porosity of the lorica is such that it may act as a filter, allowing feeding currents to flow through it and retaining bacteria transported along the currents. It is possible that scarcely mineralized loricas (Figs. 4A-D) are sufficiently porous for that purpose. In the case of loricas not acting as filters, we hypothesise that food particles transported along the outermost flow lines could still be retained by the lorica wall as a simple consequence of its adhesive properties.

CONCLUSIONS

Cells of *Ollicola vangoorii* have an apical cytostome, substantiating an existing observation that the cells are able to ingest bacteria. In the examined plankton populations it is possible that the ecological rôle of this nanoflagellate is partly that of a carbon consumer. The lorica morphology is very variable and several species considered to be distinct from *O. vangoorii* appear to be simple morphological variants along a continuous variability spectrum. Perhaps the presence of a lorica may increase the probability of intercepting food bacteria. The ultrastructure of the lorica is of interest not only *per se* but also in relation to the attachment of loricas to SPM, either by means of long, sticky fibrils extending beyond the confines of the lorica itself, or directly over the entire sticky surface of the lorica wall. Owing to the fact that *O. vangoorii* may or may not be associated with SPM, and that SPM may settle periodically to the sea bottom (Eisma, 1993), we hypothesize that this nanoflagellate constitutes a functional link between the pelagic and benthic microbial communities. This idea is in need of further investigation.

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APPENDIX

The taxonomy and nomenclature of *Ollicola* and other nomenclaturally related genera are complex. The genus was described by Vørs (1992b) based on *Ollicola vangoorii* (Conrad) Vørs 1992, a new combination introduced to accommodate a species known until then as *Calycomonas Van Goorii* (Conrad) Lund 1959, itself based on *Codonomonas Van Goorii* Conrad 1938, the first known description of this species even though it was originally thought to lack chloroplasts and possess a single flagellum rather than two. The new genus introduced by Vørs was justified by the fact that the genus name *Calycomonas* was reduced into synonymy because the type species*, C. gracilis* Lohmann (designated by Lund, 1959), was to be regarded as a testate amoeba of the genus *Paulinella* Lauterborn (Vørs, 1992b), rather than a chrysomonad, i.e. *Paulinella gracilis* (Lohmann emend. Lund) Vørs 1992, analogous to *Calycomonas ovalis* Wulff 1919, which had been also recombined under the genus *Paulinella* (Johnson *et al.*, 1988). Even in the absence of a re-examination of any preserved type material (see below), this is a valid argument and the generic vehicle *Ollicola* ought to be preferred to *Calycomonas* and *Codonomonas*.

That *Ollicola* is a preferable vehicle is also supported by the fact that the generic diagnosis is based on electron rather than light microscopy. This makes it possible to observe the key features unequivocally, as per the emended diagnosis provided by Vørs (1992b). Fortunately there are few if any reports under the generic name *Codonomonas*. These would be hardly verifiable owing to difficulties in observing the key features by using light microscopy only. Further difficulties in verifying existing literature reports of species of *Ollicola* derive from the fact that at times they may have been identified as belonging to the genus *Kephyrion* Pascher, which is totally distinct. Here again, electron microscopy –if available– is the only tool for unequivocal verifications.

The species-level taxonomy and nomenclature are also complex. The detailed revision of *Calycomonas* by Lund (1959) was based on light microscopy, which makes it difficult to establish with certainty the true identity of species and the application of appropriate names. Based on our observations, we regard several species retained as distinct by Lund as being conspecific with *Ollicola vangoorii* because the lorica morphology is the sole distinguishing character and it is shown here to have a continuous variability spectrum. Vørs (1992b) provisionally recombined some of those species in *Ollicola* (as "*Ollicola* incertae sedis" species), whilst still retaining them as distinct. Ultrastructural examinations of flagellate type materials are sometimes feasible (Novarino and Couté, 2000), and in this case they would be useful for deciding unequivocally whether or not those species should be retained as distinct. However, it appears that no type materials are available of species of *Ollicola, Calycomonas,* or *Codonomonas.* For instance, the type materials of *Calycomonas* species examined by Lohmann (Lohmann, 1908-1909) may have been destroyed in 1943, when the Zoological Museum of Hamburg was bombed and all its protistological collections were lost. (Lohmann was appointed director of the museum in 1914 and therefore any of his collections were likely to be kept there).

Even in the absence of comparisons with type materials, based on the following considerations we feel that there is sufficient evidence to regard several separately described species as simple morphotypes of *Ollicola vangoorii*:

- The original illustrations of *Calycomonas gracilis* sensu Wulff (non *Calycomonas gracilis* Lohmann) are cleary very similar to the type figures of *Ollicola vangoorii*. In our samples this morphotype is represented by Figs. 2E-F, showing centrally constricted loricas with a pointed or slightly rounded posterior. The illustrations of *Calycomonas wulffii* Conrad et Kufferath (= *Calycomonas gracilis* sensu Wulff non Lohmann) provided by Conrad and Kufferath (1954) are also very similar to the type figures of *Ollicola vangoorii,* the only difference being the higher width:length ratio. In our samples this morphotype is represented by Fig. 4A.
- The type figures of *Ollicola cylindrica* Vørs (= *Calycomonas cylindrica* (Conrad et Kufferath) Lund, *Codonomonas cylindrica* Conrad et Kufferath) show wide, unconstricted loricas. In our samples this morphotype is represented by Figs. 2B, 4B, 5A.
- The type figures of *Ollicola dilatata* Vørs (= *Calycomonas dilatata* (Conrad et Kufferath) Lund, *Codonomonas dilatata* Conrad et Kufferath) show loricas with a wide apical opening. In our samples this morphotype is represented by Fig. 4C.

By contrast, we feel that *Ollicola pascheri* (van Goor) Vørs (basionym: *Codonomonas pascheri* van Goor) has a sufficiently characteristic morphology

(bell-shaped rather than pot-shaped lorica, with a wide apical fascia) to be retained as a separate species. In addition, we also think that it ought to be excluded from *Ollicola* and retained in *Codonomonas* based on the following nomenclatural considerations. The name *Ollicola pascheri* is based on *Codonomonas pascheri* van Goor 1925. Because *Codonomonas pascheri* is the type of the genus *Codonomonas* van Goor 1925, if it were included in the genus *Ollicola* then the names *Codonomonas* and *Ollicola* would have to be regarded as synonyms. In that case *Ollicola* would have to be rejected under both Codes of nomenclature in favour of *Codonomonas* because it is the later (junior) synonym but this prospect is highly undesirable.

Therefore, based on the present generic circumscription we propose that the genus *Ollicola* only includes the type species. The following is a *bona fide* species synonymy list within the genus, which we consider as a member of the Chrysomonadidae (William Saville Kent) stat. nov. in Cavalier-Smith (1993) under the zoological nomenclature (ICZN), and the Chrysophyceae Pascher (1914) under the botanical nomenclature (ICBN).

Genus *Ollicola* Vørs 1992 (ICBN, ICZN)
Vørs 1992b, p. 60

Type species: *Ollicola vangoorii* (Conrad) Vørs 1992 (ICBN, ICZN)
Vørs 1992b, p. 60, Figs. 28, 29.

Basionym: *Codonomonas Van Goorii* Conrad 1938, p. 4, Figs. 4-6.

Synonyms: *Calycomonas Van Goorii* (Conrad 1938) Lund 1959, p. 427. *Calycomonas gracilis* sensu Wulff 1919, p. 110, pl. II Figs. 19a, b, non *Calycomonas gracilis* Lohmann 1908-09, p. 291, pl. XVII, fig. 13A, emend. Lund 1959, p. 426. *Calycomonas gracilis* sensu Espeland & Throndsen 1986, p. 213, Figs. 12, 13, non *Calycomonas gracilis* Lohmann 1908-09, p. 291, pl. XVII, fig. 13A, emend. Lund 1959, p. 426. *Calycomonas wulffii* Conrad et Kufferath 1954, p. 183, pl. V, Figs. 3A, B (= *Calycomonas gracilis* sensu Wulff non Lohmann); Delgado & Fortuño 1991, p. 22, pl. LXXXIX, fig. c; Vørs 1992a, p. 223, Figs. 6.28, 6.29. *Ollicola cylindrica* (Conrad et Kufferath 1954) Vørs 1992b, p. 60; *Calycomonas cylindrica* (Conrad et Kufferath 1954) Lund 1959, p. 427; *Codonomonas cylindrica* Conrad et Kufferath 1954, p. 166, pl. IV, Figs. 11A, B. *Ollicola dilatata* (Conrad et Kufferath 1954) Vørs 1992b, p. 61; *Calycomonas dilatata* (Conrad et Kufferath 1954) Lund 1959, p. 427; *Codonomonas dilatata* Conrad et Kufferath 1954, p. 166, pl. IV, Figs. 13A, B. *Kephyrion* sp. 1 sensu Leadbeater 1974, p. 186, pl. VII A. *Kephyrion* sp. 2 sensu Leadbeater 1974, p. 186, pl. VII B.

Species excludenda: *Ollicola pascheri* (van Goor) Vørs 1992 (ICBN, ICZN)
Vørs 1992b, p. 61.

Basionym: *Codonomonas pascheri* van Goor 1925, p. 318, fig. 3.