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*Short Communication***New records of marine choanoflagellates off the Chilean coast****Katia Soto-Liebe¹, Gloria Collantes² & Juan Kuznar¹**¹Facultad de Ciencias, Universidad de Valparaíso, Casilla 5030 Valparaíso²Facultad de Ciencias del Mar y Recursos Naturales, Universidad de Valparaíso, Casilla 5080
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ABSTRACT. This is the first report of planktonic choanoflagellates from the Chilean coast, relating their abundance with that of bacteria and viruses. Surface water samples were taken off Antofagasta, Montemar, and Puerto Montt and samples from ballast tanks were also analyzed. The choanoflagellates were identified following morphological type descriptions. Viruses and bacteria were stained with SYBR Green and choanoflagellates, bacteria, and viruses were counted simultaneously using red autofluorescence for the former and green fluorescence for the latter two. Six species of Acanthoecidae were observed for the first time in Chilean waters: *Calliacantha multispina* ($0.3 \cdot 10^3 \text{ L}^{-1}$), *Acanthocorbis apoda*, *Stephanoeca diplocostata* ($2.0 \cdot 10^3 \text{ L}^{-1}$), *Crinolina isefjordensis*, *Parvicorbicula superpositus*, and *Pleurasiga minima* ($5.0 \cdot 10^3 \text{ L}^{-1}$). The concentrations of bacteria ($1.1 \cdot 10^6$ - $4.5 \cdot 10^6$) and viral-like particles (VLPs) ($7.9 \cdot 10^6$ - $21 \cdot 10^6$) agreed with those typically found in marine coastal waters. In addition, *Acanthocorbis asymmetrica* was found in ballast waters, where its high concentration ($20 \cdot 10^3 \text{ L}^{-1}$) likely resulted from the particular physical and biological environment therein.

Key words: Choanoflagellates, bacteria, virus, ballast water, epifluorescence, Chile.

Nuevos registros de coanoflagelados marinos de la costa de Chile

RESUMEN. Este es el primer reporte sobre coanoflagelados planctónicos de la costa chilena y su abundancia se relacionó con la de bacterias y virus. Se analizaron muestras de aguas superficiales obtenidas en Antofagasta, Montemar y Puerto Montt. Además se analizaron muestras adicionales de aguas provenientes de estanques de lastre. Los coanoflagelados se determinaron de acuerdo a descripciones morfológicas tipo. Virus y bacterias fueron teñidos con SYBR Green y la enumeración simultánea de coanoflagelados, bacterias y virus se determinó a través de autofluorescencia roja y fluorescencia verde respectivamente. Seis especies de Acanthoecidae fueron observadas por primera vez en aguas chilenas: *Calliacantha multispina* ($0,3 \cdot 10^3 \text{ L}^{-1}$), *Acanthocorbis apoda* y *Stephanoeca diplocostata* ($2,0 \cdot 10^3 \text{ L}^{-1}$), *Crinolina isefjordensis*, *Parvicorbicula superpositus* y *Pleurasiga minima* ($5,0 \cdot 10^3 \text{ L}^{-1}$). Las concentraciones de bacteria ($1,1 \cdot 10^6$ - $4,5 \cdot 10^6$) y virus ($7,9 \cdot 10^6$ - $21 \cdot 10^6$) son las típicamente descritas para aguas marinas costeras. En aguas de lastre se encontró *Acanthocorbis asymmetrica* cuya alta concentración ($20 \cdot 10^3 \text{ L}^{-1}$) debiera ser consecuencia de este particular ambiente físico y biológico.

Palabras clave: coanoflagelados, bacteria, virus, agua de lastre, epifluorescencia, Chile.

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The history of choanoflagellates was delineated by Kent (1882) and Ellis (1930). Later, the current systematic hierarchy within the order was given by Norris (1965). Studies on mitochondrial (Leadbeater, 1974) and kinetid structures (Hibberd, 1975) unequivocally clarified the phylogenetic affinity of the order, placing it in the protocist Phylum Zoomastigina (Buck, 1990; Snell *et al.*, 2001).

The Class Choanoflagellidea contains almost

invariably colorless species, oval in shape, with a single flagellum, and encircled at the base by a collar composed of fine pseudopodia. Many species are enveloped in a lorica composed of silica rods and costal strips (Thronsdon, 1993). Other species have an organic thecae (Buck, 1981). Choanoflagellate taxonomy is based almost solely on lorica or theca morphology as analyzed under light and/or electron microscopy (Leadbeater, 1972a, 1972b,

1975; Thomsen, 1976). The Order Choanoflagellida, Kent 1880, consists of three Families that have been tentatively raised to the rank of Order. The Order Codonosigida/Family Codonosigidae consists of naked cells or those having an investment invisible under light microscopy. The cells of the Order Salpingoecida/Family Salpingoecidae have a hyaline close-fitting theca, are visible under light microscopy, and are prevalent in freshwater. Finally, the Order Acanthoecida/Family Acanthoecidae is made up of cells that are surrounded by a lorica (often wide) and consisting of costae constructed from costal strips of silica; they are conspicuous in marine environments (Thomsen, 1992; Vørs, 1992; Thronsen, 1993). Progress has been made in the taxonomy of *Stephanoeca diplocostata* and *Calliacantha* sp. through partial sequences of the 18S ribosomal RNA gene (Medina *et al.*, 2003; Cavalier-Smith & Chao, 2003).

Asexual reproduction in most of these species occurs through longitudinal fission (Thomsen & Larsen, 1992). For the Acanthoecidae, juvenile cells may have component costal strips associated with them when departing from the parent lorica (tectiform replication). This particular strategy seems to be species-specific. Some sedentary species reproduce by means of a swimming mastigote (Buck, 1981).

The apparent mode of nutrition is phagotrophy (Laval, 1971; Leadbeater & Morton, 1974; Fenchel, 1982; Marchant, 1985; Sherr, 1988) with ingested material consisting of bacteria (including cyanobacteria), nanoplanktonic algae, and debris. Some species are capable of subsisting solely on dissolved organic matter in axenic cultures (Gold *et al.*, 1970), whereas others may alternate between osmotrophic or pinocytotic modes (Manton *et al.*, 1981). The role of choanoflagellates within the trophodynamics of aquatic regimes is that of an intermediary between primary producers and zooplankton (Meyer & El-Sayed, 1983). Bacteria and picoplankton were recognized as important members of a microbial food web (Graham & Wilcox, 2000). Various protists, including algal species, amoebae, and ciliates can be extremely important grazers in both fresh and marine waters. Small flagellates and ciliates can ingest as much as 100% of their own cell volume within one hour, and can double their populations in about three hours. In a lake study, small heterotrophic flagellates such as bodonids and choanoflagellates were shown to account for 84% of total protistan grazing on the picoplankton, primarily cyanobacteria such

as *Synechococcus* (Carrias *et al.*, 1996). In addition to the species mentioned previously, the list of viruses that infect aquatic microorganisms is growing continuously. Viruses have profound implications for marine biodiversity and viral lysis can significantly influence phytoplankton population dynamics and biogeochemical cycles in aquatic habitats (Raven, 2006).

Although many works on choanoflagellates indicate a cosmopolitan distribution of the group, planktonic choanoflagellates have not yet been included in Chilean biodiversity (Simonetti *et al.*, 1995), nor is the taxon mentioned in the preliminary list of Chilean plankton (Antezana, 2001).

We report here several species of choanoflagellates recorded for the first time along the temperate Pacific coast of South America. In addition, a preliminary analysis of choanoflagellate abundances relative to bacteria and viruses was also carried out.

Surface water samples were taken in November 2001 from three sites off Antofagasta (23°38'S-70°24'W), Montemar (32°57'S-71°33'W), and Puerto Montt (41°41'S-72°38'W), Chile.

The sampling sites were situated 100 m from the coast. The samples ($n = 3$) were collected with sterile propylene bottles (250 mL) at 50 cm depth. Additional triplicate samples were taken from the ballast tank water of a local ship that arrived in Puerto Montt (41°29'S-72°57'W) after a short trip from Puerto Chacabuco (45°30'S-72°57'W). Temperature and salinity were determined *in situ*. All samples were filtered through a 35 μ m net, maintained in the dark at 4°C, and kept in 1-ml aliquots containing 5% formaldehyde. Bacteria and viruses were stained with SYBR Green I (Noble 2001) after water samples were filtered through an ultra-fine filter (Whatman Anodisc, 0.02 μ m Membrane Filters; Fisher Scientific, Inc). To stain the retained material, the filter was placed over a drop of SYBR Green I standard solution (Molecular Probes Inc.) 1:1000 in Milli Q water. After 15 min in the dark at room temperature, the excess stain was eliminated by washing the filter three times with Milli Q water. The filter was removed from the filtration apparatus, placed on a glass slide, and covered with a 25-mm square glass containing a drop of anti-fade mounting solution (50% PBS / 50% glycerol with 0.1% p-phenylenediamine).

The stained samples were examined in an Olympus BX60 epifluorescence microscope. Choanofla-

gellates were identified following morphological type descriptions (Ellis, 1930, Thronsen, 1970; Thomsen, 1976; Manton & Oates, 1979ab; Hara & Takahashi, 1984; Booth, 1990). The simultaneous enumeration of choanoflagellates, bacteria, and viruses was done using the red autofluorescence of choanoflagellates and the green fluorescence of bacteria and viruses. Randomly selected fields were counted using the Image Pro Plus 4.5 (MediaCybernetics).

This study revealed six species of choanoflagellates (Acanthoecidae) observed for the first time at the three sites selected off Chile (Fig. 1, Table 1). An additional species was observed in the ballast water sample (Fig. 1, Table 1). A brief description of type specimens is presented below: lorica sizes and costa numbers for the Chilean specimens are given in parentheses.

Acanthocorbis apoda (Leadbeater) Hara & Takahashi, 1984 (Figs. 1a-1c)

Lorica length: 13-18 μm (10 μm)

Lorica width: (10 μm)

Costae: 1 transverse, 12-16 longitudinal (1 transverse, 16 longitudinal)

Characteristic features: typical waist-shaped lorica.

Parvicorbicula superpositus Booth, 1990 (Fig. 1d)

Lorica length: 23-25 μm (15 μm)

Lorica width: (10 μm)

Costae: 2 transverse, 10 longitudinal (2 transverse, 10 longitudinal)

Characteristic features: funnel-shaped lorica.

Pleurasiga minima Thronsen, 1970 (Fig. 1e)

Lorica length: 10 μm (10 μm)

Lorica width: (10 μm)

Costae: 2 transverse, 7-10 longitudinal (2 transverse, 7 longitudinal)

Characteristic features: all longitudinal costae meet at the back of the lorica.

Stephaenoeca diplocostata Ellis, 1930 (Fig. 1f)

Lorica length: 16-22 μm (6.0 μm)

Lorica width: 8-10 μm (5.0 μm)

Costae: 5 transverse, 16-20 longitudinal (5 transverse, 16 longitudinal)

Characteristic features: Paired transverse costae where the lorica diameter is greatest.

Crinolina isefjordensis Thomsen, 1976 (Fig. 1g)

Lorica length: 25-30 μm (25 μm)

Lorica width: 20-30 μm (20 μm)

Costae: 2 transverse, 15-16 longitudinal (14 longitudinal)

Characteristic features: skirt-shaped lorica, open at both ends.

Calliacantha multispina Manton & Oates, 1979a (Fig. 1h)

Lorica length: 22-32 μm (20 μm)

Lorica width: (10 μm)

Costae: 2 transverse, 4-5 longitudinal (2 transverse, 6 longitudinal)

Characteristic features: The number of longitudinal costae decreases towards the posterior end of the lorica.

Acanthocorbis asymmetrica Ellis, 1930 (Figs. 1i-1l)

Lorica length: 15-20 μm (15 μm)

Lorica width: 8 μm (7-8 μm)

Costae: 2 transverse, 15-17 longitudinal (2-3 transverse, 15-18 longitudinal)

Characteristic features: stalk deviates markedly from the log axis of the cell, giving it an asymmetrical appearance.

Bacteria and viruses were visible all around and inside the choanoflagellates (Fig. 1). The abundance of choanoflagellates, bacteria, and viruses in samples from three different sites off Chile and from ballast waters are shown in Table 1. Bacteria and VLP concentrations were as expected for coastal marine waters and VLPs always exceeded bacterial abundance. Interestingly, choanoflagellate concentrations were 4 to 10 times higher in the ballast water in spite of an apparently slight decrease in the bacterial concentration. The temperature and the salinity of the surface waters off Puerto Montt (estuarine site) were lower than those off Antofagasta and Montemar (Table 1).

The range of species found in the Chilean waters was highly similar to that reported for other coasts of the world: *C. multispina* from the coasts of South Africa and southern England (Tong, 1997); *A. apoda* from the coasts of Norway (Leadbeater, 1972a, 1972b), Taiwan, Japan (Hara & Takahashi, 1984), and southern England (Tong, 1997); *S. diplocostata* from brackish waters or near-shore marine regions

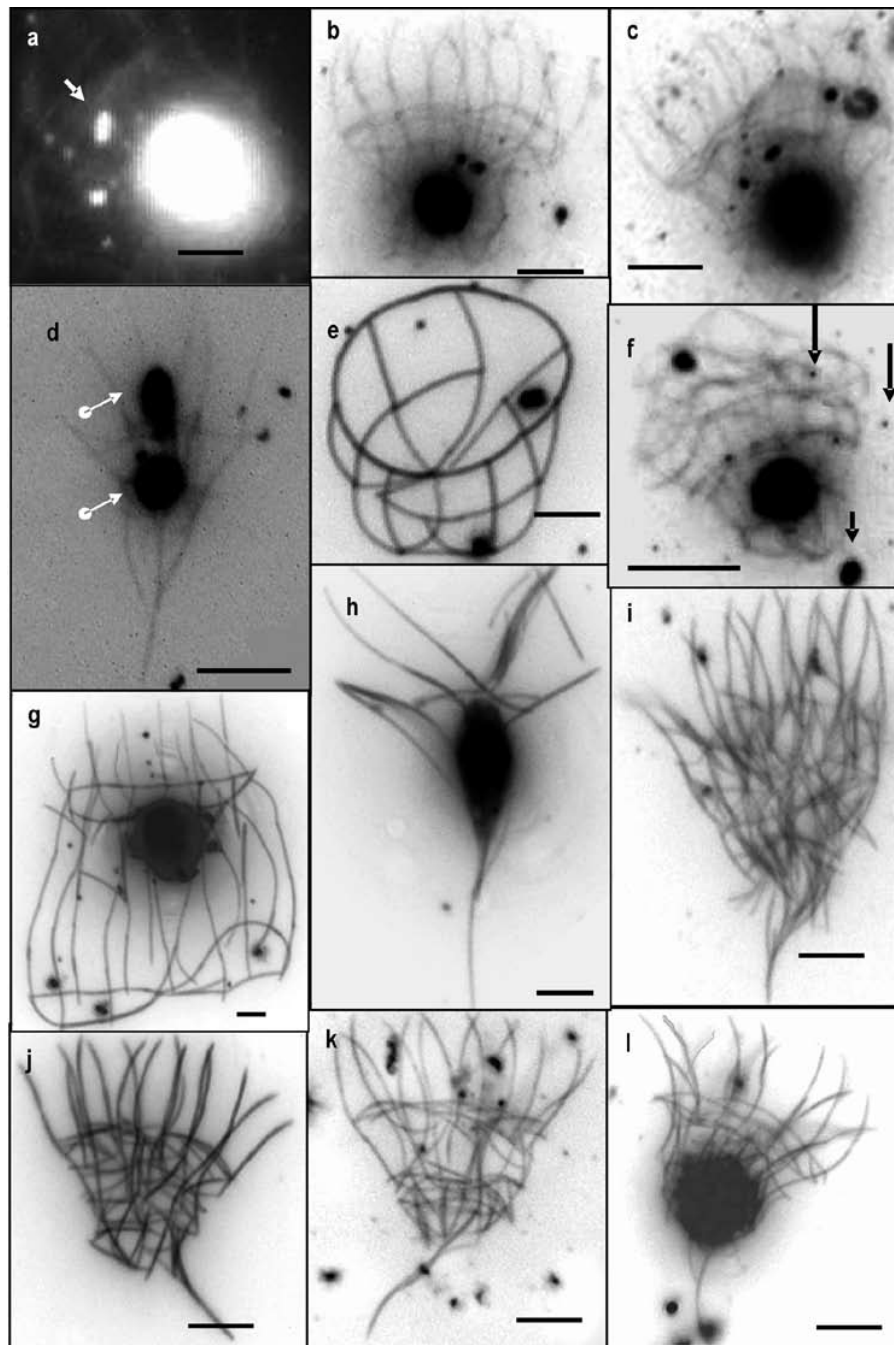


Figure 1. Marine choanoflagellates observed by means of EFM in SYBR Green stained samples. a) *Acanthocorbis apoda* showing collar with retained bacterias (short arrow), b, c) *Acanthocorbis apoda* showing loricas and the inner protoplast, d) *Parvicorvicula superpositus*, e) *Pleurasiga minima*, f) *Stephanoeca diplocostata*, g) *Crinolina isefjordensi*, h) *Calliacantha multispina*, i, j, k,) *Acanthocorbis asymmetrica* empty loricas showing variable accumulation of transversal costae, l) *Acanthocorbis asymmetrica* showing the inner protoplast. Images are the negative of the original. Scale bars: 2.5 μm .

Table 1. Abundances of choanoflagellates, bacteria and virus from three different localities from the coast of Chile and from ballast water.

Choanoflagellates	Sampling place T (°C)/ Salinity	Choanoflagellates·L ⁻¹	Bacterias·mL ⁻¹	VLP·mL ⁻¹
<i>Calliacantha multispina</i>	Antofagasta (23°38'S/70°24'W) 18.0 / 34.8	0.3·10 ³	1.1·10 ⁶	21·10 ⁶
<i>Acanthocorbis apoda</i> <i>Stephanoecca diplocostata</i>	Montemar cove (32°57'S /71°33'W) 14.5 / 34.5	5.0·10 ³	2.1·10 ⁶	14·10 ⁶
<i>Crinolina isefjordensis</i> <i>Parvicorbicula superpositus</i> . <i>Pleurasiga minima</i>	Puerto Montt, El Arenal (41°41'S/72°38'W) 13.0 / 30.5	2.0·10 ³	4.5·10 ⁶	7.9·10 ⁶
<i>Acanthocorbis asymmetrica</i>	Ballast water	20·10 ³	0.8·10 ⁶	18·10 ⁶

in the Northern Hemisphere (Ellis, 1930) and coast of southern England (Tong, 1997); *C. isefjordensis* from the coasts of Isefjord, Denmark (Thomsen, 1976) and southern England (Tong, 1997); *P. superpositus* from the coasts of the Sub-Arctic North Pacific Ocean (Booth, 1990) and southern England (Tong, 1997); and *P. minima* from the coasts of Arctic waters (Thronsdon, 1970) and southern England (Tong, 1997). The species from the temperate South Pacific coast reported in this study confirm that Acanthoeidae species have a cosmopolitan distribution. Chilean choanoflagellates showed some differences in lorica sizes and number of costae.

These species are difficult to distinguish from detritus; in addition, they are generally present only in small numbers, although they reach large numbers under eutrophic conditions such as those that can be found in ballast waters (Table 1). Acanthoeidae species also seem to be associated with estuarine or near-coastal sites (Leadbeater, 1972b), as shown by comparisons with species recorded from open water sites (Buck & Garrison, 1988; Booth, 1990; Thomsen *et al.*, 1990). The abundance of choanoflagellates sampled off Chile was rather low compared to that found in other places. In Antarctic waters, values were found ranging from 14·10³ L⁻¹ (surface) to 50·10³ L⁻¹ (20 m depth) (Chen, 1994); this is more than ten times the values shown in Table 1. These differences could be due to the fact that our samples were taken from surface waters. In addition, the data obtained by Chen (1994) was collected in summer, which offers the best conditions for a high level of primary production.

The abundance of planktonic species reflects the trophic relations between them as well as with the

environment. For example, VLPs are much more abundant than bacteria. The latter are important components of choanoflagellate food and, therefore, it was interesting to compare their abundances. However, no clear relationships between these abundances can be seen in Table 1. This kind of analysis should probably be done in deeper layers where choanoflagellates increase in number. Fenchel (1982) and Anderson & Fenchel (1985) suggested a significant influence of choanoflagellates on the bacterial abundance by judging correlational changes between the two. Ishiyama *et al.* (1993) showed no significant correlation between choanoflagellates and bacterioplankton, indicating that the ingestion of bacterioplankton by many choanoflagellate cells suggests that the biomass should be sufficient for choanoflagellates as well as for other bacteriovores in the Antarctic Ocean (Fenchel, 1982; Imai & Ito, 1984; Buck & Garrison, 1988; Marchant & Perrin, 1990).

The abundance of *A. asymmetrica* in ballast water was rather high (20·10³ L⁻¹) compared to the abundance of the choanoflagellates species from the field (0.3·10³ L⁻¹; 2.0·10³ L⁻¹; 5.0·10³ L⁻¹). This result was expected because choanoflagellates are more abundant in oxygen-deficient environments and those richer in heterotrophic bacteria (Marchant *et al.*, 1987). The abundance of bacteria (0.8·10⁶ mL⁻¹) in the ballast water samples was low compared to the abundance of bacteria from the field samples (1.1·10⁶ mL⁻¹; 2.1·10⁶ mL⁻¹; 4.5·10⁶ mL⁻¹) (Table 1, Fig. 1). In principle, high bacterial counts are expected to be associated with higher levels of choanoflagellate cells. The environmental conditions of the ballast waters may have favoured het-

erotrophic bacterial growth, promoting an active grazing of choanoflagellates.

The latter case would result in a transitorily increased population of choanoflagellates and a decreased number of bacteria. Although these findings are preliminary, casual, and based on only a few samples, two interesting speculations arise from the ballast water results: a) choanoflagellates could be considered to be putative markers in ballast water analyses and b) reproducing some of the ballast water conditions, for instance the absence of light, could improve growth conditions for choanoflagellates.

Our work has two main aims: to show the presence and the ubiquity of these interesting forms of life along the temperate Pacific coast of South America and to stimulate interest in further research on them. Choanoflagellates seem to be crucial in the evolutionary pathway leading from unicellular organisms to metazoans. Recently, these were shown to have key genes for maintaining multicellularity (King & Carrol, 2004). Additionally, the role of choanoflagellates in the microbial food web promises to be an interesting subject for further research.

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