2021

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Recommended Citation

Snyder, Richard A.; Moss, Joseph A.; Santoferrara, Luciana; Head, Marie; and Jeffrey, Wade H., Ciliate microzooplankton from the Northeastern Gulf of Mexico (2021). *ICES Journal of Marine Science*, 78(9), 3356-3371.
doi: 10.1093/icesjms/fsab002

Ciliate microzooplankton from the Northeastern Gulf of Mexico.

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Key Words: Ciliates, Microzooplankton, Gulf of Mexico, Diversity

Abstract

Microzooplankton mediate a critical juncture of autotrophic and heterotrophic microbial production in the water column. Taxonomic and ecological work on this group has been substantial, yet few reports exist for the offshore waters of the Gulf of Mexico. This report focuses on protists in the phylum Ciliophora collected at stations spanning the continental shelf in the northeastern Gulf of Mexico. We hypothesized that patterns of spatial distribution across the region would be west-east along the coast, rather than north-south coastal to offshore, reflecting major freshwater sources. Samples were obtained by 10μm plankton net for microscopy and by filtration of seawater for DNA extraction and ciliate-specific clone sequencing. Microscopy and molecular analysis recovered 46 and 156 taxa respectively. Some visually identified taxa were missing from the sequence analysis and sequences from unknown species dominated molecular results. Differences were apparent with both dominant and rare taxa between February and July sampling and across a trophic gradient from coastal influenced stations to those more representative of the offshore environment. This report provides new data on ciliate microzooplankton richness and distribution in the Gulf of Mexico, and adds to our understanding of microzooplankton diversity in the ocean.

Keywords: Gulf of Mexico, tintinnids, microzooplankton, environmental DNA sequencing

INTRODUCTION

Microzooplankton occupy a unique food web position, assimilating both autotrophic and heterotrophic production, sometimes mixotrophically, and condense microbial loop processes for consumption by meso- and macrozooplankton (Calbet and Landry, 2004; Calbet and Saiz, 2005; Sipura et al., 2003; Steinberg and Landry, 2017). In some regions of the marine environment they are dominant grazers of small autotrophic and heterotrophic eukaryotes. Holoplankton and meroplankton are beneficiaries of their presence and activity, including larvae of many commercially important species (e.g., Laiz-Carrión et al., 2015). Despite this pivotal role in the microbial loop, a significant amount of knowledge concerning the diversity, distribution, and abundance of these organisms is still missing for the Gulf of Mexico (GOM). Among the most abundant organisms in microzooplankton, ciliate protists are represented by several classes, although there are three principal groups: the loricate choreotrichs (tintinnids), the non-loricate choreotrichs, and the oligotrichs. Of these, tintinnids have been surveyed and studied the most, due largely to the stability of their shell, or lorica. General lorica morphology and oral diameter have been considered to be diagnostic of species level differences.

Advances in DNA sequence analysis have resolved phylogenetic positions for many of these planktonic ciliates (Santoferrara et al., 2017), and comparisons of morphological assessment techniques to DNA analysis have shown advantages and limitations to both (Bachy et al., 2013). For tintinnids, lorica polymorphism has led to overestimates of species diversity, and yet for others cryptic genetic diversity has been found for similar morphologies (Dolan, 2016; Santoferrara et al., 2015). A lack of resolvable morphological traits for some groups, such as the oligotrichs, also masks a great deal of the genetic diversity in planktonic ciliates. An additional issue for analysis of sequences obtained by environmental DNA extraction is the lack of annotated sequences in the databases, and despite efforts to link morphological assessment with extracted sequences (Agatha and Strüder-Kypke, 2014) and develop a curated database (Boscaro et al., 2018; del Campo et al., 2018), many sequences found in nature represent unknown phenotypes.

The GOM is a unique body of water with a strong Caribbean Sea influence mixing with a major river delta. The tropical influence dominates the southern and eastern part of the basin through the Yucatan Strait and the Loop Current, while the Mississippi River flow has greater influence to the west (Hu et al.,

2005), influencing the distribution of hypoxic zones (Obenour et al., 2013), petroleum resources, and fisheries, although wind events can push the plume eastward carrying terrestrial organic matter offshore (Da Silva and Castelao, 2018) creating a NW to SE trophic gradient along the continental shelf of the northeastern GOM. Tropical storms are frequent and result in considerable mixing of the water column. In 2010 a massive oil spill covered portions of the northeastern GOM in a gradient from the SW to the NE with polycyclic aromatic hydrocarbons (PAHs) persisting in the sediments for two years (Snyder et al., 2014). During the spill, the Mississippi River was redirected through the delta to help push oil offshore and this discharge in 2010 affected the NE Gulf in a NW to SE gradient. The plankton sampling reported in this paper followed these major perturbations in the region.

The majority of reports on the diversity of marine ciliates from the GOM cover near-shore, shallow water and estuarine environments (Coats and Clamp, 2009). Balech (1967) conducted a survey of the tintinnids and dinoflagellates in the northeastern GOM covering 31 stations in 4 cruises during warm water months (May, June, August, and September), using a 35 μm plankton net in vertical or surface tows. Using morphology of the loricae as diagnostic characteristics, Balech (1967) found 84 species in 38 genera of tintinnids. A previous environmental DNA study of plankton in the western GOM utilized 18S ribosomal ribonucleic acid (rRNA) gene cloning and sequencing (Rocke et al., 2013). However, this investigation was broad in nature due to the universal eukaryotic primers employed and thus hampered by the presence of large quantities of non-ciliate sequences from algae and metazoans recovering relatively few ciliate sequences. Functional analysis of microzooplankton in the western GOM has documented significant consumption of phytoplankton production (Fahnenstiel et al., 1995; Strom and Strom, 1996; First et al., 2009) and consumption of microzooplankton by mesozooplankton (Liu et al., 2005).

The 2010 oil spill in the northern GOM had widespread impacts, including dramatic shifts to autotrophic production and decrease in ciliate microzooplankton in the western gulf (Parsons et al., 2015) and autotropic to heterotrophic production in the offshore plankton of the northeast GOM from the degradation and assimilation of oil carbon by bacteria within the microbial loop (Grahman et al., 2010). Microzooplankton would have integrated that shift. Some reports have suggested little effect of the toxic PAH fraction of petroleum on ciliates (Lacaze, 1993; Batten et al., 1998; Dahl et al., 1983; Ozhan et al., 2014), although others have shown oil and dispersant exposure may be lethal to ciliates (Lara et al., 2007) and other microzooplankton (Almedia et al., 2014), and that open oceanic plankton may be more sensitive to these pollutants than plankton closer to the coasts (Hjorth and Nieson, 2011).

The sampling associated with this report was conducted on a series of research cruises over the northeastern GOM continental shelf in the years following the oil spill to document oil residues (Snyder et al., 2014), physical oceanography (Hamilton et al., 2015), and microbial ecology of the plankton and benthos (Moss et al., 2015; Moss et al., 2016; Nienow et al., 2016). Any persistent impacts of the disturbance on the microzooplankton of the region were unknown at the time, and little baseline data was available (Murawski et al., 2016). This investigation aimed to assess the diversity of the phylum Ciliophora in the microzooplankton 2 years after the spill. As an operational hypothesis, we presumed that the distribution of taxa from the continental shelf and slope of the northeast GOM (NE GOM) would be uniform despite an environmental gradient from coastal to offshore waters, and that investigation of this poorly explored region would add significantly to known ciliate genetic diversity. Significant novel diversity of ciliate fauna was found, adding new records for microzooplankton occurring in the GOM and the global oceans.

MATERIALS AND METHODS

Sampling and microcopy analyses

Samples for microscopy and molecular analysis were collected during cruises in February and July of 2012 (Figure 1; Table 1). Hydrographic parameters and water samples for molecular analysis were collected via a Sea-Bird CTD profiler equipped with a Niskin bottle rosette. On the down cast, water column structure was observed in real time on a shipboard computer, and 20 L Niskin bottles were electronically triggered on the upcast to capture samples at key points rather than preordained fixed depths: deep water column, midwater chlorophyll maximum (if present), and surface waters. At shallower stations that were well mixed, single samples were acquired. Ten liters from each depth were filtered onto 0.22µm Sterivex® filters using a multichannel peristaltic pump. Duplicate filters were obtained from each sample location, if possible, to provide a backup in case of sample processing difficulties and to provide archived samples. Filters were frozen at -20°C onboard ship. Between collections, bottles and tubing rigs were rinsed with 10% HCL, 95% ethanol, and 18 ohm purified water. Upon arrival to lab, all samples were kept at -80°C until processing or for archival purposes.

For microscopy, net microzooplankton samples targeting tintinnids were collected on the July cruise via oblique vertical tows through the photic zone with a WILCO 1-m long, 10-µm pore size mesh net with a stainless steel 2-mm mesh strainer fitted over the net mouth to form a dome excluding gelatinous zooplankton, *Sargassum*, and various other large organisms and particulate matter. Concentrated samples were added to 20-ml vials containing 1 ml of Lugol's fixative for a final 1:20 ratio. In the lab, samples were pipetted onto glass plates and examined with an Olympus inverted microscope (200-400x magnification). Each sample was processed until a minimum of 50 ciliates were tallied to estimate community structure as the number of taxa and their relative abundance. An ocular micrometer was used to determine lorica dimensions for tintinnids. Lorica size data combined with lorica morphology was used to bin specimens and assign species names. Works used for identifications included Marshal (1969), Bachy et al., (2012), and the images curated on the Aquaparadox website by J.R. Dolan (http://gallery.obs-vlfr.fr/gallery2/v/Aquaparadox/). Representative specimens were imaged by Spot Imaging Solutions 5.1 camera and software to assist with identifications.

Nutrient and chlorophyll concentration analyses

Dissolved and total nutrient concentrations (nitrate-nitrite and orthophosphate) were determined by standard methods. Particulate matter was removed from ambient seawater for dissolved nutrient analysis by filtration with Whatman 0.7-µm GFF filters. Filtrate was collected in 60-ml polypropylene bottles and stored at −80°C until processing. Whole seawater (100 ml) for total nutrient analysis was preserved with 0.4 ml of concentrated sulfuric acid. Nutrient concentrations were determined at the Wetlands Research Laboratory facility (UWF, Pensacola, Fla. [certification no. E71176] with a BRAN+LUEBB Auto-analyzer in accordance with EPA standard operating procedures (300.00, 300.01, 300.03). Chlorophyll samples were collected in 360 ml volumes on Whatman GF/F filters, extracted in 90% acetone and quantified fluorometrically (Welschmeyer 1994).

DNA extraction, amplification, cloning and sequencing

Filters were removed from Sterivex units with sterile hacksaw blades and forceps and inserted into separate 2ml bead-beading tubes (Powersoil; MoBio®). Lysis buffer was added and filters were subjected to 3 freeze-thaw (liquid nitrogen/75°C) cycles. Tubes were agitated using a PowerLyzer homogenizer (MoBio®) using 90 second bursts at setting S3500. DNA extraction was done according to the manufacturer's instructions. DNA concentration was estimated using a Nanodrop® 1000 and stored at -20°C.

For each sample, 763 bp fragments of the 18S rRNA gene were amplified using the ciliate-specific primer set 384F (5' YTB GAT GGT AGT GTA TTG GA 3') and 1147R (5' GAA CGA AAG WTA RGG

GAT CA 3') (Dopheide et al., 2008). PCR was performed in quadruplicate for each sample (2 to 5ng template DNA/reaction) with final reagent concentrations in 50µl volumes containing: 3.0 mM of MgCl2 (Roche), PCR buffer (FastTaq 10x/Green), 0.50µM of each primer, 0.04 U of FastStart *Taq* DNA polymerase (Roche), and 0.2 mM of PCR Nucleotide MixPlus (Roche). Reactions proceeded for 30 cycles of 94°C for 30 seconds, 60°C for 60 seconds, and 72°C for 1 minute, with a final elongation for 10 min. PCR products were verified via 0.8% agarose gels, excised using sterile scalpels, and purified with a QIAGEN Gel Extraction Kit (QIAGEN, Valencia, CA, U.S.A). Purified DNA was cloned into PCR 2.1 TOPO vectors (Life Technologies; Carlsbad, Calif.) following the manufacturer's protocol and transformed into electrocompetent cells (MegaX DH10B T1; Life Technologies) using a BIO-RAD MicroPulser set to the manufacturer's specifications. Transformed cells were incubated for 1 hour at 37° C and screened via selective growth on LB media (ampicillin 0.1%, kanamycin 0.1%, x-gal 0.08%) for 18-24 hours. For each sample, a library of 96 random clones were grown for 12-14 hours with selective LB broth and shipped in 10% glycerol stocks for Sanger Sequencing (Beckman Coulter Genomics, Danver, MA). Sequences were obtained for a total of 2194 clones. Raw sequence data are available from the GOMRI GRIIDC data servers: https://data.gulfresearchinitiative.org/data/R6.x805.000:0118, doi: 10.7266/WBVVRTXM

Sequence identification and multivariate analyses

Sequence data were trimmed and evaluated for quality using CodonCode Aligner version 4.14 (CodonCode Corporation, Centerville, MA, USA). Operational taxonomic units (OTUs) were defined by MOTHUR using the furthest neighbor algorithm with a conservative distance level of 5% (Schloss et al., 2009; Caron and Hu, 2019), resulting in 154 OTUs (Table S3). A representative sequence for each OTU was deposited in GenBank under accession numbers MT973808-MT973966 (Table S3). OTUs were used for ecological analysis of community structure, with the number of binned sequences assigned to each OTU used as a proxy of relative abundance. Relative abundance values were log transformed to account for exponential distortion of target abundance by PCR. Multivariate analyses of community structure were performed with JMP software v. 8.0.2 (SAS Institute, Inc) using Ward clustering, incorporating OTU relative abundance in each sample. Sorensen's Similarity Coefficient between samples was also calculated based on OTU distributions.

Taxonomic assignment of OTUs was done with BLAST (Camacho et al., 2009) against the EukRef-Ciliophora curated database (Boscaro et al., 2018). Additional microzooplankton sequences were added as references to flag and remove non-ciliate OTUs (5% of clones). All the remaining OTUs were classified at least into ciliate classes with a match higher than 90% in sequence similarity. OTUs were identified to the species level only based on matches with 99.7-100% similarity.

Phylogenetic analyses were done separately for 1) choreotrichs and oligotrichs, and 2) all the remaining ciliates. In each case, OTU sequences were combined with representative sequences from EukRef-Ciliophora and proper outgroups, then aligned with MAFFT v. 7 (Katoh and Standley, 2013). Ambiguous positions were removed with the guidance of Gblocks v. 0.91b under default parameters (Castresana, 2000). Maximum likelihood inferences were done with RAxML v. 8.2.10 (Stamatakis, 2014), with node support values inferred from 1,000 bootstraps and the GTRGAMMA model, as previously identified with MrModeltest v. 2 (Nylander, 2004).

RESULTS

Environmental parameters

An overview of the abiotic parameters of the sample points is given in Table 1. The shelf break occurs at approximately 100 m, so that all stations represent the NE GOM continental shelf, except for C7,

C9, P7, and P9 which were out over the continental slope in DeSoto Canyon. The light attenuation coefficients (Table 1 and Figure 2) illustrate the trophic gradient that exists in the NE GOM, as do the Chlorophyll data (Table 1), with more coastal eutrophic conditions inshore and to the west with proximity to the Mississippi River and Mobile Bay outfalls, and more oligotrophic offshore waters coming inshore over DeSoto Canyon and to the East. This same gradient is found in sediment deposits, with higher sediment nitrogen content inshore and to the west and lower enrichment offshore and to the East (data not shown). This characterization is also supported by the annual mean chlorophyll-a concentrations for the region from remote sensing (https://marinecadastre.gov/nationalviewer/) and variance in the fate of the Mississippi River plume (Da Silva and Castelao, 2018). Pensacola, Choctawhatchee, and St Andrew's Bay watersheds along the Florida Panhandle are small relative to the freshwater inputs to the west from the Mississippi River and Mobile Bay systems, and open ocean waters are found near the coast. The Loop Current extends into the southern and eastern GOM bringing Caribbean waters to the NE GOM via eddies that interact with slope topography, including minor upwelling events along the shelf break that would bring deeper waters with higher nitrogen content up onto the shelf, especially in the western part of the study region (Hamilton et al., 2015). The Spring bloom begins in February in surface waters, and a chlorophyll maximum drops to the continental shelf floor further off the coast and into deeper water off the shelf break during the summer (July Cruise) (Table 1), presumably after depletion of nutrients in surface waters. Chlorophyll concentrations were higher in February (0.9 – 3 $\mu g\ L^{-1}$) with the start of the spring bloom than in the July samples (0.7- 1.8 $\mu g\ L^{-1}$).

Salinities were fairly consistent spatially and temporally and assumed to have no effect on ciliate distributions. A low of 34.7 PSU was recorded at Station A9 on the surface and a high of 36.3 PSU recorded at several locations (P5, C5, A9) 40-90m deep in the water column. Temperature changes may have had some effect. Surface waters ranged from 19º C in February (station C5) to 28.55º C in July at station A9. Temperature at mid depths had a smaller range from 18º (C5 February) to 20-25º C (C5, C7 July). Temperature for the deep-water slope samples (P9, C9) ranged from 9-13º C. Dissolved oxygen showed low variance, with the lowest values recorded in deep stations where hypoxia limits at 2-3 $mg\ L^{-1}$ were approached. Nutrient levels (nitrate and orthophosphate) were highest in the deep-water samples and typically depleted in the mid and surface water samples.

Identifications and distribution patterns by microscopy

In this study, 39 tintinnid species were recorded by microscopy (Table 2; Table S1). Almost half of those species are shared with the previous survey by Balech (1967) in NE GOM with 19 species in common from all the Balech samples and 15 species in common with his June samples (Table 3; Table S2). Remarkably, a similar overall species richness was found by Balech (1967) on his July Cruise (42) and this study (39). Over 4 cruises, Balech (1967) recorded 68 species in our study area, reflecting the need for greater sampling effort to accomplish comprehensive surveys. The spatial distribution of species richness shows similar patterns between the previous and current data (Table 3; Table S2), likely reflecting persistent physical processes and water quality patterns in the DeSoto Canyon area. Unresolved size classes and shapes of *Eutintinus* were recorded in this study (Table 2; Table S2), and 10 species were recorded by Balech (1967), but mostly in samples collected on cruises other than his July ones (Table S2). A lorica matching the distinctive morphology of *E. apertus* was found by both Balech (1967) and this study (Table 2), although in the present case it was never found in association with *Chaetoceros* diatoms (Gómez, 2007). Samples in this study had more stations with records of *Amphoroides* spp., *Dadiella ganymedes*, *Eutintinnus stramentius*, and *Steenstrupiella* spp. than Balech's July samples, showing wider distribution of these species in the region than reported before (Table S2).

Aloricate ciliates remained mostly unidentified given the difficulty of their taxonomic identification based on unstained morphology. Plankton nets, even at 10μm, would inadequately capture these forms relative to fixed whole water samples, and loss on fixation is known (Snyder and Ohman, 1991) for many ciliates regardless the sampling method. An interesting finding was the detection of some taxa that were more typical of benthic communities rather than open waters, with several morphological types of holotrich ciliates observed. While rafting of benthic coastal or estuarine species to the offshore environment may be occurring (e.g., anecdotal observations of freshwater *Hydrilla* floating offshore), some forms were encountered frequently enough to suggest active growth in situ. Sessile species such as *Zoothamnium* sp. were likely associated with the drift weed *Sargassum* or clumps of *Trichodesmium* (Sheridan et al., 2002).

Multivariate analysis of community structure based on relative frequency of occurrence of morphologically identified taxa (Table S1; Figure 3) shows clustering based on the west-east and coastal to offshore trophic gradient (Figure 2), with NW coastal stations (P1, P2, P3, and C1) forming a subgroup, offshore P7, P9 stations grouping with C and A stations, outer C9 and mid A5 grouping together, and the furthest offshore and east (A9) as an outlier. These differences are driven not only by unique species distributions, but also changes in the relative abundance of species (Figure 4). Coastal water stations had fewer species and lower evenness than the offshore stations (Figure 4).

Dadyiella ganymedes emerged as a dominant member of the microzooplankton in the microscopic analysis, observed in every sample. It dominated coastal samples but was also the most abundant species recorded for offshore stations (Figure 4). This ciliate is known to occur in warm waters ranging from coastal to offshore regions (Pierce and Turner, 1993; Dolan and Pierce 2012; Santoferrara et al, 2018), and its distribution among our July samples would fit that characterization. The presence of *Helicostomella* as the second most abundant tintinnid in coastal waters and its absence from offshore stations also fit the characterization of that genus as a neritic organism (Pierce and Turner, 1993; Dolan and Pierce, 2012; Santoferrara et al., 2018). The same authors reported *Acanthostomella* as an offshore genus, and it was the second most abundant observed in offshore samples in this study, but also the fourth most abundant in the coastal samples (Figure 4). *Steenstupiella* has been reported for coastal and offshore waters, but more prevalent offshore (Santoferrara et al., 2018), while the reverse of that trend was found in this study. *Salpingella* was more common in our offshore samples than coastal ones, but was also found across the region. Two size classes of *Amphorides* were found, with a short form more common to offshore waters and a larger form more abundant in coastal waters. Two size classes of a *Eutintinnus* sp. were also found. It is unknown if these were polymorphic responses to food availability or real species differences.

Identifications by DNA Sequences

Clone libraries from 25 samples were processed. The ciliate specific PCR primer set used in this study (Dopheide et al., 2008), and previously by us (Moss et al., 2015), demonstrated repeated high target-specificity with roughly 95% of all cloned sequences in this study and 98% of cloned sequences in a previous report (Moss et al., 2015) corresponding to ciliate protists. The remainder corresponded to GenBank sequences of non-target organisms, mostly radiolarians and acanthareans (95% similarity), and those sequences were removed from further analysis. From 2189 remaining sequences, 156 OTUs were obtained (Table S3). Of these OTUs, 87 could be identified to family, 27 to genus, and only 13 to species (Table 4), reflecting the scarcity of morphologically-identified sequences in the available reference databases (Boscaro et al., 2018). Additional limitations of this molecular approach include uncertainties related to how comparable OTUs and species are (Caron and Hu, 2019), but OTUs have proven useful for exploration of taxonomically-resolved patterns of diversity and distribution.

The top groups of ciliates in terms of OTU richness and relative abundances were Oligotrichia, Choreotrichida, Nassaophorea, Oligohymenophorea, and Tintinnida (Table 4). EukRef (now integrated in

PR^2; Guillou et al., 2013; del Campo et al., 2018) was useful in assigning identities to sequences that remain unidentified in GenBank. Nassophorean and oligohymenophorean sequences were binned to 14 and 17 OTUs respectively (Table 4). None of these OTUs could be assigned to a family level (Table 4) despite being numerically dominant in our clone libraries. Many of these nassophorean and oligohymenophorean sequences were found to correspond to the clades OLIGO5 (Oligohymenophorea) and NASSO1 (Nassophorea), represented only by environmental sequences in the EukRef database (Boscaro et al., 2018).

Phylogenetic analysis provides additional resolution of the genetic diversity in the microzooplankton of the NE GOM (Figures 5 and 6). The dominance of oligotrichs is not surprising, but the lack of known species and deep branches of unknown clades within this genetic diversity is striking (Figure 5), especially in light of the inability of standard microscopy to resolve more than a few morphological types in unstained fixed samples. Although OTUs from this study are fairly evenly distributed across the known genetic diversity of oligotrichs, few of our OTUs align with morphologically described species (3 out of 52; Table 4), and in most cases there are few close relatives even at the genus level (Figure 5). Choreotrich OTUs were less than half the number of oligotrich OTUs. Although not as dramatic as the unknown oligotrich sequence diversity, very few morphological types could be discerned and few of the recovered sequences matched to genera or species (12 and 1, respectively, out of 25 OTUs; Table 4; Figure 5). Tintinnid sequences by number were about 12% of the oligotrich sequences, belying their usual dominance in microscopic evaluation of microzooplankton communities such as the analysis contained herein.

The molecular detection of tintinnid genera and their distribution was markedly different from our microscopy analysis (Figures 6 and 7). Part of this might have been explained by sampling effort, with random clones from each sample being dominated by oligotrichs and choreotrichs. Only 5 of the detected genera were represented in the microscopy analysis, and the most abundant sequences belonged to the genus *Stenosemella*, a genus not found by microscopy. *Stensomella pacifica* (Agatha and Tsai, 2008) was not observed microscopically, but we detected 5 OTUs that match this species. None of the most abundant tintinnids sequences found in coastal samples by microscopy were matched to named taxa in the sequence databases.

Other planktonic ciliates included members of a phylogenetically unresolved Plagiopylea + Prostomatea group (45 sequences, 12 OTUs; Figure 6). Plagiopylea + Protomatea OTUs were related to *Askenasia* (OTUs 114, 127, 132, 141, 147) and *Cyclotrichium* (108, 115, 154. Two OTUs were assigned to the Litostomatea (119 and 128; Figure 6), a group that includes typical predators of other protists, especially ciliates.

Ciliates not normally considered part of the microzooplankton but represented in our sequences included typically thigomotactic forms that may have been associated with the drift weed *Sargassum* or clumps of the cyanobacterium *Trichodesmium* (Sheridan et al., 2002), or other particulate matter in the water column. This particularly refers to the Plagiopylea + Protomatea OTUs related to *Urotrichia* (130, 155), Phyllopharygea OTUs, an OTU sister to *Licnophora macfarlandi,* an OTU related to Stichotrichia, and an OTU sister to *Protocruzia granulosa* (Table 4, Figure 6). The Phyllopharyngea did not appear to contain any known suctorian sequences that might have been associated with *Sargassum* or other surfaces. One OTU was related to *Isochona* sp., a chonotrich, typically commensal on small crustaceans such as the crabs or shrimps found in *Sargassum* or mysids and krill in the plankton (Lynn, 2016). *Licnophora* is also a known commensal on invertebrates (Lynn and Strüder-Kypke, 2002). Stichotrichs, while known to inhabit the water column when their prey is at high densities, are typically associated with surfaces such as the ones available in the GOM water column: marine snow (Alldredge and Silver, 1988), fecal pellets, the pelagic drift algae *Sargassum*, and the cyanobacteria *Trichdesmium*. Small ciliate bacterivores have been found associated with marine snow and fecal pellets (Kiørboe et al., 2003; Saba and Steinberg, 2012; Turner, 1979). The eukaryotic microbial flora of *Sargassum* has not been as closely examined for protists as it has

for bacteria (Torralba et al., 2017) and macrofauna (Huffard et al., 2014), so we cannot confirm an association with the ciliate sequences found in our water column samples. Ciliates in the genus *Euplotes* have been recorded from clumps of *Trichodesmium* in oceanic waters (Sheridan et al., 2002), though no hypotrich sequences were recovered in this investigation.

Distribution patterns by DNA sequences

OTU richness by sample and presence/absence pairwise comparisons (Tables S4 and S5) revealed a high degree of heterogeneity in OTU distributions, resulting in low similarity values between samples. For February, the number of OTUs per station ranged from 13 to 33 with a mean of 24 (Table S4), and similarity values between stations ranged from 4 to 50% with a mean of 27.4% and standard deviation of 0.13% (Table S4). The lowest similarity values (17%) were found between the deep-water samples and other stations. In July, the number of OTUs per station increased, ranging from 17 to 42 with a mean of 29 (Table S5). The similarity values between samples were lower and less variable than in February, ranging from 7 to 38% with a mean of 18.9% and a standard deviation of 0.07% (Table S5). The overall low similarity values in both cruise samples indicates a high degree of heterogeneity in the distribution of taxa across the region and the environmental gradient. As with February samples, the lowest similarity values for July were associated with comparisons to deep-water samples. In July samples, the highest similarity was 38% between two midshelf samples (C5 and A5) at a distance greater than between other stations with lower similarity. With the deep-water samples removed, the similarity values for surface and mid waters increased for February and decreased for July, but the variation remained the same, with 30.6 ± 0.13% for February and 17.8 ± 0.07% for July.

OTU richness was higher in July (115) than in February (86), with 70 OTUs unique to July and 41 OTUs unique to February, and 50 shared OTUs between seasons (Table 5). Overall OTU richness decreased slightly in surface and mid waters in July relative to February. Richness in deep waters was stable regardless of season and characterized by high richness but low relative abundance of individual OTUs. Using the relative abundance of OTUs as a proxy for community structure, cluster analysis identified a seasonal association of February samples but no consistent spatial distributions (Figure 8). The overall dissimilarity between samples was thus greater than the influence of the coastal to offshore gradient for July.

The general patterns do hide some location-specific detail. Tintinnids had perhaps the strongest seasonal response overall, and the most dramatic shift in both numbers of OTUs and sequence abundance in July was in coastal waters (Table 5; Figure 9). Non-loricate choreotrichs were more prevalent in July than February. Within Strombidiidae, OTU richness was uniformly distributed but the number of sequences was higher in February than July (Table 5), especially for surface water samples (Figure 9), perhaps reflecting population depression under grazing pressure or the loss of planktonic prey as the chlorophyll maximum dropped into midwater during July. Tontoniidae sequence numbers were also greater in February, but increased in surface waters in July relative to February (Table Figure 9), perhaps functionally replacing other Strombibiidae with mixotrophy (Stoecker et al., 2017) or by better avoiding predators.

The highest number of Apostome taxa per sample was found in July surface waters (Figure 9). Apostomes were also more abundant in coastal water samples in July than February (Figure 9). The Apostome clones were related to known parasites ciliates of planktonic krill and copepods in the genera *Collinia* (Capriulo and Small, 1986; Gómez-Gutiérrerrez et al., 2006), *Pseudocollinia* (Lynn et al., 2014), and *Vampyrophyra* (Hartley-Grimes and Bradbury, 2007). Significant presence of DNA from parasitic forms has also been reported for sediment surface samples from the deep Atlantic (Scheckenbach et al., 2010), and from the sediment surface in our NE GOM study region (Moss et al., 2015), and likely reflects recovery of encysted as well as active forms.

The uncharacterized clade NASSO1 doubled in overall sequence abundance in July samples, with the greatest change in OTU numbers occurring in July coastal waters, and the greatest increase in sequence abundance occurring in July deep waters (Table 5; Figure 9). The clade OLIGO5, which represents an uncharacterized ciliate lineage basal to scuticociliates (Figure 6), had an increase in all July sample types except surface waters for both number of OTUs and sequence abundance, with the greatest increase occurring in the deep-water samples (Table 5; Figure 9). Whether these organisms are small bacterivores, histophagous taxa in the Scuticociliatia responding to increased water column organic matter, or parasitic forms responding to an increase in water column meso- and macrofaunal, is unknown.

DISCUSSION

Identity and distribution patterns of ciliates in the NE GOM

In a 1954 review, Victor Sprague commented that " …only a very few studies on Protozoa of the GOM have been conducted" and added that "the Protozoa of the GOM, both free living and parasitic, constitute one of the great American frontiers in protozoology." Balech (1967) noted that for tintinnids, "…very little is known concerning their occurrences and distribution in the Gulf of Mexico". The situation has not much changed since those reports. A later review of Ciliophorea in the GOM by Coats and Clamp (2009) listed the species reported in the literature from published studies almost entirely representing coastal waters and estuaries and presenting a relatively depauperate representation relative to similar habitats in other regions. Moss et al., (2015) reported sequence data showing diverse benthic ciliate assemblages at the sediment water interface of the continental slope for the northeastern GOM. In offshore GOM waters, documentation of Ciliophora has been even more rare. The examination of plankton captured by 10 μm net tows in our report provided a link to the only known past comprehensive study of offshore microzooplankton in the northeastern GOM (Balech, 1967), to which we have added a survey of rRNA gene sequences. The lack of reports on the diversity and distribution of microzooplankton taxa in the GOM parallels a general dearth of scientific investigation for all aspects of the eastern Gulf, a deficit made clear with the 2010 BP oil spill and the need to compile data to assess environmental impacts. Although referring to mesozooplankton, the statement by Murawski et al., (2016) applies even more so to microzooplankton: "Despite the significance of plankton in contributing to the stability of marine food webs, there is surprisingly little pre-Deepwater Horizon baseline information on the seasonal and interannual variability in the plankton species composition and plankton dynamics in the northeastern GOM with which to evaluate the impacts of the oil spill."

Similar tintinnid species richness was recovered by microscopy in this investigation as found by Balech from his June samples (1967) although many of his records for individual species were not as widespread between stations as found in this study. While some of the differences may be accounted for by real differences in species assemblages, the variation observed over time in long range databases suggests caution in weighting this too heavily (Dolan, 2017). Some differences may be due to identification errors, and differences in the physical oceanography between these collections can only be speculated. However, the spatial distribution of tintinnid species richness found by us with microscopy shows similar patterns with Balech's results (Table 3), likely reflecting persistent physical processes and water quality patterns in the DeSoto Canyon area. Balech (1967) did not find a strong seasonal change in species distributions across the wider GOM, whereas significant seasonal differences were found by us in both microscopic and sequence analysis.

In addition to seasonal change, our microscopic results suggest spatial distribution differences relative to the environmental gradient described in the results. However, heterogeneity in our sequence data between stations was too great to define any spatial distributions based on this NW to SE environmental gradient. The lack of concordance between the net samples analyzed by microscopy and the sequence data

for tintinnids may be explained by the dominance of non-tintinnid ciliate DNA outcompeting tintinnid sequences effectively reducing their detection, and tintinnids dominating the net samples due to the poor capture and fixation of aloricate forms.

Tintinnids are one of the best annotated groups in the sequence databases, thanks to efforts such as Bachy et al., (2012), Agatha and Strüder-Kypke (2007), Santoferrara et al., (2013) and others. In our investigation, 17 of 18 OTUs could be assigned to a family, and half of our 18 OTUs could be identified to species (Table 4). *Stensomella pacifica* (Agatha and Tsai, 2008) matched to at least 5 OTUs from clones and was not observed microscopically (Table 3), although shared genetic similarity between *Codonellopsis* and *Stenosomella* (Yi et al., 2009) may account for the mismatch, especially keeping in mind that our OTU were clustered at 95% similarity, which may limit species and even genus matching (Santoferrara et al., 2016). None of the tintinnid sequences found in coastal samples were matched to named taxa in the sequence databases. No *Eutintinnus* were recovered from the cloning and sequencing, despite being prominent in the microscopic analysis. Polymorphism in lorica morphology may account for some of the variation observed microscopically, although *Eutintinnus* spp. loricae may be less variable than others (Dolan et al, 2014). Bachy et al., (2012) also found more Eutintinnidae and Cyttrocylidae species by microscopy over both cloning and pyrosequencing methods. Although dominant and found in every sample by microscopic analysis, *Dadiella ganymedes* was only recovered in one sample from molecular work but at relatively high relative abundance.

The limited depth of information provided by clones instead of high throughput (HT) sequencing may have contributed to low resolution of spatial patterns. However, a study of all eukaryotic microbes in the southern Caribbean found that cloning and sequencing gave similar results to HT sequencing and post processing fragments for higher level taxa (Edgecomb et al., 2011). We opted for cloning and sequencing to access long reads with the potential to achieve greater taxonomic resolution at lower levels, although even entire rRNA gene reads may have limited ability to resolve species level differences in some cases. The approach to bin at a 95% similarity was taken to conservatively group related taxa for their distribution and abundance patterns.

The deeper water column samples (260-450 m) would have contained lower abundances and therefore greater dispersion of ciliates. A greater sample volume might have recovered more species, but sample volume was kept constant to preserve comparability across samples, and 96 clones were processed for each sample. Even with lower population densities, we suggest that the analysis adequately captured the dominant community structure of all samples. Rarefaction curves would inform the degree of saturation in recovery of taxa within samples but was not within the scope of this work.

Holotrichs recovered by microscopy remain enigmatic but may be aligned with sequences recovered and identified as Phyllopharyngea, Nassophorea, or Oligohymenophorea. While rafting of benthic coastal or estuarine species to the offshore environment may be occurring, some forms were encountered frequently enough to suggest active growth in situ. OLIGO5 (Oligohymenophorea) and NASSO1 (Nassophorea) clades represent significant, widespread, yet unknown groups in the marine microzooplankton. The OLIGO5 clade is sister to a clade containing *Vampyrophyra pelagica* as well as being basal to the Scuticociliates (Figure 7), and though tempting to speculate they are parasitic, the group remains enigmatic.

Despite the apparent ubiquity of microorganisms over large oceanic scales, heterogeneity in the plankton at smaller scales has been well documented (Grattepanche et al., 2016; Santoferrara et al., 2016). The spatial patterns in species distributions seen in the microscopic analysis, if real, were overwhelmed by the variance between samples found by the molecular analysis. The net samples integrated the upper water column by oblique tows (~100 meters) that would have collapsed small scale spatial heterogeneity found in 10 liter samples taken from single 20 L Niskin bottle point samples. Thus, the greater taxonomic depth or resolution in the molecular analysis amplified the impact of spatial heterogeneity on similarities apparent in

Sorenson's coefficients for presence/absence (Tables S4 and S5) and multivariate cluster analysis of relative abundance of OTUs. High abundance of a few OTUs were detected in near coast samples that we interpret as small blooms. However, typical distributions falling into either coastal or offshore categories (Pierce and Turner, 1993; Dolan and Pierce 2012; Grattepanche et al., 2016; Santoferrara et al, 2018) were likely blurred in this study by the complex interactions of trophic gradients and physical oceanography of the region surrounding DeSoto Canyon in the NE GOM, where freshwater *Hydrilla* and surface salinity depressions may be found offshore and Sargassum drift weed may be found on the beaches (*personal observations*).

CONCLUSIONS

Significant widespread perturbations to the plankton that were documented from the BP oil spill in summer of 2010 had apparently dissipated by summer 2012. Similar species richness was found by microscopy as a study 30 years prior, and lack of any other microzooplankton data for the region prevents any more stringent conclusion. By molecular analysis of cloned sequences, a large amount of unknown ciliate diversity was documented for the NE GOM, as well as for marine waters at large. Lack of annotation in sequence databases continues to hamper our ability to fully understand this diversity, but distribution and abundance data for the NE GOM has been added to phenotypically unknown taxa in the databases. While microscopic analysis of tintinnids revealed temporal and some spatial patterning corresponding to known environmental gradients in the region, molecular analysis of extracted DNA from point samples was less conclusive. Clear differences were apparent between February and July samples reinforcing a seasonal change in microzooplankton distribution and abundance, but any spatial patterning was confounded by the high degree of heterogeneity between samples of ciliate microzooplankton sequences, both with depth at the station and between stations as close as 20 nautical miles apart in the open GOM.

ACKNOWLEDGEMENTS

This research was made possible by grants from BP/The Gulf of Mexico Research Initiative (GOMRI) through the Deep-C and C-Image Consortia. The assistance of the crew of the R/V Bellows, Florida Institute of Oceanography, Dr. Jim Nienow of Vladosta State University, students, and volunteers that helped with shipboard operations was invaluable. Thanks to John R Dolan and his wonderful website of tintinnid images (http://gallery.obs-vlfr.fr/gallery2/v/Aquaparadox/) for assistance with identifications. Thanks to PG Ross at VIMS ESL for GIS work. We also acknowledge the efforts of two anonymous reviewers who substantially improved the manuscript.

Data Availability Statement

Raw sequence data are available from the GOMRI GRIIDC data servers: https://data.gulfresearchinitiative.org/data/R6.x805.000:0118, doi: 10.7266/WBVVRTXM. Representative sequences of each designated OTU are available in GenBank under accession numbers MT973808-MT973966. The authors will share any other data associated with this report upon request.

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752 Table 1. Environmental parameters at the points of sample collection.

Station	Sample Date	Station Depth	Sample Depth m	Chl a[1] µg L^{-1}	Chl b[1] µg L^{-1}	Nitrate + Nitrite[1] µg L^{-1}	Ortho P[1] µg L^{-1}	K_d[2] PAR	Samples for Microsc.[3]	Samples for Molec.
C5	27-Feb-12	95	1	2.44	0.095	9.14	16.80	0.088		X
C5	27-Feb-12	95	96	1.11	0.359	10.14	17.56	0.088		X
C9	27-Feb-12	272	1	1.25	0.359	14.85	18.34			X
C9	27-Feb-12	272	263	0.18	0.058	387.90	73.46			X
A5	28-Feb-12	37	1	2.34	0.917	4.14	16.31	0.095		X
A5	28-Feb-12	37	36	2.09	0.634	4.14	16.31	0.095		X
A9	28-Feb-12	89	1	2.73	1.020	10.27	18.71	0.091		X
A9	28-Feb-12	89	75	2.76	1.160	15.11	118.34	0.091		X
P1	29-Feb-12	18	17	1.57	0.509	0.00	16.83	0.130		X
P5	29-Feb-12	48	1	0.93	0.334	0.00	16.16			X
P5	29-Feb-12	48	50	3.08	0.684	5.62	17.08			X
P9	29-Feb-12	478	450	0.08	0.067	486.60	79.79	0.077		X
P1	8-Jul-12	18	17	1.76	3.040		14.06	0.123	X	
P3	8-Jul-12	33	20					0.141	X	
P5	8 July 12	48	1					0.106	X	X
P5	8-Jul-12	48	48	1.01	1.530	6.63	14.86	0.106	X	X
P7	8-Jul-12	152	48					0.085	X	
P9	8-Jul-12	478	48	1.08	1.280		14.33	0.075	X	X
P9	8-Jul-12	478	450	0.48	0.909	90.43	22.68	0.075		X
A3	9-Jul-12	26	20					0.092	X	
A5	9-Jul-12	37	1					0.065	X	X
A5	9-Jul-12	37	36	1.70	0.167	10.48	14.23	0.065	X	X
A9	9-Jul-12	89	1	0.23	4.500		14.82	0.085	X	X
A9	9-Jul-12	89	55	0.87	0.292	6.19	14.39	0.085	X	X
A9	9-Jul-12	89	80	0.13	0.225	26.28	12.05	0.085		X
C1	10-Jul-12	25	15					0.096	X	
C3	10-Jul-12	30	20					0.084	X	
C5	10-Jul-12	95	1					0.081	X	
C5	10-Jul-12	95	40	0.73	0.876		15.03	0.081	X	X
C5	10-Jul-12	95	90	0.43	0.083	25.88	15.64	0.081		X
C7	10-Jul-12	127	50					0.065	X	
C9	10-Jul-12	272	55	1.07	0.075		15.12	0.083	X	X
C9	10-Jul-12	272	260	0.58	0.191	284.50	50.71	0.083		X

753 [1]Laboratory chlorophyll and nutrient analyses.
754 [2]line fit to light meter depth profile data.
755 [3]Oblique tows through chlorophyll maximum layer to surface incorporating multiple depths.

Table 2. Ciliates recorded by microscopy analysis. Dimensions in µm.

Tintinnids	**Oral diameter**	**Length**
Acanthostomella minutissima	18	25
Amphorellopsis acuta	28-30	100-135
Amphoroides quadrilineata (long)	28-32	125-160
Amphoroides quadrilineata (short)	35-40	55-110
Ascampbelliella sp.	30	40
Climacocylis scalaria	50	280
Climacocylis scalaroides	28-30	70-150
Codonella elongate	40	70
Codonellopsis morchella	30-35	75-100
Codonellopsis orthoceras	6	24
Dadayiella acutiformis	20	60-70
Dadayiella ganymedes	22-30	75-120
Dictyocysta lepida	38-40	60-70
Epiplocycloides ralumensis	40	60
Eutintinnus apertus	20	40
Eutintinnus lusus-endae	80	340
Eutintinnus sp. "Pint glass large"	22-25	75-80
Eutintinnus sp. "Pint glass small"	20	55-60
Eutintinnus stramentus	15-20	60-120
Eutintinnus tubulosus	30	110, 150
Helicostomella sp.	20	50-75
Leprotintinnus simplex	35	160
Leprotintinnus sp.	25	110
Metacyclis lucasensis	28	55
Proplecta claparedei	38	80
Proplecta parva	24	50
Protorhabdonella curta	22	45
Rhabdonella branditi	45	125
Salpingacantha curta	10	110
Salpingella acuminata	20	220
Salpingella faurei	10-12	35-85
Salpingella sp.	12-14	85-140
Steenstrupiella intumescens	30	155-200
Steenstrupiella steenstrupiella	20-28	60-100
Stylicaudata platensis	50	190
Tinntinopsis acuminata	18	30
Tintinnopsis kofoidi	24	135
Non-Tintinnid ciliates		
Holotrichia spp.		
Laboea sp.		
Strobilidium acuminatum		
Strobilidium large spp.		
Strobilidium small spp.		
Strombidinopsis sp.		
Strombidium spp.		
Tontonia sp.		
Zoothamnium sp.		

758 Table 3. Tintinnid species richness assessed by microscopy, comparing data from this study and
759 Balech, 1967. Stations for both are plotted in Figure 1.
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This study				Balech 1967				
Station	Lat	Long	# species	Station	Lat	Long	# species (June)	# species (all cruises)
				II	30.11	85.78		7
				VII	30.05	86.20		5
				T27	29.92	86.25		18
A3	29.99	85.87	21	III, 3	29.88	86.03	7	12
A5	29.85	85.97	19	D1	29.82	86.12		1
				D2	29.73	86.23		2
				D3	29.67	86.30	3	3
A9	29.58	86.16	12	D4	29.57	86.42		0
				D5	29.45	86.52		0
				IV, 12	29.35	86.63	13	14
				D11	29.40	86.27		17
C1	30.33	86.66	9	VI	30.12	86.43	8	8
C3	30.16	86.66	13	T1	30.00	86.50		10
C5	30.00	86.66	14	T3	29.85	86.57	11	29
C7	29.83	86.66	18	T5	29.65	86.67		1
C9	29.50	86.66	20	V	29.47	86.77	10	28
P1	30.25	87.25	17					
				T12	30.20	86.92		1
P3	30.08	87.25	10	VIII, 6	30.13	86.92	18	23
				T14	30.00	86.92		1
P5	29.92	87.25	8	T15, 7	29.90	86.92	16	19
P7	29.75	87.25	17	T17	29.78	86.92		29
				T20, 8	29.67	86.92		11
				IX	29.52	86.92		36
P9	29.42	87.25	15	T18	29.52	87.12	25	25
Total Species microscopy			**38**				**42**	**65**
Total molecular OTUs			**18**					

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764 Table 4. Taxonomic assignment of clone sequences and OTUs. Id. = identified.

Order or Class	Total clone sequences	OTUs			
		Total	Id. to family	Id. to genus	Id. to species
Oligotrichia	1183	59	52	3	3
Choreotrichida	417	25	18	12	1
Nassophorea	183	14	-	-	-
Oligohymenophorea	171	17	-	-	-
Tintinnida	138	18	17	12	9
Plagiopylea + Prostomatea	45	12	-	-	-
Phyllopharyngea	36	4	-	-	-
Litostomatea	6	2	-	-	-
Colpodea	3	1	-	-	-
Other Spirotrichea	2	2	-	-	-
other	5	2			
Total	2189	156	87	27	13

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766 Table 5. Comparison of OTUs and sequences distribution by season.

Source	February	July
Stations	7	6
Samples	12	11
Sequences	54%	46%
OTUs	55%	75%
Unique OTUs	41	70
Shared OTUs	50	50
Number of sequences / number of OTUs		
Strombidiidae	486/35	284/35
Tontoniidae	179/7	136/10
Choreotrichida	142/13	164/20
Tintinnida	12/8	83/14
NASSO1	40/3	57/12
OLIGO5	33/5	56/8
Plagiopylaea	22/9	12/6
Apostomatia	20/5	9/4
Phyllopharyngea	20/4	5/2
Peritrichia	1/1	20/2
Litostomatea	1/1	3/1
Colpodea	3/1	0
Protocruzia	1/1	1/1
Olligohymenohorea	1/1	0
Licnophora	1/1	0

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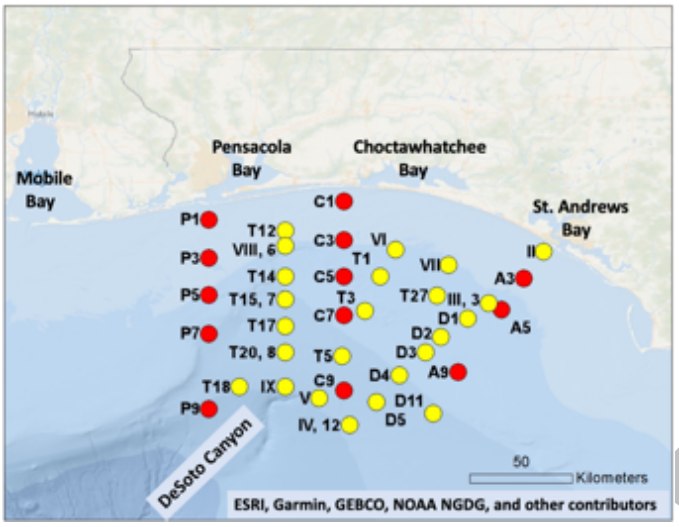

Figure 1. Stations analyzed in this study (red; see also Table 1). Sampling sites of a previous study (Balech 1967) are shown in yellow.

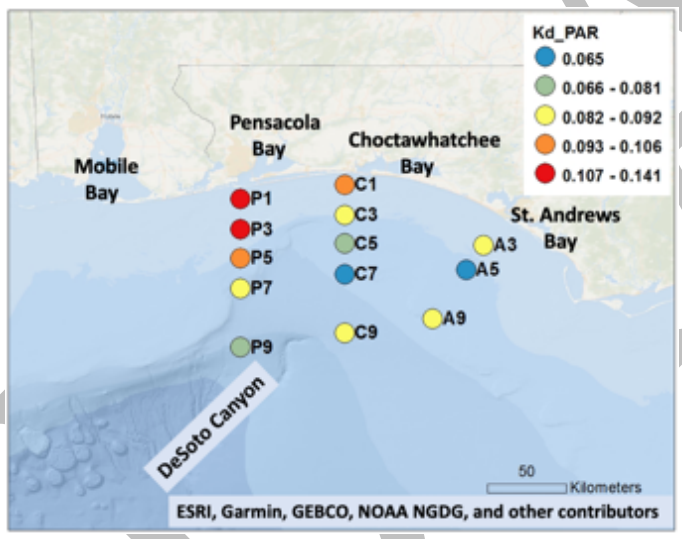

Figure 2. Distribution of photosynthetically active radiation (PAR) attenuation coefficients (K_d values) from July 8-10 (Table 2) as an indicator of water clarity.

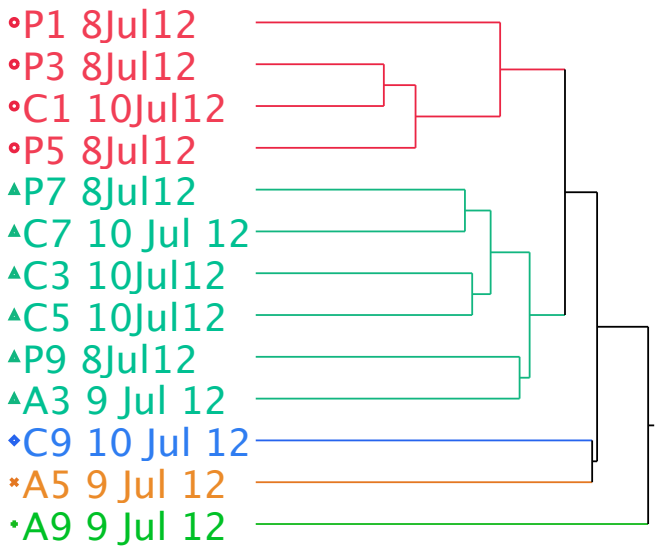

Figure 3. Community structure analysis based on microscopy data. Significant clusters emerge for coastal water stations (red) and stations influenced by both coastal and offshore water masses (blue-green). Offshore stations were more variable (bottom; blue, yellow, green). See the text for a description of the coastal to offshore gradient.

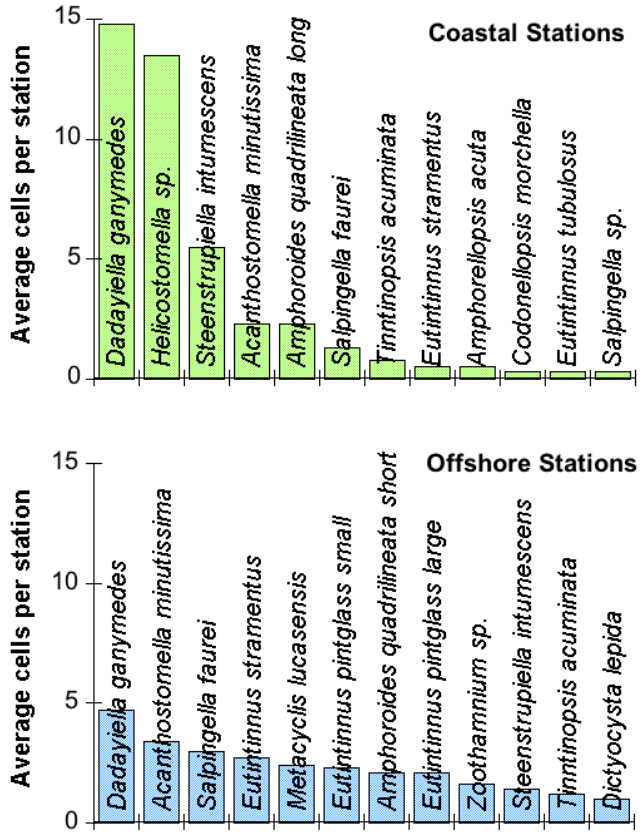

Figure 4. Ranked relative abundance of species identified by microscopy for the coastal water stations (P1, P3, P5, C1) and offshore water stations (P7, P9, C3, C5, C7, C9, A3, A5, A9) as indicated by attenuation coefficients (Figure 2). Coastal water stations were dominated by relatively few species, while offshore water stations had greater evenness in the community structure.

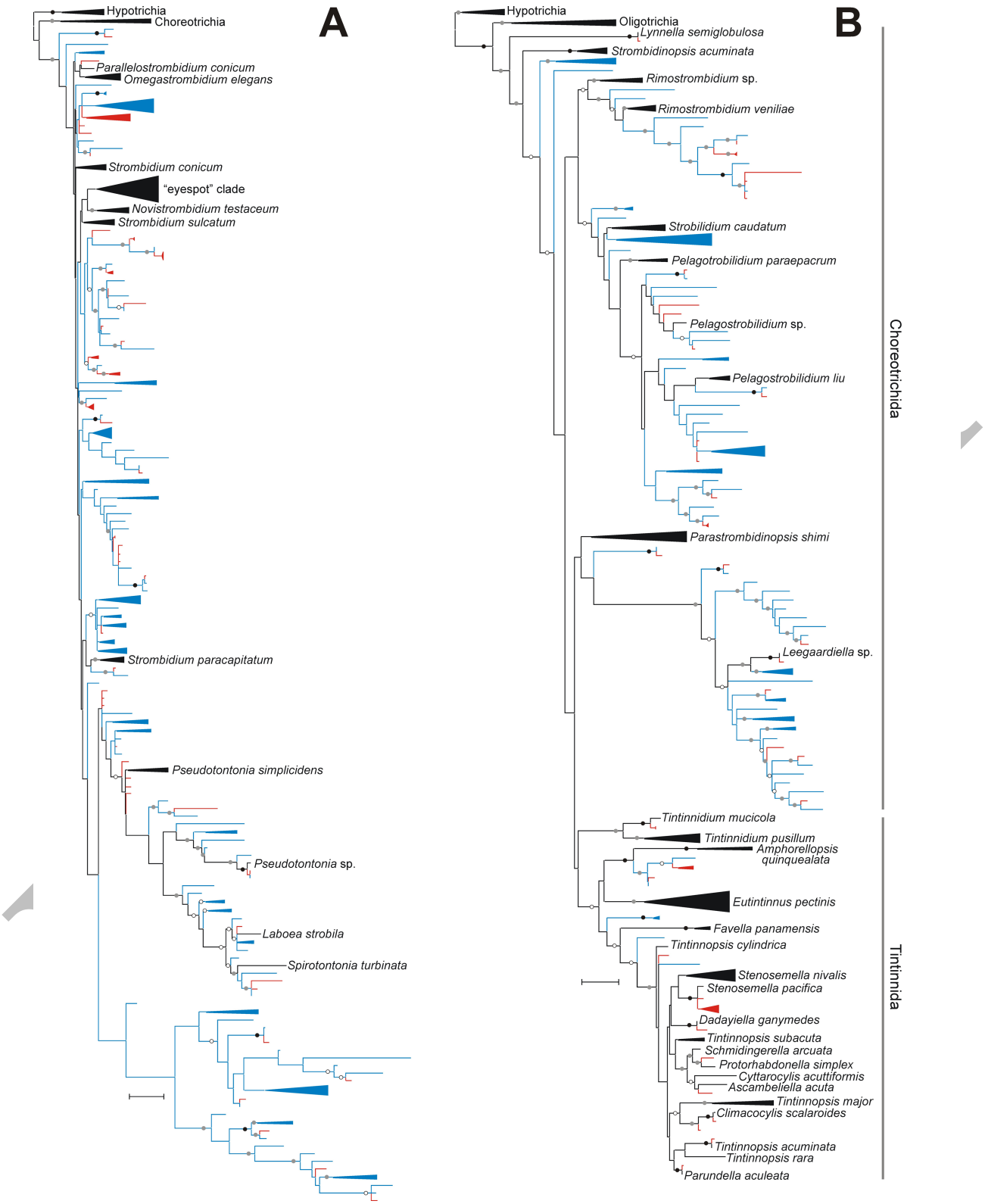

Figure 5. RAxML trees of the subclasses Oligotrichia (A) and Choreotrichia (B). OTUs from this study (red), morphologically-identified sequences (black) and unidentified environmental sequences from GenBank (blue) are shown. Clades are labeled as in Santoferrara et al., (2017).

Clades not including any OTU from this study are collapsed and exemplified by one morphologically-identified sequence, if available. RAxML bootstrap support is shown if 50% or higher (black = 100%; grey = 99-70%; white = 69-50%). The scale bar indicates 5 substitutions per 100 bp. The complete trees with GenBank accession numbers are available from the authors upon request.

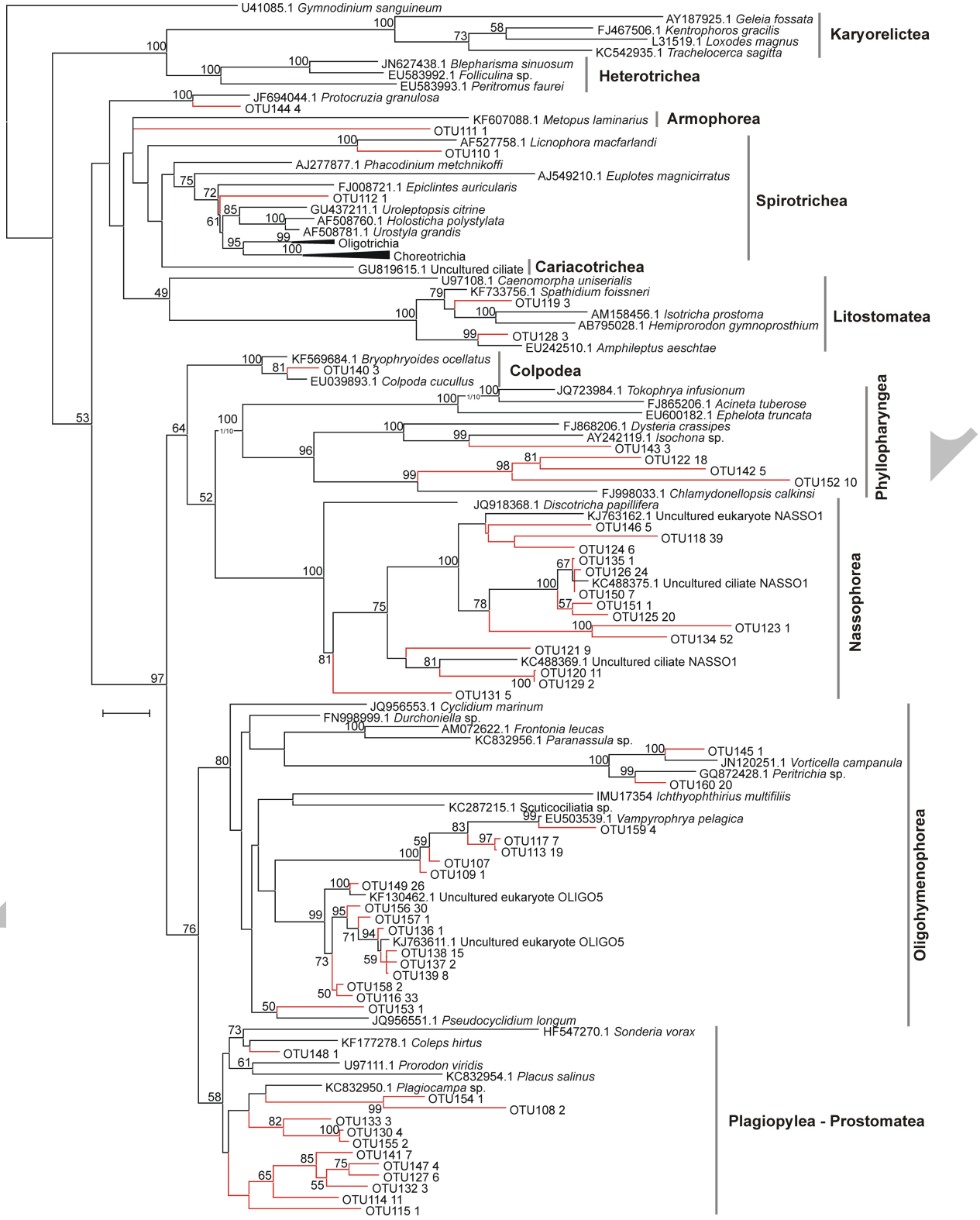

Figure 6. RAxML tree of the phylum Ciliophora (excluding Oligotrichia and Choreotrichia). OTUs from this study are shown in red. Classes are labeled as in Boscaro et al., (2018). RAxML

818 bootstrap support is shown if 50% or higher. The scale bar indicates 5 substitutions per 100 bp.
819 The complete tree is available from the authors upon request.
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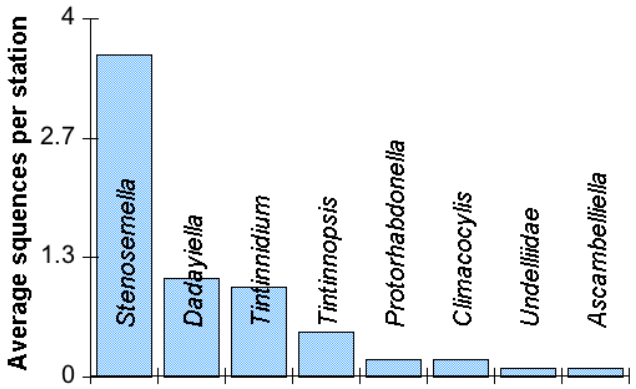

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824 Figure 7. Ranked relative abundance of Tintinnida sequences identified to genus from offshore
825 stations. Three of these were not detected by microscopic identifications: *Stenosemella*,
826 *Tintinnidium*, and Undellidae. The Tintinnida sequences from coastal waters were not matched
827 in the databases.
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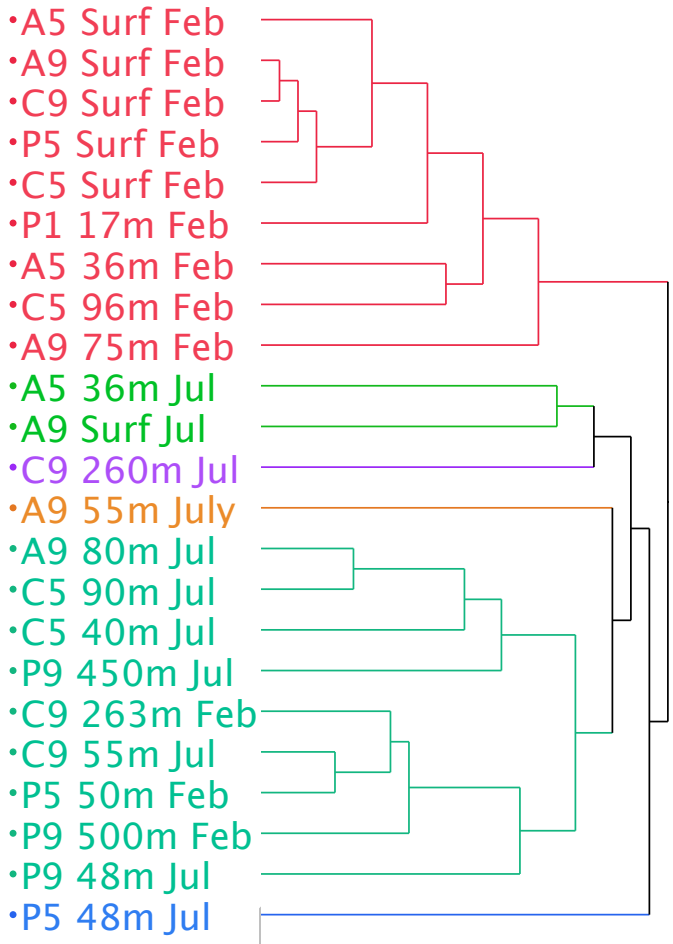

Figure 8. Community structure similarity analysis based on relative abundance of OTUs. Seasonal differences were found for February samples (top; red) and a cluster of mostly July samples (below; blue-green). Overall, July samples were more heterogeneous than February samples.

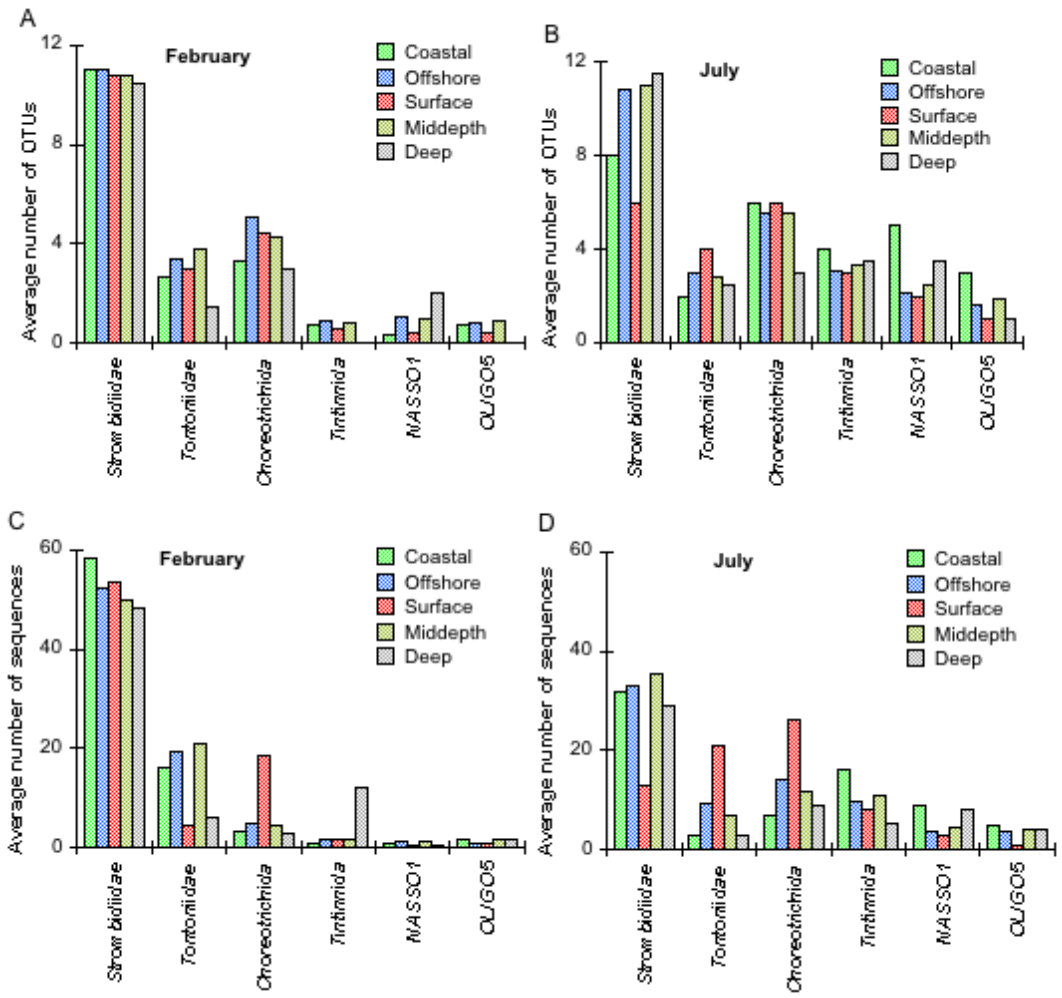

Figure 9. Distribution of dominant ciliates based on number of OTUs (A, B) and sequences (C, D) grouped by distance to shore or depth. Mid-depth samples represent the chlorophyll maximum in the water column.