Available online at www.sciencedirect.com
ScienceDirect
European Journal of Protistology xxx (2017) xxx–xxx
European Journal of PROTISTOLOGY
www.elsevier.com/locate/ejop

Integrating dimensions of biodiversity in choreotrichs and oligotrichs of marine plankton

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Abstract

Choreotrichs and oligotrichs are the main ciliate groups in marine plankton, where they play major roles as trophic intermediaries. We have studied these groups with a variety of approaches to combine the three dimensions of biodiversity—taxonomy, genetics and function. Here we revisit our findings with an integrative perspective, and highlight future directions. In our studies, the correspondence between classical taxonomy (mostly based on morphology) and the increasingly available genetic data (DNA sequences) is examined at the individual, population, species, and assemblage levels. We use a combination of single-cell and environmental sequencing to quantify diversity, track distribution patterns, and explain biogeography processes. Comparatively, we know little about how the morphological and genetic estimates of diversity relate to function, but we expect to better link these aspects by incorporating modern -omics approaches. For example, we have pioneered functional transcriptomic analyses in these groups by contrasting a heterotrophic choreotrich and a mixotrophic oligotrich. These data provide a tremendous resource to start building reference databases needed to measure differential expression of key functional genes, either experimentally or directly in the environment.

Keywords: Ciliates; Environmental sequencing; Single-cell sequencing; Tintinnids; Transcriptomics

Introduction

The subclasses Choreotrichia and Oligotrichia (class Spirotrichea) are among the most abundant and speciose ciliate groups in marine plankton (Lynn 2008). They play important roles in the food web, where they act as a link between plankters of different sizes and trophic statuses, as they ingest a spectrum of prey from bacteria to diatoms, and are themselves ingested by small metazoans (McManus and Santoferrara 2013; Pierce and Turner 1992; Sanders and Wickham 1993). As key components of the heterotrophic and mixotrophic plankton, they participate in a range of ecological and biogeochemical processes, including trophic interactions, energy flux and nutrient remineralization (Caron and Goldman 1988; Worden et al. 2015). The importance of choreotrichs and oligotrichs has been confirmed also by recent molecular surveys, which documented their ubiquity in marine plankton at a global scale (Gimmler et al. 2016; Guidi et al. 2016).

The taxonomy of these groups has a long tradition based on morphology (e.g., Kahl 1932; Kofoid and Campbell 1929), but has been marked by different methods of study. While the loricate choreotrichs (tintinnids) have been classified mostly on the basis of their distinctive external loricae, the aloricate choreotrichs and the oligotrichs can only be distinguished after elaborate staining procedures. Even though the lorica is

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http://dx.doi.org/10.1016/j.ejop.2017.04.004
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easier to study (it is fairly stable and ornamented), the cytology as revealed by staining provides more reliable diagnostic characters. Currently, there is a disproportion in the number of species described (>1000 and <200 for loricates and aloricates, respectively), their true diversity is unknown, and a natural classification is lacking (Agatha 2011). Although the classification of choreotrichs and oligotrichs is still based mostly on morphology, the incorporation of genetics (mainly sequences of the ribosomal RNA gene = rDNA) is slowly complementing taxonomy and evolutionary studies in these groups (Agatha and Strüder-Kypke 2013, 2014; Santoferrara et al. 2017).

Compared to the relationship between morphological and genetic approaches for taxa identification, less is known about how these diversity criteria relate to functional differences in these groups, as it happens with other protists. The degree of correlation between traits currently used for species delimitation (morphology, DNA sequences) and functional differences has been mostly ignored (Caron 2013). An additional layer of complexity appears when functional differences observed experimentally have to be extrapolated to the environment (Weisse et al. 2016).

Because most choreotrichs and oligotrichs are difficult to culture, existing data on physiology and behavior are based on only a minor fraction of the species present in nature (e.g., Montagnes 2013; Stoecker and Michaels 1991). But in some cases, the invaluable information collected for half a century has been challenged by the discovery of classification inconsistencies. For example, some tintinnid species that used to be identified within *Favella* have served as model for functional studies of microzooplankton (Echevarria et al. 2014), but they are now known to belong to two distant families (Agatha and Strüder-Kypke 2012). The temporal and spatial occurrence of these taxa is difficult to disentangle (Ganser and Agatha 2016), and, more importantly, the impact of their phylogenetic (un)relatedness on physio-behavioral traits is unknown. This exemplifies the need for integrated approaches at the intraspecific, interspecific and community levels in order to understand ciliate diversity and ecological roles, an obvious but demanding requisite that entails multidisciplinary and cooperative efforts (e.g., Santoferrara et al. 2016a). Beyond the combination of traditional methods, the incorporation of modern sequencing technologies for microbial -omics in cultured specimens (e.g., Keeling et al. 2014) or directly in the environment (e.g., Louca et al. 2016) is transforming our opportunities to link taxonomy, genetics and function.

We have studied choreotrichs and oligotrichs using multiple approaches in order to combine the different dimensions of biodiversity (Fig. 1). Taking advantage of the dynamic interactions among taxonomic, genetic, and functional facets, our analyses have targeted from individuals to populations, and from cultures to natural assemblages. Here we review our and other recent findings with an integrative perspective and highlight future directions in studying (1) the diversity, (2) the patterns of biogeography and the processes that explain them, and (3) the functional genes and associated ecological roles in these two keystone groups.

Diversity

Choreotrichs and oligotrichs are among the most well-studied ciliate groups, but their diversity is not clear, even at the taxonomic level. They remain unexplored in huge areas of the World Ocean and many of their species need revalidation using modern methods (Agatha 2011); thus, their global species richness is still uncertain. Analysis of all the sequences available in GenBank for these subclasses (both from known morphospecies and environmental surveys) has suggested that many of their aloricate lineages remain partially or completely unknown from the morphological point of view (Santoferrara et al. 2017).

The fact that aloricate diversity is deeply underestimated was confirmed by the discovery of cryptic species (Katz et al. 2005; McManus and Katz 2009). For example, wild populations of a distinctive morphospecies were found to include at least four abundant, genetically-distinct phylotypes, only two of which are linked to named species (McManus et al. 2010). For loricates, although the lorica has been a valuable means for taxonomic and ecological studies for more than two centuries, phenotypic plasticity in some species (Laval-Peuto and Brownlee 1986) and lack of clearly distinct features in others (Alder 1999; Dolan 2016) have led to uncertainties. Using single-cell sequencing, we found general agreement between lorica morphology and rDNA sequences for species delimitation (Santoferrara et al. 2013, 2015). However, cases of crypticity/pseudo-crypticity (identical/almost identical morphologies corresponding to divergent sequences) and polymorphism (a gradient of different morphologies corresponding to identical sequences) leave unresolved whether tintinnid diversity has overall been under- or overestimated (Fig. 2). Unfortunately, genetic data is not perfect either, as we also found cases of low molecular resolution: morphospecies that are identical in the most commonly-sequenced marker (SSU rDNA) sometimes differ in faster-evolving markers (ITS regions and LSU rDNA; Santoferrara et al. 2013, 2015). Thus, “genetic crypticity” also exists if single or unsuitable loci are used.

Given the inconsistencies in defining species morphologically or genetically, we questioned how this would influence the way we perceive the diversity of choreotrichs and oligotrichs in natural assemblages (Santoferrara et al. 2014a, 2016b). Our comparisons have used environmental samples examined by both microscopy and metabarcoding (high-throughput sequencing of a variable SSU rDNA region). Due to the conflicts mentioned above, we concluded that none of these methods provide absolute estimations of species richness at present. Additional issues in metabarcoding include diversity inflation caused by intraspecific variants and sequencing errors, as well as poor quantification power due

Please cite this article in press as: Santoferrara, L.F., McManus, G.B., Integrating dimensions of biodiversity in choreotrichs and oligotrichs of marine plankton. Eur. J. Protistol. (2017), http://dx.doi.org/10.1016/j.ejop.2017.04.004