

ORIGINAL PAPER

Phylogeny and Morphology of New Diplonemids from Japan



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Diplonemids were recently found to be the most species-rich group of marine planktonic protists. Based on phylogenetic analysis of 18S rRNA gene sequences and morphological observations, we report the description of new members of the genus *Rhynchopus* – *R. humris* sp. n. and *R. serpens* sp. n., and the establishment of two new genera – *Lacrimia* gen. n. and *Sulcionema* gen. n., represented by *L. lanifica* sp. n. and *S. specki* sp. n., respectively. In addition, we describe the organism formerly designated as *Diplonema* sp. 2 (ATCC 50224) as *Flectonema neradi* gen. n., sp. n. The newly described diplonemids share a common set of traits. Cells are sac-like but variable in shape and size, highly metabolic, and surrounded by a naked cell membrane, which is supported by a tightly packed corset of microtubules. They carry a single highly reticulated peripheral mitochondrion containing a large amount of mitochondrial DNA, with lamellar cristae. The cytopharyngeal complex and flagellar pocket are contiguous and have separate openings. Two parallel flagella are inserted sub-apically into a pronounced flagellar pocket. *Rhynchopus* species have their flagella concealed in trophic stages and fully developed in swimming stages, while they permanently protrude in all other known diplonemid species.

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Introduction

Diplonemids are colorless heterotrophic, predominantly marine protists, equipped with two flagella. They belong to Euglenozoa and are thus related to ecologically important euglenids and econom-

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ically and medically relevant kinetoplastids (Adl et al. 2012; Maslov et al. 1999; Moreira et al. 2001). For a long time, diplomemids were considered a small and rare group of flagellates with only three genera and less than a dozen species formally described (Massana 2011; Simpson 1997; Vickerman 2000; von der Heyden et al. 2004). However, they emerged from obscurity thanks to molecular analysis of hundreds of planktonic samples collected across the globe by several research expeditions (de Vargas et al. 2015; Lara et al. 2009). The recent Tara Oceans 18S rRNA-based metabarcoding survey revealed remarkable diversity and abundance of marine diplomemids: with over 45,000 operational taxonomic units (OTUs), they qualify as the most species-rich marine planktonic eukaryotes (David and Archibald 2016; Flegontova et al. 2016). Furthermore, diplomemids show cosmopolitan distribution with different lineages being abundant and often dominant in most ocean niches, from shallow littoral sediments (Larsen and Patterson 1990) to deep aphotic pelagic waters (de Vargas et al. 2015; Flegontova et al. 2016; Gawryluk et al. 2016; Lara et al. 2009), hydrothermal vents (López-García et al. 2007), and down to poorly studied abyssopelagic zones (Eloe et al. 2011; Scheckenbach et al. 2010).

According to 18S rRNA-based phylogeny, diplomemids represent a monophyletic group which can be subdivided into four robustly supported lineages: (i) the so-called classic diplomemids, hereafter referred to as Diplonemidae, consisting of the genera *Diplonema* and *Rhynchopus*; (ii) a small planktonic clade containing the genus *Hemistasia*; (iii) a deep-sea pelagic diplomemids (DSPD) clade I and (iv) DSPD clade II (Flegontova et al. 2016). DSPD I diplomemids were recently formally described as Eupelagonemidae (Okamoto et al., submitted; this work). While this phylogeny shows a clear support for diplomemid monophyly, the relationships between these major lineages are unclear. Most of the species richness is apparently confined within the hyperdiverse Eupelagonemidae clade which, together with DSPD II, was until recently known exclusively from environmental sequences, and we still lack any cultured representative (Flegontova et al. 2016; Lara et al. 2009; Lukeš et al. 2015). However, the single-cell approach provided a first glance at morphological diversity and genomic characteristics of members of the Eupelagonemidae, although it was stressed that poor quality of genomic assemblies and possible contamination necessitates establishment of stable cultures (Gawryluk et al. 2016).

Despite their abundance, huge diversity and consequently importance in marine food webs, very little is known about the behavior as well as molecular, morphological and biochemical traits of diplomemids (David and Archibald 2016). Indeed, there are only four species altogether for which both morphological observations and sequence data, albeit limited, is available: two members of the genus *Diplonema* – *D. papillatum* (Maslov et al. 1999; Porter 1973) and *D. ambulator* (Busse and Preisfeld 2002; Montegut-Felkner and Triemer 1996; Triemer and Ott 1990; Triemer 1992), and a single species for each of the genera *Rhynchopus* and *Hemistasia*, namely *Rhynchopus euleeides* (von der Heyden et al. 2004; Roy et al. 2007) and *Hemistasia phaeocysticola* (Elbrächter et al. 1996; Yabuki and Tame 2015), respectively. However, virtually all molecular studies, such as the analysis of mitochondrial RNA editing and *trans*-splicing, were performed solely on *D. papillatum* (Kiethega et al. 2013; Marande et al. 2005; Marande and Burger 2007; Moreira et al. 2016; Vlcek et al. 2011;). Otherwise, a few other species known from early morphological studies, namely *Diplonema breviciliata* (Griessmann 1913), *D. nigricans* (Schuster et al. 1968), *D. metabolicum* (Larsen and Patterson 1990), *Rhynchopus amitus* (Skuja 1948), and *R. coscinodiscivorus* (Schnepf 1994), lack any molecular data and are unavailable in culture. Moreover, several putative species have been classified according to their 18S rRNA gene sequences and are available at the American Type Culture Collection (ATCC) but lack proper morphological description (von der Heyden et al. 2004).

Diplonemids belonging to the genera *Diplonema* and *Rhynchopus*, or Diplonemidae, were only marginally present in the global metabarcoding dataset (Flegontova et al. 2016). Still, the number of putative diplomemid OTUs revealed by environmental molecular barcodes available on public databases greatly exceeds the currently recognized number of species. Despite their newly discovered abundance and diversity, to the best of our knowledge, no new classic diplomemid species have recently been described. Therefore, we attempted to establish axenic cultures by manual picking of diplomemid-like cells from samples collected in surface waters around Japan. Based on phylogenetic analysis of nearly full-size 18S rRNA gene sequences and morphological observations using electron and light microscopy, we report the description of four new diplomemid species and two new genera. In addition to this, we create a novel

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