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ORIGINAL ARTICLE

Molecular phylogenetics of sessile *Dolium sedentarium*, a petalomonad euglenid

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Abstract

The euglenids are a species-rich group of flagellates with varying modes of nutrition that can be found in diverse habitats. Phagotrophic members of this group gave rise to phototrophs and hold the key to understanding the evolution of euglenids as a whole, including the evolution of complex morphological characters like the euglenid pellicle. Yet to understand the evolution of these characters, a comprehensive sampling of molecular data is needed to correlate morphological and molecular data, and to estimate a basic phylogenetic backbone of the group. While the availability of SSU rDNA and, more recently, multigene data from phagotrophic euglenids has improved, several "orphan" taxa remain without any molecular data whatsoever. *Dolium sedentarium* is one such taxon: It is a rarely-observed phagotrophic euglenid that inhabits tropical benthic environments and is one of few known sessile euglenids. Based on morphological characters, it has been thought of as part of the earliest branch of euglenids, the Petalomonadida. We report the first molecular sequencing data for *Dolium* using single-cell transcriptomics, adding another small piece in the puzzle of euglenid evolution. Both SSU rDNA and multigene phylogenies confirm it as a solitary branch within Petalomonadida.

KEYWORDS
euglenozoa, flagellate, phylogenomics, single cell, SSU, transcriptomics

INTRODUCTION

EUGLENIDS are a diverse group of flagellated cells, where the best known taxa are phototrophic (e.g. *Euglena*), but many are phagotrophic (Leander et al., 2017). The latter are pivotal in understanding euglenid evolution, since they gave rise to the phototrophic Euglenophyceae through a secondary endosymbiotic event (Jackson et al., 2018; Turmel et al., 2009). Increased taxon sampling for molecular phylogenetics, particularly for multigene analyses, but also SSU rDNA, have recently furthered our understanding of phagotrophic euglenid evolution (Cavalier-Smith et al., 2016; Lax et al., 2021, 2023; Paerschke et al., 2017; Schoenle et al., 2019). Yet euglenid identification and systematics still heavily rely on morphological characters, such as the number of pellicle strips a cell has, or how many flagella emerge from the flagellar pocket. The pellicle is a synapomorphous character of euglenids, consisting of proteinaceous strips underlying the cell membrane (Cavalier-Smith, 2017; Leander et al., 2017). Only a handful of studies exist that have been able to correlate these morphological characters with molecular phylogenies, thanks to the wider breadth of molecular data sampling (Chan et al., 2013; Lax & Simpson, 2013, 2020; Lee & Simpson, 2014a, 2014b).

Using multigene phylogenetics, one species-rich group, the Petalomonadida, has been identified as the earliest-branching euglenid subgroup (Lax et al., 2021), and as such carry special relevance to our understanding of early euglenid evolution. Petalomonads have less than 10 pellicle strips, rendering them rigid. They have either one or two emergent flagella, the main classical

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distinction between the two main genera of this group are that *Petalomonas* has a single emergent flagellum, whereas *Notosolenus* has two flagella (Leander et al., 2017; Lee & Simpson, 2014a). All other euglenids have 10+ pellicle strips, and some phototrophic and phagotrophic euglenids within Spirocuta have more than 100 strips, rendering them flexible and able to undergo euglenoid metaboly. This transition from "primitive" few-stripped to "complex" many-stripped euglenids has been an intriguing subject of character evolution (Cavalier-Smith, 2017; Esson & Leander, 2006; Leander, Triemer, & Farmer, 2001; Leander, Witek, & Farmer, 2001; Yubuki & Leander, 2012). But despite their key position in the tree, there are relatively few molecular resources from petalomonads with which we might infer their character diversity and evolution.

Petalomonads also constitute a large portion of biomass in many marine and freshwater benthic ecosystems (Arndt et al., 2000), and are thought to be important predators of bacteria (Boenigk & Arndt, 2002; Lee & Patterson, 2002). Interestingly they are also the only euglenids that have a somewhat large number of environmental sequences assigned to them (Kolisko et al., 2020). Considering they appear to be highly abundant in microscopy-based ecology studies, one would expect there to be even more environmental sequences from benthic environments, but their divergent SSU rDNA sequences appear to lead to them being undersampled in environmental molecular data (Busse et al., 2003; Lax & Simpson, 2013; Łukomska-Kowalczyk et al., 2016).

There are over 100 described petalomonad species to date, yet only 13 species from four genera (*Petalomonas*, *Notosolenus*, *Scytomonas*, and *Sphenomonas*) have SSU rDNA sequences available. Three of these genera also have multigene data from transcriptomes (*Scytomonas* does not). Unfortunately, however, defining genus boundaries of petalomonads, particularly between *Notosolenus* and *Petalomonas*, are still highly dependent on probably uninformative morphological characters—such as the number of emergent flagella, which is not a good identifier for at least some petalomonads, with *N. urceolatus* branching among *Petalomonas*, and not with other *Notosolenus* in SSU phylogenies (Lee & Simpson, 2014a). With more taxa of both genera sequenced, it is becoming clear that neither genus should be considered monophyletic and that the taxonomy needs to be reworked (Lee & Simpson, 2014a). To do this, an exhaustive molecular sampling for additional petalomonad taxa is likely needed.

Almost all petalomonads are motile and glide on their anterior flagellum (Lax & Simpson, 2020; Leander et al., 2017). One exception for which we have molecular data is *Scytomonas saepesedens*, which is sessile in one of its life cycle stages (Cavalier-Smith et al., 2016). Another prominent sessile euglenid is *Dolium sedentarium* Larsen & Patterson, 1990, a relatively large euglenid (40–70 × 20–30 μm) with two flagella, one of which is emergent (Larsen & Patterson, 1990). Its pellicle has six prominent ridges extending longitudinally. Based on these morphological observations, it has been considered a petalomonad (Larsen & Patterson, 1991; Lee & Simpson, 2014a), but this has not been tested with molecular data.

To examine the phylogenetic position of this species, we imaged, isolated, and generated a transcriptome from a single cell of *D. sedentarium*, and place it in a previously published 19-gene phylogeny, as well as a comprehensive SSU rDNA phylogeny, which confirms its position among other petalomonads with strong support.

MATERIALS AND METHODS

Sampling, imaging, and isolation

A sediment sample mainly consisting of dead, broken, and finely fragmented *Halymeda* pieces was collected from the Spaanse Water Mangrove in Curaçao in April 2022 (12.070621, −68.860269). Following a coverslip method described previously (Larsen & Patterson, 1990), the sediment was spread out in a container, a kimwipe added, and 20 × 50 mm coverslips placed on top. After 2 days the coverslips were examined and a single *D. sedentarium* cell was discovered and imaged with a Leica DM IL inverted microscope at 630× magnification and a Sony alpha7RIII camera. The cell was isolated using a glass micropipette and deposited in Smart-seq2 lysis buffer (Picelli et al., 2014) and frozen at −20°C. Lysis was carried out by four freeze–thaw cycles, alternating between room temperature and −80°C.

Sequencing and transcriptome assembly

We generated a single-cell transcriptome with the Smart-seq2 protocol (Picelli et al., 2014), but used 24 cycles in the cDNA amplification step. The generated cDNA was used as input for Illumina DNA library prep, and sequenced on an Illumina MiSeq instrument with paired end 2 × 250 bp reads, multiplexed with six unrelated organisms (both done by the Sequencing and Bioinformatics Consortium at the University of British Columbia).

Raw reads were read-corrected with Rcorrector version 1.0.4 (Song & Florea, 2015). Corrected reads were adapter-trimmed using Nextera (5′-CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-3′, 5′-CTGTCTCTTATACACATCTGACGCTGCCGACGA-3′) and Smart-seq2 adapter sequences (TSO: 5′-AAGCAGTGGTATCAACGCAGAGTACATGGG-3′, oligo-dT: 5′-AAGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTTT-3′, ISPCR: 5′-AAGCAGTGGTATCAACGCAGAGT-3′), and quality-trimmed (ILLUMINACLIP: 2:30:10 LEADING:5 SLIDINGWINDOW:5:16 MINLEN:60) with Trimmomatic version 0.39 (Bolger et al., 2014).

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Trimmed paired and unpaired reads were used as input for rnaSPAdes version 3.15.1 (Bushmanova et al., 2018) and assembled using default parameters. Amino acid sequences of coding regions were predicted with TransDecoder version 5.5.0 (Grabherr et al., 2011). General transcriptome metrics and completeness scores were collected with QUAST version 5.0.2 (Mikheenko et al., 2018) and BUSCO version 5.4.3 (Manni et al., 2021) using the eukaryota_odb10 and euglenozoa_odb10 databases. Trimming and assembly metrics were visualized with multiQC (Ewels et al., 2016) and can be found in the DataDryad supplements.

Phylogenetics

SSU rDNA sequences were extracted from the rnaSPAdes assembly using barrnap version 0.9 (https://github.com/tseemann/barrnap/). Sequences were then blasted against NCBI GenBank nr/nt, to determine and discard contaminant or prey SSU sequences. The remaining single euglenid SSU rDNA sequence (2044 bp) was added to an existing SSU alignment (Lax et al., 2023), and phylogenetic analysis confirmed the new sequence branched within petalomonads (not shown). The alignment was modified to remove excess non-petalomonad sequences. A blastn search against GenBank's nr/nt database using the *D. sedentarium* SMS11 SSU rDNAs as query yielded some additional environmental sequences (as of January 2023) that were included. We also added environmental sequences from the metaPR2 database (Vaulot et al., 2022; using the clustered ASVs database), identified by using blastn with petalomonad sequences as queries.

The alignment was realigned using MAFFT E-INS-I version 7.481 (Katoh & Standley, 2013), manually corrected, and then trimmed with trimAI version 1.2rev59 (Capella-Gutiérrez et al., 2009; −gt 0.5 −st 0.001). A Maximum-likelihood tree of the final alignment of 167 taxa with 1327 nucleotides was estimated with RAxML-NG version 1.1.0 (Kozlov et al., 2019) under GTR+GAMMA with 1000 nonparametric bootstrap replicates. Table S1 lists all accession numbers of SSU rDNA sequences used, and Figure S1 shows the same tree as Figure 1A, without any clades collapsed.

The multigene analysis used a previously described 19-gene dataset (Lax et al., 2021). Briefly, coding sequences of the transcriptome were determined with TransDecoder version 5.5.0 (Grabherr et al., 2011), and relevant marker genes extracted with a pipeline described previously (Lax et al., 2023). After screening of single gene trees, single orthologuous sequences from nine genes were retained and added to the original dataset. Table S2 lists all taxa used in the multigene dataset, and their sources.

This 19-gene, 63-taxa dataset was analyzed with IQ-TREE2 version 2.0.7 (Minh et al., 2020) under the LG+C60+F+G site-heterogeneous mixture model and 1000 UltraFast Bootstraps (UFB; Minh et al., 2013). We used the resulting tree as a guide tree to run a posterior mean site frequency analysis (PMSF) under the same model with 500 nonparametric bootstrap replicates in IQ-TREE 2 (Wang et al., 2018).

RESULTS

Morphology

Our single-cell isolate SMS11 of *D. sedentarium* was 45.1 × 19.5 µm in size and had a slender shape with a pointed posterior end, like an amphora (Figure 1B,C; Movie S1). The anterior end was rounded and centrally hollow. The cell had two flagella, visible in the flagellar pocket, one emergent (~1× cell length), and the other only ~5 µm long (Figure 1C). Six prominent ridges ran longitudinally along the cell. The cell was sessile and did not move. The general morphology and cell dimensions are consistent with the original description of *D. sedentarium* Larsen & Patterson, 1990.

Phylogenetics

In our SSU rDNA phylogenetic tree, *D. sedentarium* SMS11 branches within Petalomonadida, which is maximally supported (Figure 1A; Figure S1). Petalomonadida is composed of the genera *Petalomonas*, *Notosolenus*, *Scytomonas*, *Sphenomonas*, and *Dolium*. *Sphenomonas* is the deepest branch (99% BS), followed by a clade that includes *Notosolenus ostium* and *N.* c.f. *mediocanellatus* (88% BS). The following largest group (100% BS) can be separated into two main clades: "Clade 1" (96% BS) contains *P. sphagnophila* and *Notosolenus urceolatus*, 24 several environmental sequences, and *D. sedentarium* SMS11. "Clade 2" (76% BS) includes *P. cantuscygni*, *P. planus*, *P. acorensis*, and *Scytomonas saepesedens*, and several environmental sequences. *D. sedentarium* forms the earliest branch on Clade 1, and is on its singular branch without any environmental sequences.

From our single-cell transcriptome assembly we recovered nine out of 19 genes used in our multigene analysis, representing 34.6% of sites (out of a total 6194 aa; Table S2). *D. sedentarium* SMS11 branches within a maximally supported Petalomonadida in our multigene trees (Figure 2), with the latter being recovered as the deepest branch of euglenids (97% UFB, 93% PMSF). Its immediate sister is *Notosolenus urceolatus*, with 53% (UFB) and 58% (PMSF).

DISCUSSION

Dolium sedentarium is one of only two known sessile phagotrophic euglenids, the other being *Scytomonas saepesedens* (Cavalier-Smith et al., 2016). The sessile form of *S. saepesedens* is a lifestage, alternatively gliding on its

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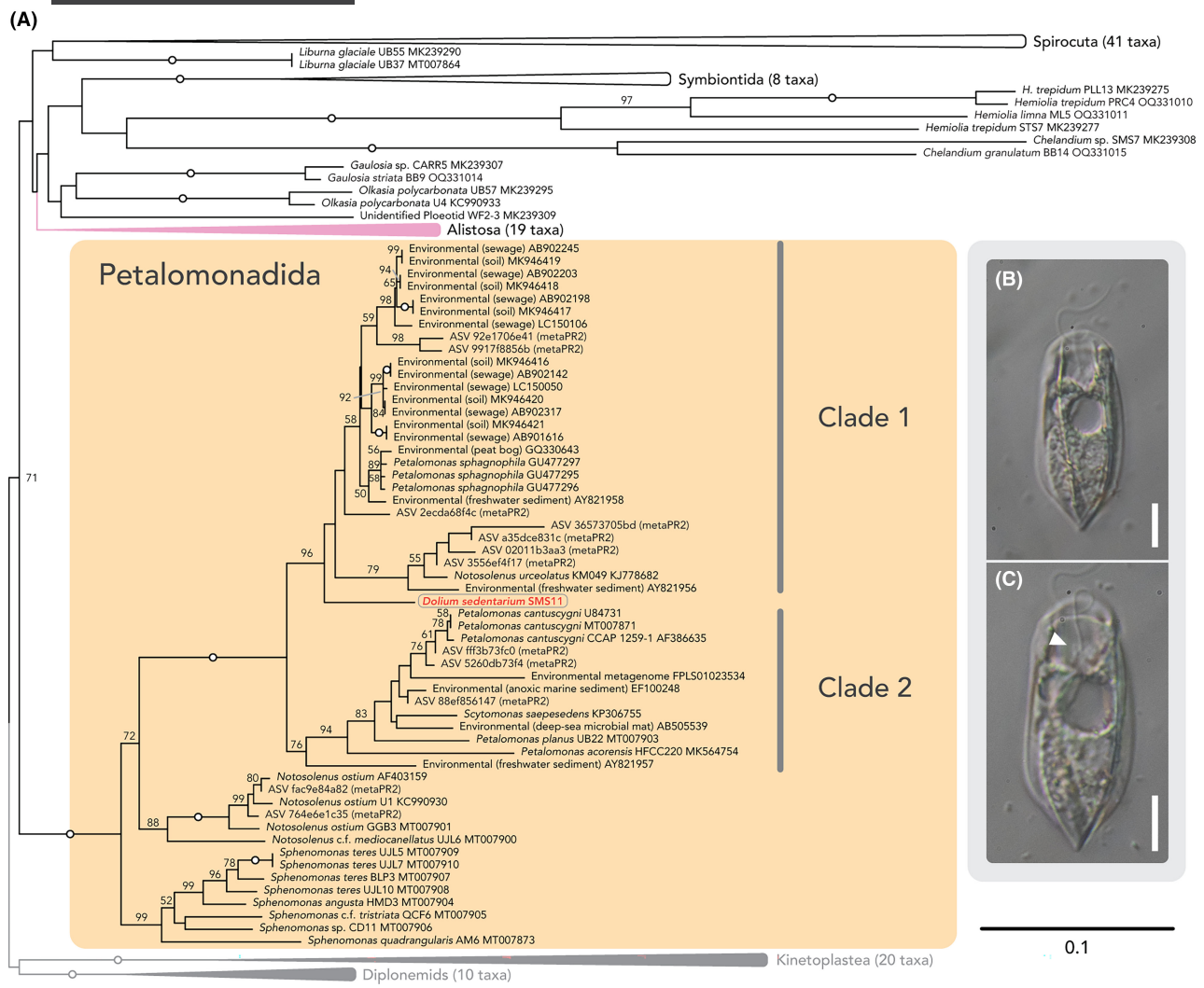

FIGURE 1 SSU rDNA phylogeny and microscopy of *Dolium sedentarium*. (A) Maximum likelihood SSU rDNA phylogeny of Euglenozoa estimated under GTR+GAMMA and 1000 nonparametric bootstrap replicates, with a focus on petalomonad taxa, rooted on outgroups Kinetoplastea and Diplonemea. Bootstrap values under 50% are not shown and nodes with 100% support are denoted by a circle. (B) Micrograph of single cell of *D. sedentarium* used for transcriptome sequencing. (C) Same cell, with an arrowhead showing the second, non-emergent flagellum. Scale bars for (B) and (C) are 10 μm.

anterior flagellum or forming a rounded resting stage. It is currently unclear whether the sessile form is just a life-stage in *Dolium*, or a permanent state, but the original description and all observations since then have noted that observed *D. sedentarium* is not mobile (Al-Qassab et al., 2002; Ekebom et al., 1995; Larsen & Patterson, 1990; Patterson & Simpson, 1996). Continuous observation of wild cells or a culture of *Dolium* are needed to learn more about its habits and life cycle, unfortunately it has been rarely observed, and usually as a solitary cell only (Larsen & Patterson, 1990).

Among phototrophic euglenids, the genus *Colacium* is known to be sessile in one of its life stages (Al-Dhaheri & Willey, 1996; Rosowski & Kugrens, 1973). It is also the only colonial euglenid known, and connects to the substrate and other cells with mucus on its anterior end (Rosowski & Kugrens, 1973). While *Dolium* may use mucus to attach to the substrate, we did not see any indication of this in the reported *Dolium* cell, neither was it reported previously. Considering that the mechanism of attachment in *Colacium* is completely different than in *Dolium* and *Scytomonas*—both are non-colonial and attach on their posterior end—sessile stages in euglenids have arisen at least three times. Temporary or continuous attachment to the substrate may be beneficial for some euglenids in certain circumstances, waiting to ambush their prey (*Dolium* and *Scytomonas*) or use their host to move around in the environment to more beneficial conditions (*Colacium*).

Dolium sedentarium is morphologically similar to other petalomonads, it has six pellicle strips, other petalomonads always have less than 10 pellicle strips (Leander et al., 2017; Lee & Simpson, 2014a). We could neither observe any feeding apparatus with our light microscopy, nor did any other study report such an observation (Al-Qassab et al., 2002; Ekebom et al., 1995; Larsen & Patterson, 1990; Patterson & Simpson, 1996). Our

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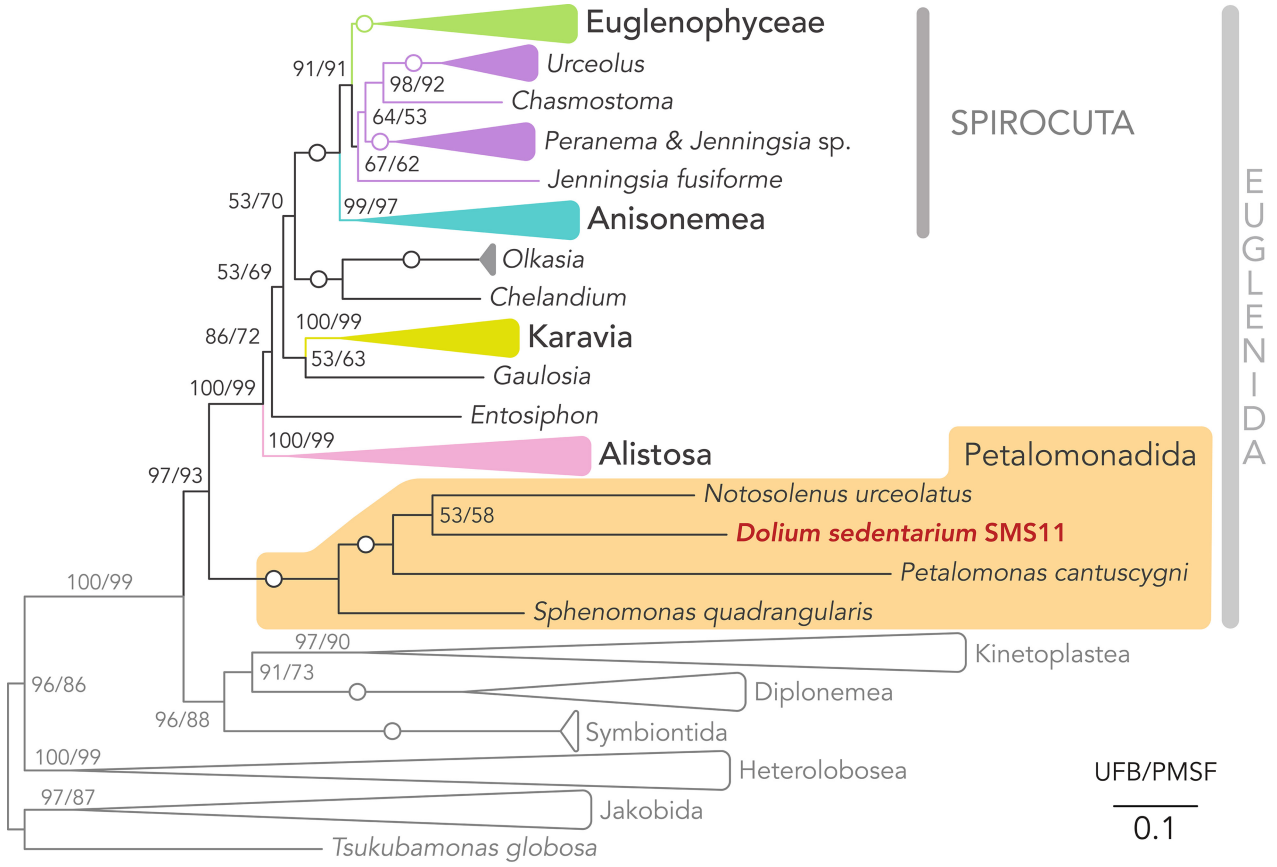

FIGURE 2 Maximum likelihood tree of 63 discobid taxa using 19 genes, estimated under LG+C60+F+G, outgroup-rooted on Jakobids and *Tsukubamonas*. *Dolium sedentarium* is marked in bold and red. Nodes that received full support from both ultrafast bootstraps (UFB) and posterior mean site frequency (PMSF) analyses are marked with a circle.

isolate had some small orange inclusions in the posterior, and other observations noted ingested diatom frustules (Al-Qassab et al., 2002; Larsen & Patterson, 1990). *D. sedentarium* might be an ambush predator (Leander et al., 2017), so it could in theory catch and ingest slow-moving diatoms. No feeding behavior has been observed in *D. sedentarium*, likely due to its observed rarity. Considering most other petalomonads have a "type I" feeding apparatus structure (Triemer & Farmer, 1991), we can assume *Dolium* has the same, but there is currently no ultrastructural study available to investigate this. Any ultrastructural study of *Dolium* would be valuable, considering we still understand very little about character evolution in euglenids, particularly in early-branching petalomonads (Lee & Simpson, 2014a). Any insights into feeding behavior require either continued observation in the wild or an established culture of *D. sedentarium*, the latter also being required for any ultrastructural data and information on its general biology.

In our SSU rDNA phylogeny *D. sedentarium* branches sister to a large clade ("Clade 1") that includes two subclades: one with *Petalomonas sphagnophila* and 19 environmental sequences, and another with *Notosolenus urceolatus* and five environmental sequences. *P. sphagnophila*, an anterior flagellum glider with four pellicle strips, is relatively large with dimensions of 40×20 μm and has only been found in *Sphagnum*-dominated peatlands in temperate zones (Kim et al., 2010; Schnepf et al., 2002), it also harbors a complex diversity of endosymbiotic bacteria. Meanwhile *Dolium* has only been found in tropical and subtropical marine benthic environments, and we did not see any evidence of endosymbionts with light microscopy or in our sequence data.

Curiously there are no environmental sequences that branch with *Dolium*. Euglenids are somewhat of an abnormality in environmental sequencing studies investigating eukaryotes, since they are rarely recovered in any meaningful sense because of primer biases (Forster et al., 2016; Kolisko et al., 2020; Łukomska-Kowalczyk et al., 2016). Petalomonads are one of the few euglenid groups for which short environmental sequencing data are available (Kolisko et al., 2020), seemingly because while "universal" eukaryotic primers fail to work on many euglenids (Łukomska-Kowalczyk et al., 2016), petalomonads appear to be the exception. Both of these factors likely contribute to the availability of some environmental sequence data for petalomonads, but to the absence in other euglenid groups.

Even if there are comparatively few petalomonad environmental sequences, only four such sequences were

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recovered for all ploeotids, a phylogenetically much more diverse clade (Lax et al., 2019, 2023). While the majority of euglenids have divergent and often expanded SSU rDNA sequences (often >2300 bp), all currently named petalomonads have SSU rDNA sequences ranging between 2000 and 2300 bp (Busse et al., 2003; Lax et al., 2019; Lax & Simpson, 2020).

It is now abundantly clear that both *Petalomonas* and *Notosolenus* are not true monophyletic genera, but that each will likely be broken up into several genera. This splitting will be highly dependent on the molecular characterization of type species *Petalomonas abscissa* and *Notosolenus apocamptus*, since it is currently unclear what clade should be considered representing "true" *Petalomonas* or *Notosolenus*, respectively. It should also be noted that we still know comparatively little about both the diversity and ultrastructure of petalomonads, the earliest-branching euglenids to date.

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DATA AVAILABILITY STATEMENT

The SSU rDNA sequence of *D. sedentarium* SMS11 is deposited under NCBI GenBank accession OQ389522, and the raw reads under NCBI BioProject PRJNA930043. Additional images, all alignments and trees, and assembly with predicted proteome can be found under DataDryad DOI doi.org/10.5061/dryad.34tmpg4qb.

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REFERENCES

Al-Dhaheri, R.S. & Willey, R.L. (1996) Colonization and reproduction of the epibiotic flagellate Colacium vesiculosum (Euglenophyceae) on Daphnia pulex. *Journal of Phycology*, 32, 770–774. Available from: https://doi.org/10.1111/j.0022-3646.1996.00770.x

Al-Qassab, S., Lee, W.J., Murray, S., Simpson, A.G.B. & Patterson, D.J. (2002) Flagellates from stromatolites and surrounding sediments in Shark Bay, Western Australia. *Acta Protozoologica*, 41, 91–144.

Arndt, H., Dietrich, D., Auer, B., Cleven, E.-J., Gräfenhan, T., Weitere, M. et al. (2000) Functional diversity of heterotrophic flagellates in aquatic ecosystems. In: Leadbeater, B.S.C. & Green, J.C. (Eds.) *The flagellates: Unity, diversity and evolution*. London: Taylor & Francis Press, pp. 240–268.

Boenigk, J. & Arndt, H. (2002) Bacterivory by heterotrophic flagellates: community structure and feeding strategies. *Antonie van Leeuwenhoek Journal of Microbiology*, 81, 465–480.

Bolger, A.M., Lohse, M. & Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30, 2114–2120.

Bushmanova, E., Antipov, D., Lapidus, A. & Prjibelski, A.D. (2018) rnaSPAdes: a de novotranscriptome assembler and its application to RNA-Seq data. *bioRxiv*, 32, 1009–1015. Available from: https://doi.org/10.1101/420208

Busse, I., Patterson, D.J. & Preisfeld, A. (2003) Phylogeny of Phagotrophic Euglenids (Euglenozoa): a molecular approach based on culture material and environmental samples. *Journal of Phycology*, 39, 828–836. Available from: https://doi.org/10.1046/j.1529-8817.2003.02178.x/pdf

Capella-Gutiérrez, S., Silla-Martínez, J.M. & Gabaldón, T. (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, 25, 1972–1973. Available from: https://doi.org/10.1093/bioinformatics/btp348

Cavalier-Smith, T. (2017) Euglenoid pellicle morphogenesis and evolution in light of comparative ultrastructure and trypanosomatid biology: semi-conservative microtubule/strip duplication, strip shaping and transformation. *European Journal of Protistology*, 61, 137–179. Available from: https://doi.org/10.1016/j.ejop.2017.09.002

Cavalier-Smith, T., Chao, E.E. & Vickerman, K. (2016) New phagotrophic euglenoid species (new genus Decastava; Scytomonas saepesedens; Entosiphon oblongum), Hsp90 introns, and putative euglenoid Hsp90 pre-mRNA insertional editing. *European Journal of Protistology*, 56, 147–170. Available from: https://doi.org/10.1016/j.ejop.2016.08.002

Chan, Y.-F., Moestrup, Ø. & Chang, J. (2013) On Keelungia pulex nov. gen. Et nov. sp., a heterotrophic euglenoid flagellate that lacks pellicular plates (Euglenophyceae, Euglenida). *European Journal of Protistology*, 49, 15–31. Available from: https://doi.org/10.1016/j.ejop.2012.04.003

Ekebom, J., Patterson, D.J. & Vørs, N. (1995) Heterotrophic flagellates from coral reef sediments (great barrier reef, Australia). *Archiv für Protistenkunde*, 146, 251–272. Available from: https://doi.org/10.1016/S0003-9365(96)80013-3

Esson, H.J. & Leander, B.S. (2006) A model for the morphogenesis of strip reduction patterns in phototrophic euglenids: evidence for heterochrony in pellicle evolution. *Evolution & Development*, 8, 378–388. Available from: https://doi.org/10.1111/j.1525-142X.2006.00110.x

Ewels, P., Magnusson, M., Lundin, S. & Käller, M. (2016) MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, 32, 3047–3048.

Forster, D., Dunthorn, M., Mahé, F., Dolan, J.R., Audic, S., Bass, D. et al. (2016) Benthic protists: the under-charted majority. *FEMS Microbiology Ecology*, 92, fiw120.

Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I. et al. (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology*, 29, 644–652. Available from: https://doi.org/10.1038/nbt.1883

Jackson, C., Knoll, A.H., Chan, C.X. & Verbruggen, H. (2018) Plastid phylogenomics with broad taxon sampling further elucidates the distinct evolutionary origins and timing of secondary green plastids. *Scientific Reports*, 8, 1523. Available from: https://doi.org/10.1038/s41598-017-18805-w

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772–780. Available from: https://doi.org/10.1093/molbev/mst010

Kim, E., Park, J.S., Simpson, A.G.B., Matsunaga, S., Watanabe, M., Murakami, A. et al. (2010) Complex array of endobionts in Petalomonas sphagnophila, a large heterotrophic euglenid protist from sphagnum-dominated peatlands. *The ISME Journal*, 4, 1108–1120. Available from: https://doi.org/10.1038/ismej.2010.40

Kolisko, M., Flegontova, O., Karnkowska, A., Lax, G., Maritz, J.M., Pánek, T. et al. (2020) EukRef-excavates: seven curated SSU ribosomal RNA gene databases. *Genome Biology and Evolution*,

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2020, 1–11. Available from: https://doi.org/10.1093/database/baaa080/5996027

Kozlov, A.M., Darriba, D., Flouri, T., Morel, B. & Stamatakis, A. (2019) RAxML-NG: a fast, scalable, and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics*, 35, 4453–4455. Available from: https://doi.org/10.1093/bioinformatics/btz305

Larsen, J. & Patterson, D.J. (1990) Some flagellates (Protista) from tropical marine sediments. *Journal of Natural History*, 24, 801–937.

Larsen, J. & Patterson, D.J. (1991) The diversity of Euglenoid flagellates. In: Larsen, J., Patterson, D.J., Systematics Association & Durham, G.B. (Eds.) *The biology of free-living heterotrophic flagellates*. Oxford, UK: Clarendon Press, pp. 205–217.

Lax, G., Cho, A. & Keeling, P.J. (2023) Phylogenomics of novel ploeotid taxa contribute to the backbone of the euglenid tree. *Journal of Eukaryotic Microbiology*, 7, e12973. Available from: https://doi.org/10.1111/jeu.12973

Lax, G., Kolisko, M., Eglit, Y., Lee, W.J., Yubuki, N., Karnkowska, A. et al. (2021) Multigene phylogenetics of euglenids based on single-cell transcriptomics of diverse phagotrophs. *Molecular Phylogenetics and Evolution*, 159, 107088. Available from: https://doi.org/10.1016/j.ympev.2021.107088

Lax, G., Lee, W.J., Eglit, Y. & Simpson, A. (2019) Ploeotids represent much of the phylogenetic diversity of euglenids. *Protist*, 170, 233–257. Available from: https://doi.org/10.1016/j.protis.2019.03.001

Lax, G. & Simpson, A.G.B. (2013) Combining molecular data with classical morphology for uncultured Phagotrophic Euglenids (Excavata): a single-cell approach. *Journal of Eukaryotic Microbiology*, 60, 615–625. Available from: https://doi.org/10.1111/jeu.12068/full

Lax, G. & Simpson, A.G.B. (2020) The molecular diversity of Phagotrophic Euglenids examined using single-cell Methods. *Protist*, 171, 125757. Available from: https://doi.org/10.1016/j.protis.2020.125757

Leander, B.S., Lax, G., Karnkowska, A. & Simpson, A.G. (2017) Euglenida. In: Archibald, J.M., Simpson, A.G. & Slamovits, C.H. (Eds.) *Handbook of Protists*, Vol. 2, 2nd edition. New York City: Springer Cham, pp. 1047–1088. Available from: https://doi.org/10.1007/978-3-319-28149-0_13

Leander, B.S., Triemer, R.E. & Farmer, M.A. (2001) Character evolution in heterotrophic euglenids. *European Journal of Protistology*, 37, 337–356.

Leander, B.S., Witek, R.P. & Farmer, M.A. (2001) Trends in the evolution of the euglenid pellicle. *Evolution*, 55, 2215–2235. Available from: https://doi.org/10.1111/j.0014-3820.2001.tb00737.x/abstract

Lee, W.J. & Patterson, D.J. (2002) Abundance and biomass of heterotrophic flagellates, and factors controlling their abundance and distribution in sediments of Botany Bay. *Microbial Ecology*, 43, 467–481. Available from: https://doi.org/10.1007/s00248-002-2000-5

Lee, W.J. & Simpson, A.G.B. (2014a) Morphological and molecular characterisation of Notosolenus urceolatus Larsen and Patterson 1990, a member of an understudied deep-branching Euglenid group (Petalomonads). *Journal of Eukaryotic Microbiology*, 61, 463–479. Available from: https://doi.org/10.1111/jeu.12126

Lee, W.J. & Simpson, A.G.B. (2014b) Ultrastructure and molecular phylogenetic position of Neometanema parovale sp. nov. (Neometanema gen. Nov.), a marine Phagotrophic Euglenid with skidding motility. *Protist*, 165, 452–472. Available from: https://doi.org/10.1016/j.protis.2014.05.001

Łukomska-Kowalczyk, M., Karnkowska, A., Krupska, M., Milanowski, R. & Zakryś, B. (2016) DNA barcoding in autotrophic euglenids: evaluation of COI and 18s rDNA. *Journal of Phycology*, 52, 951–960. Available from: https://doi.org/10.1111/jpy.12439

Manni, M., Berkeley, M.R., Seppey, M., Simão, F.A. & Zdobnov, E.M. (2021) BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. *Molecular Biology and Evolution*, 38, 4647–4654. Available from: https://doi.org/10.1093/molbev/msab199

Mikheenko, A., Prjibelski, A., Saveliev, V., Antipov, D. & Gurevich, A. (2018) Versatile genome assembly evaluation with QUAST-LG. *Bioinformatics*, 34, i142–i150. Available from: https://doi.org/10.1093/bioinformatics/bty266

Minh, B.Q., Nguyen, M.A.T. & Haeseler, A. (2013) Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution*, 30, 1188–1195. Available from: https://doi.org/10.1093/molbev/mst024

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., Haeseler, A. et al. (2020) IQ-TREE 2: new models and efficient Methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, 37, 1530–1534.

Paerschke, S., Vollmer, A.H. & Preisfeld, A. (2017) Ultrastructural and immunocytochemical investigation of paramylon combined with new 18S rDNA-based secondary structure analysis clarifies phylogenetic affiliation of Entosiphon sulcatum (Euglenida: Euglenozoa). *Organisms Diversity & Evolution*, 59, 1–12. Available from: https://doi.org/10.1007/s13127-017-0330-x

Patterson, D.J. & Simpson, A.G.B. (1996) Heterotrophic flagellates from coastal marine and hypersaline sediments in Western Australia. *European Journal of Protistology*, 32, 423–448. Available from: https://doi.org/10.1016/S0932-4739(96)80003-4

Picelli, S., Faridani, O.R., Björklund, Å.K., Winberg, G., Sagasser, S. & Sandberg, R. (2014) Full-length RNA-seq from single cells using smart-seq2. *Nature Protocols*, 9, 171–181. Available from: https://doi.org/10.1038/nprot.2014.006

Rosowski, J.R. & Kugrens, P. (1973) Observations on the euglenoid Colacium with special reference to the formation and morphology of attachment material. *Journal of Phycology*, 9, 370–383. Available from: https://doi.org/10.1111/j.1529-8817.1973.tb04110.x

Schnepf, E., Schlegel, I. & Hepperle, D. (2002) Petalomonas sphagnophila (Euglenophyta) and its endocytobiotic cyanobacteria: a unique form of symbiosis. *Phycologia*, 41, 153–157.

Schoenle, A., Živaljić, S., Prausse, D., Voss, J., Jakobsen, K. & Arndt, H. (2019) New phagotrophic euglenids from deep sea and surface waters of the Atlantic Ocean (Keelungia nitschei, Petalomonas acorensis, Ploeotia costaversata). *European Journal of Protistology*, 69, 102–116. Available from: https://doi.org/10.1016/j.ejop.2019.02.007

Song, L. & Florea, L. (2015) Rcorrector: efficient and accurate error correction for Illumina RNA-seq reads. *GigaScience*, 4, 1–8. Available from: https://doi.org/10.1186/s13742-015-0089-y

Triemer, R.E. & Farmer, M.A. (1991) The ultrastructural organization of the heterotrophic euglenids and its evolutionary implications. In: Larsen, J., Patterson, D.J., Systematics Association & Durham, G.B. (Eds.) *The biology of free-living heterotrophic flagellates*. Oxford: Clarendon Press, pp. 185–204.

Turmel, M., Gagnon, M.-C., O'Kelly, C.J., Otis, C. & Lemieux, C. (2009) The chloroplast genomes of the Green algae Pyramimonas, Monomastix, and Pycnococcus shed new light on the evolutionary history of Prasinophytes and the origin of the secondary chloroplasts of Euglenids. *Molecular Biology and Evolution*, 26, 631–648. Available from: https://doi.org/10.1093/molbev/msn285

Vaulot, D., Sim, C.W.H., Ong, D., Teo, B., Biwer, C., Jamy, M. et al. (2022) metaPR2: a database of eukaryotic 18S rRNA metabarcodes with an emphasis on protists. *Molecular Ecology Resources*, 22, 3188–3201. Available from: https://doi.org/10.1111/1755-0998.13674

Wang, H.-C., Minh, B.Q., Susko, E. & Roger, A.J. (2018) Modeling site heterogeneity with posterior mean site frequency profiles accelerates accurate Phylogenomic estimation. *Systematic Biology*, 67, 216–235.

15507408, 2023, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jeu.12991 by Cochrane Canada Provision, Wiley Online Library on [12/09/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Yubuki, N. & Leander, B.S. (2012) Reconciling the bizarre inheritance of microtubules in complex (euglenid) microeukaryotes. *Protoplasma*, 249, 859–869. Available from: https://doi.org/10.1007/s00709-011-0340-z

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