

Putatively asexual chrysophytes have meiotic genes: evidence from transcriptomic data

Diana Kraus¹, **Jingyun Chi**¹, **Jens Boenigk**², **Daniela Beisser**², **Nadine Graupner**², **Micah Dunthorn**^{Corresp. 1}

¹ Department of Ecology, University of Kaiserslautern, Kaiserslautern, Germany

² Department of Biodiversity, University of Duisburg-Essen, Essen, Germany

Corresponding Author: Micah Dunthorn
Email address: dunthorn@rhrk.uni-kl.de

Chrysophytes are a large group of heterotrophic, phototrophic, or even mixotrophic protists that are abundant in aquatic as well as terrestrial environments. Although much is known about chrysophyte biology and ecology, it is unknown if they are sexual or not. Here we use available transcriptomes of 18 isolates of 15 putatively asexual species to inventory the presence of genes used in meiosis. Since we were able to detect a set of nine meiosis-specific and 29 meiosis-related genes shared by the chrysophytes, we conclude that they are secretively sexual and therefore should be investigated further using genome sequencing to uncover any missed genes from the transcriptomes.

Putatively asexual chrysophytes have meiotic genes: evidence from transcriptomic data

**Diana Kraus¹ • Jingyun Chi¹ • Jens Boenigk^{2,3} • Daniela Beisser² • Nadine
Graupner² • Micah Dunthorn^{1,3,4}**

¹ Department of Ecology, University of Kaiserslautern, Erwin Schrödinger Straße 14, D-67663
Kaiserslautern, Germany

² Department of Biodiversity, University of Duisburg-Essen, Universitätsstraße 5, D-45117
Essen, Germany

³ Centre for Water and Environmental Research (ZWU), University of Duisburg-Essen,
Universitätsstraße 2, D-45141 Essen, Germany

⁴ Department of Eukaryotic Microbiology, University of Duisburg-Essen, Universitätsstraße 5,
D-45117 Essen, Germany

Email: Micah Dunthorn

micah.dunthorn@uni-due.de

Abstract Chrysophytes are a large group of heterotrophic, phototrophic, or even mixotrophic protists that are abundant in aquatic as well as terrestrial environments. Although much is known about chrysophyte biology and ecology, it is unknown if they are sexual or not. Here we use available transcriptomes of 18 isolates of 15 putatively asexual species to inventory the presence of genes used in meiosis. Since we were able to detect a set of nine meiosis-specific and 29 meiosis-related genes shared by the chrysophytes, we conclude that they are secretively sexual and therefore should be investigated further using genome sequencing to uncover any missed genes from the transcriptomes.

Keywords Asexuality . Meiosis . Crossover pathways . Sex

Introduction

The Chrysophyceae Pascher 1914 are a morphologically diverse group of flagellates that are among the dominant protists in aquatic and terrestrial habitats (Boenigk & Arndt 2002; Foissner 1987; Kristiansen & Preisig 2001; Kristiansen & Škaloud 2017; Sandgren 1988). These protists serve as excellent models in ecology, ecophysiology, and evolution (Boenigk 2008; Graupner et al. 2018), because of their wide range of nutritional strategies. Their ecological importance of the chrysophytes is derived from the heterotrophic and mixotrophic taxa being important grazers of bacteria (del Campo & Massana 2011; Ekelund et al. 2001; Finlay & Esteban 1998), and the phototrophic and mixotrophic taxa being a large component of the primary producers in oligotrophic freshwaters (Kristiansen & Škaloud 2017; Wolfe & Siver 2013).

Despite their known ecological importance, chrysophyte taxon richness and species boundaries are difficult to infer. For example, there are some taxa with morphological characters of high diagnostic value such as in *Paraphysomonas* (Scoble & Cavalier-Smith) and *Synura* (Siver & Lott 2016), taxa with morphological characters of uncertain taxonomic value such as in the *Dinobryon divergens* complex (Jost et al. 2010), and taxa that are largely missing much morphological characters such as many colorless non-scaled taxa (Grossmann et al. 2016).

Assessing reproductive isolation in these taxa may offer a starting point for a consistent taxonomic revision and recognition of species boundaries based on mating abilities. In general, chrysophytes are assumed to be capable of sex, even though conclusive evidence has not been demonstrated for either meiosis or the fusion of meiotic products from different individuals (Kristiansen & Škaloud 2017). Possible formation of zygotes was observed in *Dinobryon* and *Synura* using morphological observations, but changes in ploidy were not evaluated (Bourrelly

1957; Fott 1959; Sandgren 1983; Wawrik 1972). These morphological studies are also restricted to a handful of taxa and the distribution of sex within the chrysophytes remains unknown.

Meiotic sex is assumed to be retained in most macro-organismic eukaryotes because asexuality can lead to extinction over time (Bell 1982; Maynard Smith 1978). However, sex is often not easily observable in many microbial eukaryotic groups, which can lack distinctive morphological differences between the sexes or we do not know the right environmental conditions to induce sex in the laboratory (Dunthorn & Katz 2010; Schurko et al. 2009; Speijer et al. 2015). In the absence of direct observations of sex (e.g., O’Gorman et al. 2009) and in the absence of known sexual mating types (e.g., Corradi & Brachmann 2017), one of the strongest molecular signatures of secretive sex in putative asexual protists is the presence of meiotic genes. If the meiotic genes are found in their genomes, then the protein products are likely being used for sex, otherwise they would have been lost over evolutionary time (Normark et al. 2003; Schurko & Logsdon 2008). While genomic data are usually used for such meiotic gene inventories in protists (Chi et al. 2014a; Dunthorn et al. 2017; Malik et al. 2008; Patil et al. 2015; Ramesh et al. 2005; Tekle et al. 2017), expressed sequence tag (EST) have also been used, although genes can be missing from an EST library if they are not being expressed at the time the protist was collected and analyzed for a secretive sexual stage (Chi et al. 2014b).

Transcriptomic data from 18 chrysophyte isolates, representing 15 different species that were either photo-, mixo-, or hetero-trophic, were recently used to gain insights into nutritional strategies and phylogenetic relationships (Beisser et al. 2017). Within the chrysophytes able to perform photosynthesis, the transcriptomes revealed a higher expression of genes participating in photosynthesis, photosynthesis-antenna proteins, porphyrin and chlorophyll metabolism, carbon fixation and carotenoid biosynthesis, while in the heterotrophic strains there was a higher

expression of genes involved in nutrient absorption, environmental information processing and various transporters (e.g., monosaccharide, peptide, lipid transporters). Here we used those same 18 chrysophyte transcriptomes from Beisser et al. (2017) for a meiotic gene inventory to evaluate if these putatively asexual protists are capable of sex. Following Chi et al. (2014a), the presence and absence of these genes were placed into the context that there are two meiotic crossover pathways: class I pathway, which relies on meiotic-specific genes and can include a synaptonemal complex; and class II pathway, which uses meiotic-related genes that are also involved in mitosis (Loidl 2016).

Materials and methods

From Beisser et al. (2017), sequenced and cleaned transcriptomic data were taken for 18 chrysophytes strains of 15 species: *Acrispumella msimbaziensis* (strain JBAF33), *Apoikiospumella mondseeiensis* (strain JBM08), *Cornospumella fuschlensis* (strain A-R4-D6), *Dinobryon* sp. (strain FU22KAK), *Dinobryon* sp. (strain LO226KS), *Epipyxis* sp. (strain PR26KG), *Ochromonas* or *Spumella* sp. (strain LO244K-D), *Pedospumella encystans* (strain JBMS11), *Poterioochromonas malhamensis* (strain DS), *Poteriospumella lacustris* (strain JBC07), *Poteriospumella lacustris* (strain JBM10), *Poteriospumella lacustris* (strain JBNZ41), *Pedospumella sinomuralis* (strain JBCS23), *Spumella bureschii* (strain JBL14), *Spumella lacusvadosi* (strain JBNZ39), *Spumella vulgaris* (strain 199hm), *Synura* sp. (strain LO234KE), and *Uroglena* sp. (strain WA34KE). The data are available at the European Nucleotide Archive accession PRJEB13662.

Here these data were compared to a query database of nine meiosis-specific and 30 meiosis-related genes established by Chi et al. (2014a). This database was originally established using literature and keyword searches of the NCBI protein database and the Uniprot Knowledgebase. Using local scripts, two methods were used for comparing the transcriptomic data to the query database of meiotic genes: BlastP (Altschul et al. 1990) and HMMER v3.0 (Eddy 2011). Reciprocal BLAST analysis were also performed using BLASTP against the non-redundant protein sequence database of NCBI. The parameters for BLASTp and HMMER is default, except sequences were retained if they had hits with E-values <10E-4. Following *Saccharomyces cerevisiae* nomenclature, gene names are signified in italic capital letters, and proteins in lowercase except first letter.

Results

Out of the 39 meiotic genes, 38 were identified in the transcriptomes of 18 chrysophytes strains (Table 1, Supplementary File 1). For the nine meiosis-specific genes, all of them were found in at least six transcriptomes. In particular, *SPO11*, which initiates meiosis through double-strand DNA breaks in most eukaryotes (Keeney et al. 1997) except in some amoebae (Bloomfield 2018), was found in seven strains. The following other meiosis-specific genes were found: *DMC1* in 15 strains, is important for recombination homolog bias (Bugreev et al. 2011); *HOP2* in 18 strains, stabilizes the association of the protein Dmc1 with DNA (Chen et al. 2004); *MND1* in 12 strains, also stabilizes the association of the protein Dmc1 with DNA (Chen et al. 2004); *HOP1* in six strains, forms part of the synaptonemal complex (Hollingsworth et al. 1990); *REC8* in 12 strains, forms part of the sister chromatid cohesin complex (Howard-Till et al. 2013); *MER3* in 16 strains, is a DNA helicase (Nakagawa & Kolodner 2002); and *MSH4* in 14 strains and *MSH5* in 13 strains, which are heterodimers that stabilize recombination intermediates (Nishant et al. 2010; Snowden et al. 2004).

For the 30 meiosis-related genes, 29 were found in at least five out of the 18 transcriptomes. The only gene that was not found in any transcriptome was REC114. The meiosis-related gene *MMS4* was found in the smallest amount of five transcriptomes. The seven meiosis-related genes *MPH1*, *PMS1*, *RAD23*, *RAD50*, *SGS1*, *SMC5* and *SMC6* were found in all 18 transcriptomes. Nine of the other meiosis-related genes were only not present in two or three chrysophyte transcriptomes.

Many of the missing meiotic genes could really be missing from the genomes, or the genes could be missing because of how the data was generated. In transcriptomes, just like in ESTs (Chi et al. 2014b), missing genes are expected because only genes being actively expressed

130 will be sequenced. These differences between the sequences of strains of the same species here
 131 suggest that indeed the transcriptomes are likely missing a lot of non-expressed genes. For
 132 example, *HOP1* is only found in two of three strains of *Poterospumella lacustris*, and *MSH4*
 133 and *MSH5* is only found in one of two stains of *Dinobryon* sp.

Discussion

In this gene inventory of chrysophyte transcriptomes, we found evidence for the presence of many meiosis-specific and meiosis-related genes. If we assume a use-it-or-lose-it view of these genes (Normark *et al.* 2003; Schurko & Logsdon 2008), then the chrysophytes are using the protein products of these genes to construct functional meiotic machinery. As with most other eukaryotes (Dunthorn & Katz 2010; O'Malley *et al.* 2013), the chrysophytes are therefore likely sexual, which supports earlier microscopic observations that potentially indicated sex (Bourrelly 1957; Fott 1959; Sandgren 1983; Wawrik 1972). If this is the case, and even if sex has not yet been directly observed, the genetic diversity and adaptive evolution of the chrysophytes would benefit from this secretive sex. And this benefit could occur even if sex was a rare event in the chrysophytes (D'Souza & Michiels 2010; Green & Noakes 1995).

Additionally, we found meiotic genes involved in both crossover pathways, including genes involved in making the synaptonemal complex in class I pathway. Although these pathways have been differentially lost in various eukaryotic groups (Chi *et al.* 2014a; Loidl 2016), chrysophyte potentially use both of these pathways. Given the phylogenetic placement across the chrysophyte tree of life of the 15 species sampled here (Beisser *et al.* 2017), these results supporting secretive sex and the presence of both crossover pathways should be applicable for all, or most, other chrysophyte species.

Here we used transcriptomic data to show that there are meiotic genes in the putative asexual chrysophytes. These genes are likely being used for sex. This finding suggests that more thorough *de novo* genome sequencing of different chrysophyte species should be performed to uncover the meiotic genes possibly missed in the transcriptomes. This finding also suggests that targeted mating attempts of different chrysophyte species in the laboratory should be attempted,

157 as these observations will offer the best evidence that the chrysophyte are truly sexual in nature
 158 and that meiosis in these protists is not being used just for automixis.

159 **Funding**

160 The Deutsche Forschungsgemeinschaft provided support to MD (grant # DU1319/1- 1) and JB
161 (grant #s BO3245/17 and BO3245/19).

162

163 **Grant Disclosures**

164 The following grant information was disclosed by the authors:

165 Deutsche Forschungsgemeinschaft: #DU1319/1-1.

166 Deutsche Forschungsgemeinschaft: #BO3245/17.

167 Deutsche Forschungsgemeinschaft: # BO3245/19.

168

169 **Competing Interests**

170 The authors declare there are no competing interests.

171

172 **Author Contributions**

173 • Diana Kraus, Jingyun Chi, and Micah Dunthorn conceived and designed the experiments,
174 performed the experiments, analyzed the data, wrote the paper, prepared figures and tables.

175 • Jens Boenigk, Daniela Beisser, and Nadine Graupner contributed data, analyzed the data, wrote
176 the paper, and reviewed drafts of the paper.

References

- Altschul SF, Fish W, Miller W, Myers EW, and Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403-410.
- Andrews S. 2012. FastQC a quality control tool for high throughput sequence data. Available at [http:// www.bioinformatics.babraham.ac.uk/projects/fastqc/](http://www.bioinformatics.babraham.ac.uk/projects/fastqc/).
- Beisser D, Graupner N, Bock C, Wodniok S, Grossmann L, Vos M, Sures B, Rahmann S, and Boenigk J. 2017. Comprehensive transcriptome analysis provides new insights into nutritional strategies and phylogenetic relationships of chrysophytes. *PeerJ* 5:e2832. 10.7717/peerj.2832
- Bell G. 1982. *The masterpiece of nature: the evolution and genetics of sexuality*. Berkeley: University of California Press.
- Bloomfield G. 2018. Spo11-independent meiosis in social amoebae. *Annu Rev Microbiol* 72:293-307. 10.1146/annurev-micro-090817-062232
- Boenigk J. 2008. Nanoflagellates: functional groups and intraspecific variation. *Denisia* 23:331-335.
- Boenigk J, and Arndt H. 2002. Bacterivory by heterotrophic flagellates: community structure and feeding strategies. *Antonie Van Leeuwenhoek* 81:465-480.
- Bourrelly P. 1957. Recherches sur les Chrysophycées. *Revue Algologique Mémoire Hors Série* 1:1-412.
- Bugreev DV, Pezza RJ, Mazina OM, Voloshin ON, Camerini-Otero RD, and Mazin AV. 2011. The resistance of DMC1 D-loops to dissociation may account for the DMC1 requirement in meiosis. *Nature Struct Mol Biol* 18:56-61.
- Chen YK, Leng CH, Olivares H, Lee MH, Chang YC, Kung WM, Ti SC, Lo YH, Wang AHJ, Chang CS, Bishop DK, Hsueh YP, and Wang TF. 2004. Heterodimeric complexes of Hop2 and Mnd1 function with Dmc1 to promote meiotic homolog juxtaposition and strand assimilation. *Proc Natl Acad Sci USA* 101:10572-10577.
- Chi J, Mahé F, Loidl J, Logsdon J, and Dunthorn M. 2014a. Meiosis gene inventory of four ciliates reveals the prevalence of a synaptonemal complex-independent crossover pathway. *Mol Biol Evol* 31:660-672.
- Chi J, Parrow MW, and Dunthorn M. 2014b. Cryptic sex in *Symbiodinium* (Alveolata, Dinoflagellata) is supported by an inventory of meiotic genes. *J Eukaryot Microbiol* 61:322-327.
- Corradi N, and Brachmann A. 2017. Fungal mating in the most widespread plant symbionts? *Trends Plant Sci* 22:175-183. <http://dx.doi.org/10.1016/j.tplants.2016.10.010>

- 211 **D'Souza TG, and Michiels NK. 2010.** The costs and benefits of occasional sex: theoretical
212 predictions and a case study. *J Heredity* 101:S34-S41. 10.1093/jhered/esq005
- 213 **del Campo J, and Massana R. 2011.** Emerging diversity within chrysophytes,
214 choanoflagellates and bicosoecids based on molecular surveys. *Protist* 162:435-448.
- 215 **Dunthorn M, and Katz LA. 2010.** Secretive ciliates and putative asexuality in microbial
216 eukaryotes. *Trends Microbiol* 18:183-188.
- 217 **Dunthorn M, Zufall RA, Chi J, Paszkiewicz K, Moore K, and Mahé F. 2017.** Meiotic genes
218 in colpodean ciliates support secretive sexuality. *Genome Biol Evol* 9:1781-1787.
- 219 **Eddy SR. 2011.** Accelerated profile HMM searches. *PLOS Comput Biol* 7.
- 220 **Ekelund F, Ronn R, and Griffiths BS. 2001.** Quantitative estimation of flagellate community
221 structure and diversity in soil samples. *Protist* 152:301-314.
- 222 **Finlay BJ, and Esteban GF. 1998.** Freshwater protozoa: biodiversity and ecological function.
223 *Biodiversity and Conservation* 7:1163-1186.
- 224 **Foissner W. 1987.** Soil protozoa: fundamental problems, ecological significance, adaptations in
225 ciliates and testaceans, bioindicators, and guide to the literature. *Prog Protistol* 2:69-212.
- 226 **Fott B. 1959.** Zur Frage der Sexualität bei den Chrysomonaden. *Nova Hedwigia* 1:115-129.
- 227 **Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L,
228 Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di
229 Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, and Regev A.
230 **2011.** Full-length transcriptome assembly from RNA-Seq data without a reference
231 genome. *Nature Biotechnol* 29:644-652.**
- 232 **Graupner N, Jensen M, Bock C, Marks S, Rahmann S, Beisser D, and Boenigk J. 2018.**
233 Evolution of heterotrophy in chrysophytes as reflected by comparative transcriptomics.
234 *FEMS Microbiol Ecol* 94: fty039.
- 235 **Green RF, and Noakes DLG. 1995.** Is a little bit of sex as good as a lot? *J Theor Biol* 174:87-
236 96. <http://dx.doi.org/10.1006/jtbi.1995.0081>
- 237 **Grossmann L, Bock C, Schweikert M, and Boenigk J. 2016.** Small but manifold: hidden
238 diversity in 'Spumella-like flagellates'. *J Eukaryot Microbiol* 63:419-439.
- 239 **Hollingsworth NM, Goetsch L, and Byers B. 1990.** The *HOP1* gene encodes a meiosis-
240 specific component of yeast chromosomes. *Cell* 61:73-84.
- 241 **Howard-Till RA, Lukaszewicz A, Novatchkova M, and Loidl J. 2013.** A single cohesin
242 complex performs mitotic and meiotic functions in the protist *Tetrahymena*. *PLoS Genet*
243 9:e1003418. 10.1371/journal.pgen.1003418

- 244 **Jost S, Medinger R, and Boenigk J. 2010.** Cultivation independent species identification of
245 *Dinobryon* sp. (Chrysophyceae) by means of multiplex single cell PCR (MSC-PCR). *J*
246 *Phycol* 46:901-906.
- 247 **Keeney S, Giroux CN, and Kleckner. 1997.** Meiosis-specific DNA double-strand breaks are
248 catalyzed by Spo11, a member of a widely conserved protein domain. *Cell* 88:375-384.
- 249 **Kristiansen J, and Preisig HR. 2001.** *Encyclopedia of chrysophyte genera*. Berlin: J. Cramer.
- 250 **Kristiansen J, and Škaloud P. 2017.** Chrysophyta. In: Archibald JM, Simpson AGB, Slamovits
251 CH, Margulis L, Melkonian M, Chapman DJ, and Corliss JO, eds. *Handbook of the*
252 *Protists*. Cham: Springer.
- 253 **Loidl J. 2016.** Conservation and variability of meiosis across the eukaryotes. *Annu Rev Genet*
254 50:293-316. 10.1146/annurev-genet-120215-035100
- 255 **Malik S-B, Pightling AW, Stefaniak LM, Schurko AM, and Logsdon JM. 2008.** An
256 expanded inventory of conserved meiotic genes provides evidence for sex in
257 *Trichomonas vaginalis*. *PLoS ONE* 3:e2879.
- 258 **Martin M. 2011.** Cutadapt removes adapter sequences from high-throughput sequencing reads.
259 *EMBnetjournal* 17:1012. 10.14806/ej.17.1.200
- 260 **Maynard Smith J. 1978.** *The evolution of sex*. Cambridge: Cambridge University Press.
- 261 **Nakagawa T, and Kolodner RD. 2002.** *Saccharomyces cerevisiae* Mer3 Is a DNA helicase
262 involved in meiotic crossing over. *Mol Cellular Biol* 22:3281-3291.
- 263 **Nishant KT, Chen C, Shinohara M, Shinohara A, and Alani E. 2010.** Genetic analysis of
264 baker's yeast Msh4-Msh5 reveals a threshold crossover level for meiotic viability. *PLoS*
265 *Genetics* 6:e1001083.
- 266 **Normark BB, Judson OP, and Moran NA. 2003.** Genomic signatures of ancient asexual
267 lineages. *Biol J Linn Soc* 79:69-84.
- 268 **O’Gorman CM, Fuller HT, and Dyer PS. 2009.** Discovery of a sexual cycle in the
269 opportunistic fungal pathogen *Aspergillus fumigatus*. *Nature* 457:471-474.
- 270 **O’Malley MA, Simpson AGB, and Roger AJ. 2013.** The other eukaryotes in light of
271 evolutionary protistology. *Biol Philos* 28:299-330.
- 272 **Patil S, Moeys S, von Dassow P, Huysman MJJ, Mapleson D, De Veylder L, Sanges R,**
273 **Vyverman W, Montresor M, and Ferrante MI. 2015.** Identification of the meiotic
274 toolkit in diatoms and exploration of meiosis-specific SPO11 and RAD51 homologs in
275 the sexual species *Pseudo-nitzschia multistriata* and *Seminavis robusta*. *BMC Genomics*
276 16:930. 10.1186/s12864-015-1983-5

- Ramesh MA, Malik S-B, and Longsdon JM. 2005.** A phylogenomic inventory of meiotic genes: evidence for sex in *Giardia* and an early eukaryotic origin of meiosis. *Curr Biol* 15:185-191.
- Sandgren CD. 1983.** Survival strategies of chrysophyte flagellates: reproduction and formation of resistant spores. In: Fryxell G, ed. *Survival strategies in the algae* Cambridge: Cambridge University Press, 23-48.
- Sandgren CD. 1988.** The ecology of chrysophyte flagellates: their growth and perennation strategies as freshwater phytoplankton. In: Sandgren CD, ed. *Growth and reproductive strategies of freshwater phytoplankton*. Cambridge: Cambridge University Press, 9-104.
- Schurko AM, and Logsdon JM. 2008.** Using a meiosis detection toolkit to investigate ancient asexual "scandals" and the evolution of sex. *BioEssays* 30:579-589.
- Schurko AM, Neiman M, and Logsdon JM. 2009.** Signs of sex: what we know and how we know it. *Trends Ecol Evol* 24:208-217.
- Scoble JM, and Cavalier-Smith T. 2014.** Scale evolution in Paraphysomonadida (Chrysophyceae): sequence phylogeny and revised taxonomy of *Paraphysomonas*, new genus *Clathromonas*, and 25 new species. *Eur J Protistol* 50:551-592.
- Siver PA, and Lott AM. 2016.** Descriptions of two new species of Synurophyceae from a bog in Newfoundland, Canada: *Mallomonas baskettii* sp. nov. and *Synura kristiansenii* sp. nov. *Nova Hedwigia* 102:501-511.
- Snowden T, Acharya S, Butz C, Berardini M, and Fishel R. 2004.** hMSH4-hMSH5 recognizes Holliday junctions and forms a meiosis-specific sliding clamp that embraces homologous chromosomes. *Mol Cell* 15:437-451.
- Speijer D, Lukeš J, and Eliáš M. 2015.** Sex is a ubiquitous, ancient, and inherent attribute of eukaryotic life. *Proc Natl Acad Sci USA* 112:8827-8834.
- Tekle YI, Wood FC, Katz LA, Cerón-Romero MA, and Gorfu LA. 2017.** Amoebozoans are secretly but ancestrally sexual: evidence for sex genes and potential novel crossover pathways in diverse groups of amoebae. *Genome Biol Evol* 9:375-387. 10.1093/gbe/evx002
- Wawrik F. 1972.** Isogame Hologamie in der Gattung Mallomonas Perty. *Nova Hedwigia* 23:353-362.
- Wolfe AP, and Siver PA. 2013.** A hypothesis linking chrysophyte microfossils to lake carbon dynamics on ecological and evolutionary time scales. *Global Planet Change* 111:189-198.

Table 1(on next page)

Meiosis genes inventoried in the transcriptomes of 18 strains of 15 species of chrysophytes.

Gene	Chrysophyte species													
	<i>Uroglena</i> sp.	<i>Synura</i> sp.	<i>Spumella vulgaris</i>	<i>Spumella lacusvadosi</i>	<i>Spumella bureschii</i>	<i>Potriospumella lacustris</i> (strain JBNZ41)	<i>Potriospumella lacustris</i> (strain JBM10)	<i>Potriospumella lacustris</i> (strain JB07)	<i>Potriochromonas malhamensis</i>	<i>Pedospumella sinomuralis</i>	<i>Pedospumella encystans</i>	<i>Ochromonas</i> or <i>Spumella</i> sp.	<i>Epipyxis</i> sp.	<i>Dinobryon</i> sp. (strain LO226KS)
Double-strand break formation														
REC114	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SPO11	-	+	+	-	+	-	-	-	-	+	+	-	+	-
Crossover regulation														
DMC1	-	-	+	+	+	+	+	+	+	+	+	+	+	+
HOP1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HOP2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MER3	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MND1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MSH4	-	-	+	+	+	+	+	+	+	+	+	+	+	+
MSH5	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Double-strand break repair														
REC8	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Bouquet formation														
SAD1	-	-	+	+	+	+	+	+	+	+	+	+	+	+
DNA damage sensing/response														
MRE11	-	-	+	+	+	+	+	+	+	+	+	+	+	+
RAD17	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RAD23	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RAD24	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RAD50	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NBS1	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Double-strand break repair (nonhomology end join)														
KU	-	-	+	+	+	+	+	+	+	+	+	+	+	+
LIG4	-	-	+	+	+	+	+	+	+	+	+	+	+	+
LIF1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Recombinational repair														
DNA2	-	+	+	+	+	+	+	+	+	+	+	+	+	+
MMS4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EME1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EXO1	-	-	+	+	+	+	+	+	+	+	+	+	+	+
FEN1	+	-	+	+	+	+	+	+	+	+	+	+	+	+
MLH1	+	-	+	+	+	+	+	+	+	+	+	+	+	+
MLH3	-	+	+	+	+	+	+	+	+	+	+	+	+	+
MPH1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MSH2	-	-	+	+	+	+	+	+	+	+	+	+	+	+
MSH6	+	-	+	+	+	+	+	+	+	+	+	+	+	+
MUS81	-	-	+	+	+	+	+	+	+	+	+	+	+	+
PMS1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RAD51	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RAD52	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RAD54	-	+	+	+	+	+	+	+	+	+	+	+	+	+
RTEL	-	-	+	+	+	+	+	+	+	+	+	+	+	+
SAE2	+	-	+	+	+	+	+	+	+	+	+	+	+	+
SLX1	-	-	+	+	+	+	+	+	+	+	+	+	+	+
SLX4	+	-	+	+	+	+	+	+	+	+	+	+	+	+
SMC5	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMC6	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GEN1	-	-	+	+	+	+	+	+	+	+	+	+	+	+