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Genome size of colonial chrysophytes

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ABSTRACT
Genome size is a fundamental characteristic of the cell and is associated with a number of key features of the organism, such as cell size, division rate or metabolic rate. Knowledge of the genome size is also a prerequisite for many areas of research (e.g. selection of suitable organisms for whole genome sequencing or cell cycle analysis). However, genome size analysis in microalgae is often difficult and involves many methodological challenges. As a result, genome size data for microalgae are largely lacking. In this study, we focused on fragile, poorly growing colonial chrysophytes. We analysed their genome size using flow cytometry and tested the difference between colonial and solitary living chrysophytes on all published genome size data using analysis of variance. We successfully established nine cultures that were further determined to belong to six species of colonial chrysophytes. We estimated their genome size to be on average 0.24 pg·cell^{-1} for *Chrysosphaerella longispina* Lauterborn, 1.70 pg·cell^{-1} for *Neotessella lapponica* (Skuja) B.Y.Jo, J.I.Kim, W.Shin, P.Škaloud & P.A.Siver, 0.25 pg·cell^{-1} for *Uroglenopsis turfosa* (Skuja) R.H.Thompson & D.E.Wujek, 0.31 pg·cell^{-1} for *Urostipulosphaera articulata* (Korshikov) Pusztai & Škaloud, 0.22 pg·cell^{-1} for *U. granulata* Pusztai & Škaloud and 0.19 pg·cell^{-1} for *U. lindiae* (Bourrelly) Pusztai & Škaloud. It was also shown that colonial chrysophytes have larger genomes compared to solitary living species. This study further revealed the smallest genome among colonial chrysophytes, belonging to *Urostipulosphaera lindiae*. Nevertheless, colonial chrysophytes have lower variance in genome size, possibly due to evolutionary constraints on cell size (and genome size) variation to maintain the functionality of the whole colony movement.

KEY WORDS
Colonial chrysophytes, genome size, *Urostipulosphaera*, *Uroglenopsis*, *Chrysosphaerella*, *Neotessella*.

RÉSUMÉ

Taille du génome des chrysophytes coloniales.

La taille du génome est une caractéristique fondamentale de la cellule et est associée à un certain nombre de caractéristiques clés de l'organisme, telles que la taille de la cellule, le taux de division ou le taux métabolique. Connaître la taille du génome est également nécessaire à de nombreuses applications de recherche (par exemple, la sélection d'organismes pour le séquençage de génome entier ou l'analyse du cycle cellulaire). Cependant, l'analyse de la taille du génome des microalgues est souvent difficile et implique de nombreux défis méthodologiques. Par conséquent, les données sur la taille du génome des microalgues font largement défaut. Dans cette étude, nous nous sommes concentrés sur les chrysophytes coloniales fragiles et à faible croissance. Nous avons analysé la taille de leur génome par cytométrie en flux. Nous avons également évalué les différences de tailles de génome entre les chrysophytes coloniales et les chrysophytes solitaires sur la base de toutes les données de taille de génome publiées, au moyen d'une analyse de variance. Nous avons établi avec succès neuf cultures, déterminées comme appartenant à six espèces de chrysophytes coloniales. La taille du génome mesurée était en moyenne de 0,24 pg·cellule^{-1} pour *Chrysosphaerella longispina* Lauterborn, de 1,70 pg·cellule^{-1} pour *Neotessella lapponica* (Skuja) B.Y.Jo, J.I.Kim, W.Shin, P.Škaloud & P.A.Siver, de 0,25 pg·cellule^{-1} pour *Uroglenopsis turfosa* (Skuja) R.H.Thompson & D.E.Wujek, de pg·cellule^{-1} pour *Urostipulosphaera articulata* (Korshikov) Pusztai & Škaloud, de 0,22 pg·cellule^{-1} pour *U. granulata* Pusztai & Škaloud et de 0,19 pg·cellule^{-1} pour *U. lindiae* (Bourrelly) Pusztai & Škaloud. Nous avons de plus démontré que les chrysophytes coloniales ont de plus grands génomes que les espèces solitaires. Cette étude a enfin révélé le plus petit génome parmi les chrysophytes coloniales, qui appartient à *Urostipulosphaera lindiae*. Néanmoins, les chrysophytes coloniales ont une taille du génome moins variable que les chrysophytes solitaires, probablement en raison de contraintes évolutives sur la variation de la taille des cellules (et de la taille du génome) pour maintenir la fonctionnalité du mouvement de l'ensemble de la colonie.

MOTS CLÉS
Chrysophytes coloniales, taille du génome, *Urostipulosphaera*, *Uroglenopsis*, *Chrysosphaerella*, *Neotessella*.

INTRODUCTION

The nuclear genome is a fundamental component of the cell, and the knowledge of its size has become a prerequisite for many areas of research. Firstly, the DNA amount is essential for selecting suitable strains for whole-genome sequencing, since the DNA content directly influences the cost of a sequencing project (Gregory 2005). The DNA amount is also key to designing an optimal sequencing strategy (e.g. sequencing coverage or sequencing technology selection). Furthermore, the nuclear DNA content, at least in relative units, is essential for cell cycle determination (Lemaire *et al.* 1999; Reinecke *et al.* 2018). Genome size can also be used as a tool to distinguish cryptic species based on their differences in genome size (Figueroa *et al.* 2010). Although genome size does not correlate with overall biological complexity, it is known to correlate with many features at the cellular and organismal level. The amount of nuclear DNA determines nucleus size, and this also affects the size of the cell (Cavalier-Smith 2005). The genome size-cell size correlation may have further consequences for metabolic rates, the duration of mitosis and meiosis, or generation time (Gregory 2002b). In recent years, there has been an increasing number of studies linking genome size to species ecophysiology (e.g. Pandit *et al.* 2014; Leinaas *et al.* 2016; Womack *et al.* 2019; Stelzer *et al.* 2021). Genome size may be associated with tolerance to stressful environmental conditions (Nardon *et al.* 2005), ecological niche breadth (Pyšek *et al.* 2018) or even speciation and diversification rates (Igea *et al.* 2017). Taken this together, genome size is one of the most fundamental and irreducible traits of an organism.

The most suitable method for precise and rapid estimation of nuclear DNA content is flow cytometry (FCM). Using FCM enables us to detect fluorescent-stained particles (nuclei) in a stream of fluid (Doležel *et al.* 2007). While genome size data are available and relatively easy to obtain for many plant and animal species, they are largely lacking for algae, especially microalgae (Čertnerová 2021a; Čertnerová & Galbraith 2021). One of the reasons for this is that FCM of microalgae comes with many methodological challenges. Microalgae very often possess various complex cell walls and secondary metabolites. Sample preparation therefore often requires extraction of protoplasts specifically optimized for the studied group of microalgae (Čertnerová & Galbraith 2021). In addition, a unialgal, clonal (and if possible axenic) culture with a considerable amount of biomass is needed. It has been shown that 10^5 cells is the minimum number of cells necessary for the successful analysis (Lemaire *et al.* 1999; Parrow & Burkholder 2002; Olefeld *et al.* 2018). For microalgae cultivated in a liquid medium which can reach high population densities, only 1 mL of well-grown culture can be used for successful FCM analysis (e.g. haptophyte *Prymnesium parvum* N.Carter or some chrysophyte species; [Čertnerová *et al.* 2022; Kuhl *et al.* 2024]). However, for some microalgal species that cannot grow to such high concentrations, much higher culture volumes are required to achieve sufficient biomass. For example, 15 ml of well-grown culture was needed for FCM analysis of some diatom species (Connolly *et al.* 2008). Even

TABLE 1. — Collection details for the strains of colonial chrysophytes used in this study.

Species	Strain	Collection site	GPS coordinates	Sampling date	*in situ* measured environmental variables: pH	Conductivity (µS cm-1)	Water temperature (°C)
	JAP	Nishionuma, Kameda District, Hokkaidó, Japan	41°59'36.62"N, 140°37'59.86"E	10.IX.2016	6.5	68	20.1
Chrysosphaerella longispina Lauterborn	J70	Little Gull Lake, Newfoundland, Canada	48°22'27.336"N, 55°28'15.996"W	28.V.2017	6.8	44	19
	IR34Ch	Lough Nagladary, Ireland	55°0'4.972"N, 8°18'32.969"W	02.X.2011	5.6	119	15.5
Neotessella lapponica (Skuja) B.Y.Jo, J.I.Kim, W.Shin, P.Škaloud & P.A.Siver	CZ38O	Mariánský pond, Czech Republic	50°32'43.836"N, 14°40'36.084"E	04.V.2020	6.4	121	10.8
Uroglenopsis turfosa (Skuja) R.H.Thomps. & Wujek	UK-81 CAN	Expoits River oxbow lake, Newfoundland, Canada	48°56'32.450"N, 55°46'9.344"W	29.V.2017	7.8	37	11
Urostipulosphaera articulata (Korshikov) Pusztai & Škaloud	U5-5 CZE	Kříž pond, Na Plachtě, Czech Republic	50°10'58.015"N, 15°52'12.972"E	03.XII.2014	8.4	704	2
	U7-1 CZE	Pool in Botanical Garden, Prague, Czech Republic	50°4'15.901"N, 14°25'14.311"E	06.II.2015	6.9	605	0.4
Urostipulosphaera granulata Pusztai & Škaloud	U33 CZE	Small pond near Milíčovský pond, Prague, Czech Republic	50°1'31.912"N, 14°32'6.521"E	06.III.2017	NA	NA	NA
Urostipulosphaera lindiae (Bourr.) Pusztai & Škaloud	UP-34 POR	Lagao do Viriato, Portugal	40°18'48.768"N, 7°33'58.003"W	04.IV.2015	7.4	38	NA

more, 50 ml of culture, was necessary to successfully analyse the DNA content of some dinoflagellate species (Parrow & Burkholder 2002; Kremp & Parrow 2006). Obtaining enough biomass may be particularly challenging for microalgae, as they are often difficult to grow and/or maintain under long-term cultivation. This may be particularly true for some extremely fragile, poorly growing colonial chrysophytes (e.g. species of the genera *Uroglena* Ehrenberg, *Uroglenopsis* Lemmermann and *Urostipulosphaera* Pusztai & Škaloud; [Wujek & Thompson 2002; Pusztai & Škaloud 2019]). These taxa are therefore very rare in the world's algal collections. In this study, we aim to estimate the genome size of nine extremely fragile colonial chrysophytes and to compare these rare data with other published genome size data for representatives of the Chrysophyceae.

MATERIAL AND METHODS

Origin, cultivation and species determination of the investigated strains

To establish new cultures of colonial chrysophytes, water samples were collected from various freshwater bodies using a 20 µm mesh plankton net. At each site, abiotic factors including water pH, temperature and specific conductivity were measured using a combined pH/conductometer (WTW 340i; WTW GmbH, Weilheim, Germany). Collected samples were kept in a polystyrene box with a cooling gel pad for a few hours until they were processed in the laboratory. Individual colonies were captured by micropipetting and transferred into separate culture wells filled with TES-buffered WC medium (Guillard & Lorenzen 1972). Cultures were maintained at 15°C (cooling box Pol-Eko Aparatura Sp.J., model ST 1, Wodzisław Śląski, Poland) with a 24-hour light mode under illumination of 30 µmol m^{-2} s^{-1} (TLD 18 W/33 fluorescent lamps, Philips, Amsterdam, The Netherlands).

The species of the new chrysophyte strains were determined using a transmission electron microscope (TEM). The samples were transferred onto Formvar-coated copper grids. The grids with the sample were then subsequently washed in four drops of distilled water and air dried before further examination. The grids were examined using a TEM Jeol 1011 with integrated CCD camera Velvet (Olympus Soft Imaging Solution GmbH, Münster, Germany).

To broaden our dataset, four *Urostipulosphaera* and one *Uroglenopsis* strain from Pusztai & Škaloud (2019) and Pusztai & Škaloud (2022) were included in this study. Subsequently, all the investigated strains were transferred into Erlenmeyer flasks filled with 50 mL of TES-buffered WC medium and kept for longer cultivation. The sampling details of all strains used in this study are listed in Table 1.

Genome size estimation

To estimate genome size of the investigated strains, we employed propidium iodide flow cytometry (PI FCM). Approximately four to six weeks before the planned FCM analyses, cultures were inoculated into fresh medium. Either 20 mL (in the case of *Chrysosphaerella longispina* Lauterborn)

TABLE 2. — Genome size of nine strains of colonial chrysophytes analysed in this study.

Species	Strains	Genome size (pg)	Genome size (*c.* Mbp)
Chrysosphaerella longispina Lauterborn	JAP	0.24 ± 0.002	233
	J70	0.19 ± 0.003	186
	IR34Ch	0.29 ± 0.004	281
Neotessella lapponica (Skuja) B.Y.Jo, J.I.Kim, W.Shin, P.Škaloud & P.A.Siver	CZ38O	1.70 ± 0.020	1662
Uroglenopsis turfosa (Skuja) R.H.Thomps. & Wujek	UK-81 CAN	0.25 ± 0.005	246
Urostipulosphaera articulata (Korshikov) Pusztai & Škaloud	U5-5 CZE	0.31 ± 0.004	303
Urostipulosphaera granulata Pusztai & Škaloud	U7-1 CZE	0.21 ± 0.004	208
	U33 CZE	0.22 ± 0.004	217
Urostipulosphaera lindiae (Bourr.) Pusztai & Škaloud	UP-34 POR	0.19 ± 0.004	182

or 50 mL of culture in its exponential phase of growth was used for a sample preparation. Each sample was centrifuged (5 min, 2040 *g*; Eppendorf) and the superfluous medium was removed by pipetting. Consequently, 350 µL of ice-cold nuclei isolation buffer Otto I (0.1 M citric acid, 0.5% Tween 20; Otto 1990) was added to the algal pellet, causing an osmotic rupture of the cells and release the sample nuclei. The resulting suspension was thoroughly shaken and kept on ice. The plant *Solanum pseudocapsicum* L., commercial clone (2C = 2.59 pg; Temsch *et al.* 2010) was used as a (pseudo) internal standard for *Neotessella lapponica* (Skuja) B.Y.Jo, J.I.Kim, W.Shin, P.Škaloud & P.A.Siver and the plant *Carex acutiformis* Ehrh., wild clone (2C = 0.82 pg; Veselý *et al.* 2012) was used as a (pseudo) internal standard for the remaining samples. To release the nuclei of the standard, *c.* 20-mg piece of fresh leaf tissue was chopped with a razor blade in a plastic Petri dish containing 250 µL of ice-cold Otto I buffer. Both suspensions (with algal and standard nuclei) were thoroughly mixed and filtered through a 42 µm nylon mesh into a special 3.5-mL cuvette for direct use with the flow cytometer. After 20 min incubation at room temperature, the sample was mixed with 1 mL of staining solution consisting of Otto II buffer (0.4 M Na2HPO4 × 12H2O; Otto 1990), 50 µg × mL^{-1} PI, 50 µg × mL^{-1} RNase IIA and 2 µL × mL^{-1} β-mercaptoethanol. The stained sample was immediately analysed using a Partec CyFlow SL cytometer (Partec GmbH, Münster, Germany) equipped with a green solid-state laser (Cobolt Samba, 532 nm, 100 mW). In each sample, 5000 particles were measured and the resulting FCM histograms were analysed using FloMax ver. 2.4d (Partec). The first sample peak in the FCM histogram was identified as G1 (vegetative cells) and a second peak with twice the relative fluorescence (if visible) as G2 (dividing cells). The absolute nuclear DNA content (C-value) was calculated as sample G1 peak mean fluorescence / standard G1 peak mean fluorescence × standard 2C DNA content (according to Doležel 2005). To minimize the effect of random instrumental shift, each strain was analysed at least three times on separate days and the estimates averaged. Each time the three independent DNA content estimates differed by >4 %, the most outlying measurement was discarded, and a new measurement was carried out. Nuclear DNA content is reported in absolute units per cell (pg of DNA and equivalent values in Mbp).

Genome size of colonial versus solitary living chrysophytes

Statistical analyses were conducted in the R programming language v.4.3.3 (R Core Team 2022). To test the difference in genome size between colonial chrysophytes and solitary living chrysophytes, we summarized the published genome size data for chrysophytes. The data for nuclear DNA content are listed in absolute units per cell (pg·cell^{-1}). For the following analysis, we averaged genome size data for the same species and removed taxa that were identified only to genus and where it was not clear whether they could belong to the same species (Appendix 1). Analysis of variance (ANOVA) was used to test the difference between genome size of colonial and solitary living chrysophytes.

RESULTS

In this study, we established long-term cultivations of nine colonial chrysophytes and taxonomically determined new strains as three strains of *Chrysosphaerella longispina* and one strain of *Neotessella lapponica* (Fig. 1). We successfully estimated the absolute nuclear DNA amount in all investigated strains (Table 2; Appendix 2). The flow cytometric measurements were precise, resulting in clearly delimited peaks in FCM histograms, with relatively low CVs for both sample and standard G_1 nuclei peaks (mean CV = 1.84 and 3.34 %, respectively) and the average coefficient of variation among repeated estimates was 3.1 % (see Figure 2 for a representative analysis).

The genome size of the analysed strains ranged from 0.19 pg (*c.* 182 Mbp) in *Urostipulosphaera lindiae* to 1.7 pg (*c.* 1662 Mbp) in *Neotessella lapponica*.

The analysis of variance (ANOVA) detected significant differences in average genome size between colonial and solitary living chrysophytes, showing that colonial chrysophytes have higher DNA contents ($F_{1.72}$ = 20.06, P <0.001, Fig. 3).

DISCUSSION

Microalgae (and other unicellular eukaryotes) are a very promising group to study genome size variability and its consequences because of their low body complexity, short

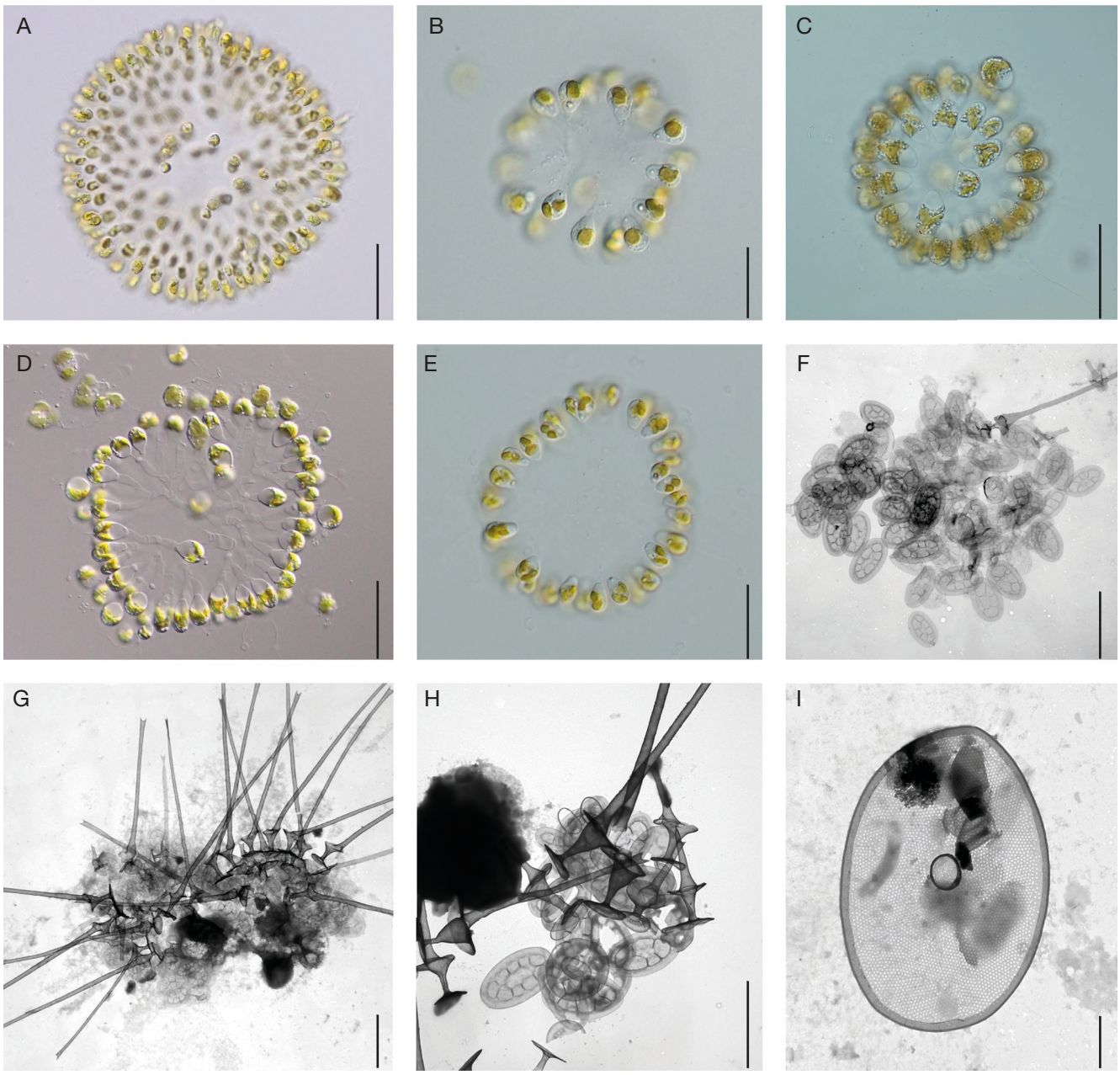

Fig. 1. — Microphotographs of colonies and scales of nine colonial chrysophytes analysed in this study: **A**, *Uroglenopsis turfosa* (Skuja) R.H.Thompson & D.E.Wujek (strain UK-81 CAN); **B**, *Urostipulosphaera articulata* (Korshikov) Pusztai & Škaloud (strain U5-5 CZE); **C**, *U. granulata* Pusztai & Škaloud (strain U7-1 CZE); **D**, *U. granulata* (strain U33 CZE); **E**, *U. lindiae* (Bourrelly) Pusztai & Škaloud (strain UP-34 POR); **F**, *Chrysosphaerella longispina* Lauterborn (strain JAP); **G**, *C. longispina* (strain J70); **H**, *C. longispina* (strain IR34Ch); **I**, *Neotessella lapponica* (Skuja) B.Y.Jo, J.I.Kim, W.Shin, P.Škaloud & P.A.Siver (strain CZ38O). Scale bars: A, 50 µm; B-E, 20 µm; F-H, 5 µm; I, 1 µm.

generation time, large population sizes, quick response to environmental changes, or because they can be easily maintained in the laboratory under highly controlled conditions (Lynch & Conery 2003; Foissner 2007; Ribeiro *et al.* 2013). Despite these advantages, there is a widespread lack of studies on this topic, and genome size data for microalgae are largely lacking. Although data for chrysophytes are also very limited (especially lacking for the difficult to cultivate species), they are becoming the most intensively studied group of microalgae due to the increasing number of genome size studies in recent years (e.g. Olefeld *et al.* 2018; Čertnerová & Škaloud 2020; Majda *et al.* 2021; Čertnerová *et al.* 2022; Čertnerová *et al.* 2025). To date, it has been shown that the genome size among Chrysophyceae varies widely, from 0.09 pg·cell^{-1} (*c.* 88 Mbp) in unknown heterotrophic chrysophyte flagellate to almost 25 pg·cell^{-1} (*c.* 24.3 Gbp) in *Mallomonas caudata* Iwanoff [Ivanov] (Olefeld *et al.* 2018). Although the largest genome belongs to the solitary living chrysophyte, the colonial species tend to have larger genomes compared to the solitary living species (Fig. 2). This is despite the fact that the solitary living chrysophytes have a greater variance in genome size. This may be linked to the strong positive correlation between genome

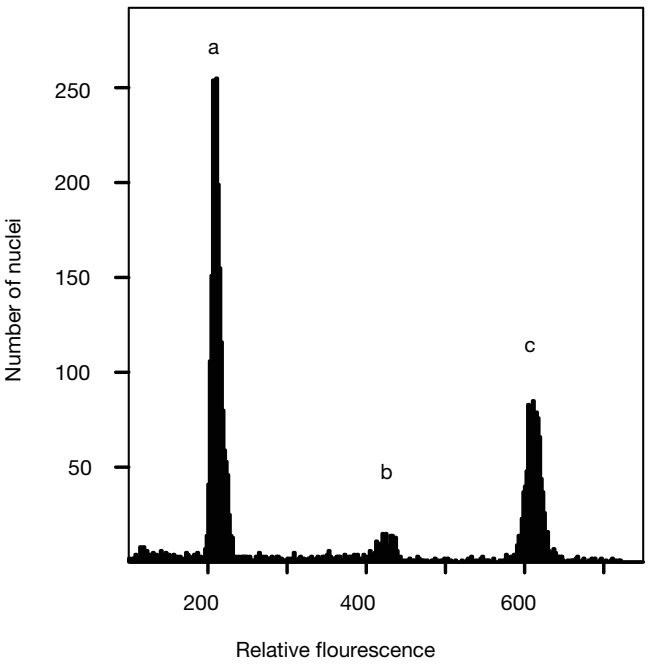

Fig. 2. – A representative flow cytometric analysis showing relative fluorescence of propidium iodide-stained nuclei of chrysophyte *Chrysosphaerella longispina* Lauterborn (strain IR34Ch) with G1 (**a**) and G2 phase (**b**) nuclei and the reference standard (*Carex acutiformis* Ehrh.; **c**).

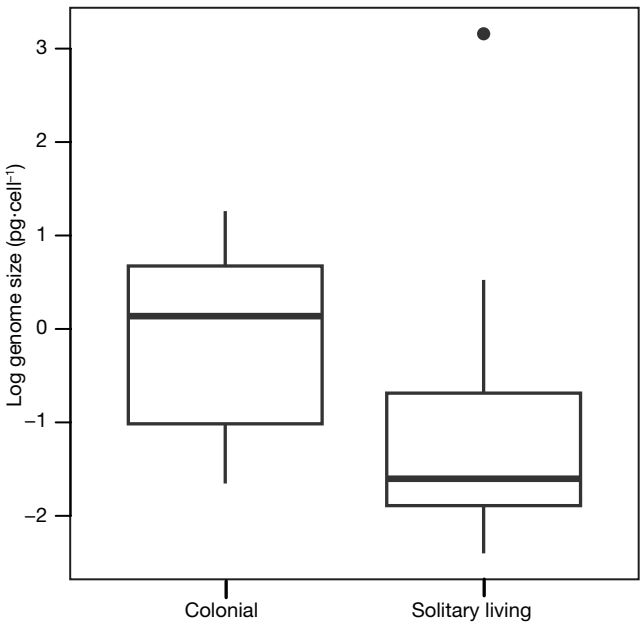

Fig. 3. — Boxplot showing logarithmically transformed genome sizes of colonial and solitary 429 living chrysophytes.

size and cell size (e.g. Čertnerová & Škaloud 2020 and the references therein) and the movement of the whole colony. Excessive changes in cell size could act as a constraint on the functional movement of the colony. In contrast, solitary living chrysophytes are not under the same evolutionary pressure and may therefore allow their cells (and their genomes) to vary more extensively.

The lack of genome size data makes it difficult to compare this trend with other groups of microalgae. For example, the green microalgae Chlorophyceae also include both solitary living and colonial taxa. However, from a handful of species analysed for genome size within this group, there does not seem to be the same trend. The solitary living species such as *Chlamydomonas noctigama* Korschikov or *Dunaliella tertiolecta* Butcher have slightly larger genomes (0.33 and 1.38 pg·cell^{-1}, respectively; Čertnerová 2021b; Veldhuis *et al.* 1997) compared to their colonial counterparts such as *Volvox carteri* f. *nagariensis* M.O.P.Iyengar or *Eudorina californica* (Shaw) Goldstein (0.14 and 0.17 pg·cell^{-1}, respectively; Prochnik *et al.* 2010; Tautvydas 1976). However, much more genome size data would be needed to determine whether there is a general tendency for small or large genomes in colonial *v.* solitary living species among all microalgae.

In this study, we also revealed the smallest genome among colonial chrysophytes. It belongs to *Urostipulosphaera lindiae* with a genome size of 0.19 pg·cell^{-1} (*c.* 182 Mbp). It seems that *Uroglena*-like taxa tend to have small genomes. Two other strains analysed for *Urostipulosphaera granulata* (U7-1 CZE, U33 CZE) also have very small genomes and very similar genome sizes (0.21 and 0.22 pg·cell^{-1}, *c.* 208 and 217 Mbp, respectively). However, it should be noted that both strains were collected from geographically close localities in Central Europe (although they were collected more than two years apart). Except for the *Uroglena*-like taxa analysed in this study, *Uroglena* sp. (strain WA34K-E) was previously analysed in Olefeld *et al.* (2018) and also has a small genome (0.32 pg·cell^{-1}, *c.* 318 Mbp).

The second smallest genome among colonial chrysophytes belongs to *Synura leptorrhabda* (Asmund) K.H.Nicholls (strain H92) and to *Chrysosphaerella longispina* (strain J70) with the identical genome size of 0.19 pg·cell^{-1} (*c.*186 Mbp; Čertnerová *et al.* 2025, this study). Interestingly, three strains of *Chrysosphaerella longispina* analysed in this study originate from three geographically very distinct locations (North America, Europe, East Asia), yet their genome size differs relatively insignificantly (from 0.19 to 0.29 pg·cell^{-1} *c.*186 – 281 Mbp). The genome size of another *Chrysosphaerella longispina* strain (E17) was previously analysed in the study by Čertnerová *et al.* (2022), which focused on the alternation of life stages within the haploid-diploid life cycle. The strain was shown to alternate between 1C with 0.23 pg·cell^{-1} and 2C with 0.50 pg·cell^{-1}. Th e DNA content of the strains analysed in this study therefore corresponds to the 1C stage of the life cycle. Interestingly, while the 2C stage predominated in the life cycle of strain E17 (Čertnerová *et al.* 2022), no life stage alternation was observed in the strains analysed in this study.

Genome size analysis of these poorly growing and fragile colonial chrysophytes would not be possible without establishing at least a temporary culture and a large amount of input culture (tens of mL). Alternatively, genome size analysis of these colonial chrysophytes could be carried out directly from the bloom in the field (skipping the cultivation step),

although species identification using molecular markers (which is very often required) could then be difficult due to frequent contamination with other protists and bacteria.

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APPENDICES

Appendix 1. — List of published data on the chrysophyte genome size, averaged for each species, with the addition of the new data provided in this study.

		Genome size		
Taxon	**Type**	**(pg)**	**(Mbp)**	**Original reference**
Acrispumella msimbasiensis Boenigk & Grossmann	solitary living	0.15	147	Olefeld *et al.* 2018
Bitrichia sp. Woloszynska	solitary living	0.60	584	Olefeld *et al.* 2018
Cornospumella fuschlensis Boenigk & Grossmann	solitary living	0.15	143	Olefeld *et al.* 2018
Dinobryon bavaricum O.E.Imhof	colonial	0.32	317	Olefeld *et al.* 2018
Dinobryon divergens O.E.Imhof	colonial	0.33	322	Olefeld *et al.* 2018
Dinobryon pediforme (Lemmerm.) Steinecke	colonial	0.23	227	Olefeld *et al.* 2018
Dinobryon sociale Ehrenb.	colonial	0.27	268	Olefeld *et al.* 2018
Dinobryon sociale Ehrenberg var. americana Brunthaler cf. div. *schauinslandii*	colonial	0.36	355	Olefeld *et al.* 2018
Epipyxis sp. Ehrenberg	solitary living	0.20	193	Olefeld *et al.* 2018
Chlorochromonas danica I.F.Lewis	solitary living	0.22	214	Gibbs *et al.* 1974; Cattolico & Gibbs 1975; Charles 1977; Olefeld *et al.* 2018
Chromulinospumella sphaerica (Valkanov) Boenigk & Grossmann	solitary living	0.16	157	Olefeld *et al.* 2018
Chrysosaccus sp. Pascher	solitary living	0.13	128	Veldhuis *et al.* 1997
Chrysosphaerella longispina Lauterborn	colonial	0.24	233	this study
Kephyrion sp. Pascher	solitary living	0.14	141	Olefeld *et al.* 2018
Mallomonas annulata (Bradley) Harris	solitary living	0.69	672	Olefeld *et al.* 2018
Mallomonas caudata Iwanoff [Ivanov]	solitary living	23.43	22910	Olefeld *et al.* 2018
Mallomonas cf. *tonsurata*	solitary living	1.68	1639	Olefeld *et al.* 2018
Mallomonas kalinae Mallomonas Perty	solitary living	0.59	581	Olefeld *et al.* 2018
Melkoniania moestrupii R.A.Andersen & H.-S.Yoon	solitary living	0.50	490	Veldhuis *et al.* 1997
Neotessella lapponica B.Y.Jo, J.I.Kim, W.Shin, P.Škaloud & P.Siver	colonial	1.70	1662	this study
Ochromonas sp. Vysotskii	colonial	0.57	559	Veldhuis *et al.* 1997
Pedospumella encystans Boenigk & Findenig	solitary living	0.17	159	Olefeld *et al.* 2018
Pedospumella sinomuralis Boenigk & Findenig	solitary living	0.32	309	Olefeld *et al.* 2018
Posterioochromonas malhamensis Scherffel	solitary living	0.15	151	Olefeld *et al.* 2018
Posteriospumella lacustris Boenigk & Findenig	solitary living	0.15	143	Olefeld *et al.* 2018
Segregatospumella dracosaxi Boenigk & Grossmann	solitary living	0.09	89	Olefeld *et al.* 2018
Spumella lacusvadosi Cienkowsky	solitary living	0.31	302	Olefeld *et al.* 2018
Spumella rivalis Cienkowsky	solitary living	0.10	98	Olefeld *et al.* 2018
Spumella vulgaris Cienkowski	solitary living	0.30	294	Olefeld *et al.* 2018
Synura americana Ehrenberg	colonial	2.24	2187	Čertnerová *et al.* 2022; Čertnerová *et al.* 2025
Synura bjoerkii Ehrenberg	colonial	3.09	3022	Čertnerová *et al.* 2025
Synura borealis Ehrenberg	colonial	2.02	1978	Čertnerová *et al.* 2025
Synura conopea Kynclova & Škaloud	colonial	1.95	1909	Čertnerová *et al.* 2025
Synura cornuta Ehrenberg	colonial	1.78	1736	Čertnerová *et al.* 2025
Synura curtispina (J.B.Petersen & J.B.Hansen) Asmund	colonial	1.92	1875	Čertnerová *et al.* 2025
Synura echinulata Korshikov	colonial	0.30	292	Čertnerová *et al.* 2025
Synura fluviatilis Ehrenberg	colonial	0.89	867	Čertnerová *et al.* 2025
Synura glabra Ehrenberg	colonial	1.96	1921	Čertnerová *et al.* 2022
*Synura heteropora*Škaloud, Škaloudova & Prochazkova	colonial	1.47	1436	Olefeld *et al.* 2018; Čertnerová *et al.* 2022
Synura hibernica Ehrenberg	colonial	1.80	1757	Čertnerová *et al.* 2025
Synura lanceolata Ehrenberg	colonial	1.02	993	Čertnerová *et al.* 2025
Synura laticarina Ehrenberg	colonial	1.83	1790	Čertnerová *et al.* 2025
Synura leptorrhabda (Asmund) K.H.Nicholls	colonial	0.28	277	Čertnerová *et al.* 2025
Synura macropora Škaloud & Kynclova	colonial	3.24	3164	Čertnerová *et al.* 2022
Synura petersenii Korshikov	colonial	1.29	1257	Čertnerová & Škaloud 2020; Škaloud *et al.* 2024
Synura praefracta Ehrenberg	colonial	1.08	1056	Čertnerová *et al.* 2025
Synura rubra Ehrenberg	colonial	0.96	934	Čertnerová *et al.* 2025
Synura sp. Ehrenberg	colonial	0.90	880	Čertnerová *et al.* 2025
Synura sp.	colonial	0.98	958	Čertnerová *et al.* 2025
Synura sp.	colonial	1.06	1037	Čertnerová *et al.* 2025
Synura sp.	colonial	1.09	1066	Čertnerová *et al.* 2025
Synura sp.	colonial	1.46	1428	Čertnerová *et al.* 2025
Synura sp.	colonial	1.46	1428	Čertnerová *et al.* 2025
Synura sp.	colonial	1.61	1575	Čertnerová *et al.* 2025
Synura sp.	colonial	1.96	1917	Čertnerová *et al.* 2025
Synura sp.	colonial	2.15	2103	Čertnerová *et al.* 2025
Synura sp.	colonial	2.21	2161	Čertnerová *et al.* 2025
Synura sp.	colonial	2.22	2171	Čertnerová *et al.* 2025

Appendix 1. — Continuation.

Taxon	Type	Genome size (pg)	Genome size (Mbp)	Original reference
Synura sp.	colonial	2.25	2201	Čertnerová *et al.* 2025
Synura sp.	colonial	2.27	2220	Čertnerová *et al.* 2025
Synura sp.	colonial	2.41	2357	Čertnerová *et al.* 2025
Synura sp.	colonial	3.51	3433	Čertnerová *et al.* 2025
Synura sphagnicola (Korshikov) Korshikov	colonial	0.43	425	Čertnerová *et al.* 2022
Synura spinosa Korshikov	colonial	1.73	1687	Čertnerová *et al.* 2025
Synura splendida Korshikov	colonial	1.11	1086	Čertnerová *et al.* 2025
Synura synuroidea (Prowse) Pusztai, Čertnerová, Škaloudová & Škaloud	colonial	0.30	293	Čertnerová *et al.* 2025
Synura truttae Ehrenberg	colonial	1.36	1330	Čertnerová *et al.* 2025
Synura uvella Korshikov	colonial	0.80	782	Čertnerová *et al.* 2025
Synura vinlandica Ehrenberg	colonial	1.14	1115	Čertnerová *et al.* 2025
Uroglena sp. Ehrenberg	colonial	0.32	318	Olefeld *et al.* 2018
Uroglenopsis turfosa (Skuja) R.H.Thomps. & Wujek	colonial	0.25	246	this study
Urostipulosphaera articulata (Korshikov) Pusztai & Škaloud	colonial	0.31	303	this study
Urostipulosphaera granulata Pusztai & Škaloud	colonial	0.22	213	this study
Urostipulosphaera lindiae (Bourr.) Pusztai & Škaloud	colonial	0.19	182	this study

Appendix 2. — Summary statistics of the flow cytometry data of colonial chrysophytes analysed in this study. Abbreviation: **CV**, coefficient of variation of nuclear fluorescence intensity.

Species	Strains	Replicate	Sample/standard ratio of relative fluorescence	Genome size (pg)	Genome size (~Mbp)	Sample CV (%)	Standard CV (%)
Chrysosphaerella longispina Lauterborn	JAP	1	0.287	0.24	230	1.63	4.14
		2	0.290	0.24	232	1.38	2.80
		3	0.293	0.24	235	1.34	2.75
	J70	1	0.234	0.19	188	1.76	2.95
		2	0.233	0.19	187	1.61	1.88
		3	0.227	0.19	182	1.53	3.10
	IR34Ch	1	0.354	0.29	284	2.01	1.93
		2	0.345	0.28	276	1.80	2.52
		3	0.352	0.29	282	2.21	4.79
Neotessella lapponica B.Y.Jo, J.I.Kim, W.Shin, P.Škaloud & P.Siver	CZ38O	1	0.650	1.68	1645	1.36	2.44
		2	0.665	1.72	1683	2.36	3.43
		3	0.654	1.69	1657	1.92	2.94
Uroglenopsis turfosa (Skuja) R.H.Thomps. & Wujek	UK-81 CAN	1	0.302	0.25	242	1.93	4.28
		2	0.307	0.25	246	3.53	4.96
		3	0.313	0.26	251	2.25	6.45
Urostipulosphaera articulata (Korshikov) Pusztai & Škaloud	U5-5 CZE	1	0.383	0.31	307	1.72	2.80
		2	0.376	0.31	302	1.74	2.48
		3	0.372	0.31	299	2.32	2.79
Urostipulosphaera granulata Pusztai & Škaloud	U7-1 CZE	1	0.263	0.22	211	1.66	3.49
		2	0.253	0.21	203	2.13	3.03
		3	0.261	0.21	209	2.27	4.41
	U33 CZE	1	0.265	0.22	213	1.24	4.30
		2	0.275	0.23	221	1.66	3.66
		3	0.269	0.22	216	2.05	3.03
Urostipulosphaera lindiae (Bourr.) Pusztai & Škaloud	UP-34 POR	1	0.224	0.18	180	1.87	3.56
		2	0.223	0.18	179	1.36	2.99
		3	0.233	0.19	187	1.14	2.32