





https://doi.org/10.11646/phytotaxa.609.1.5

Coccolithophores in the algal flora from South Urals (Russia) with the description of a new *Hymenomonas* species

MARINA IGNATENKO^{1,2*} & TATYANA YATSENKO-STEPANOVA^{1,3}

¹ Institute for Cellular and Intracellular Symbiosis of the Ural Branch of the Russian Academy of Sciences of the Orenburg Federal Research Center RAS, Pionerskaya Street, 11, 460000, Orenburg, Russia

² = ignatenko_me@mail.ru; ⁶ https://orcid.org/0000-0002-4451-7816

³ scenkostn@gmail.com; ^b https://orcid.org/0000-0001-6168-9516

**Corresponding author:* \blacksquare *ignatenko_me@mail.ru*

Abstract

This paper focuses on the study of the diversity of coccolithophores in the reservoirs in the steppe zone of the South Urals (Russia). Four species of coccolithophores were identified in the studied reservoirs by scanning electron microscopy, namely *Hymenomonas roseola, Jomonlithus littoralis, Chrysotila carterae* and *C. roscoffensis*. Of these, *H. roseola* is registered in freshwater, *J. littoralis*—in the salinity range from 0.6 to 5.7‰, *C. carterae* and *C. roscoffensis*—at salinity 3.8–8.1‰. *J. littoralis, C. carterae* and *C. roscoffensis* are reported for the first time in Russia. A wide range of halotolerances of *J. littoralis, C. carterae* and *C. roscoffensis* is suggested. We noted morphological variability in the ornamentation of placoliths of *C. roscoffensis*. The description and microphotographs of unidentified coccoliths found in freshwater, which represent a species new to science, are also given.

Keywords: Coccolithophyceae, coccoliths, diversity, Haptophyta, scanning electron microscopy

Introduction

Coccolithophores belong to a diverse group of protists of the phylum Haptophyta, a distinctive feature of which is the presence of calcified scales (coccoliths) covering the cell. Coccolithophorids emerged in the late Triassic (about 220-225 million years ago) and by the Cretaceous Period became the main component of algae communities of the world ocean (Nicholls 2015; Godrijan et al. 2022). Currently, 300-400 species of haptophyte algae known to date, about two-thirds reside within the clade of calcifying haptophytes (Nicholls 2015; Eikrem et al. 2016; Henderiks et al. 2022). Coccolithophores are distributed all over the world. Their greatest species diversity is noted in subtropical and tropical waters (over 100 species), diversity decreasing toward the high latitudes (<10 species in subpolar waters) (Jordan 2009; Balch et al. 2019). These are mainly marine organisms, that supply from 10 to 20% of the total volume of primary production of phytoplankton of the ocean, according to various estimates, they are also responsible for about half of all modern carbonate sedimentation in the ocean (Tyrrell & Young 2009; Nicholls 2015; Pautova et al. 2020; Meng et al. 2022). Among coccolithophores, there are also known species inhabiting brackish and salt coastal and inland waters, including estuaries, lagoons, lakes, ponds (Johansen et al. 1988; Reifel et al. 2001; Seoane et al. 2009; Nicholls 2015). Only one species, Hymenomonas roseola F. Stein (1878: legend to pl. XIV), is found in freshwater (there are data about another freshwater coccolithophores species detected from a stream in Colorado, USA, but this species has not been described) (Dashiell 2010; Nicholls 2015). Some coccolithophores form large and sometimes regularly occurring "blooms" both in the world ocean and inland waters (Reifel et al. 2001; Tyrrell & Young 2009; Eikrem et al. 2016; Pautova et al. 2020).

The identification of this group of microorganisms is based on the study of the morphology of coccoliths using scanning electron microscopy, therefore, the diversity of coccolithophores is often underestimated in studies using only by light microscopy (Seoane *et al.* 2009).

This study is aimed at identifying the diversity of calcifying haptophytes in the reservoirs of the steppe zone of the South Urals (Russia) using scanning electron microscopy.

Material and methods

Integrated water samples (plankton, epipelon and epilithon) were collected from four different types of reservoirs from South Urals, Russia (Fig. 1, Table 1). The samples were preserved with 4% formaldehyde solution. Environmental variables (temperature, pH and salinity) measurements were performed using HANNA HI98127 (HANNA Instruments Inc., USA) portable device and ANION 4100 laboratory analyzer (Russia).

TABLE 1. List of the sampling sites with environment	tal variables (T-temperature	e, S-salinity and n/a-	-no parameters
were measured).			

N₂	Site name	Location	Date	Coordinates	T ⁰C	pН	S, ‰
1	Ural River	Ural River in the vicinity of the Belyaevka	27.07.2021	51°25'55"N,	22.7	n/a	0.2
		village, Orenburg Region		56°28'58"E			0.5
2	Ural River	A backwater of the Ural River in the vicinity of	27.07.2021	51°20'59"N,	24.7	n/a	0.6
		the Nikolskoye village, Orenburg Region		57°06'18"'E			0.0
3	Ushkotinsky	Orenburg Region	09.11.2022	50°43'46"N,	1.3	7.52	0.3
	Reservoir			59°57'54"E			
4	Zhetykol Lake	"Svetlinsky" Biological Reserve, Orenburg	02.06.2022	51°05'22"N,	19.8	7.53	5.7
				60°53'07"E			
5 Z	Zhetykol Lake	Region	00.06.0000	51°05'67"N,	22.5	9.1	3.8
			02.06.2022	60°29'25"'E			
6	Aschisaysky Pond	Ashchisai steppe, "Orenburgskiy" State Nature	27.08.2021	51°02'35"N,	n/a	8.9	0.1
		Reserve, Orenburg Region 27.		61°11'33"E			8.1

For electron microscopical studies, an aliquot of the water sample was washed with deionized water several times to remove the fixative, placed onto aluminum stubs, air-dried and sputter-coated with gold using Quorum Q150R S Plus sputter coater. Morphology of coccoliths was studied using TESCAN Mira 3 scanning electron microscope ("Gagarin" Center for the identification and support of talented children, Orenburg Region, Russia).

For descriptors of heterococcolith shape we use the terms proposed by Young et al. (2003):

a) placolith—a disc-shaped coccolith with two shields connected by a tube; formerly, the term "cricolith" was used to describe coccoliths of a similar shape (Young *et al.* 1997);

b) murolith—a bowl-shape coccolith with well-defined base, open central-area and subvertical sides; formerly, the term "tremalith" was used to describe coccoliths of a similar shape (Young *et al.* 1997).

The specimens deposited at the Herbarium of the Steppe Institute of the Ural Branch of the Russian Academy of Sciences, Orenburg (ORIS).

Results

In the studied samples we found characteristic coccoliths of the species from the genera *Chrysotila* P.L. Anand (1936: 282) (*=Pleurochrysis* E.G. Pringsheim (1955: 409)), *Hymenomonas* F. Stein and *Jomonlithus* I. Inouye & M. Chihara (1983: 387).

Below we give a list of Coccolithophyceae species identified in the reservoirs of the steppe zone of the South Urals (Table 2).

TABLE 2. List of taxa observed at the investigated waterbodies (site numbers correspond to those indicated in Fig. 1).

Taxon		Site number						
		2	3	4	5	6		
Chrysotila carterae (Braarud & Fagerland) R.A. Andersen, J.I. Kim, Tittley & H.S. Yoon (2014: 471)				+	+	+		
C. roscoffensis (P.A. Dangeard) R.A. Andersen, J.I. Kim, Tittley & H.S. Yoon (2015: 321)				+	+	+		
Hymenomonas roseola F. Stein		+	+					
H. uralensis Ignatenko & Yatsenko-Stepanova sp. nov.	+	+						
Jomonlithus littoralis Inouye & Chihara (1983: 374)		+		+				

Haptophyta Hibberd ex Edvardsen & Eikrem Coccolithophyceae Rothmaler Coccolithales Schwarz Hymenomonadaceae Senn Hymenomonas F. Stein

Hymenomonas roseola F. Stein (Figs. 2-4)

Coccolith is bowl-shape (murolith), with more or less straight sides. The funnel-shaped part of the coccolith expands towards the top and has a jagged edge. The number of calcite "teeth" (crystal units) of coccoliths belonging to single cell varies from 11 to 15. The height of coccoliths in the discovered specimens varies within $0.50-1.12 \mu m$; the height of the base and the height of the funnel are approximately the same.

This species was observed in Ural River and Ushkotinsky Reservoir (see Table 2).



FIGURE 1. Schematic map of the study area and sampling sites: 1, 2—Ural River, 3—Ushkotinsky Reservoir, 4, 5—Zhetykol Lake, 6—Aschisaysky Pond.

Jomonlithus I. Inouye & M. Chihara

Jomonlithus littoralis Inouye & Chihara (Figs. 5-7)

The diameter of the coccolith-bearing cell is $10.5-12.0 \mu m$. The surface of the cell is covered by small oval-shaped coccoliths (muroliths), $1.07-1.39 \times 0.77-0.92 \mu m$; rim width $0.11-0.17 \mu m$, rim height $0.2-0.29 \mu m$. The coccolith structure corresponds to the description of Probert *et al.* (2014) to that which is composed of an organic base plate scale with a lightly calcified rim made up of small rectangular inner and outer sub-elements.

This species was observed from a backwater of the Ural River and Zhetykol Lake (see Table 2).



FIGURES 2–7. Coccolithophores from the reservoirs of the steppe zone of the South Urals (SEM). Figs. 2–4: *Hymenomonas roseola*. Figs. 5–7: *Jomonlithus littoralis*. The arrows mark the preserved areas of the organic basal plate. Scale bars: 2, 6–5 μ m, 3, 4, 7–2 μ m, 5–1 μ m.

Pleurochrysidaceae Fresnel & Billard

Chrysotila P.L. Anand

Chrysotila carterae (Braarud & Fagerland) R.A. Andersen, J.I. Kim, Tittley & H.S. Yoon (Figs. 8, 9) *≡Pleurochrysis carterae* (Braarud & Fagerland) T. Christensen (1978: 68)



FIGURES 8–14. Species of *Chrysotila* from the reservoirs of the steppe zone of the South Urals (SEM). Figs. 8, 9: *Chrysotila carterae*. Figs. 10–14: *C. roscoffensis*. The arrows mark the different types of ornamentation of the distal shield of the placoliths belonging to the single cell. Scale bars: 8–10, 12–14–2 µm, 11–5 µm.

A disc-shaped coccolith (placolith), composed of two interlocking cycles of crystal units, V and R units, which represent the calcite crystals with subvertical (V units) and subradial (R units) orientations of the c-axis relative to the base plate; $2.2-6 \times 1.4-1.7 \mu m$ in size.

This species was recorded from Zhetykol Lake and Aschisaysky Pond (see Table 2).

Chrysotila roscoffensis (P.A. Dangeard) R.A. Andersen, J.I. Kim, Tittley & H.S. Yoon (Figs. 10-14)

≡Pleurochrysis roscoffensis (P.A. Dangeard) Fresnel & Billard (1991: 77)

A disc-shaped coccolith (placolith), that consists of a central vertical tube bounded on each side by shield, there are small nodes on the distal shield of the placolith and within the tube, extending into the central area; $2.46-2.85 \times 1.74-1.98 \,\mu$ m in size.

This species was recorded from Zhetykol Lake and Aschisaysky Pond (see Table 2).

In this study, we found coccoliths in freshwater (Fig. 15), which we could not correlate with any of the described species. We described their as *Hymenomonas uralensis sp. nov*.

Hymenomonas uralensis Ignatenko & Yatsenko-Stepanova sp. nov. (Fig. 15)



FIGURE 15. Hymenomonas uralensis sp. nov. (SEM). The arrows mark the preserved areas of the organic basal plate. Scale bars: 2 µm.

Coccoliths are elliptical muroliths. Each coccoliths composed of an organic base plate and calcified elements. The base of the coccolith $(0.93-1.2 \times 0.8-0.96 \ \mu\text{m}, n=15)$ has a jagged edge, followed by a tubular part with more or less straight sides and the expanding part $(1.3-1.6 \times 0.97-1.3 \ \mu\text{m})$ is formed by overlapping elements. The height of the coccolith is $0.36-0.51 \ \mu\text{m}$, the width of the rim is $0.22-0.3 \ \mu\text{m}$. In most coccoliths a single rim element at each end of the coccolith is extended distally as a rectangular block protruding above the rim (width $0.18-0.33 \ \mu\text{m}$, height $0.25-0.43 \ \mu\text{m}$).

Type:—The specimen with *Hymenomonas uralensis* coccoliths on SEM stub number 49_I_3 deposited at the Herbarium of the Steppe Institute of the Ural Branch of the Russian Academy of Sciences, Orenburg (ORIS). Material from the Ural River (51°25′55″N, 56°28′58″E), Orenburg Region, Russia. Sample collected 27th of July, 2021, by M. Ignatenko.

Type Locality:—RUSSIA: Orenburg region, Ural River. 51°25'55"N, 56°28'58"E. 27 July 2021.

Etymology:—Hymenomonas uralensis is named after the Ural River, where this species was discovered.

Distribution:—Besides the type locality this species was found from the backwater of the Ural River in the vicinity of the Nikolskoye village (see Table 2).

Discussion

Five species of Coccolithophyceae were found in different types of reservoirs in the steppe zone of the South Urals. *Jomonlithus littoralis, Chrysotila carterae* and *C. roscoffensis* are reported for the first time in Russia. We also found a taxon, which we described as a new species to science.

The new species presented in this paper was placed by us in *Hymenomonas* based on the coccolith morphology. But our diagnosis needs to be improved. Since we worked only with fixed samples, without coccolith-bearing cells into cultures, we give only a description of the morphology of coccoliths. We do not know about the size and shape of coccolith-bearing cells, the haploid phase of the life-cycle of this organism, the ability to move, the morphology of haptonema, etc. Additional studies are needed to compile a complete and correct description of this organism. At the same time, our finding is undoubtedly of great interest, since today only two species of coccolithophores are known to have been found in freshwater habitats, *Hymenomonas roseola* and "*Pleurochrysis dimidius*" (*nom. inval.*) (Dashiell 2010)

H. roseola is the only freshwater representative of the genus *Hymenomonas* (Young *et al.* 2003; Nicholls 2015; Henderiks *et al.* 2022). It has been found in ponds, lakes and backwaters of rivers, in phytoplankton and epilithon (Stoermer & Sicko-Goad 1977). The species is widespread in Europe, found in North America and Cuba (Stoermer & Sicko-Goad 1977; Guiry & Guiry 2023), in Russia (Kotkova *et al.* 2023).

Data on the distribution of *Jomonlithus littoralis* are scarce. Only a few findings are known for today. For the first time the species was discovered in samples from the mouth of Nakagawa river, in the coastal zone of Japan (Inouye & Chihara 1983). Long after, *J. littoralis* was registered in El Perelló, approximately 25 km south of Valencia on the Spanish Mediterranean coast (Probert *et al.* 2014). Probert *et al.* (2014) suggested in their study, that the species is probably widely distributed over the world, but it is restricted to coastal (littoral or brackish water) locations. In our study coccoliths of *J. littoralis* were detected in reservoirs of various types at salinity significantly lower than sea (5.7%), as well as in freshwater (0.6%) (Tables 1, 2). Studies by Simon *et al.* (2014) confirms the possibility of the existence of *J. littoralis* in freshwater habitats. In the research of the diversity of communities of small protists (0.2–5.0 µm cell size fraction) from five small and shallow freshwater ecosystems at the Natural Regional Park of the Chevreuse Valley (France, South of Paris) by amplification of 18S rRNA gene fragments and direct high-throughput 454-pyrosequencing, they found a sequence on 99% identical to *J. littoralis* (Simon *et al.* 2014).

In two previous studies (Inouye & Chihara 1983; Probert *et al.* 2014), the authors did not indicate the salinity values of reservoirs in which *J. littoralis* was found. However, in both cases, cells of *J. littoralis* were isolated into culture and cultivate in a medium with a seawater base (salinity ~ 20.0‰) (Inouye & Chihara 1983; Probert *et al.* 2014). Therefore, guided by our own and literature data, we can conclude that this species has a wide halotolerance range from freshwater to brackish water (from 0.6 to ~ 20.0‰).

Dashiell (2010) provides an example of the possibility of coccolithophores inhabiting the entire salinity range from freshwater to brackish water. From a freshwater stream in Colorado, USA, the author isolated putatively new species and invalidly published it as *Pleurochrysis dimidius*. In the experiment it was shown that despite the fact that the species was originally isolated from freshwater, it retained its viability for 60 days at salinity of 5, 8, 10, 15, 20, 22, 25, 30, 33, 36‰ (Dashiell 2010).

Chrysotila (=*Pleurochrysis*) is one of the best studied genera of Coccolithophyceae (Meng *et al.* 2022). They have a wide distribution, and they are found in coastal, estuarine, brackish waters (Johansen *et al.* 1988; Fresnel & Billard 1991; Reifel *et al.* 2001; Seoane *et al.* 2009; Meng *et al.* 2022). *C. carterae* is the object of numerous researches aimed at studying the mechanisms of calcification and formation of coccoliths, as well as the possibility of its use for biodiesel production since it efficiently accumulates lipids (Casareto *et al.* 2009; Saruwatari *et al.* 2011; Endo *et al.* 2016; Walker *et al.* 2020; Liu *et al.* 2021). *C. roscoffensis* is also considered as a promising biotechnological object due to its ability to accumulate lipids, polyunsaturated fatty acids and fucoxanthin (Liu *et al.* 2023).

In our study, we report the discovery of *C. carterae* and *C. roscoffensis* in shallow lakes with low salinity (3.8–8.1‰). We also noted morphological variability of placoliths of *C. roscoffensis*. According to the original description, *C. roscoffensis* differs from the morphologically similar species *C. pseudoroscoffensis* (Gayral & Fresnel) R.A. Andersen, J.I. Kim, Tittley & H.S. Yoon (2015: 321) by the presence of small nodes on the distal shield of the placolith (Gayral & Fresnel 1983). During the study, we observed cells carrying both placoliths with nodes on the distal shield and without nodes at the same time (Figs. 10–14). We agree with the observation of Young *et al.* (2003), indicating the existence of intermediate forms of *C. roscoffensis*, and that the separation of species based on the presence or absence of nodes on the distal shield of the placolith may be artificial.

This study is not only a contribution to our knowledge of the diversity of coccolithophores in the reservoirs in the steppe zone of the South Urals (Russia), but also to our understanding of the distribution and ecology of this group of microorganisms.

Acknowledgements

The authors are grateful to Aleksei A. Urzhumov, the Head of "Gagarin" Center for the identification and support of talented children, Orenburg Region, for his help with sampling. The authors are also grateful to two anonymous reviewers for their valuable remarks that improved the manuscript.

This study was supported by a grant from the Russian Science Foundation (project No. 23-24-10056).

References

- Anand, P. (1936) Seven new Chrysophyceae from the south-east coast of England. In: Proceedings of the Twenty-third Indian Science Congress, Indore, 1936 (Fourth Circuit). Royal Asiatic Society of Bengal, Calcutta, pp. 282–283.
- Andersen, R.A., Kim, J.I., Tittley, I. & Yoon, H.S. (2014) A re-investigation of *Chrysotila* (Prymnesiophyceae) using material collected from the type locality. *Phycologia* 54: 463–473. https://doi.org/10.2216/14-016.1
- Andersen, R.A., Kim, J.I., Tittley, I. & Yoon, H.S. (2015) *Chrysotila dentata* comb. nov., *Chrysotila roscoffensis* comb. nov. and *Chrysocapsa wetherbeei* sp. nov. *Phycologia* 54: 321–322.

https://doi.org/10.2216/15-13.1

- Balch, W.M., Bowler, B.C., Drapeau, D.T., Lubelczyk, L.C., Lyczkowski, E., Mitchell, C. & Wyeth, A. (2019) Coccolithophore distributions of the North and South Atlantic Ocean. *Deep-Sea Research Part I* 151: 1–23. https://doi.org/10.1016/j.dsr.2019.06.012
- Casareto, B.E., Niraula, M.P., Fujimura, H. & Suzuki, Y. (2009) Effects of carbon dioxide on the coccolithophorid *Pleurochrysis carterae* in incubation experiments. *Aquatic biology* 7: 59–70. https://doi.org/10.3354/ab00182
- Christensen, T. (1978) Annotations to a textbook of phycology. *Botanisk Tidsskrift* 73: 65–70.
- Dashiell, C. (2010) A Review of the Prymnesiophyta, Emphasizing the Morphology and Systematics of Hymenomonas Stein (1878) and Pleurochrysis Pringsheim (1955). Dissertation to the University of North Carolina Wilmington, pp. 78.
- Eikrem, W., Medlin, L.K., Henderiks, J., Rokitta, S., Rost, B., Probert, I., Throndsen, J. & Edvardsen, B. (2016) Haptophyta. *In:* Archibald, J.M., Simpson, A.G.B., Slamovist, C.H., Margulis, L., Melkonian, M., Chapman, D.J. & Corliss, J.O. (Eds.) *Handbook of the Protists.* Springer International Publishing, Switzerland, pp. 1–61. https://doi.org/10.1007/978-3-319-32669-6 38-1
- Endo, H., Yoshida, M., Uji, T., Saga, N., Inoue, K. & Nagasawa, H. (2016) Stable Nuclear Transformation System for the Coccolithophorid Alga *Pleurochrysis carterae. Scientific Reports* 6: 22252.

https://doi.org/10.1038/srep22252

- Fresnel, J. & Billard, C. (1991) *Pleurochrysis placolithoides* sp. nov. (Prymnesiophiceae), a new marine coccolithophorid with remarks on the status of Cricolith-bearing species. *British Phycological Journal* 26: 67–80.
- Gayral, P. & Fresnel, J. (1983) Description, sexualité et cycle de développement d'une nouvelle Coccolithophoracée (Prymnesiophyceae): *Pleurochrysis pseudoroscoffensis* sp. nov. *Protistologica* 19: 245–261.
- Godrijan, J., Drapeau, D.T. & Balch, W.M. (2022) Osmotrophy of dissolved organic carbon by coccolithophores in darkness. New Phytologist 233 (2): 781–794.

https://doi.org/10.1111/nph.17819

- Guiry, M.D. & Guiry, G.M. (2023) *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org (accessed 2 July 2023).
- Henderiks, J., Sturm, D., Šupraha, L. & Langer, G. (2022) Evolutionary rates in the Haptophyta: exploring molecular and phenotypic diversity. *Journal of Marine Science and Engineering* 10: 798–827.

https://doi.org/10.3390/jmse10060798

- Inouye, I. & Chihara, M. (1983) Ultrastructure and taxonomy of *Jomonlithus littoralis* gen. et sp. nov. (Class Prymnesiophyceae), a coccolithophorid from the Northwest Pacific. *Botanical Magazine, Tokyo* 96: 365–376.
- Johansen, J.R., Doucette, G.J., Barclay, W.R. & Bull, J.D. (1988) The morphology and ecology of *Pleurochrysis carterae* var. *dentata* var. nov. (Prymnesiophyceae), a new coccolithophorid from an inland saline pond in New Mexico, USA. *Phycologia* 27 (1): 78–88. https://doi.org/10.2216/i0031-8884-27-1-78.1
- Jordan, R.W. (2009) Coccolithophores. *In:* Schaechter, M. (Ed.) *Encyclopedia of Microbiology*. San Diego State University, San Diego, USA, pp. 593–605.

https://doi.org/10.1016/b978-012373944-5.00249-2

Kotkova, V.M., Czernyadjeva, I.V., Davydov, E.A., Doroshina, G.Ya., Efimov, D.Yu., Efimova, L.A., Frolov, I.V., Gabiger, YA., Glustahenko, M.Yu., Gorbunova, I.A., Himelbrant, D.E., Ignatenko, M.E., Kalinina, L.B., Kurbatova, L.E., Kushnevskaya, H.V., Lashchinsky, N.N., Lotiev, K.Yu., Moroz, E.L., Notov, A.A., Novozhilov, Yu.K., Otmakhov, Yu.S., Plikina, N.V., Popova, N.N., Potemkin, A.D., Putilina, V.A., Ryzhkova, P. Yu., Sambyla, Ch.N., Smirnova, E.V., Stepanchikova, I.S., Storozhenko, Yu.V., Troeva, E.I., Tsurykau, A.G., Vishnyakov, V.S., Vlasenko, A.V., Vlasenko, V.A., Volkova, E.A., Volosnova, L.F., Yakovchenko, L.S., Yatsenko-Stepanova, T.N., Zhuykov, K.A. & Zueva, A.S. (2023) New cryptogamic records. 11. *Novosti sistematiki nizshikh rastenii* 57 (1): 155–204. [In Russian]

https://doi.org/10.31111/nsnr/2023.57.1.155

- Liu, J., Sun, Y., Zhang, L., Li, X., He, Z., Zhou, C. & Han, J. (2023) Screening of antibiotics to obtain axenic cell cultures of a marine microalga *Chrysotila roscoffensis*. *Frontiers in Bioengineering and Biotechnology* 11: 1218031. https://doi.org/10.3389/fbioe.2023.1218031
- Liu, Y.-W., Rokitta, S.D., Rost, B. & Eagle, R.A. (2021) Constraints on coccolithophores under ocean acidification obtained from boron and carbon geochemical approaches. *Geochimica et Cosmochimica Acta* 315: 317–332. https://doi.org/10.1016/j.gca.2021.09.025
- Meng, R., Zhang, L., Zhou, C., Liao, K., Xiao, P., Luo, Q., Xu, J., Cui, Y., Hu, X. & Yan, X. (2022) Genome sequence of *Chrysotila roscoffensis*, a coccolithphore contributed to global biogeochemical cycles. *Genes* 13: 40. https://doi.org/10.3390/genes13010040
- Nicholls, K.H. (2015) Haptophyte Algae. In: Wehr, J.D., Sheath, R.G. & Kociolek, J.P. (Eds.) Freshwater Algae of North America. Ecology and Classification. Elsevier Academic Press, USA, pp. 587–605. http://dx.doi.org/10.1016/B978-0-12-385876-4.00013-X
- Pautova, L.A., Silkin, V.A., Kravchishina, M.D., Chultsova, A.L. & Lisitzin, A.P. (2020) The Biological Calcium Carbonate Pump in the Norwegian and Barents Seas: Regulation Mechanisms. *Doklady Earth Sciences* 490 (1): 55–60. https://doi.org/10.1134/S1028334X20010079
- Pringsheim, E.G. (1955) Kleine Mitteilungen über Flagellaten und Algen I. Algenartige Chrysophyceen in Reinkultur. Archiv für Mikrobiologie 21: 401–410.

https://doi.org/10.1007/BF00413002

Probert, I., Fresnel, J. & Young, J. (2014) The life cycle and taxonomic affinity of the coccolithophore *Jomonlithus littoralis* (Prymnesiophyceae). *Cryptogamie, Algologie* 35 (4): 389–405.

https://doi.org/10.7872/crya.v35.iss4.2014.389

- Reifel, K.M., McCoy, M.P., Tiffany, M.A., Rocke, T.E., Trees, C.C., Barlow, S.B., Faulkner, D.J. & Hurlbert, S.H. (2001) *Pleurochrysis pseudoroscoffensis* (Prymnesiophyceae) blooms on the surface of the Salton Sea, California. *Hydrobiologia* 466: 177–185.
- Saruwatari, K., Nagasaka, S., Ozaki, N. & Nagasawa, H. (2011) Morphological and crystallographic transformation from immature to mature coccoliths, *Pleurochrysis carterae. Marine Biotechnology* 13 (4): 801–809.

https://doi.org/10.1007/s10126-010-9342-7

Seoane, S., Eikrem, W., Arluzea, J. & Orive, E. (2009) Haptophytes of the Nervión River estuary, northern Spain. *Botanica Marina* 52: 47–59.

https://doi.org/10.1515/bot.2009.027

Simon, M., Jardillier, L., Deschamps, P., Moreira, D., Restoux, G., Bertolino, P. & López-García, P. (2014) Complex communities of small protists and unexpected occurrence of typical marine lineages in schallow freshwater systems. *Environmental Microbiology* 17 (10): 3610–3627.

https://doi.org/10.1111/1462-2920.12591

Stein, F.R. von (1878) Der Organismus der Infusionsthiere. Abt. III. Verlag von Wilhelm Engelmann, Leipzig, pp. 1–154.

Stoermer, E.F. & Sicko-Goad, L. (1977) A new distribution record for *Hymenomonas roseola* Stein (Prymnesiophyceae, Coccolithophoraceae) and *Spiniferomonas trioralis* Takahashi (Chrysophyceae, Synuraceae) in the Laurentian Great Lakes. *Phycologia* 16 (4): 355–358.

https://doi.org/10.2216/i0031-8884-16-4-355.1

- Tyrrell, T. & Young, J.R. (2009) Coccolithophores. In: Steele, J.H., Thorpe, S.A. & Turekian, K.K. (Eds.) Encyclopedia of Ocean Sciences. Elsevier Academic Press, Boston, USA, pp. 606–614. https://doi.org/10.1016/b978-012374473-9.00662-7
- Walker, J.M., Marzec, B., Ozaki, N., Clare, D. & Nudelman, F. (2020) Morphological development of *Pleurochyrsis carterae* coccoliths examined by cryo-electron tomography. *Journal of Structural Biology* 210: 107476. https://doi.org/10.1016/j.jsb.2020.107476
- Young, J.R., Geisen, M., Cros, L., Kleijne, A., Sprengel, C., Probert, I. & Østergaard, J.B. (2003) A guide to extant coccolithophore taxonomy. *Journal of Nannoplankton Research* Special Issue 1: 1–125.
- Young, J.R., Bergen, J.A., Bown, P.R., Burnett, J.A., Fiorentino, A., Jordan, R.W., Kleijne, A., Niel, B.E.V., Romein, A.J.T. & Salis, K.V. (1997) Guidelines for coccolith and Calcareous nannofossil terminology. *Palaeontology* 40: 875–912.