

# ELEMENT COMPOSITION OF *TRACHELOMONAS* ENVELOPES (EUGLENOPHYTA)

MAŁGORZATA PONIEWOZIK

**Abstract.** Cells of euglenoid *Trachelomonas* Ehrenb. vary in the size, shape and appearance of the external envelope's ornamentation, as well as internal structure. The loricae are generally similar in color, ranging from light (hyaline, yellowish, golden, reddish, brownish) to dark (brown, black). This study examined the loricae of twelve trachelomonad species, some light (yellow to golden) and some dark (brown). There were no black loricae, suggesting that little or no Mn was taken up by the cells. Though the envelopes differed in surface ornamentation and color, comparative SEM-EDS analyses of all the material studied showed high iron content, silicon mineralization, and no manganese. The degree of iron and silicon mineralization varied. Some loricae had high silicon content (e.g., *T. compacta* Middelhoeck) and others were more saturated by iron ions (*T. manginii* Deflandre, *T. lacustris* Drezep.).

**Key words:** element composition, euglenoids, loricae, SEM, *Trachelomonas*, X-ray analysis

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## INTRODUCTION

The euglenophytes are a heterogeneous group of freshwater and marine flagellates. Some of them are naked, covered only by the periplast (e.g., the genera *Euglena* Ehrenb., *Phacus* Dujard., *Astasia* Ehrenb.), and others (*Trachelomonas* Ehrenb., *Strombomonas* Deflandre, *Ascoglena* F. Stein) have an external lorica (envelope) which encloses the cell. The envelopes of euglenophytes, built of mucopolysaccharides and initially flexible, become mineralized by mineral salts and turn into solid structures (Leedale 1975; Conforti *et al.* 1994). Envelopes in this form are used for taxonomic identification. That manner of identification was initiated by Ehrenberg in the 19<sup>th</sup> century (Ehrenberg 1833). A hundred years later, Deflandre defined a new loricated genus, *Strombomonas* (Deflandre 1930). In later studies of lorica structure and its formation process, Brosnan *et al.* (2005) maintained *Strombomonas* and *Trachelomonas* as separate genera. At present, some cytological characteristics, in addition to cell size, are used for taxonomic diagnoses. The number and shape of chloroplasts as well as the presence and structure of pyrenoids

are most important, but the principal criteria are still the appearance of loricae – their shape, the presence or lack of a collar or thickening around the apical pore, and external structure. In *Trachelomonas* the envelope surface presents great diversity. Many studies have employed scanning electron microscopy to explore the external structure of envelopes in detail (e.g., Leedale 1975; West & Walne 1980; Dunlap *et al.* 1983; Conforti *et al.* 1994; Wang *et al.* 2000; Pereira & Azeiteiro 2003; Pereira *et al.* 2003; Wołowski & Hindák 2004; Wołowski & Walne 2007; Duangjan & Wołowski 2013; Poniewozik 2016; Wołowski *et al.* 2016). The shape and appearance of loricae are characteristic features of species but the chemical composition and proportions of individual elements of the envelope wall may vary to some extent. Despite all the research on trachelomonad loricae, there is still relatively little information on the chemical composition of their envelopes. The main elements that build the envelope include iron (Fe) and manganese (Mn). An increase of Fe or Mn in the environment can alter

the microarchitecture of loricae, increasing the number of granular formations or spicules (Dunlap & Walne 1987). Carbon, oxygen and nitrogen are usually in great abundance; other constituents of loricae are Si, P, S, K, Mg, Al and Cl (West *et al.* 1980; Dunlap *et al.* 1986). Some minerals (e.g., Ti, Ba, Ni, Cu, Pb, Mo) occur in lesser amounts (Mann & Fyfe 1988).

In this study I examined the chemical composition of *Trachelomonas* envelopes, using loricae from field samples. An aim of this work was to determine whether loricae differing in wall surface (smooth, spiny, warty) also differed in chemical composition.

#### MATERIALS AND METHODS

Samples were netted (20 µm mesh) from two natural freshwater bodies near each other in eastern Poland: Nadrybie Reservoir (51°20'N, 23°3'E) and a small abandoned clay-pit (51°22'N, 23°1'E). Both waterbodies had densely overgrown shores and were rich in organic matter. The organisms were analyzed and identified, first by light microscopy (Nikon Eclipse E600, Nomarski differential interference contrast) and then by scanning electron microscopy. All LM observations were of living material. Taxonomic identification followed Starmach (1983), Wołowski and Hindák (2005) and Dillard (2000). Material for further analyses was fixed with glutaraldehyde/formaldehyde. Samples for SEM were prepared as described by Bozzola and Russel (1995). The fixed material was washed several times in distilled water to remove buffer salts and then dehydrated in a graded ethanol series. Then drops of dehydrated material were transferred on slides mounted on aluminum stubs (Ø 10.0 mm) with graphite paint and air-dried overnight at ca 20°C. After drying, the stubs were coated with a 20 nm carbon layer using a Cressington sputter-coater. SEM observations of carbon-coated material employed a Hitachi S-4700 microscope (Laboratory of Field Emission Scanning Electron Microscopy and Microanalysis, Jagiellonian University, Kraków, Poland). Element composition was microanalyzed with the same unit operating at 20 keV and equipped with an X-ray detector (ThermoNORAN Vantage energy-dispersive spectrometer). The

X-ray spectra were generated over 50 s life-times from 0 to 15 keV and with a take-off angle of 30°.

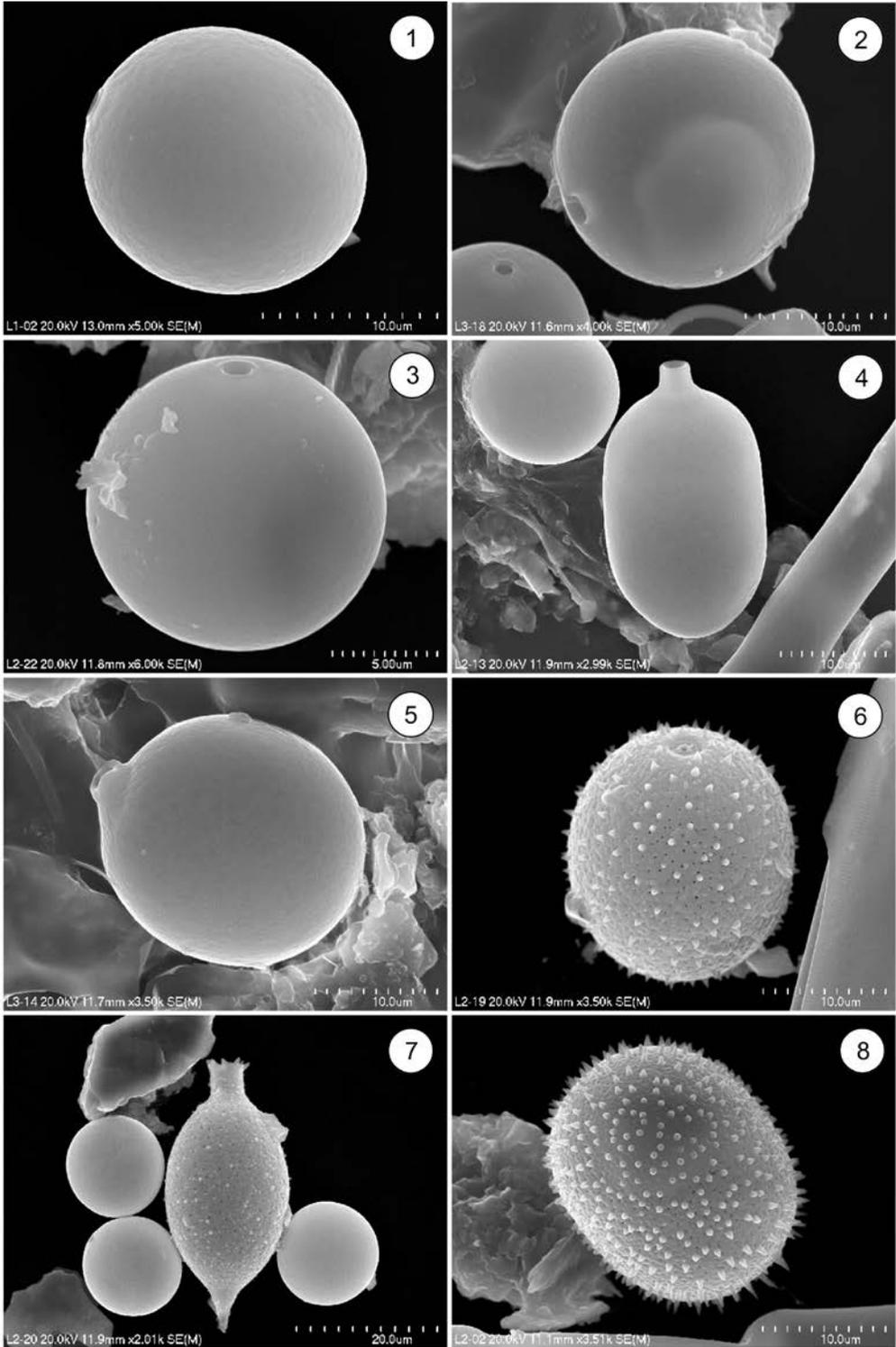
#### RESULTS

In this study the ultrastructure and chemical composition of *Trachelomonas* loricae of selected species were analyzed in a number of taxa. Five were species with smooth loricae: the slightly oval loricae of *Trachelomonas volvocina* Ehrenb. var. *volvocina* (Fig. 1), the consistently round loricae of *T. volvocinopsis* Svirenko fo. *volvocinopsis* (Fig. 2), and the transverse oval loricae of *T. curta* A. M. Cunha fo. *curta* (Fig. 3), *T. dubia* Svirenko emend. Deflandre (Fig. 4) and *T. manginii* Deflandre (Fig. 5). The loricae of *T. dubia* and *T. curta* were the palest (yellowish to golden), and those of *T. volvocina* and *T. volvocinopsis* were reddish to brownish. The next group consisted of five spiny loricae: *T. hispida* (Perty) F. Stein var. *hispida* (Fig. 6), *T. caudata* (Ehrenb.) F. Stein fo. *caudata* (Fig. 7), *T. cf. hirta* A. M. Cunha var. *duplex* Deflandre (Fig. 8), *T. lacustris* Drezep. (Fig. 9) and *T. verrucosa* A. Stokes var. *granulosa* (Playfair) W. Conrad (Fig. 10). The latter had short, thick, blunt spines, and the rest had fairly narrow, sharp spines. *T. caudata* had the darkest loricae; they were brown, not black or blackish. Also studied was *T. compacta* Middelhoeck (Fig. 11), having small depressions on the lorica wall surface, and *T. intermedia* P. A. Dangeard (Fig. 12), having small pores on a smooth surface.

SEM-EDS analyses showed that all the examined loricae were of generally similar composition. The main components were silicon and iron (Table 1). The analysis did not indicate the presence of manganese in any of the studied envelopes.

The external surfaces of the studied *Trachelomonas volvocina* var. *volvocina*, *T. volvocinopsis* fo. *volvocinopsis*, *T. curta* fo. *curta* and *T. dubia* were all smooth, without any external structures or processes, but energy-dispersive X-ray analysis showed differences in the content of basic

**Figs 1–8.** External views of the studied loricae. 1 – *Trachelomonas volvocina* Ehrenb. var. *volvocina*, 2 – *T. volvocinopsis* Svirenko fo. *volvocinopsis*, 3 – *T. curta* A. M. Cunha fo. *curta*, 4 – *T. dubia* Svirenko, 5 – *T. manginii* Deflandre, 6 – *T. hispida* (Perty) F. Stein var. *hispida*, 7 – *T. caudata* (Ehrenb.) F. Stein fo. *caudata*, 8 – *T. cf. hirta* var. *duplex* Deflandre.



**Table 1.** Relative amounts of the main elements in envelopes of the studied trachelomonads. 0 – EDS analysis of basal glass, 1 – *Trachelomonas volvocina* Ehrenb. var. *volvocina*, 2 – *T. volvocinopsis* Svirenko fo. *volvocinopsis*, 3 – *T. curta* A. M. Cunha fo. *curta*, 4 – *T. dubia* Svirenko, 5 – *T. manginii* Deflandre, 6 – *T. hispida* (Perty) F. Stein var. *hispida*, 7 – *T. caudata* (Ehrenb.) F. Stein fo. *caudata*, 8 – *T. cf. hirta* var. *duplex* Deflandre, 9 – *T. lacustris* Drezep., 10 – *T. verrucosa* A. Stokes var. *granulosa* (Playfair) W. Conrad, 11 – *T. compacta* Middelhoeck, 12 – *T. intermedia* P. A. Dangeard.

	Al		Si		P		K		Fe		Na	
	Atom [%]	Err.+/- [%]										
0	4.63	0.19	67.65	0.36	–	–	8.70	0.23	–	–	10.47	0.17
1	3.06	0.10	31.04	0.27	13.51	0.15	3.32	0.15	29.59	0.50	12.99	0.32
2	3.73	0.18	49.81	0.50	8.10	0.25	7.23	0.34	11.09	0.43	7.29	0.43
3	6.38	0.18	37.26	0.46	15.73	0.46	3.47	0.12	23.41	0.69	6.58	0.45
4	3.36	0.18	26.74	0.46	18.62	0.49	3.31	0.13	36.63	0.90	2.75	0.50
5	2.48	0.26	13.74	0.33	28.96	0.69	0.94	0.17	41.63	1.20	3.63	0.46
6	4.04	0.14	43.22	0.49	12.34	0.48	4.96	0.28	17.33	0.70	12.01	0.23
7	2.73	0.17	39.23	0.53	11.47	0.50	8.06	0.41	20.53	0.97	8.16	0.25
8	3.98	0.15	29.7	0.24	17.12	0.24	3.28	0.26	27.2	0.79	9.16	0.25
9	6.51	0.25	21.73	0.30	18.09	0.32	–	–	39.75	1.27	2.83	0.46
10	5.81	0.34	34.88	0.47	17.22	0.25	2.76	0.23	26.08	0.68	5.55	0.22
11	13.39	0.17	73.56	0.54	–	–	3.58	0.29	4.07	0.53	–	–
12	5.14	0.09	56.99	0.30	4.02	0.12	7.05	0.18	9.18	0.37	11.46	0.21

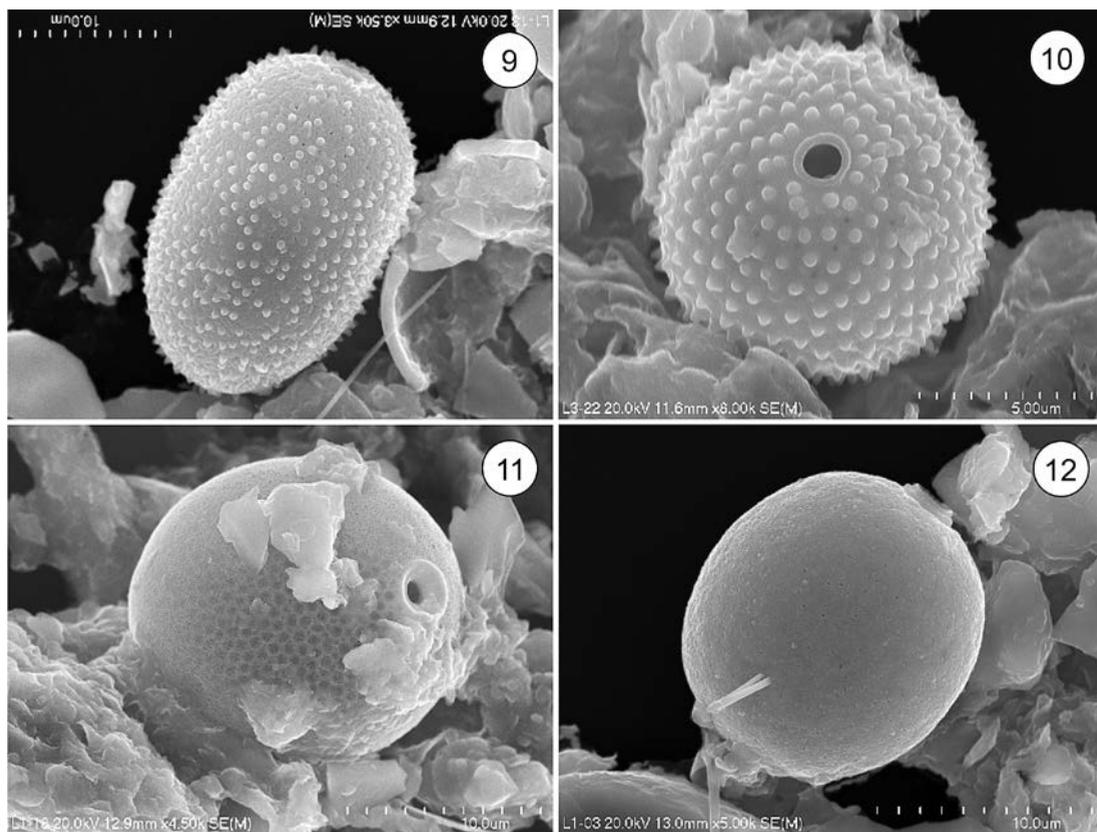
components of these envelopes (Table 1). The loricae of *T. dubia* had comparable amounts of silicon and iron and were slightly mineralized. Si was the main mineralizing element in the other three species; Fe was present in smaller amounts, least in *T. volvocinopsis* (Table 1). In the lorica of *T. manginii*, phosphorus (P) was an important chemical component in addition to the Si and Fe (Table 1).

In the group of trachelomonads represented by *T. hispida* var. *hispida*, *T. caudata* fo. *caudata* and *T. verrucosa* var. *granulosa*, the envelope wall was fairly rough and covered with spines or granules. EDS showed Si to be a very important element in these species. Fe usually occurred in smaller amounts. In addition to silicon and iron, the spines covering the loricae of *T. cf. hirta* var. *duplex* and *T. lacustris* also contained a large amount of phosphorus (Table 1). The lorica of *T. compacta*, which showed scrobiculate external structure, was composed mainly of silicon compounds, with negligible content of the other elements (Table 1). The lorica of *T. intermedia* was very strongly mineralized by Si, with smaller amounts of the remaining elements (Table 1). The high amounts of carbon (C) in the initial dataset are

ignored because the SEM samples were carbon-coated. Carbon is known to be a component of loricae but in this analysis it was not possible to factor out the amount of carbon in the coating.

## DISCUSSION

These analyses indicated that, apart from iron, silicon was the main component of the wall in all loricae. Previous studies have detected Si in the chemical composition of envelopes (West 1977; Donnelly 1979; West *et al.* 1980) but noted it as an accompanying element. Later, Steinberg and Klee (1984) showed silicon to be the main element in the loricae of some trachelomonad species, (e.g., *T. volvocina*, *T. hispida*, *T. hispida* var. *punctata* Lemmer), accompanied by smaller amounts of Fe, Ca, Cu, Zn and even Co; Si was the only element they found in the loricae of *T. rasumowskoensis* Dolgoff. Si was the only element I detected in *T. compacta* loricae. It should be mentioned that the *T. rasumowskoensis* specimens Steinberg and Klee (1984) studied probably were chrysophycean cysts, not the trachelomonad representative. Wołowski (2005) discussed the taxonomic position of that species, suggesting it to be a chrysophyte



**Figs 9–12.** External views of the studied loricae. 9 – *Trachelomonas lacustris* Drezep., 10 – *T. verrucosa* A. Stokes var. *granulosa* (Playfair) W. Conrad, 11 – *T. compacta* Middelhoeck, 12 – *T. intermedia* P. A. Dangeard.

cyst. Wang *et al.* (2003) reported high Si content in several loricae of *Trachelomonas*; they found Si in all the loricae they studied, but it dominated in those that had a rough surface (*T. scabra* Playfair, *T. silvatica* Svirenko); smooth loricae (*T. volvocina*) had high amounts of iron, with Si also occurring in significant amounts. Barnes *et al.* (1986) found small amounts of Si occurring in granular regions along the margins of *T. lefevrei* Deflandre envelopes, Fe being the major element (Barnes *et al.* 1986). Possibly some of the silicon in the loricae I analyzed came from the cover slips used for preparing the material, but other work on similar trachelomonad taxa has shown silicon to be a component of the lorica, along with iron and/or manganese (Poniewozik 2015; Wołowski *et al.* 2016). In *Strombomonas*, Conforti *et al.* (1994) found Si to be the main component

of loricae, with much lower Fe content and a trace amount of Mn; they attributed their findings to the specific structure of *Strombomonas* loricae, which were rough on the surface and had accumulated extraneous particles, including sand grains. In my study, smooth as well as rough loricae of *Trachelomonas* were saturated with silicon. According to Wang *et al.* (2003), the Si in *Strombomonas* loricae comes from exogenous particles like sand grains but is also evenly distributed in envelopes of *Strombomonas* and *Trachelomonas* and is a constitutive element of the envelope. The high amount of silicon in loricae might be explained in part by the possibility that the brown compounds of the trachelomonad loricae are iron silicates or phosphosilicates of iron or manganese (Pereira & Azeiteiro 2003). Steinberg and Klee (1984) suggested that the large amount of Si in trachelomonad

loricae indicates that the formation of loricae impregnated with inorganic compounds starts with a silicification process, and may be a common process in organisms of different taxonomic groups that produce external structures. Silicon is well known as the main component in diatoms and chrysophyte stomatocysts, but also in chlorophytes, haptophytes and dinoflagellates (Preisig 1994; Pla 2001). In my study, I found that iron usually occurred in considerable amounts, in addition to silicon. Conforti *et al.* (1994) found that the loricae of *T. volvocina* and *T. similis* A. Stokes consisted mainly of iron, with Si present in small amounts. In *Trachelomonas lefevrei* loricae, Fe as well as Mn were detected, in varying proportions depending on the color and age of the cells (Dunlap *et al.* 1983). Manganese was not present in the envelopes of any of the loricae I studied. This is surprising, because smaller or larger amounts of Mn have usually been found in other work on the chemical composition of envelopes (Dunlap *et al.* 1983; 1986; Barnes *et al.* 1986; Pereira *et al.* 2003). Only Wang *et al.* (2000, 2003) detected no manganese in loricae. Among the heavy metals, Mn is known to be an essential micronutrient for plant organisms, required for redox reactions as an enzyme cofactor and for protection against oxidative stress (Bowler *et al.* 1994; Büchel *et al.* 1999). The acquisition of this metal is based on active transport. The uptake of manganese is determined by cell activity (Dunlap *et al.* 1983) and, as in the case of other ions, is affected by pH and other environmental conditions. Richardson *et al.* (1988) reported that manganese oxidation occurred in a dense population of phytoplankton that generated microenvironment conditions exceeding pH 9. In experiments by Ferroni *et al.* (2004), the X-ray emission peak corresponding to Mn did not differ between *Euglena gracilis* G. A. Klebs cells exposed to manganese excess and control cells, even though the internal concentration of Mn was two times higher in the Mn-exposed cells. They attributed this finding to the presence of Mn in diluted form throughout the cells, not detectable by the X-ray method. Pereira *et al.* (2003) found that *T. hispida* var. *coronata* Lemmerm. loricae contained a large amount of Fe and no manganese,

despite the high amount of dissolved manganese recorded in the water, which was half the amount of dissolved Fe recorded. Phytoplankton cells take up and accumulate manganese (II) intracellularly (Sunda & Huntsman 1986), and in some cases this may explain why manganese ions were not detected. The lack of manganese in the chemical composition of loricae might also be explained by selective mineralization of Mn or Fe, resulting in differences in the chemical composition of trachelomonad envelopes, as suggested by some authors (Leedale 1975; Dunlap & Walne 1985). Heinrich *et al.* (1987) suggested that the chemical composition of *Trachelomonas* envelopes is species-specific to some extent. They analyzed trachelomonads (*T. lefevrei*, *T. volvocina*, *T. hispida*, *T. volvocinopsis*) from natural sites. Specimens from the same water sample (e.g., *T. lefevrei*) varied significantly: some had iron dominant and small amounts of manganese, and others had manganese dominant and only traces of iron. Others (*T. volvocina*, *T. hispida*) accumulated only iron. Dunlap *et al.* (1986) reported no significant difference in the chemical composition of the envelopes they studied: both *Strombomonas conspersa* (Pasccher) Tell & Conforti and some *Trachelomonas* species (*T. lefevrei*, *T. hispida* var. *coronata*, *T. zorensis* Deflandre) had manganese as the predominant element; all the loricae had needle-like structures and had dark, mineralized envelopes.

There is little information on P as the main component of trachelomonad loricae. I found high concentrations of phosphorous in *T. manginii*. In some *Trachelomonas* species, Pereira *et al.* (2003) detected phosphorus accompanied by sulphur, potassium and calcium, with iron dominant. In other work, phosphorus occurred in small or very small amounts in the lorica of *T. hispida* var. *coronata* and *T. lefevrei*, and never as the main component (West 1977; Dunlap *et al.* 1983; Pereira *et al.* 2003). Donnelly (1979) found large amounts of Fe, P and Cl in *T. bulla* F. Stein.

Such a chemical composition is not limited to species that form loricae. *Lepocinclis spyrogyroides* B. Marin & Melkonian (formerly *Euglena spirogyra* Ehrenb.) showed a chemical composition similar to that of *Trachelomonas* envelopes

– warts of *L. spirogyroides* covering the pellicle in longitudinal rows were composed mainly of Fe and P, suggested to occur as a complex – iron phosphate (Dawson *et al.* 1988). Similar mechanisms of ion accumulation have been reported in other species that produce mineralized structures, such as *Lepocinclis fusca* (G. A. Klebs) Kosmala & Zakryś [formerly *Euglena fusca* (G. A. Klebs) Lemmerm.], *Lepocinclis spinosa* M. S. Bennett & Triemer (formerly *Phacus horridus* Pochm.) and *Lepocinclis ovum* (Ehrenb.) Lemmerm. (Pereira & Azeiteiro 2003).

Although representatives of the genus *Colacium* Ehrenb. do not produce loricae, warts or spines, they often form gelatinous stalks and cushion holdfasts which facilitate their attachment to substrate. *C. vesiculosum* Ehrenb. stalks sometimes have characteristic dark brown Fe and Mn deposits which stiffen their surface and turn them brown, but deposition of that material is not an obligatory part of their normal metabolism (Rosowski & Kugrens 1973; Rosowski & Willey 1977). In cultured *Colacium*, Rosowski and Kugrens (1973) showed the role of Fe and Mn compounds in cell growth and stalk production. The organisms developed well and formed stalks in Fe- and Mn-enriched medium. Martin (1970) suggested that Fe and Mn compounds contained in copepod skeletons play a role in the stalk development of *Colacium* that settle on them. The same was suggested by Wołowski *et al.* (2015), who observed the growth and development of *Colacium minimum* Fott & Komárek on *Trachelomonas* and *Strombomonas* loricae, which are known to contain iron and manganese, though not always. Similar mechanisms of mineral salts uptake and mineralization may operate in organisms that build iron-enriched external structures. What is obvious is that species that differ from each other in their pellicle ornamentation – warts, spines or other formations – also differ in their chemical composition to some extent. There have been attempts to explain the problem of wall chemical composition in *Trachelomonas*, especially in taxa with smooth loricae, but no comprehensive hypothesis has emerged.

Some authors have shown or suggested that the color of loricae resulted from their chemical

composition, especially from high levels of manganese (Dunlap *et al.* 1983; Dawson *et al.* 1988; Wołowski & Hindák 2005). I did not find a clear connection between lorica color (hyaline, yellow, dark) and element composition; the samples included both light (e.g., *T. dubia*, *T. curta*, *T. lacustris*) and dark (e.g., *T. caudata*, *T. volvocinopsis*) loricae, but EDS analyses did not detect Mn in any of them. None of the studied loricae were black. At most they were brown or reddish brown. According to Dawson *et al.* (1988), low concentrations of Mn in *Lepocinclis spirogyroides* can be masked by Fe and detected only occasionally. Dunlap *et al.* (1983) observed dark golden to brown loricae in *Trachelomonas* which were Mn-enriched and had dense needle-like structures, as well as hyaline to light golden ones which were Fe-enriched and had granular structure. Dunlap and Walne (1987) showed that an increase in Fe or Mn in the environment led to modification of the microarchitecture of loricae in *Trachelomonas lefevrei*, seen as an increase of granular or needle-like crystalline formations, but other work of theirs (Donnelly & Walne 1979) found no clear correlation between the color and chemical composition of loricae treated with additional Fe or Mn, nor any effect on envelope microarchitecture or color. In *Trachelomonas hispida* var. *coronata* cultured in iron-enriched standard medium, West and Walne (1980) observed specimens with colorless as well as dark-colored loricae, both spiny and smooth. In material from freshwaters of Argentina and Brazil, Conforti *et al.* (1994) found no relationship between the dark color of trachelomonad loricae and their element composition, which lacked Mn. Wang *et al.* (2003) reached the same conclusion in a comparison of the color and chemical composition of *Strombomonas* and *Trachelomonas* loricae from waterbodies in northeastern China; those specimens mainly showed high Si and Fe content and a lack of Mn.

It is difficult to pinpoint one well-defined pattern of lorica chemistry. The presence of Fe in loricae wall structure is clear, but other elements occur irregularly (Mn and other ions) and seem to be strongly influenced by environmental factors. Van Veen *et al.* (1978) demonstrated the

role of pH in Fe oxidation processes in bacteria. Work by Dunlap and Walne (1985) showed that Fe accumulation is efficient under high  $\text{NH}_4\text{OH}$  in the culture medium, which was associated with increased medium/water pH; they also stressed the importance of physiological conditions such as cellular activity, the types of mucilage and mucopolysaccharides produced by a cell, and the presence of enzymes required to convert inorganic forms to organic ones. My results point to the need for further analyses of the chemical composition of trachelomonad loricae and the effects of a host of environmental and physiological conditions on the uptake of minerals.

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