Cryptogamie, Algologie, 2016, 37 (2): 97-108

Morphological variability of loricae in *Trachelomonas caudata* complex (Euglenophyta)

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Abstract – This study documented lorica ultrastructure variation in *Trachelomonas caudata* and related taxa from natural populations. On that basis, inaccuracies in the taxonomic system of euglenoid *Trachelomonas* are pointed out. Loricae of *T. caudata* and taxa very similar to it were examined by light and scanning electron microscopy and by energy-dispersive X-ray spectroscopy (EDS). The latter observations showed similarity of chemical composition between loricae of different putative taxa, with silicon and iron as the main components, and the absence of a chemical element considered to be a very important component of trachelomonad envelopes – manganese. All observed morphotypes were classified in one complex based upon envelope configuration, and the "caudatae complex" was established. Our morphological analyses led us to conclude that f. *pseudocaudata* of *T. caudata* should be subsumed in the typical form or treated as a synonym of *T. caudata*, as the morphological differences between them are within the typical range of phenotypic variability of species from natural environments. The same view applies to the species *T. bernardinensis*, *T. fusiformis*, *T. allorgei* and *T. molesta*, which should be considered synonyms of *T. caudata*.

Euglenoids / lorica / morphology / SEM / *Trachelomonas* / X-ray analysis

Résumé – Cette étude présente les variations ultra-structurelles de la lorica chez les populations naturelles de *Trachelomonas caudata* et des taxons proches. En s'appuyant sur ces variations, des incohérences dans le traitement taxonomique des euglénöides ont été mises en évidence. Les loricae de *T. caudata* et des taxons proches ont été examinés au microscope photonique, microscopie électronique à balayage et par spectroscopie à rayon X à dispersion d'énergie (EDS). Cette dernière approche a montré des similarités dans la composition chimique entre les loricae de différents taxons putatifs, avec comme éléments principaux fer et silicium, et l'absence d'un élément chimique considéré comme constitutif important des enveloppes de *Trachelomonas*, le manganèse. L'ensemble des morpho-types observés ont été classé dans un complexe basé sur la structure de l'enveloppe, le complexe « caudatae ».

Euglenoides / lorica / morphologie / MEB / *Trachelomonas* / analyse aux rayons X

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doi/10.7872/crya/v37.iss2.2016.97

INTRODUCTION

Trachelomonas caudata F.Stein was first described as having an elongate-ellipsoid lorica covered with blunt or sharp spines, with a wide collar at the apical end and a small cauda terminating in two or three processes at the posterior end (Stein, 1878). Almost a hundred years later, based on the description of *T. pseudocaudata* Deflandre (Deflandre, 1927), Popova (1966) emended and moved that species to *T. caudata* f. *pseudocaudata* (Deflandre) T.G.Popova, which, however, was not clearly distinguished from the typical form of *T. caudata*. Concurrently with the work of Stein, Stokes (1894) described *T. fusiformis* A.Stokes from waterbodies in the United States. These taxa had very similar descriptions. Several decades after Stein and Stokes published, Deflandre (1927) gave a description of another species, *T. bernardinensis*, with its form *africana* Deflandre. These taxa were also very similar to *T. caudata*, though apparently a bit smaller. Also in the 1920s, Deflandre described two taxa, *T. allorgei* Deflandre and *T. molesta* Deflandre, which are very close to *T. caudata* (Deflandre, 1926; 1927). Those two taxa are not widespread: there are only two records for *T. allorgei* (Romania, given by Cărăuş, 2012; Brazil, given by Alves-da-Silva & Menezes, 2010) and one for *T. molesta* (Brazil, given by Alves-da-Silva & Menezes, 2010). *Trachelomonas caudata* seems to be polymorphic, with these mentioned taxa assembling around it. Deflandre (1926) separated two groups: one with elliptically shaped lorica ornamented by short spines and having a widened neck at the rim (*T. caudata s. st.*, *T. pseudocaudata*), and the second with an elliptical or longitudinally elliptical lorica and ornamented with sparse dispersed spines and having a cylindrical collar (*T. fusiformis*, *T. molesta*, *T. bernardinensis*). Some details of lorica structure are difficult to observe with a light microscope but well distinguishable by SEM. However, the differences are still not significant enough to separate all those taxa as species. This presents a challenge, since, despite the huge development of cytological, genetic and molecular methods, the lorica of the euglenoid genus *Trachelomonas* is still used for diagnosis.

Trachelomonas caudata has been reported from virtually the whole world (Tell & Conforti, 1986; Conforti, 1993; Duque, 1995; Tell, 1998; Dillard, 2000; Zalocar de Domitrovic, 2014; Onuoha *et al*., 2014) and is commonly reported from Europe (Wołowski, 1998; Barone, 2003; Kočárková *et al*., 2004; Poniewozik, 2009). It has frequently been described as cosmopolitan and as a good indicator of β-mesosaprobic waters (Sládeček & Sládečková, 1996). The LM and SEM documentation of this species is meager: a few micrographs were published from Poland (Wołowski, 1998), and from Argentina for its variety *intermedia* Yacubson (Conforti, 1999).

Here we present the variability of loricae in *Trachelomonas caudata*: that is, of their organization, ornamentation and surface ultrastructure observed by SEM, and chemical composition. We also suggest changes in the taxonomic rank of *Trachelomonas caudata* and related species.

MATERIALS AND METHODS

Representatives of *Trachelomonas caudata* were obtained from different natural habitats. The analyzed material came from a puddle at a wayside shrine in

Bukowina Miejska glade near Mount Turbacz (Gorce Mts, SE Poland, 49°32'N, 20°05'E, ca. 1000 m a.s.l) and from a lake (51°23'N, 23°01'E) and a pond (51°22'N, 23°01'E) in the Lublin Upland (E Poland). We examined more than 30 samples containing *T. caudata* specimens. During the geobotanical study of desmids of the Gorce Mts (Wayda, 2016 in print) a sample from the puddle was taken. Studies in E Poland were carried out from August 2002 to July 2004 in monthly intervals. Water column and sediment samples were collected using a 30 ml pipette. When present, aquatic plants (primarily mosses) were sampled by squeezing them out. Only living material was used for taxonomic identification, lorica measurements and LM photographs (Nikon Eclipse E600 microscope, Nomarski differential interference contrast). All representatives of *T. caudata* from all the water bodies were regarded as a whole group. We did not focus on exceptionality of each specimen coming from particular water body. We wanted to catch diversity and variability of taxa that met a broad definition as *T. caudata* and we claimed that they should have been classified just as *T. caudata*, not as many independent species. Samples for SEM were prepared according to Bozzola & Russel (1995). Material fixed with glutaraldehyde/formaldehyde was rinsed in distilled water several times to remove buffer salts, after which the samples were dehydrated in a graded ethanol series and small drops of material were transferred to slides mounted on SEM stubs and then air-dried. 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) operating at 20 keV, coupled with a Thermo NORAN Vantage energy dispersive X-ray spectrometer (EDS analyses). The X-ray spectra were generated over 50 s live-time from 0 to 15 keV (30° take-off angle). Lorica ultrastructure was analyzed twice for each loriceae morphotype of *T. caudata* and for each one the EDS analysis was done. Physicochemical properties of the source water were measured with an Elmetron CP-401 pH meter and a CC-411 conductivity meter. Basic physicochemical properties of the water and names of accompanying species are presented in Table 1.

The ranges of lorica length and width were calculated using Statistica 10 for Windows (StatSoft).

Table 1. Basic physicochemical properties of water and accompanying species in habitats with species of the *Trachelomonas caudata* group

	T *[°C]*	*pH*	*EC* *[mS cm^{-1}]*	*ORP* *[mV]*	*Accompanying species*
Bukowina Miejska	21.0	6.0	31.0	291.0	*T. volvocina* Ehrenb., *T. hispida* (Perty) F.Stein, *T. abrupta* var. *minor* Deflandre, *T. stokesii* Drezep., *Phacus anomalus* F.E.Fritsch & M.F.Rich, *Lepocinclis ovum* (Ehrenb.) Lemmerm.
Lublin Upland	18.3-19.8	5.8-9.7	177.0-284.0	25.0-42.1	*T. volvocinopsis* Svirenko, *T. volvocina, T.rugulosa* F.Stein, *T. sabra* var. *labiata* (Teiling) Hub.-Pest., *Phacus pusillus* Lemmerm., *Phacus smulkowskianus* (Zakryś) Kusber, *Euglena texta* (Dujard.) Hübner

RESULTS

Light and scanning electron microscopy

Lorica appearance and ultrastructure. We examined many samples of loricae belonging to *Trachelomonas caudata* and the group of related species. Lorica length ranged from 26.1 μm to 38.3 μm, most often ca. 31.0-31.5 μm (median 31.18 μm) (Fig. 1). Width ranged from 14.1 μm to 20.0 μm, most often 17.0-18.0 μm (median 17.73, mean 17.41 μm) (Fig. 2). Some of the loricae were broadly oval in outline (Figs 3-15, 23-25) and some were narrower (Figs 16, 34) to even broadly fusiform (Fig. 17).

The surface of loricae showed one pattern of external structure: all envelopes had a rough wall covered with spines or warts. They varied in some details. The lorica wall was perforated but there were smaller or larger punctae regularly spread on the surface or, in some cases, holes combined with larger, irregularly distributed perforations (Figs 30-31). The spines covering the wall were either densely arranged (Figs 3-8, 23, 26, 28, 32) or sparsely distributed (Figs 31, 34), and either sharp (Figs 3-9) or blunt (Fig. 32). In some cases there were warts instead of spines on the lorica surface (Figs 13-15). By LM, some loricae seemed devoid of spines (Figs 10-11, 16). In several specimens found in the puddle at Bukowina, the upper part of the lorica was unnaturally developed, with large folds surrounding the collar and growing around it (Figs 18-22); the remaining external features and internal monad organization were the same as observed in other specimens.

Collar. The collar showed some differences between specimens. Most were cylindrical and denticulate at the rim (Figs 3-12, 16-17, 23, 25, 27, 29-30). Some were slightly widened at the rim and differently toothed (with shorter or longer teeth; e.g. Figs 3, 8-10, 32-34). Some were slightly curved to one side (Figs 4, 30), and low or asymmetrical collars were seen (Figs 14, 18-22, 24, 26). A few loricae had no collars (Figs 15, 31).

Posterior end. The end of the lorica projection was of different shapes and sizes (Figs 35-41). Most were conical, and ranged in length from 1.5 to 4.5 μm. The top of the projection terminated in three small teeth, considered typical of *T. caudata* f. *caudata* (Figs 35, 36). Some loricae possessed a projection with no additional structures (Fig. 37), in agreement with description or drawings of *T. fusiformis*, *T. bernardinensis*, *T. molesta*, *T. allorgei* and also *T. caudata* f. *caudata*. Other specimens had a blunt projection with small or distinct folds (Figs 38-40) or even a truncated end (Fig. 41).

Element composition (EDS analyses)

SEM-EDS analyses indicated that, though sourced from different water bodies, all the examined loricae generally had similar chemical composition, the main components being silicon (Si) and iron (Fe) (Table 2). The loricae from the Lublin Upland in eastern Poland contained more silicon, but iron levels were similar in all the studied loricae. Potassium (K) and phosphorus (P) were also present in relatively high amounts in the loricae of *T. caudata*. The phosphorus concentration in the loricae from eastern Poland was several times higher than in those from the puddle in Bukowina Miejska (SE Poland). Interestingly, our analyses did not detect manganese (Mn) in any of the studied envelopes, although that metal is considered to be one of the main ions, besides iron, forming the chemical composition of

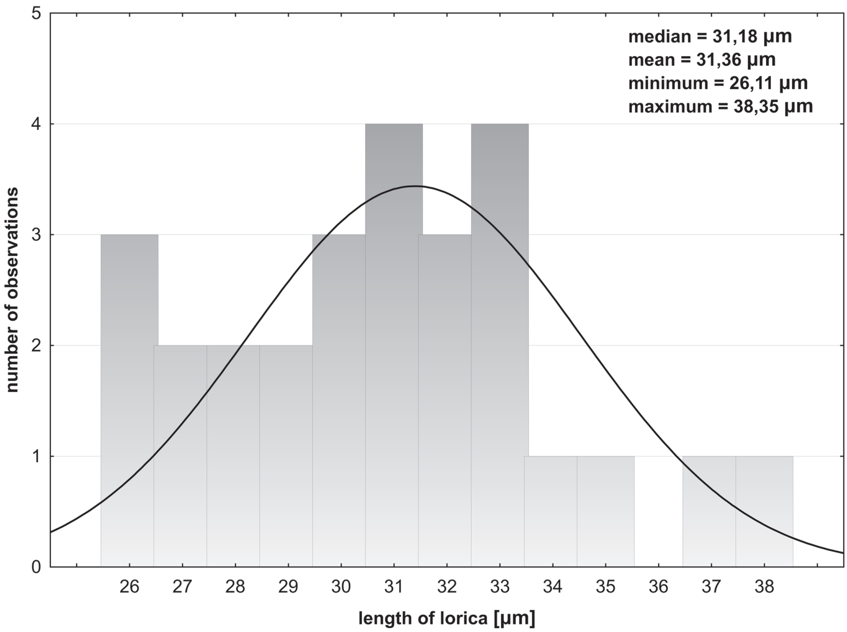


Fig. 1. Length of the studied loricae of specimens of *Trachelomonas caudata* group.

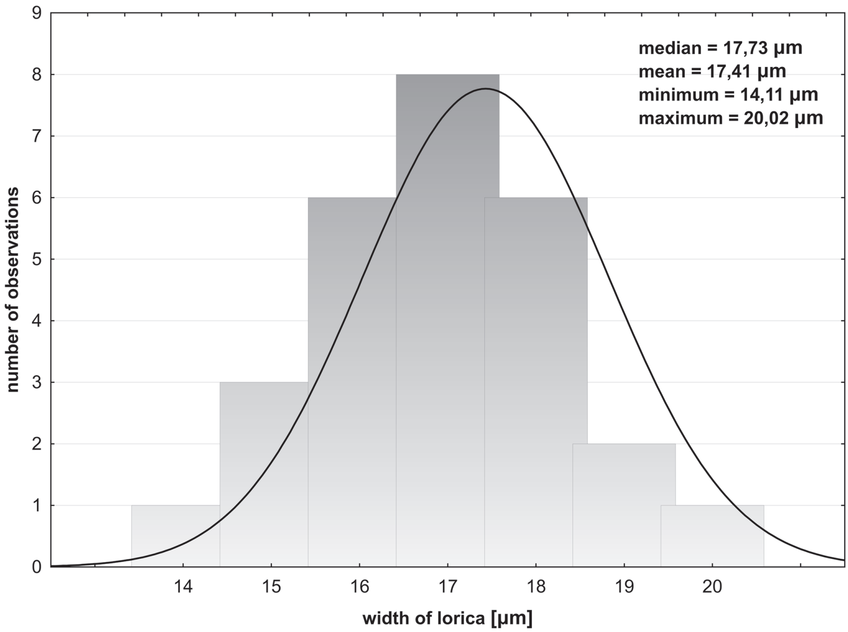
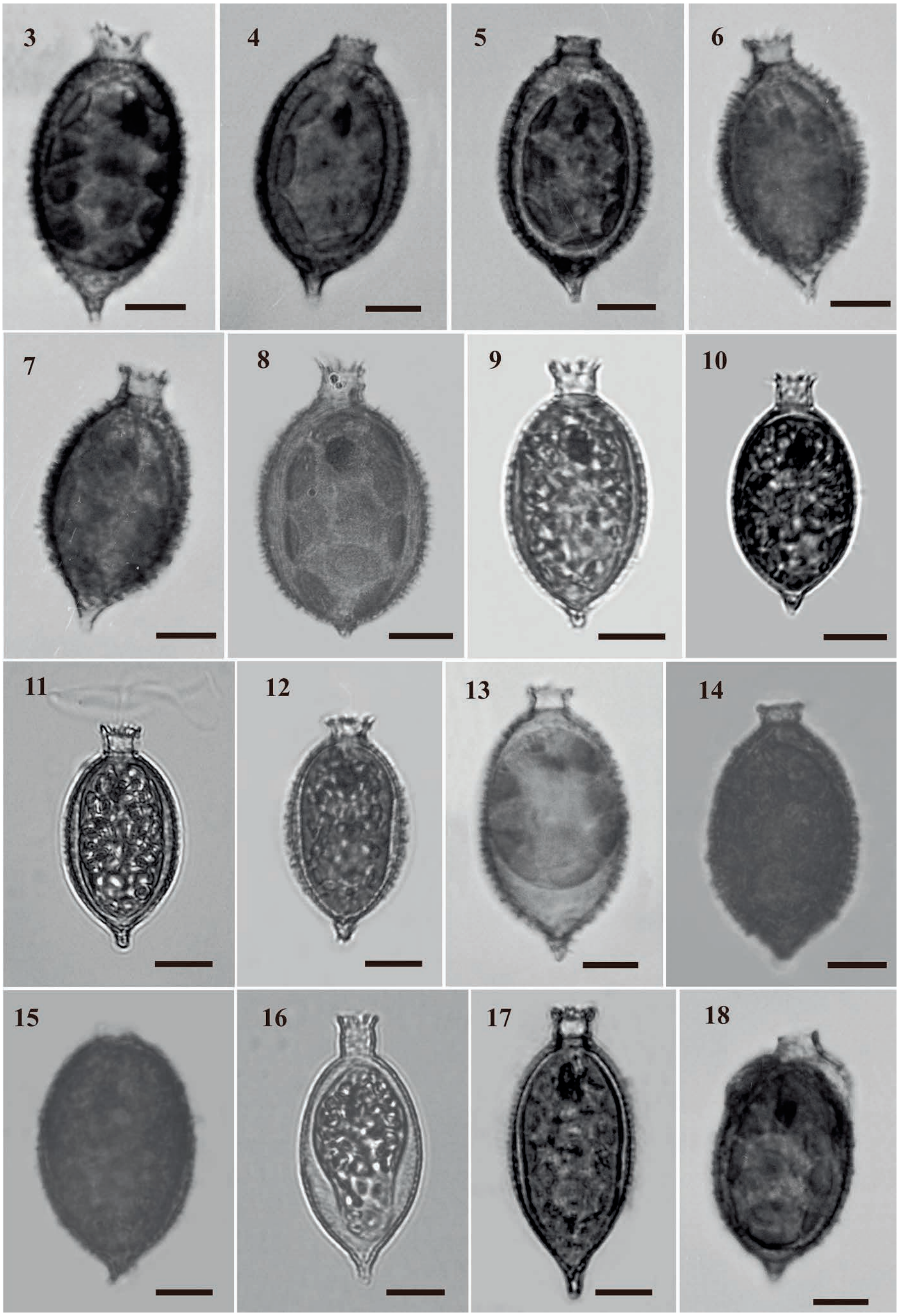
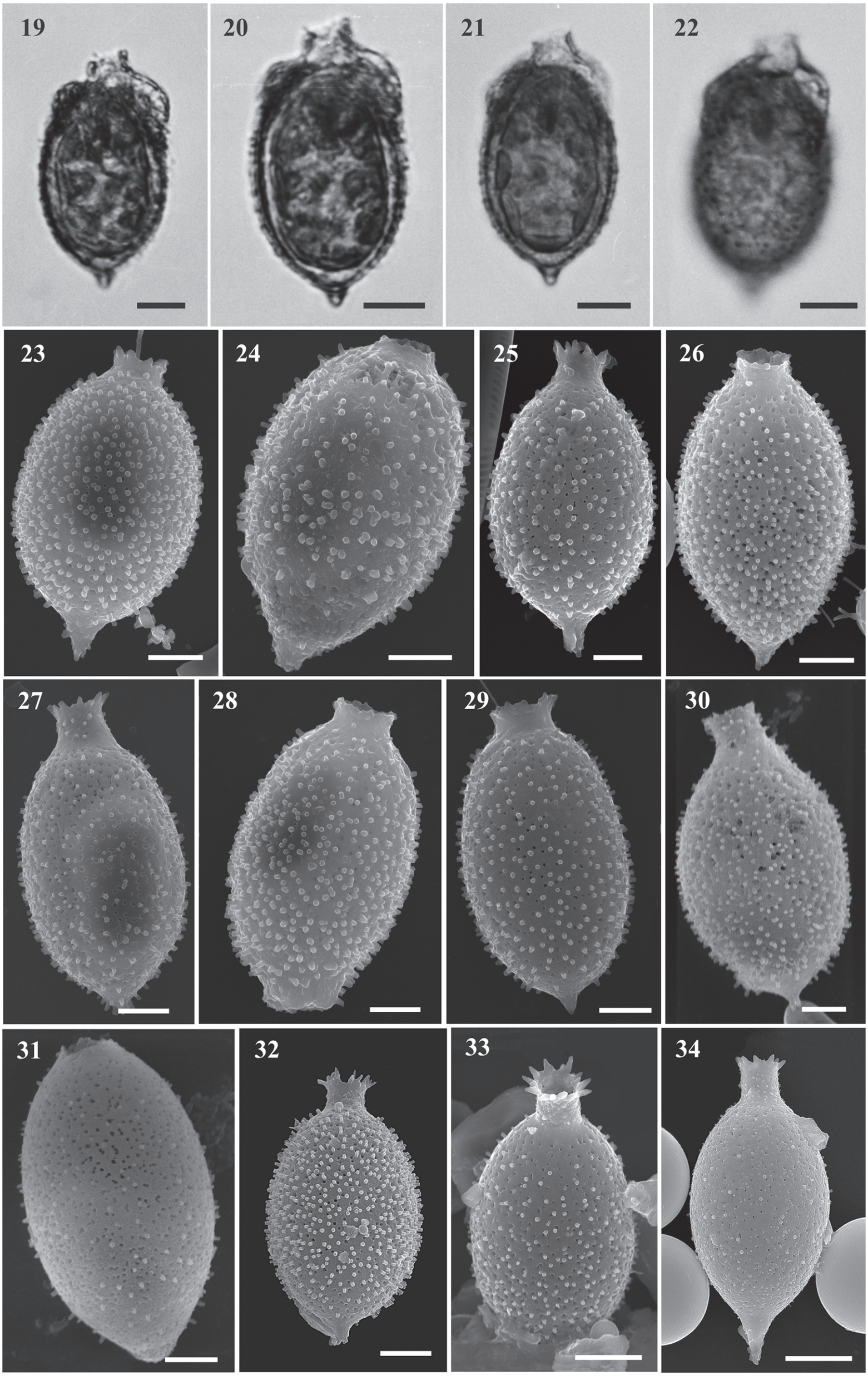


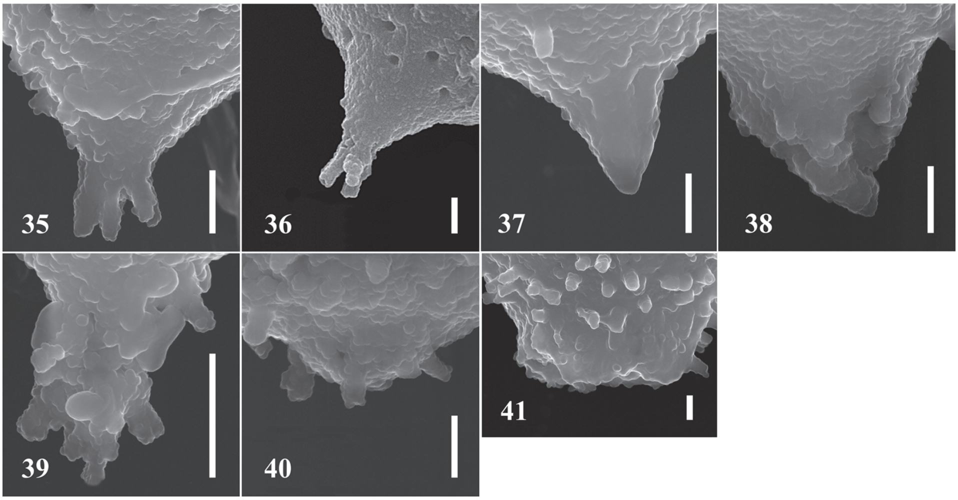
Fig. 2. Width of the studied loricae of specimens of *Trachelomonas caudata* group.



Figs 3-18. Morphological variability of *Trachelomonas caudata* loricae (LM micrographs).



Figs 19-34. Morphological variability and structural details in *Trachelomonas caudata* (LM and SEM micrographs).



Figs 35-41. Structural details of posterior end in *Trachelomonas caudata* loricae (SEM micrographs).

Table 2. Chemical composition of the studied loricae of specimens of the *Trachelomonas caudata* group. Sample "0" presents results of the EDS analysis for basal glass. Specimens 1-3 came from the puddle in Bukowina Miejska (SE Poland), 4 from the lake and 5 from the pond (both from Eastern Poland)

	Al		*Si*		*Ca*		*K*		*Fe*		*Na*		*P*	
	Atom [%]	*Err. +/– [%]*	*Atom [%]*	*Err. +/– [%]*	*Atom [%]*	*Err. +/– [%]*	*Atom [%]*	*Err. +/– [%]*	*Atom [%]*	*Err. +/– [%]*	*Atom [%]*	*Err. +/– [%]*	*Atom [%]*	*Err. +/– [%]*
0	4.63	0.19	67.65	0.36	–	–	8.70	0.23	–	–	10.47	0.17	–	–
1	4.61	0.17	41.93	0.46	5.18	0.21	6.51	0.21	15.82	0.94	15.54	0.94	1.29	0.18
2	6.62	0.34	43.41	0.52	3.72	0.40	5.97	0.39	23.20	1.14	7.25	0.27	2.10	0.21
3	3.98	0.24	39.14	0.56	6.25	0.53	6.25	0.27	15.65	0.82	13.98	0.38	3.47	0.29
4	5.42	0.19	50.34	0.69	2.53	0.18	5.64	0.37	15.43	0.93	2.20	0.38	9.99	0.59
5	4.04	0.14	43.22	0.49	2.55	0.13	4.96	0.28	17.33	0.70	12.01	0.23	12.34	0.48

trachelomonad loricae. Other components of the envelope wall, present in small amounts, were aluminium, calcium and sodium (Table 2).

On the basis of this work we believe that *T. caudata* f. *pseudocaudata* and *T. bernardinensis* should be treated as synonyms of *T. caudata*. The differences between the investigated specimens were very narrow and within the typical range of phenotypic variability within such species. We also judge *T. fusiformis*, *T. allorgei* and *T. molesta* to be synonyms of *T. caudata*. Our LM observations of live monads (Figs 3-22) showed that these representatives of the *T. caudata* group had very similar intracellular organization: they usually had 8-11 plate-shaped chloroplasts without pyrenoids. Thus we suggest that there is no need to distinguish several separate species; the specimens have the same external and internal organization of the monad and lorica.

DISCUSSION

Trachelomonas caudata and similar species such as *T. bernardinensis*, *T. fusiformis*, *T. allorgei* and *T. molesta* were described for the first time about a century ago. Up to now only *T. caudata* has been reported as a widespread and well known species (Wołowski, 1998; Dillard, 2000; Wołowski & Hindák, 2003). The other species have not been recorded for many years. This seems to mean that their first descriptions as new species were erroneous. They have very similar descriptions of the external appearance and the internal structure of the monad. The morphological features of loricae given for these taxa, presented in Table 3, are very similar: the loricae of all the taxa are described as elliptical (broadly elliptical, longitudinally elliptical, or just elliptical) or egg-shaped, or broadly fusiform. The external structure of the lorica is also similar: it is described as covered by spines (sparsely or densely distributed). Only *T. allorgei* has no spines on the lorica surface (Table 3). There are also insignificant differences in the shape of the collar, which is cylindrical and more or less denticulate at the rim. In a few cases we observed loricae that had no collar and the apical pore was jagged at the rim (Figs 15 and 31). We presume that there is a possibility that the collars might just have broken off, but, on the other hand, it raises an intriguing question why had they broken off as a whole? Nevertheless, we do not preclude such a case. The sizes given for the loricae are similar, especially for width: between 18 µm and 24 µm. Length is from 26 µm to 75 µm (Table 3), but such a large range of lorica length has been observed in other species such as *T. caudata* f. *pseudocaudata* or *T. caudata* f. *caudata* (Table 3). Most of the features

Table 3. Summary of major characteristics of "caudatae complex" species reported by different authors

Species	*Shape of lorica*	*Shape of collar*	*External structure*	*Width [µm]*	*Length [µm]*
T. caudata f. *caudata*	elliptical or egg-shaped	cylindrical, widened at rim	lorica covered by small spines or punctate, wall light to dark brown	(18)-19-24	30-45-(50)
T. caudata f. *pseudocaudata*	regularly elliptical	widened and denticulate at rim	lorica thickly covered by obtuse spines	(18)-21-24	30-36-56
T. fusiformis	longitudinally elliptical or egg-shaped	cylindrical, denticulate at rim	punctate, covered by sparse spines, wall yellow-brown	19-22	45-63
T. bernardinensis	elliptical	cylindrical or funnel-shaped, smooth or denticulate at rim	covered by small papillae, rarely by thin spines, wall light or dark brown	17-22	26-38
T. allorgei	elliptical or broadly fusiform	widened and denticulate at rim	lorica punctate, wall yellow-brown	20-22	52-61
T. molesta	broadly fusiform	cylindrical, smooth at rim	lorica slightly punctate, light yellow-brown	20-22	65-75

1 – *T. caudata* f. *caudata*, 2 – *T. caudata* f. *pseudocaudata*, 3 – *T. fusiformis*, 4 – *T. bernardinensis*, 5 – *T. allorgei*, 6 – *T. molesta*.

of the remaining species (*T. bernardinensis*, *T. fusiformis*, *T. allorgei*, *T. molesta*) are contained in the description of *T. caudata*. Having the benefit of more comparative data at our disposal, we find it difficult to see what the differences between these species could be. The authors of the taxa described the appearance of the lorica more or less precisely, using different definitions for morphological features. When we look at the original drawings we do not see important differences between that structures on the lorica wall that were described variously as small blunt spines, thin spines, or small papillae. Moreover, the original drawings of some of the mentioned taxa look the same. Lorica size raises another question: what does it mean to say that species x is a few microns longer than species y? Considering the shape, size, colour and external surface of the lorica, as well the shape and size of the collar in published descriptions and iconography, we conclude that the species have no strong, distinguishable features (Table 3) and are likely synonymous with *T. caudata*. The mentioned differences in lorica arrangement and size are not enough to distinguish and maintain these taxa as separate species. Moreover, some of them have monads bearing the same description (e.g., *T. caudata*, *T. bernardinensis*), and there is no description of monad arrangement for *T. fusiformis*, *T. allorgei* or *T. molesta*.

Our morphological analyses led us to conclude that f. *pseudocaudata* of *T. caudata* should be subsumed in the typical form or treated as a synonym of *T. caudata*, as the morphological differences between them are within the typical range of phenotypic variability of species from natural environments. The same view applies to the species *T. bernardinensis*, *T. fusiformis*, *T. allorgei* and *T. molesta*, which should be considered synonyms of *T. caudata*. However, independent molecular studies are needed to verify our morphology-based preliminary conclusion. It may be difficult to obtain material of *T. fusiformis*, *T. allorgei* and *T. molesta* for molecular work; they were found decades ago and have not been banked in any culture collections anywhere. In any event, on the basis of *Trachelomonas caudata* morphological features, our results allowed us to establish a complex taxon.

This is the first study of the element composition of *T. caudata* envelopes. Unexpectedly we recognized the main component of the studied loricae was silicon. We presume that some amount of silicon might have come from the cover slip that was used for the preparation of the material, but according to extended studies, silicon is one of the components of loricae like iron and manganese (Poniewozik, personal report). Dominance of Si in lorica walls is not a common finding; silicon generally is found as an accompanying element (West *et al*., 1980). Only Steinberg & Klee (1984) reported silicon as the main element in the loricae of some trachelomonad species. Iron, known to be a fundamental component of loricae ultrastructure, also occurred in high amounts. The envelopes also contained small amounts of P and K compounds. Pereira *et al*. (2003) reported that the loricae of *T. hispida* var. *coronata* Lemmerm., *T. similis* A.Stokes and *T. volvocinopsis* largely contained iron compounds (1-49% of the total weight of loricae). Dunlap *et al*. (1983) found iron to be the main component of hyaline lorica of *T. lefevrei* Deflandre, while specimens of the same species with dark loricae had a high percentage of manganese. Iron as a dominant component was detected in loricae of *T. volvocina* and *T. similis* (Conforti *et al*., 1994). Our results on the chemical composition of "caudata group" loricae are in line with previous findings that envelopes having granular structure are saturated mainly by iron and are lighter in colour – pale yellow or almost hyaline (Dunlap *et al*., 1983). Our analyses did not detect manganese in any of the studied loricae, although it is considered to be one of the predominant elements in trachelomonad envelopes (West & Walne, 1980; Dunlap *et al*., 1983). It is known that the chemical composition of loricae somewhat depends on which chemical compounds are available to organisms in the water (Pereira & Azeiteiro, 2003; Pereira *et al*., 2003). Testing the composition of trachelomonad loricae from natural environments, Heinrich *et al*. (1987) found that it varied from iron dominance to manganese dominance, and some *Trachelomonas* species from the same natural conditions accumulated only iron, indicating that the chemical composition of loricae is species-specific to some extent (Heinrich *et al*., 1987).

Acknowledgements. We thank Dr Maciej Wayda for providing material for research and Michael Jacobs for line-editing the manuscript for submission. We also thank our friend Kady Du for her help in French abstract preparation. This work received support from the W. Szafer Institute of Botany of the Polish Academy of Sciences through its statutory funds (F-010811) and from the Polish Ministry of Sciences and Higher Education (grant no. 2P04C 043 30).

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