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A Taxonomic and Ultrastructural Study of *Trachelomonas* spp. (Euglenophyta) from Agricultural Area Pond, Lamphun Province

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Abstract

A taxonomic and ultrastructure study of *Trachelomonas* spp. from agricultural area pond in Thailand are presented. Forty-nine taxa are briefly described, among them thirty eight are recorded for the first time from Thailand and nine are reported as rare worldwide. Light and scanning electron microscope were used to show a complete structure for identification of *Trachelomonas* taxa. The physico-chemical parameters of water in sampling sites are given.

Background

Trachelomonas Ehrenberg is a genus in Euglenophyta division which is often abundant in polluted environments. They are characterized as free-swimming *Euglena*-like cells, phototrophic and completely enclosed in a spherical to ovoid mineralized envelope (lorica). The lorica has small, apical opening, usually with an abrupt collar. Lorica tends to be porous and variously ornamented with papilla or spines [1]. The genus *Trachelomonas* was first described by Ehrenberg [2] and studied worldwide e. g.: [3,4,5,6]. The present database includes 306 species that have been recorded as currently accepted taxonomically using light (LM) and scanning electron (SEM) microscope [7]. Information about *Trachelomonas* in Thailand is still scarce, so this study was focused on a taxonomy, ultrastructure and biodiversity of *Trachelomonas* spp. from agricultural area pond as natural environment for this species.

Materials and Methods

Samples were collected in February-March 2010 using plankton net of 10 µm pore size. Material was taken into a plastic flask ca. 100 ml and divided into two parts, one was preserved with Lugol's solution and studied with scanning electron microscope (SEM), while the second one was transported as fresh material and studied in laboratory using light microscope (LM). The samples were identified according to [3,4,8,9].

The physico-chemical properties of water such as alkalinity, BOD, conductivity, DO, nutrients (nitrate, ammonium and soluble reactive phosphorus, SRP), pH, air and water temperature were analyzed [10]. In term of water quality, AARL PC-Score [11] was followed.

Ultrastructure of lorica was studied using a Hitachi S-4700 SEM. Sample fixed with Lugol's solution was rinsed in distilled water several times and then the small drop of each sample was transferred onto the surface of the slides mounted on SEM stubs and air-dried. The samples were coated with carbon [7].

Results and Discussion

Forty-nine taxa were found and the description is shown below (Table 1); the illustration is shown in Figs 1 and 2. New taxa for Thailand is marked with asterisk. The physico-chemical properties of the water of agricultural area pond were shown (Table 2.).Water quality was mesotrophic.

Figure 1. LMs of lorica of *Trachelomonas* spp. 1. *T. atrata* (Skvortzov) Deflandre*, 2. *T. atrata* var. *pustulosa* Conrad*, 3. *T. bulla* Stein*, 4. *T. felix* Skvortzov*, 5. *T. grandis* Singh*, 6. *T. hirta* var. *duplex* Deflandre*, 7. *T. hispida* var. *spinulosa* Skvortzov *, 8. *T. hispida* var. *volicensis* Drežepolski*, 9. *T. planctonica* Swirenko*, 10. *T. similis* var. *hyalina* Skvortzov.
" * " = new record of Thailand.

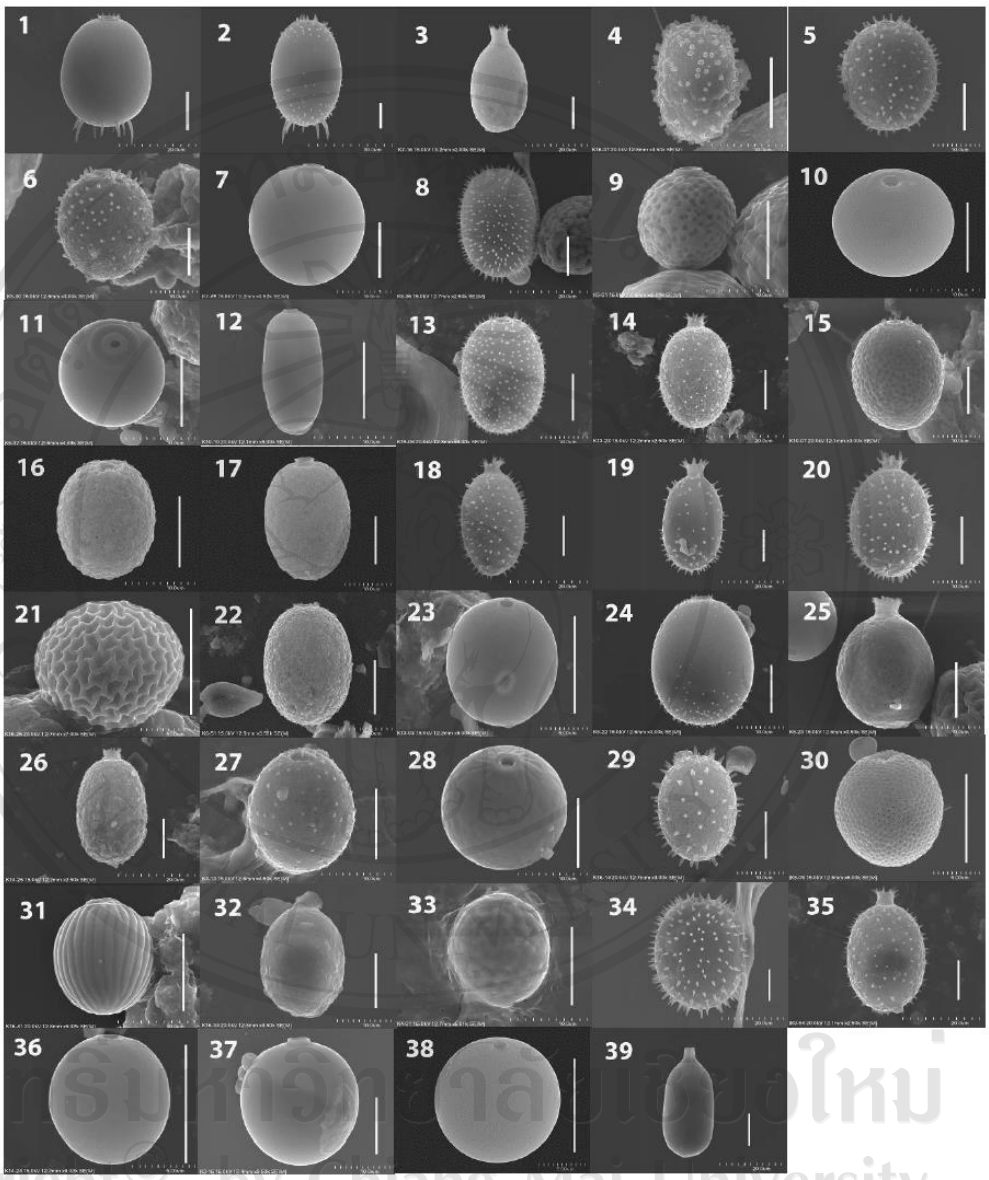

Figure 2. SEMs of *Trachelomonas spp*. 1. *T. armata* (Ehrenberg) Stein, 2. *T. armata* var. *steinii* Lemmermann, 3. *T. arnoldiana* Skvortzov*, 4. *T. australica* var. *rectangularis* Deflandre*, 5. *T. bacillifera var. minima* Playfair*, 6. *T. bacillifera f. sparsispina* Deflandre*, 7. *T. cervicula* Stokes*, 8. *T. cingeri* Roll*, 9. *T. compacta* Middelhoek*, 10. *T. curta* Da Cunha*, 11. *T. curta* var. *castrensis* (Palmer) Deflandre*, 12. *T. cylindrica* Ehrenberg, 13. *T. hispida* (Perty) Stein, 14. *T. hispida var. cremulatocollis* (Maskell) Lemmermann*, 15. *T. intermedia f. papillifera* (Popowa) Popowa*, 16. *T. irregularis* Swirenko*, 17. *T. lefevrei* Deflandre*, 18. *T. lotharingiae* De Pouques*, 19. *T. mirabilis* var. *helvetica* Huber-Pestalozzi*, 20. *T. mirabilis var. obesa* (Messikommer) Conrad*, 21. *T. nexilis* Palmer*, 22. *T. nigra* Swirenko*, 23. *T. oblonga* Lemmermann*, 24. *T. oviformis var. duplex* V. Conforti*, 25. *T. pavlovskoensis* (V. Poljanskij) Popowa*, 26. *T. planctonica f. oblonga* (Drežepolski) Popowa, 27. *T. pulchella* Drežepolski*, 28. *T. radiosa* Fritsch*, 29. *T. robusta* Swirenko emend. Deflandre*, 30. *T. rotunda* Swirenko*, 31. *T. rugulosa var.minima* V.Conforti & L.Ruiz*, 32. *T. similis* Stokes*, 33. *T. spirillifera* Schkorbatov*, 34. *T. superba* Swirenko, 35. *T. sydneyensis var. grandicollis* Deflandre*, 36. *T. volvocina* Ehrenberg, 37. *T. volvocina var. derephora* Conrad*, 38. *T. volvocinopsis* Swirenko*, 39. *T. volzii var. cylindracea* Playfair*. " * " = new record of Thailand.

Table 1 Morphological character of forty-nine taxa from agricultural area pond, Lamphun province

Species	Lorica width (µm)	Lorica length (µm)	Lorica Shape	Apical pore	Cell wall
T. armata	29-32	28-45	Broadly ellipsoidal	With thickening or with low tooth	Wall punctate or smooth, with long spines (8-10 µm long) at posterior end
T. armata var. *steinii*	23-25	30-40	Broadly ellipsoidal	Without collar	Finely punctate or smooth, short spines at the anterior end, stout long spines at posterior end, up to 9 µm long
T. arnoldiana *	20	25	Pear-shaped	Collar cylindrical, toothed on the top, 5 µm high	Wall finely punctate, with rare granular
T. atrata *	16-24	26-33	Oval	Collar cylindrical, curved or straight	With row loose-standing papilla at posterior end
T. atrata var. *pustulosa* *	10.5-13	13 – 16	Oval	Collar cylindrical, bent	With group of papilla (2-4 papillae)
T. australica var. *rectangularis* *	22	32	Oval	Without collar	With blunt spine
T. bacillifera var. *minima**	15-15.5	16.5-17.5	Broadly oblong	With thickening	Wall punctate, with short, obtuse spines
T. bacillifera f. *sparsispina* *	20	25	Broadly oblong	With thickening	With rarely rod-shaped spine
T. bulla *	21-25.5	39-46.4	Oval	Collar conical-shaped, serrate or curved edge	With irregular short spines
T. cervicula *	20-25	20-25	Broadly ovoid	Collar cylindrical extending inwards to lorica	Wall smooth; chloroplasts plate-shaped without pyrenoid
T. cingeri *	21.6-22-(23.6)	40.6-42	Elliptically cylindrical	With thickening	Wall densely punctate, with rare sharp spine, 3.5-4 µm long
T. compacta *	15-16	15-19	Nearly spherical	With ring-like thickening or low collar	Wall ca. 3 µm thick, with fine holes
T. curta *	15-18 (-22)	9-12 (-20)	Compressed globose	With thickening	Wall smooth
T. curta var. *castrensis* *	20	16	Compressed globose	With wide thickening	Wall smooth
T. cylindrica	8-10	14-21	Cylindrical	Without or with very low collar	Wall Smooth; few, plate-shaped chloroplasts without pyrenoid
T. felix *	15	20	Oval	Without collar	Wall punctate, with some elongated papilla
T. grandis *	25-32	30-43	Large ellipsoidal	Collar short, narrow	With short irregularly granules; chloroplasts with haplopyrenoids
T. hirta var. *duplex* *	16.5	24	Oval	Without collar	Wall radically spine, arranged irregularly at both end; plate-shaped chloroplasts
T. hispida	16.5-22	20-26	Oval	Without collar	Wall densely punctate, spines; 8-10 plate-shaped chloroplasts, with pyrenoid
T. hispida var. *crenulatocollis*	19-22	28-30	Oval	With toothed collar, 2 µm high	Wall densely punctate, spines; 8-10 plate-shaped chloroplasts, with pyrenoid
T. hispida var. *spinulosa**	16.2-19.5	24-28	Oval	With toothed collar, 2 µm high	Wall densely punctate, densely spine; 8-10 plate-shaped chloroplasts, with pyrenoid
T. hispida var. *volicensis* *	18-21	23-26	Broadly ellipsoidal, slightly flattened at the anterior end	Without collar	Wall punctate, with densely short spines
T. intermedia f. *papillifera* *	16.8-21	19.6-22	Oval	Without collar	With 2 row papillae at anterior end, punctate
T. irregularis *	11-18	16-18	Irregularly elliptical	Collar low, with toothed or regular edge	With finely granular
T. lefevrei *	22-24	27-31	Cylindrical to ellipsoidal	Collar low, with toothed edge	Wall densely punctate, chloroplasts with haplopyrenoids

Journal of the Microscopy Society of Thailand **5** (1-2), 23-27 (2012)

Table 1 Morphological character of forty-nine taxa from agricultural area pond, Lamphun province (continued)

Species	Lorica width (µm)	Lorica length (µm)	Lorica Shape	Apical pore	Cell wall
T. lotharingiae *	22-25	30-34	Obovoid	Collar cylindrical, 4-5 µm high, with short spines on edge	Wall punctate, with rare spines, 2-3 µm long
T. mirabilis var. *obesa* *	21	32-33	Oval	Collar cylindrical, with spines, 7 µm long	Wall punctate, with strong spines
T. mirabilis var. *helvetica**	20-21	31.5-33	Oval	Collar cylindrical, with 5 spine on top	Wall with strong spines at the anterior and posterior part, middle part of lorica covered with short spines and punctate
T. nexilis *	20	20	Almost spherical	Collar low in small depression	With broken slat forming type lateral netting
T. nigra *	19-19.5	23-23.5	Broadly ellipsoidal	With several large verrucae	Wall punctate, with small, single verrucae
T. oblonga *	10.5-12	11.5-19	Broadly ellipsoidal	Without collar	Wall smooth, chloroplasts with pyrenoids
T. ovitormis var. *duplex* *	25-27	30-32	Oval	Without collar	Wall with very short spines around both ends, middle part punctate with densely papillae, short spines on the edge
T. pavlovskoensis *	20-26	26-32	Oval, anterior end slightly narrowed	Collar cylindrical, 3.5-6-(7) µm wide, 3-6 µm high with serrated edge	Wall densely punctate
T. planctonica *	18-19.5	20.3-27.5	broadly ellipsoidal	Collar cylindrical, irregularly toothed on edge	Wall densely punctate
T. planctonica f. *oblonga*	17-19	22-24	Oval	Collar cylindrical, irregularly toothed on edge	Wall densely punctate
T. pulchella *	15-17	18-21	Oval	Without collar	Wall with loosely papilla or short spines
T. radiosa *	20	17-18	Oval	With high ring-like thickening	Wall with 12 longitudinal ribs extend from the top to the middle part
T. robusta	17-22	20-25	Broadly ellipsoidal	Without collar	Wall with rare, strong spines, smooth or punctate
T. rotunda *	13.8	16.6	Oval	Without collar	Wall minutely punctate (4-5 points per 1 μm^2), densely scrobiculated
T. rugulosa var. minima *	9-10	10-11	Oval	With ring-like thickening	Wall with ridges longitudinally, branched or not, starting near apical pore
T. similis	12-22.5	19-32	Oval	Collar cylindrical, bent, irregularly toothed	Wall scattered punctuate, chloroplasts numerous, without pyrenoids
T. similis var. *hyalina* *	15.5	18.7-23	Broadly ellipsoidal to ovoid	Collar bent, irregularly toothed	Wall scattered punctuate, hyaline
T. spirillifera *	13	17.5	Slightly elongated	Collar low, wide apical pore	With spiral ridges, broken, not connected with each other
T. superba	30-36	38-45	Broadly ellipsoidal	Without collar, with stout spines	Wall punctate, with sharp spines, 2-5 µm long
*T. sydneyensis** var. *grandicollis*	24-28	34-48	Oval	Collar cylindrical, 3-6 high, spine at the edge	Wall punctuate, with sparsely spines
T. volvocina	6-23(-32)	6-23(-32)	Spherical	Without collar or collar very low	Wall smooth; 2 chloroplasts with pyrenoids
T. volvocina var. *derephora*	12-21	12-21	Globular	Collar short, stout	Wall smooth; 2 chloroplasts with pyrenoids
T. volvocinopsis *	15.5-24	15.5-24	Spherical	With ring-like thickening	Wall smooth; several, small, plate-shaped chloroplasts, without pyrenoids
T. volzii var. *cylindracea*	15-16	34-38	Cylindrical, anterior end narrowed, posterior end broadly rounded	Collar conical, ring-like thickening at the base	Wall smooth

Table 2 The physico-chemical properties of the water of agricultural area pond, Lamphun province.

Date	25/2/2010	4/4/2010
Air temperature (°C)	33.0	41.0
Water temperature (°C)	25.6	30.1
pH	7.4-7.6	7.3-7.5
Conductivity (µs/cm)	294-296.0	290.0-294.0
Alkalinity (mg/l as CaCO3)	147.0-150.0	94.0-96.0
DO (mg/L)	5.0-5.2	3.6-4.0
BOD (mg/L)	2.8-3.0	3.2-3.4
NH4-N (mg/L)	0.0	0.0
NO3-N (mg/L)	0.7-0.8	0.3-0.4
SRP (mg/L)	0.0	0.1

Conclusion

All together 49 taxa of *Trachelomonas* were found which dominated among other euglenoids genera. Especially *T. armata* (Ehrenberg) Stein, *T. cervicula* Stokes, *T. hispida* (Perty) Stein, *T. oblonga* Lemmermann, *T. planctonica* Swirenko and *T. volvocinopsis* Swirenko occurred frequently. All of those taxa are well known all over the world and usually can be found in polluted water. Earlier similar reports were published from e.g. Europe: Wołowski [12,13], Safonova [14]; Asia Yamagishi [15]; North America: Dillard [5], Wołowski, [8]; Australia Playfair [6]. Some data about *Trachelomonas* from Thailand were elaborated by Lewmanomont *et al.* [16] who included information about 13 taxa. Recently detailed information was published by Yamagishi [15]. He reported data about 31 *Trachelomonas* species occurring in different ponds of Thailand. He included well elaborated LM documentation for all studied taxa. Our study gives information about 38 new taxa for Thailand. The high efficiency equipment as LM and SEM provided more information, helped with identification and increased the opportunity to discover more species. However, this study includes only Lamphun province, expanding the investigation to wider area could provide more information about the species that will sustain the biodiversity database of Thailand.

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NEW TAXA OF LORICATE EUGLENOIDS *STROMBOMONAS* AND *TRACHELOMONAS* FROM THAILAND

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Abstract. Five new species and one new variety of loricate euglenoid taxa were discovered in Thailand: *Strombomonas starmachii* Duangjan & Wołowski, *S. chiangmaiensis* Duangjan, *Trachelomonas peerapornpisalii* Duangjan & Wołowski, *T. thailandicus* Duangjan & Wołowski, *T. reticulato-spinifera* Duangjan and *T. hystrix* var. *paucispinosa* Prowse. We propose to raise the variety to species level [*T. paucispinosa* (Prowse) Duangjan & Wołowski, *stat. et comb. nov.*]. The morphology and fine lorica ultrastructure of the species are described. All taxa are documented by SEM images and some by LM micrographs.

Key words: Thailand, Euglenophyta, *Strombomonas*, *Trachelomonas*, new taxa

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INTRODUCTION

The lorica is a protective envelope surrounding the protoplast in some euglenoid genera such as *Strombomonas*, *Trachelomonas* and *Ascoglena*. The mature individual has its own taxon-specific lorica. Lorica characters can facilitate identification but the lorica also frequently obscures the cell (monad) inside it, and the range of intraspecific variation of its morphology needs to be determined in clonal culture. Nor can cytological and genetic studies be assured of clear results when only a small sample is available. So far the lorica development process is not sufficiently known for the majority of taxa, despite the importance of the morphological characters of mature loricas for identifying and describing new loricate euglenoid taxa. According to several researchers (Ciugulea *et al.* 2008; Brosnan *et al.* 2005) the identification of *Strombomonas* and *Trachelomonas* taxa from field collections alone is nearly impossible, but despite that we believe that the morphological diversity should be recorded. The lack of molecular data should not exclude morphological descriptions as a valid taxonomical observation.

Some of the first descriptive information about loricate taxa was published by the eminent phycologists Ehrenberg (1833) and Stein (1878), and later by Lemmermann (1913), Playfair (1915), Deflandre (1926, 1930) and Conrad (1932), Popova and Safonova (1976), Starmach (1983). Lorica development was studied and described in cultured *Trachelomonas* species by Pringsheim (1953), Singh (1956) and Lidale (1975). More recent years have brought detailed studies of lorica ultrastructure (e.g., Conforti 1999; Conforti & Tell 1986; Conforti & Perez 2000; Wołowski & Hindák 2004, 2005; Wołowski & Walne 2007; Da *et al.* 2009 and Ciugulea & Triemer 2010). West *et al.* (1980), Dunlap *et al.* (1983), Dunlap & Walne (1985) and Dunlap *et al.* (1986) made detailed studies of the element composition and microarchitecture of the lorica in selected *Trachelomonas* and *Strombomonas* taxa. Recently, Brosnan *et al.* (2003, 2005) and Ciugulea *et al.* (2008) addressed problems related to separation of those genera based on differences in lorica morphology and

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development. Ciugulea *et al.* (2008), Linton *et al.* (2010) demonstrated the very high diagnostic value of cell morphology (number of chloroplasts, type of pyrenoid).

Information about the euglenoids of Thailand is scarce. The main sources of information about the group are references given by Lewmanomont *et al.* (1995) and Peerapornpisal *et al.* (2004) in publications on different taxonomic groups of algae. Yamagishi (2010) published information, with good documentation, about 31 *Trachelomonas* and 7 *Strombomonas* euglenophyte species occurring in Thai ponds. Recently, Duangjan *et al.* (2012) gave descriptions accompanied by LM and SEM documentation for 49 taxa of *Trachelomonas*.

This paper presents the results of a study on euglenoids from various small water bodies in northern Thailand. Most parts of the country are under tropical wet and dry climate.

Materials and Methods

Each month from April 2009 to March 2010, samples were collected from different types of ponds in northern Thailand. The material was taken from open water with a plankton net (10 µm mesh) and from the bottom with a slime aspirator, and then placed in plastic flasks (*ca* 100 ml) and divided into two parts: one preserved with Lugol's solution, and the other transported as fresh material and studied in the laboratory with an Olympus CX31 light microscope.

Taxonomic studies were based on live and preserved material observed by LM with an Olympus BX51 and a Nikon Eclipse E600 with Nomarski phase contrast. For SEM, samples were prepared according to the procedures described by Bozzola & Russell (1991) and then studied with a Hitachi S-4700 SEM in the Scanning Microscopy Laboratory of Biological and Geological Sciences, Jagiellonian University, Kraków, Poland.

Water pH and conductivity were determined with a pH/ORP electrode SMS125. Alkalinity was measured by the phenolphthalein methyl orange indicator method (Greenberg *et al.* 2005). Nutrient concentrations (PO_4, NO_3, NH_4) were measured in the laboratory: nitrate nitrogen analysis by cadmium reduction method, ammonium nitrogen analysis by Nesslerization method, soluble reactive phosphorus (SRP) by ascorbic acid method (Greenberg *et al.* 2005).

Results

All descriptions are based on phenotype observations of living specimens by LM. Detailed observations of lorica structure were made by SEM. The new taxa described below were found in a fishpond and a garden pond. Samples were collected from the pond bottom or as plankton and in some cases scraped from plant parts.

Strombomonas starmachii Duangjan & Wołowski, ***sp. nov.*** Figs 1–5

Lorica irregularly oval, 19.2–22.5 µm wide, 29.8–30.9 µm long, collar very low, extensive, surrounding apical pore (1.7 × 1.2 µm) located at top of diagonally truncated tube 3 µm high moved to right side of rim, wall irregular, scrobiculate, covered by small sand grains. Cell ovoid, chloroplasts numerous, disc-shaped with pyrenoids, paramylon bodies small, cylindrical or ellipsoidal. Species similar to *S. amphoraeformis* (Hortobagy) Huber-Pestalozzi (1955) in general view and lorica structure, but our specimens have a characteristic well developed collar; the shape of the lorica also resembles that of monads of some *Urceolus* taxa (*U. cyclostomus* or *U. macromastix*) which are colorless euglenoids and have no lorica.

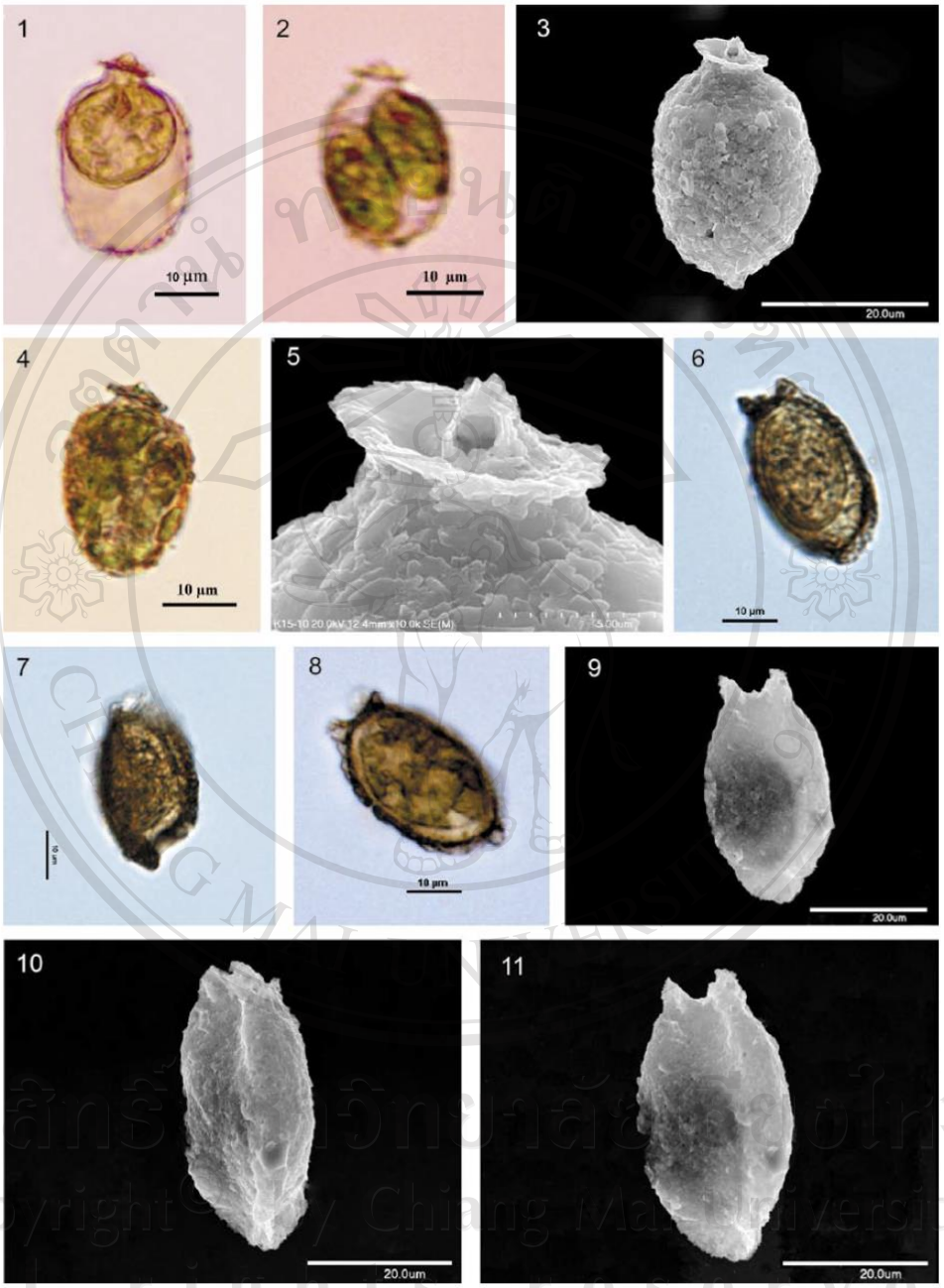
Holotype: slide number 15, deposited in Applied Algal Research Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Thailand (Iconotype: Fig. 3).

Etymology. Named to honor the memory of the eminent phycologist and hydrobiologist Karol Starmach.

Distribution. Found in fishpond, Chiang Rai Province, Cabbages and Condoms (C&C) Restaurant (19.26635°N, 99.51508°E) with the following water parameters: conductivity 191–193 µS cm^{-1};

→

Figs 1–11. 1–5 – *Strombomonas starmachii* Duangjan & Wołowski, *sp. nov.* 1, 2 & 4 – live specimens inside lorica by LM, 3 & 5 – lorica ultrastructure by SEM; 6–11 – *S. chiangmaiensis* Duangjan, *sp. nov.* 6–8 – live specimens inside lorica in various positions by LM, 9–11 – lorica ultrastructure in various views by SEM.



pH 6.75–6.97; alkalinity 60 mg l^{-1} as $CaCO_3$; PO_4 1.42–1.47 mg l^{-1}; NO_3 1.20–2.10 mg l^{-1}; NH_4 5.10–5.21 mg l^{-1}. Several specimens were observed.

Strombomonas chiangmaiensis Duangjan, ***sp. nov***. Figs 6–11

Lorica triple-walled, longitudinally oval in outline, 17.4–26.5 µm wide, 33.3–43.3 µm long, *ca* 9.7 µm thick, no collar, incised at top, posterior part slightly narrowed, ended with fin, wall irregular-scrobiculate, slightly concave on 3 sides, tapered at edges, yellow to brown. Cell obovoid, chloroplasts numerous, disc-shaped with pyrenoids, paramylon bodies small, cylindrical or ellipsoidal. Species similar to *S. scabra* Tell & Conforti var. *labiata* but our specimens were triple-walled, and to *S. triguetra* Playfair 1915 Deflandre 1930 which differs from our specimens by its regular shape and short collar.

HOLOTYPE: slide number 13, deposited in Applied Algal Research Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Thailand (ICONOTYPE: Fig. 11).

ETYMOLOGY. The epithet *chiangmaiensis* refers to Chiang Mai, where the material was collected.

DISTRIBUTION. Found in fishpond, Chiang Rai Province, Cabbages and Condoms (C&C) Restaurant (19.26635 N, 99.51508 E) with the following water parameters: conductivity 120–133 µS cm^{-1}; pH 5.6–5.9; alkalinity 45–50 mg l^{-1} as $CaCO_3$; PO_4 0.71–1.07 mg l^{-1}; NO_3 0.1–1.0 mg l^{-1}; NH_4 1.76 mg l^{-1}. Several specimens were observed.

Trachelomonas peerapornpisalii Duangjan & Wołowski, ***sp. nov***. Figs 12–19

Lorica broadly ellipsoidal in side view, spherical from top view, wider than longer, 15.3–17.9 µm wide, 11.6–12.3 µm long, surrounded by three rings, collar small, apical pore 3.5 µm in diameter, surrounded by a thickening, wall densely punctate, 17 punctae in 1 μm^2, yellow to brown. Cell oblong, chloroplasts numerous, disc-shaped with pyrenoids, paramylon bodies small, cylindrical or ellipsoidal. Species similar to *T. peridiniformis* Skvortzov (1917) but our specimens are smaller and ellipsoidal in side view. The rings are broader than in *T. peridiniformis*. In shape it also resembles *T. olla* Conrad (1932), which has two rings.

HOLOTYPE: slide number 3, deposited in Applied Algal Research Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Thailand (ICONOTYPE: Fig. 18).

ETYMOLOGY. Named in honor of Associate Professor Yuwadee Peerapornpisal for her many and varied contributions to world algological research.

DISTRIBUTION. Found in a village pond in Chiang Mai Province, Faculty of Agriculture, Chiang Mai University (18.79233°N, 98.96438°E), containing several water lilies and having the following water parameters: conductivity 146–151 µS cm^{-1}; pH 6.0–6.2; alkalinity 35–59 mg l^{-1} as $CaCO_3$; PO_4 0.07–0.18 mg l^{-1}; NO_3 0.2–0.3 mg l^{-1}; NH_4 0.01–0.02 mg l^{-1}. Several specimens were observed.

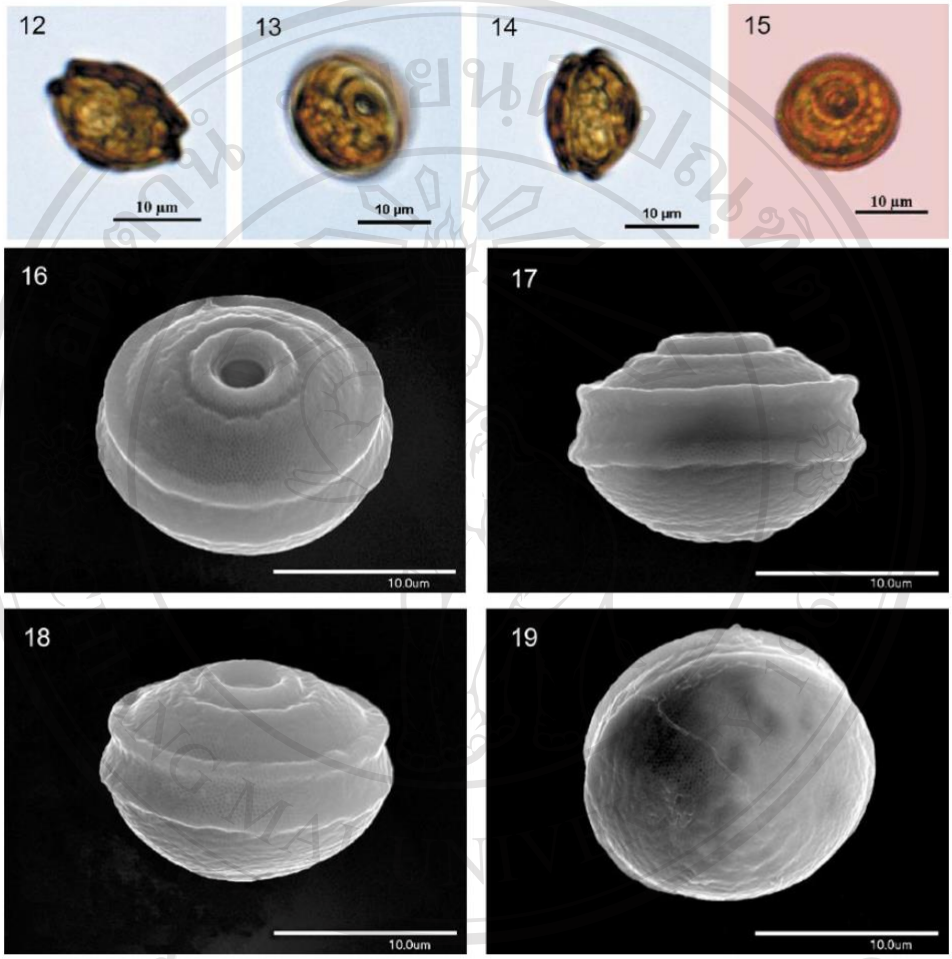
Trachelomonas thailandicus Duangjan & Wołowski, ***sp. nov.*** Fig. 20

Lorica oblong, *ca* 14 µm wide, 24.2 µm long, apical pore surrounded by 8 rods (blunt spines) 3 µm long, making at the base a low collar *ca* 1 µm high, wall irregularly punctate, 1 punctum in 1 μm^2, and covered by short sparse rods irregularly dispersed. Monads were not observed.

HOLOTYPE: slide number 12, deposited in Applied Algal Research Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Thailand (ICONOTYPE: Fig. 20).

ETYMOLOGY. The epithet *thailandicus* refers to Thailand, where the material was collected.

DISTRIBUTION. Found in a village pond in Chaing Rai Province, Pa Ko Dam Tobacco Station (19.78423°N, 99.748045°E), with the following water parameters: conductivity 95 µS cm^{-1}; pH 6.9–7.1; alkalinity 51–53 mg l^{-1} as $CaCO_3$; PO_4 0.01–0.07 mg l^{-1}; NO_3 0.1–0.4 mg l^{-1}; NH_4 0.7–0.9 mg l^{-1}. Only one specimen was observed.



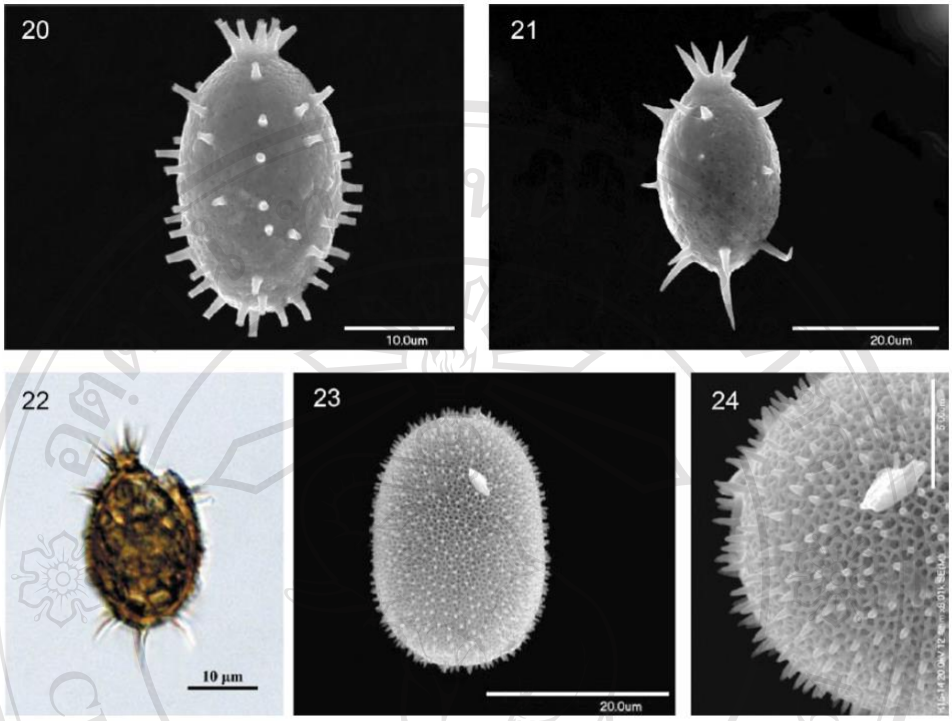
Figs 12–19. *Trachelomonas peerapornpisalii* Duangjan & Wołowski, *sp. nov*. 12–15 – live specimens inside lorica in various positions by LM, 16–19 – lorica ultrastructure in various views by SEM.

Trachelomonas paucispinosa (Prowse) Duangjan & Wołowski, ***stat. et comb. nov***. Figs 21 & 22

BASIONYM: *Trachelomonas hystrix* Teilings var. *paucispinosa* Prowse, The Gardens Bulletin Singapore **16**: 183, Figs $6b_1$, c_1. 1958.

Lorica ellipsoidal, 17.1–18.0 µm wide, 25.5–26.8 µm, collar very low, 1.5 µm high with 4 sharp spines (5.8 µm long) well set on the rim, wall punctate, 3 punctae in 2 µm^2, a few spines at both ends, 4.0–8.3 µm long, single short spines covering middle part of lorica, one well developed sharp spine 8.5–9.0 µm long at posterior end. Cell elliptical, chloroplasts numerous, disc-shaped, paramylon bodies small, cylindrical or rod-like.

NOTE: Specimens described by Prowse (1958) as *Trachelomonas hystrix* var. *paucispinosa* differ



Figs 20–24. 20 – *Trachelomonas thailandicus* Duangjan & Wołowski, *sp. nov*., lorica ultrastructure by SEM; 21, 22 – *T. paucispinosa* Duangjan & Wolowski, *sp. nov*.: 21 – lorica ultrastructure by SEM, 22 – live specimen inside lorica by LM; 23, 24 – *T. reticulato-spinifera* Duangjan, *sp. nov*., detail of lorica ultrastructure by SEM.

from the type taxa *T. hystrix*. The latter is egg-shaped to oblong and has a high (3.0–5.5 µm), well developed collar with several spines at the rim; the whole lorica is densely punctate, covered by thin spines, and sometimes has one long spine at the posterior end.

DISTRIBUTION. Found in a village pond in Chaing Rai Province, Pa Ko Dam Tobacco Station (19.78423°N, 99.74804°E), with the following water parameters: conductivity 95 µS cm^{-1}; pH 6.9–7.1; alkalinity 51–53 mg/l^{-1} as $CaCO_3$; PO_4 0.01–0.07 mg l^{-1}; NO_3 0.1–0.4 mg l^{-1}; NH_4 0.7–0.9 mg l^{-1}. A few specimens were observed. Earlier described from Malaysia as *T. hystrix* var. *paucispinosa* by Prowse (1958). Yamagishi (2010) reported it from Malaysia (Alor Setar, ditch) and from Cambodia: Bayon (pond), Preah Ko (paddy field) Baray (paddy field) and Siem Reap (ditch). Conforti and Perez (2000) described it from Uruguay (Rio Negro) as *T. mirabilis* var. *obesa* but the picture of the specimen and the description resemble rather *Trachelomonas paucispinosa*.

Trachelomonas reticulato-spinifera Duangjan, ***sp. nov.*** Figs 23 & 24

Lorica oval, without collar 18.8 µm wide, 27.8 µm long, round at both ends, apical pore 3 µm in diameter, wall reticular, thickly covered with spines (1.0–1.5 µm long), by LM the reticulation resembles pores. Cells were not observed. Species similar to *T. allia* Drężepolski but our specimens have a reticulated wall and are not punctate.

HOLOTYPE: slide number 15, deposited in Applied Algal Research Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Thailand (ICONOTYPE: Fig. 23).

ETYMOLOGY. The epithet *reticulate-spinifera* refers to the lorica ultrastructure.

DISTRIBUTION. Found in a fishpond in Chiang Rai Province, Cabbages and Condoms (C&C) Restaurant (19.26635°N, 99.51508°E), with the following water parameters: conductivity 191–193 μS cm^{-1}; pH 6.8–7.0; alkalinity 60 mg l^{-1} as $CaCO_3$; PO_4 1.42–1.47 mg l^{-1}; NO_3 1.2–2.1 mg l^{-1}; NH_4 5.1–5.21 mg l^{-1}. Only one specimen was observed.

DISCUSSION

The described taxa were among the 136 species of *Trachelomonas* and 58 of *Strombomonas* found during long-term study. All of them occurred in shallow polluted ponds.

The two new *Strombomonas* taxa are described from mature specimens with well formed loricas which are species-specific and identified for the first time. Live cells observed by LM had the same type of lorica as seen in SEM. Several specimens were used for both types of observations, excluding *Trachelomonas reticulato-spinifera*, only one specimen of which was observed. Surprisingly, the shape of the lorica in *Strombomonas starmachii* resembles that of monads of the colorless genus *Urceolus*, which is naked. According to Mereschkowsky (1877) the pellicle of *Urecolus* sp. has thick spiral striae. Our LM observations indicated (and the micrographs show) two cells after division, which have several chloroplasts, inside the lorica.

One new *Trachelomonas* species has a smooth lorica. It is similar to *T. peridiniformis* reported from Europe and Asia, described by Skvortzov (1917), which has three rings, but its lorica is longer than wider (22.4 μm long, 20.8 μm wide). The described taxon is also similar to *T. olla* described by Conrad (1932) from brackish water in Belgium, but it is larger than *T. peerapornpisalii* and has only one thickening and two rings. We observed several well developed live specimens (Figs 12–15). The other three new *Trachelomonas* taxa are ornamented by variously developed spines. One of them, *Trachelomonas thailandicus*, is documented by a SEM image (Fig. 20) showing a lorica type not known previously. *Trachelomonas paucispinosa* sp. nova was observed several times and we also documented a live specimen (Fig. 22). Our specimens were reported earlier from Malaysia (Prowse 1958; Yamagishi 2010) but as *T. hystrix* var. *paucispinosa*. In view of the large differences between the putative variety and the type, we reclassified it as a separate taxon. This taxon is one of a group of taxa connected with the tropical zone, such as the newly described *S. chiangmaiensis*. *Trachelomonas reticulate-spinifera* presents very interesting lorica structure: its wall is reticulate and densely covered with short sharp spines. It is an example of a mixed pattern of lorica development combining the simplest pattern, observed in *T. reticulate*, with the more complicated pattern observed in *T. hispida*, forming a mature lorica with spinney ornamentation.

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