

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, am-

monium 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

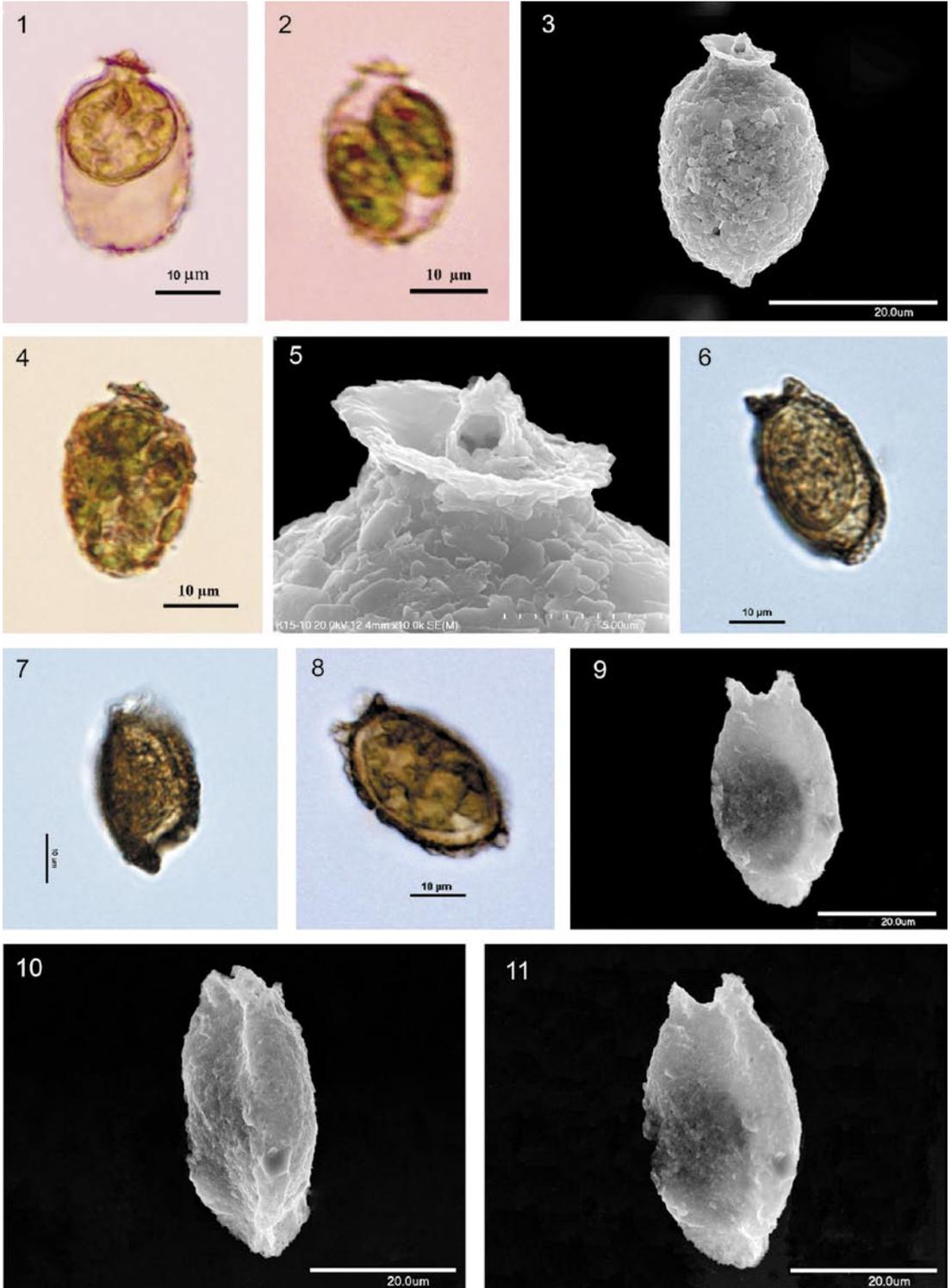
Lorica irregularly oval, 19.2–22.5 μm wide, 29.8–30.9 μm long, collar very low, extensive, surrounding apical pore (1.7 \times 1.2 μm) located at top of diagonally truncated tube 3 μm high moved to right side of rim, wall irregular, scrobiculate, covered by small sand grains. Cell ovoid, chloroplasts 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 $\mu\text{S cm}^{-1}$;

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 CaCO₃; PO₄ 1.42–1.47 mg l⁻¹; NO₃ 1.20–2.10 mg l⁻¹; NH₄ 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. trigueta* 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 CaCO₃; PO₄ 0.71–1.07 mg l⁻¹; NO₃ 0.1–1.0 mg l⁻¹; NH₄ 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 µm², 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 CaCO₃; PO₄ 0.07–0.18 mg l⁻¹; NO₃ 0.2–0.3 mg l⁻¹; NH₄ 0.01–0.02 mg l⁻¹. Several specimens were observed.

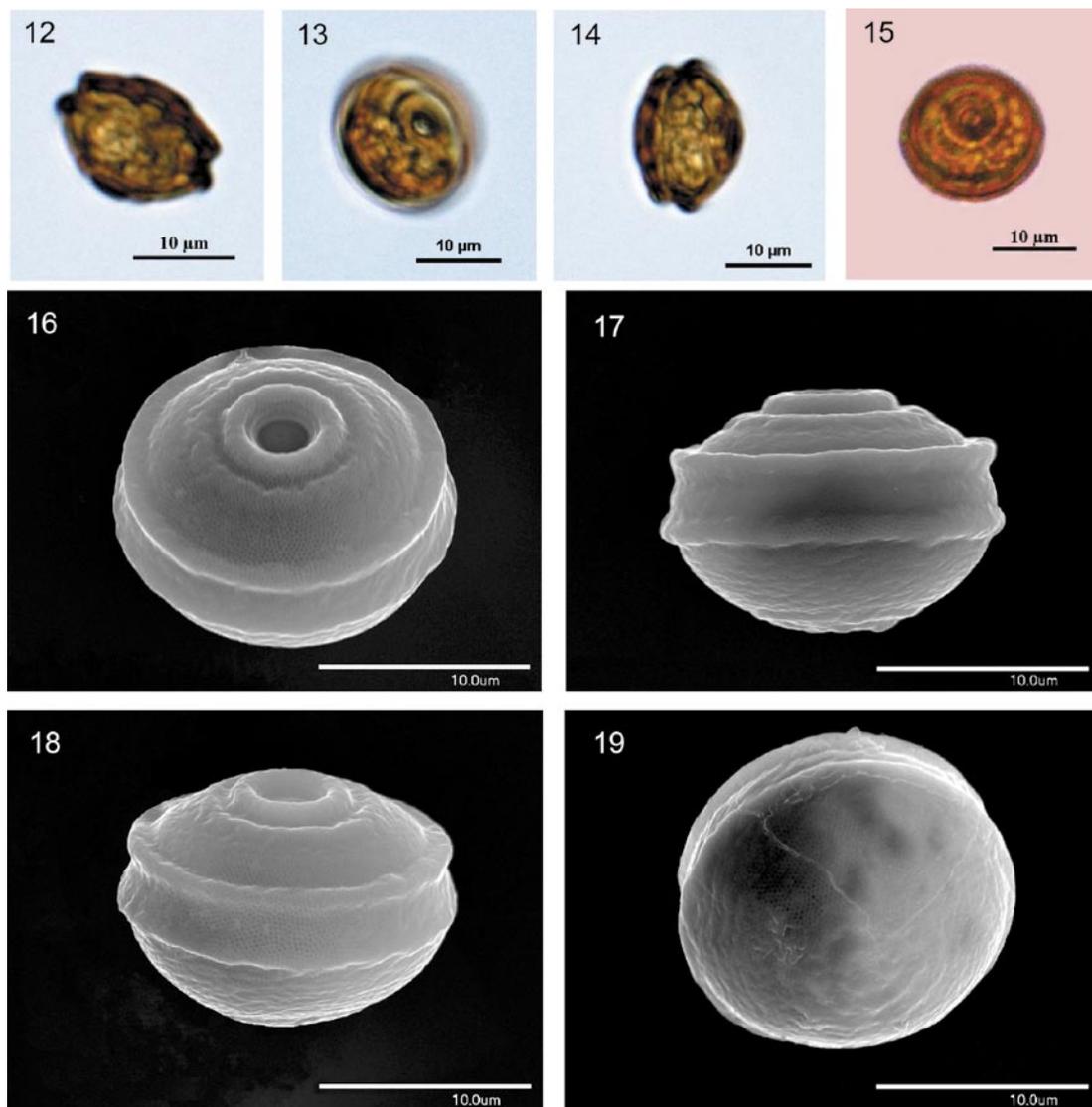
Trachelomonas thailandicus Duangjan
& Wołowski, *sp. nov.* Fig. 20

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 µm², 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 CaCO₃; PO₄ 0.01–0.07 mg l⁻¹; NO₃ 0.1–0.4 mg l⁻¹; NH₄ 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.

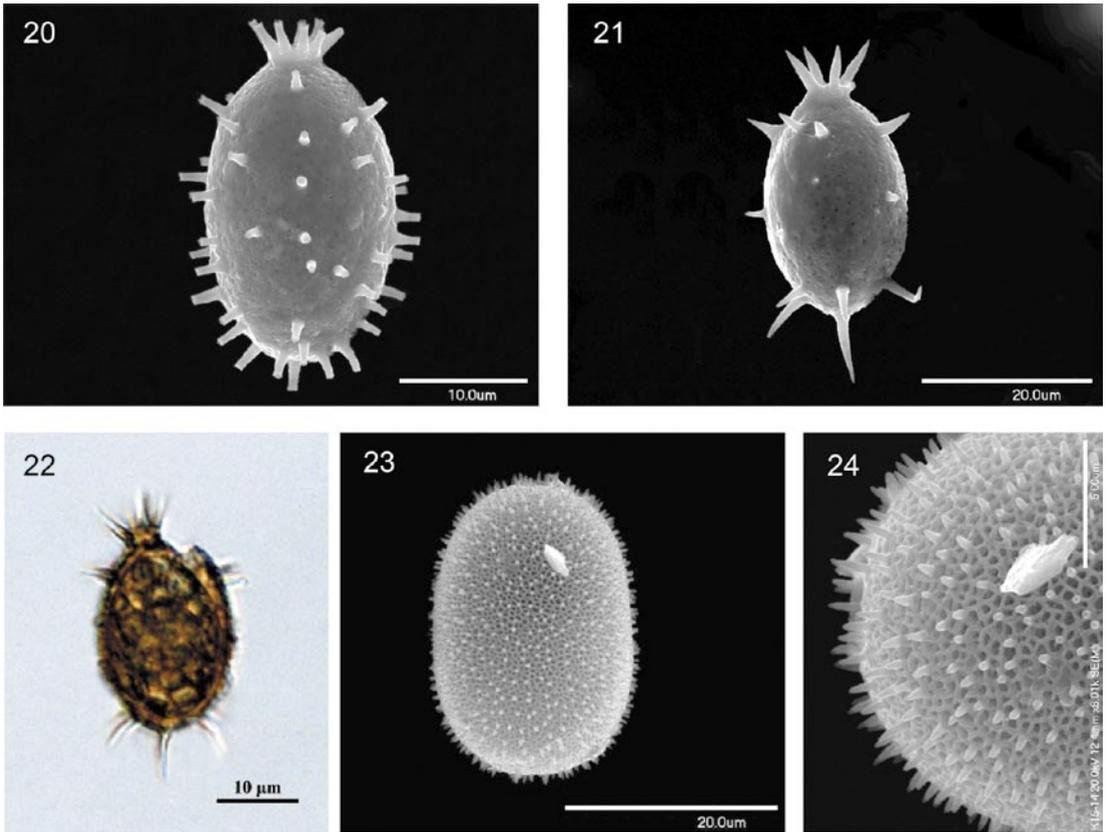
Trachelomonas paucispinosa (Prowse) Duangjan & Wołowski, *stat. et comb. nov.* Figs 21 & 22

BASIONYM: *Trachelomonas hystrix* Teulings var. *paucispinosa* Prowse, The Gardens Bulletin Singapore **16**: 183, Figs 6b₁, c₁. 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 µm², 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 & Wołowski, *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 $\mu\text{S cm}^{-1}$; 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* Drezepolski 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 $\mu\text{S cm}^{-1}$; 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 *Urceolus* 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 ob-

served 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. reticulata*, with the more complicated pattern observed in *T. hispida*, forming a mature lorica with spinney ornamentation.

ACKNOWLEDGEMENTS. We are especially grateful to Associate Professor Yuwadee Peerapornpisal for her help and consultations. We thank our many colleagues for their assistance, and the Applied Algal Research Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Thailand, for making its database available and for providing access to equipment. We thank Anna Łatkiewicz for help with SEM studies and Michael Jacobs for improving the English version of this work and Professor Pertti Eloranta for valuable remarks on the manuscript. This study was supported by the Royal Golden Jubilee Ph.D. Program of Thailand and through the statutory fund of the W. Szafer Institute of Botany, Polish Academy of Sciences.

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Received 18 May 2013