

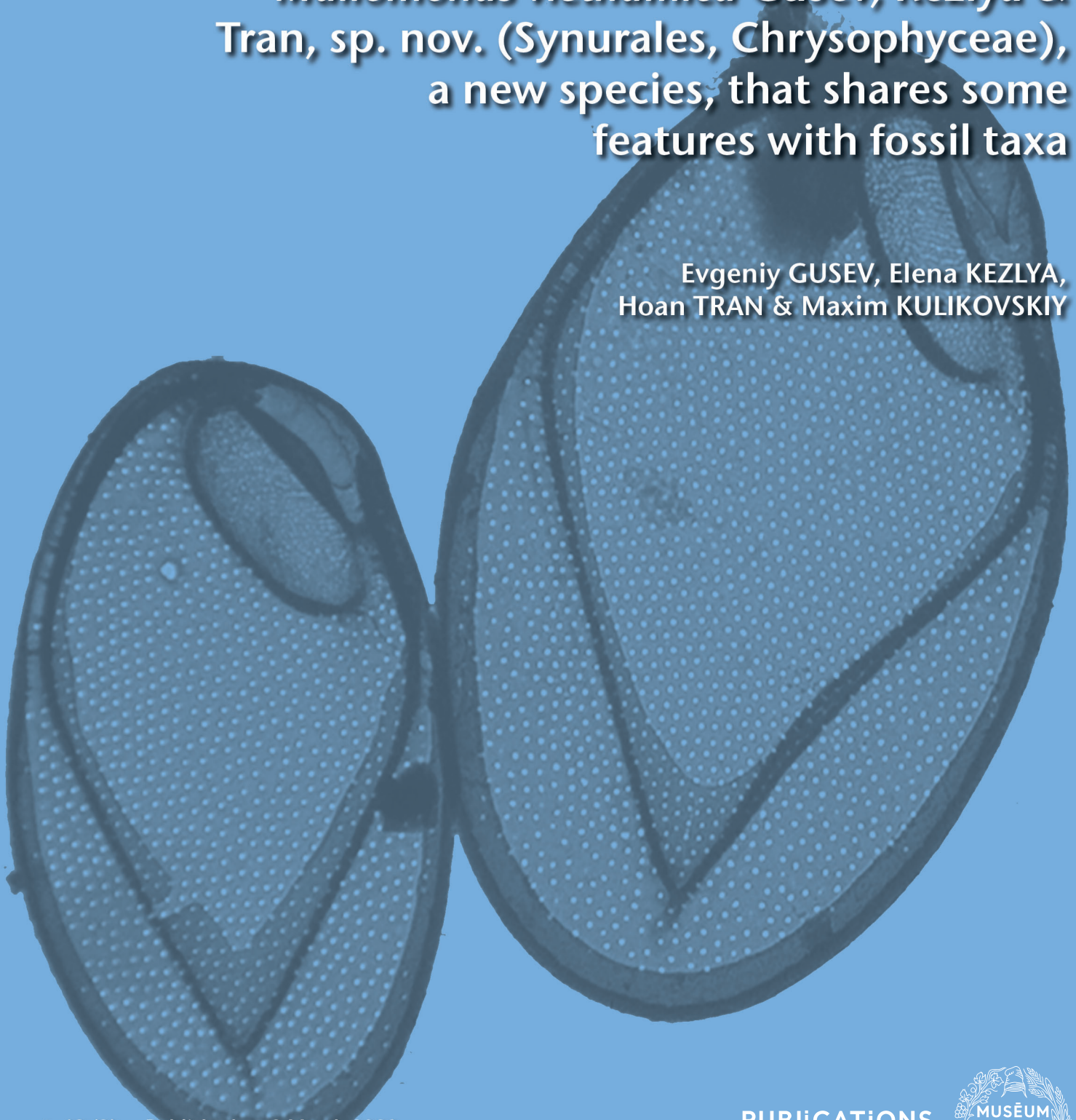
cryptogamie

Algologie

2021 • 42 • 3

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diff.pub@mnhn.fr / <http://sciencepress.mnhn.fr>

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ISSN (imprimé / *print*): 0181-1568 / ISSN (électronique / *electronic*): 1776-0984

***Mallomonas vietnamica* Gusev, Kezlya & Tran, sp. nov.
(Synurales, Chrysophyceae), a new species,
that shares some features with fossil taxa**

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Submitted on 30 September 2020 | Accepted on 16 December 2020 | Published on 3 March 2021

Gusev E., Kezlya E., Tran H. & Kulikovskiy M. 2021. — *Mallomonas vietnamica* Gusev, Kezlya & Tran, sp. nov. (Synurales, Chrysophyceae), a new species, that shares some features with fossil taxa. *Cryptogamie, Algologie* 42 (3): 39–46. <https://doi.org/10.5252/cryptogamie-algologie2021v42a3>. <http://cryptogamie.com/algologie/42/3>

ABSTRACT

A new species, *Mallomonas vietnamica* Gusev, Kezlya & Tran, sp. nov., is described from Vietnam based on silica-scale morphology elucidated by transmission and scanning electron microscopies. The species belongs in section *Mallomonas* Perty and is characterized by large oval scales with a wide and deep dome, which is recessed from the scale perimeter. The domes are shifted to the right side of scales and oriented at different angles to the scale's longitudinal axis. Bristles of the newly described taxon are characterized by a distinctly hooked foot. Scales of *M. vietnamica* Gusev, Kezlya & Tran, sp. nov. were found at only one locality in Hanoi, at the border of Ba Vi National Park in the fresh-water Da River (Sông Đà).

RÉSUMÉ

Mallomonas vietnamica Gusev, Kezlya & Tran, sp. nov. (Synurales, Chrysophyceae), une nouvelle espèce, qui partage quelques caractéristiques avec les taxons fossiles.

Une nouvelle espèce, *Mallomonas vietnamica* Gusev, Kezlya & Tran, sp. nov., est décrite au Vietnam sur la base de la morphologie à l'échelle de la silice élucidée par microscopie électronique à transmission et balayage. L'espèce appartient à la section *Mallomonas* Perty et se caractérise par de grandes écailles ovales avec un dôme large et profond, qui est en retrait par rapport au périmètre de l'écaille. Les dômes sont décalés vers le côté droit des écailles et orientés selon différents angles par rapport à l'axe longitudinal de l'écaille. Les soies du taxon nouvellement décrit sont caractérisées par un pied nettement crochu. Des écailles de *M. vietnamica* Gusev, Kezlya & Tran, sp. nov. ont été trouvées dans une seule localité de Hanoi, à la limite du parc national de Ba Vi, dans la rivière d'eau douce Da (Sông Đà).

KEY WORDS

Synurales,
Mallomonas,
Vietnam,
scale ultrastructure,
SEM & TEM,
new species.

MOTS CLÉS

Synurales,
Mallomonas,
Vietnam,
ultrastructure à l'échelle,
SEM & TEM,
espèce nouvelle.

INTRODUCTION

Mallomonas Perty is the most diverse genus in the order Synurales (Stramenopiles, Chrysophyceae). Species of this genus are widely distributed in various freshwater habitats from the polar regions to the tropics (Kristiansen 2000; Kristiansen & Preisig 2007). Cells of *Mallomonas* are covered with siliceous scales and bristles, which are specific for each taxon. According to the most recent multigene phylogenies of Synurales (Siver *et al.* 2015; Čertnerová *et al.* 2019), *Mallomonas* is divided into two major lineages, with scales characterized by either the presence or absence of a V-rib.

The oldest records of *Mallomonas* scales and bristles found so far are from middle Eocene deposits (Siver & Wolfe 2005). Fossil taxa of the genus include both extinct and modern lineages (Siver *et al.* 2015). Many of the extinct species had large and robust scales, often four times larger than those of most modern species (Siver 2015, 2018a, b). Remains of other species show clear evolutionary stasis with respect to scale and bristle morphology (Siver *et al.* 2009, 2013). Some taxa observed in Eocene deposits, like *Mallomonas insignis* Penard, *M. intermedia* Kisselev, *M. peronoides* (K. Harris) Momeu & L.S.Péterfi, *M. lychenensis* Conrad, *M. asmundiae* (Wujek & van der Veer) Nicholls and some others, are present in modern flora (Siver & Wolfe 2005; Siver *et al.* 2009, 2015, 2019). Some recent tropical findings, like *M. neoampla* Gusev & Siver, represent a transitional form between fossil and modern taxa (Gusev & Siver 2017). The diversity of the genus *Mallomonas* in the tropics is quite high with many rare and endemic taxa in South and Central America (Cronberg 1989; Wujek 1984), Africa (Compère, 1974; Hansen 1996; Hansen & Kristiansen 1995), Asia (Dürschmidt & Croome 1985; Dürschmidt & Cronberg 1989; Neustupa & Řezáčová 2007; Gusev & Nguyen 2011; Gusev 2013a; Gusev *et al.* 2017a, 2019a) and Indonesia (Vyverman & Cronberg 1993; Kapustin & Gusev 2019). More than 20 new tropical species have been described in the last decade from African countries (Němcová *et al.* 2011; Němcová & Kreidlová 2013; Piątek 2015), Indonesia (Kapustin *et al.* 2019) and Vietnam (Gusev 2012, 2015; Gusev *et al.* 2018, 2019d; Gusev & Kulikovskiy 2020 and others).

During phytoplankton studies in the northern part of Vietnam we observed scales and bristles of unknown *Mallomonas* species with some ultrastructure features more characteristic for fossil taxa. We describe this species here.

MATERIAL AND METHODS

Water sample was collected from the Da river (Sông Đà) in northern Vietnam (Ba Vi District, Hanoi) (Fig. 1). Sampling occurred during an expedition of the Joint Russian–Vietnamese Tropical Research and Technological Centre (“Ecolan 3.2” project). The Ba Vi district is in the semi-mountainous region to the northwest of Hanoi. Ba Vi has a monsoon tropical climate, with a rainy season from April to October and a dry season from November to March. The average annual rain-

fall in the region is approximately 1800 mm, the average air temperature is approximately 22°C, there are approximately 1400–1500 sunny hours per year, and mean relative humidity of 70–85%.

Samples were collected using a plankton net with 20 µm mesh, 30 cm mouth diameter. For electron microscopy studies, an aliquot of a sample was washed three times with deionized water by repeated centrifugation. After washing, an aliquot of the sample was dried on stubs for the Scanning Electron Microscope (SEM) or grids for the Transmission Electron Microscope (TEM) or digested for 4 to 5 minutes in sulfuric acid with potassium dichromate before mounting. For SEM studies, samples were dried onto aluminum stubs, coated with gold for 10 minutes with a JEE-4X (JEOL) sputter coater and observed with a JEOL 6510 LV scanning electron microscope. For TEM studies, samples were dried onto formvar coated grids (EMS FF200-Cu-50, Electron Microscopy Sciences) and observed with a JEM-1011 TEM. Specific conductance, pH and temperature measurements were made at one occasion using a Hanna HI 9828 device, Hanna Instruments, Inc., United States.

RESULTS

We found a *Mallomonas* species with large cells in a planktonic sample collected from the Da river. Studies using transmission and scanning electron microscopy showed that this species has large scales with a unique ultrastructure that has not been previously reported. This taxon was abundant in the sample alongside another *Mallomonas* species – *M. caudata* Iwanoff. There is a large reservoir, Hoa Binh, about 25 km upstream from the sampling site. It seems likely that both species developed in the reservoir and were discharged with the bottom waters, because the water temperature in the river was 3–5°C lower than in the nearby reservoirs. This assumption is supported by the fact it is typical for *Mallomonas caudata* to vegetate in the meta- and hypolimnion of water bodies and survive with lower saturating light intensities than other synurophytes (Kim *et al.* 2009; Gusev 2013b).

Below we give a formal description of this species.

Class CHRYSOPHYCEAE
Order SYNURALES Andersen
Family MALLOMONADACEAE Diesing
Genus *Mallomonas* Perty

Mallomonas vietnamica Gusev, Kezlya & Tran, sp. nov.
(Figs 2; 3)

HOLOTYPE SPECIMEN. — Portion of a single gathering of cells on SEM stub No. BaVi 17 deposited at the Herbarium, Papanin Institute for Biology of Inland Waters RAS, Borok (IBIW). Material from the Da river (Sông Đà), Hanoi, Vietnam, collected by E.S. Gusev on 7.V.2019. Figure 2A illustrates a representative scale from the holotype specimen.

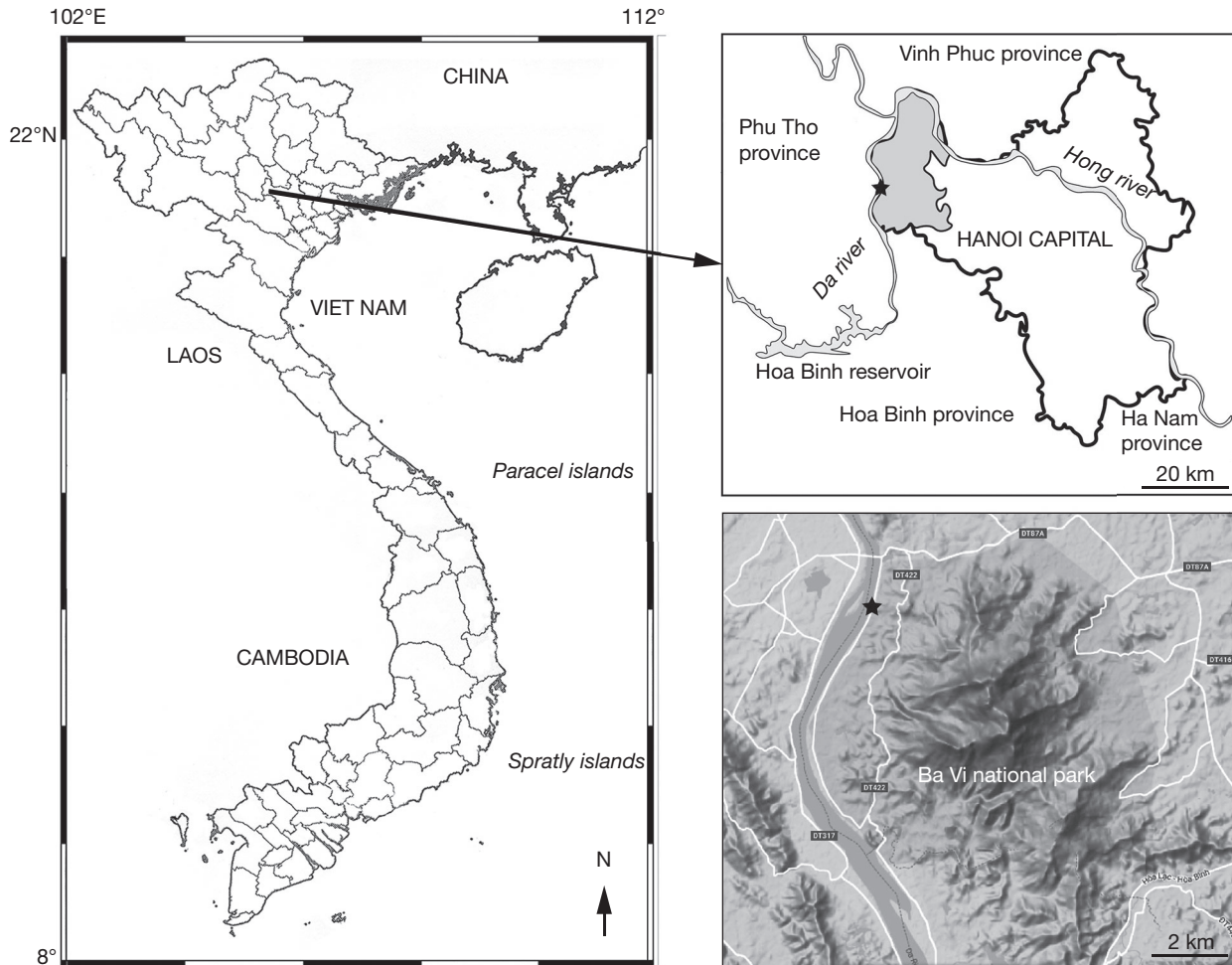


FIG. 1. — Map of the study area (stars indicates sampling site).

TYPE LOCALITY. — Vietnam, Hanoi, the Da river (Sông Đà), latitude/longitude: 21°5.673'N, 105°19.149'E.

EPITHET. — Species name derived from “Vietnam”, country from which this taxon was described.

DISTRIBUTION. — To date, *Mallomonas vietnamica* Gusev, Kezlya & Tran, sp. nov. has only been observed in the type locality. At the time of collection, the pH was 7.82, specific conductance 153 $\mu\text{S}\cdot\text{cm}^{-1}$, and temperature 25°C.

SEM AND TEM DESCRIPTION

Scales are oval, large, tripartite, $7.5\text{--}10.1 \times 4.1\text{--}6.2 \mu\text{m}$ in size. A dome is elongated and large ($2.2\text{--}2.5 \times 0.7\text{--}1.2 \mu\text{m}$), deep, with smooth surface. The domes are shifted to the right part of the scales, recessed from the distal margin, and oriented at different angles to the longitudinal axis. A V-rib is acute, continuous with the anterior submarginal rib. Arms of the anterior submarginal rib either fully or partially surround the dome. In the latter case, there is a hyaline area between the dome and the edge of a scale. Posterior flange and shield without secondary layer and with pores, evenly spaced on all surfaces. A narrow, upturned rim encircles

the entire perimeter of the scale. It is wider in the posterior portion than in the anterior part, where it is joined with the arms of the anterior submarginal rib by transverse struts. Anterior flange is narrow. Bristles are of two types: long straight bristles ($37\text{--}72 \mu\text{m}$) and short curved bristles ($23\text{--}35 \mu\text{m}$). The margins of the bristles are rolled to form an open groove. The distal end of the bristle is expanded, with two apical and one subapical large tooth. The foot of the bristle is expanded and distinctly hooked, and the lower portion of the shaft flattened. Cysts unknown.

DISCUSSION

We propose placement of *Mallomonas vietnamica* Gusev, Kezlya & Tran, sp. nov. in section *Mallomonas* (Kristiansen & Preisig 2007). This species is characterized by large oval tripartite scales with a few unique features. First, the scales of *M. vietnamica* Gusev, Kezlya & Tran sp. nov. have large and deep domes, which are recessed from the scale perimeter. All sixty-nine examined scales have domes; it is likely that this spe-

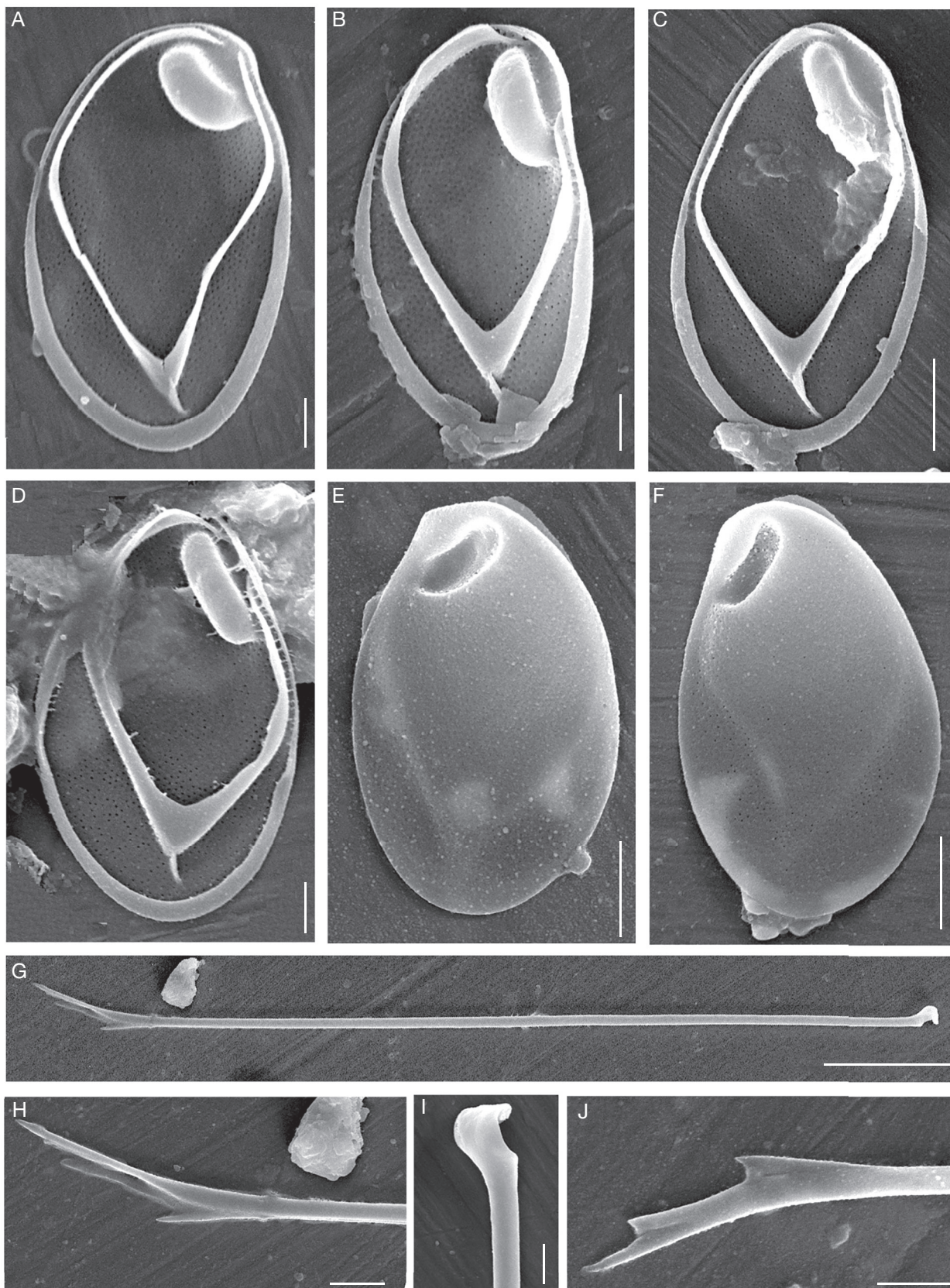


FIG. 2. — *Mallomonas vietnamica* Gusev, Kezlya & Tran sp. nov. scanning electron microscopy images (SEM: A–J): **A–D**, scales with recessed domes, shifted to the right part of the scales, oriented at different angles to the longitudinal axis (Fig. 2A is a representative scale from the holotype specimen); **E–F**, undersurface view of the scales; **G**, long straight bristle; **H, J**, tips of bristles with teeth; **I**, foot of the bristle. Scale bars: A, B, D, I, 1 µm; C, E, F, H, J, 2 µm; G, 10 µm.

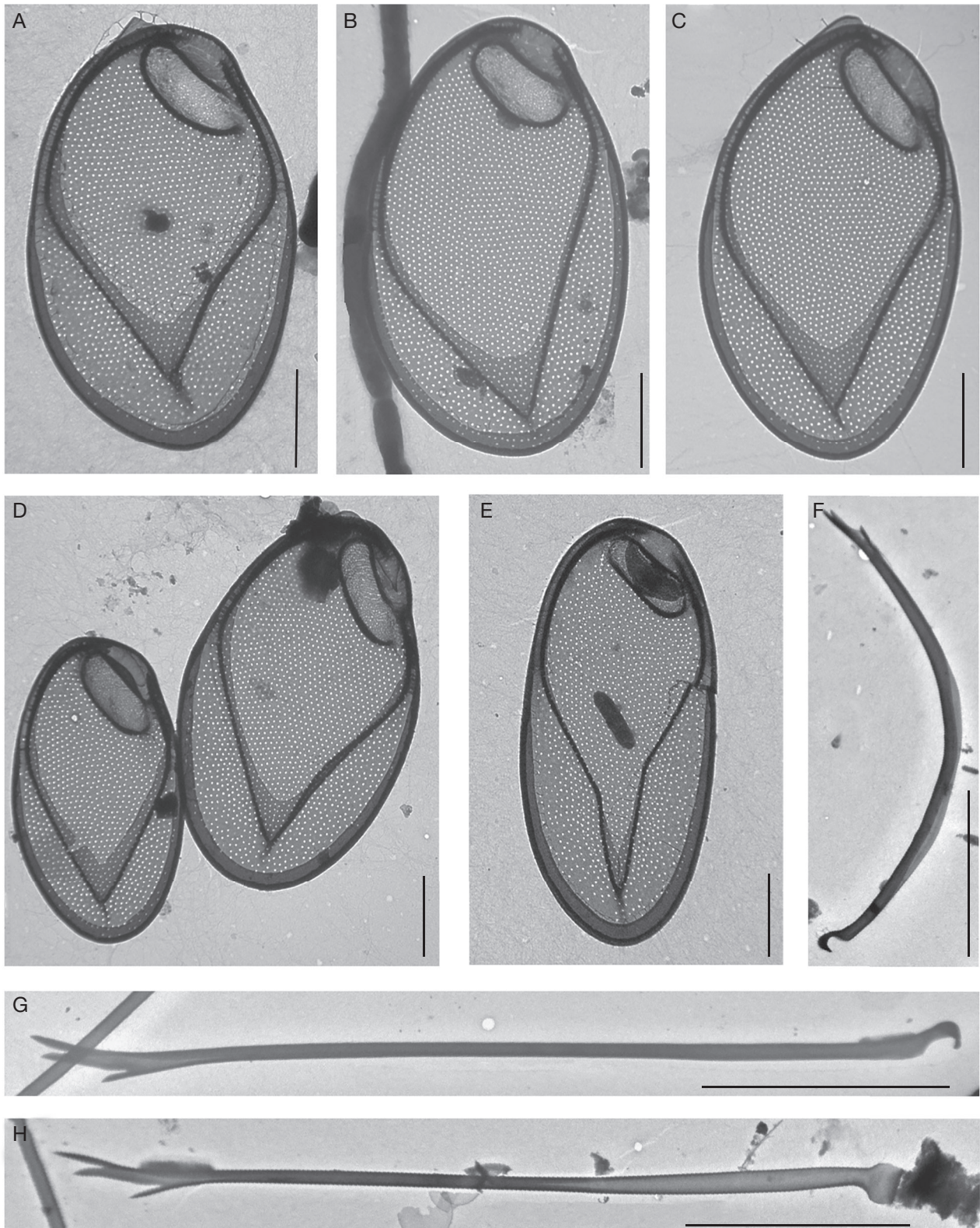


FIG. 3. — *Mallomonas vietnamica* Gusev, Kezlya & Tran sp. nov., transmission electron microscopy images (TEM: A-H): **A-E**, scales with different shape and size; **F**, curved bristle; **G, H**, straight bristles. Scale bars: A-E, 2 µm; F-H, 10 µm.

cies does not have domeless scales. One more specific feature is that the domes are shifted to the right side of scales, and oriented at different angles to the scale's longitudinal axis. A

narrow, upturned rim encircling the whole scale perimeter, is another unique feature of the new taxon. Usually, in *Mallomonas* upturned rim surrounds one half of the scale perimeter,

or rarely up to two-thirds. There are short transverse ribs on the anterior flanges of *M. vietnamica* Gusev, Kezlya & Tran, sp. nov. scales, which are joined arms of the anterior rib and anterior part of the upturned rim. This feature was not observed in other taxa from the section *Mallomonas*. Bristles of the newly described taxon are characterized by a distinctly hooked foot, which is probably aids in the attachment of the bristle to the recessed dome.

The ultrastructure of *M. vietnamica* Gusev, Kezlya & Tran sp. nov. scales can be compared with taxa from the section *Mallomonas*, such as *M. alpina* Pascher & Ruttner (Pascher 1913), *M. areolata* Nygaard (Nygaard 1949), *M. kuzminii* Gusev & Kulikovskiy (Gusev & Kulikovskiy 2013) and *M. elongata* Reverdin (Reverdin 1919). All of these taxa lack a secondary layer on the scale shield and have pores on the shield and posterior flange and a V-rib continuous with the anterior submarginal rib. Moreover, *M. alpina*, *M. areolata* and *M. elongata* form a monophyletic lineage on the phylogenetic tree (Čertnerová *et al.* 2019). *Mallomonas vietnamica* Gusev, Kezlya & Tran sp. nov. differs from all these taxa by the position and shape of a dome, which is elongated, recessed from the scale perimeter, and shifted to the right portion of the scale. The domes of *M. alpina*, *M. areolata*, *M. kuzminii* and *M. elongata* are rounded and positioned near the anterior scale margin at the center. Moreover, all of these taxa have domeless scales, which are not observed for *M. vietnamica* Gusev, Kezlya & Tran sp. nov. Another feature that distinguishes *M. vietnamica* Gusev, Kezlya & Tran, sp. nov. from these taxa is the presence of the anterior flange with struts. Only *M. elongata* has scales of up to 9 µm long, are comparable in size with those of *M. vietnamica* Gusev, Kezlya & Tran, sp. nov. However, the scales of *M. elongata* are asymmetrical and differ from those of *M. vietnamica* Gusev, Kezlya & Tran, sp. nov. in outline. The scales of *M. alpina*, *M. areolata*, and *M. kuzminii* do not exceed 7 µm in length. Bristles of *M. vietnamica* Gusev, Kezlya & Tran, sp. nov. also clearly differs from the bristles of the mentioned taxa in the presence of a double bifurcate tip, and distinctly hooked foot.

Some key features of the scale ultrastructure of *Mallomonas vietnamica* Gusev, Kezlya & Tran, sp. nov. are similar to fossil taxa from the genus. First is the dome, recessed from the distal scale margin. Scales with recessed domes were common in the Eocene (Siver & Lott 2012). At least three Eocene *Mallomonas* taxa from different sections have recessed domes: *M. preisigii* Siver, attributed to section *Papillosae*; *M. ampla* Siver & Lott, from section *Multisetigerae* and *M. media* Siver & Lott from section *Planae* (Siver & Lott 2012). As mentioned by Siver & Lott (2012), a dome recessed from the distal scale perimeter would decrease rotational flexibility, since part of the shaft would underlie a portion of the scale, while a dome positioned on the scale perimeter allows flexibility in bristle rotation. This explains why these earlier dome structures were replaced with those that position the dome on the scale perimeter, and are not present in modern flora. However, one extant species with a recessed dome was described recently from Vietnam – *M. neoampla* (Gusev &

Siver 2017). This taxon shares some features of the scale morphology with the fossil species *M. ampla*, and others with the modern and widely distributed *M. multisetigera* Dürschmidt. Thus, *M. vietnamica* Gusev, Kezlya & Tran sp. nov. and *M. neoampla* indicate that species with recessed domes are still represented in the modern flora. Notably, the bristles of this new species have a hooked foot, similar to the structure in *M. ampla* and *M. neoampla*.

A comparison of scales sizes between species from the Eocene and modern flora shows that large scales are more typical for fossil (presumably extinct) taxa (Siver *et al.* 2015). Fossil species with large scales (up to 8–10 µm) found in the Giraffe locality (Canada), are known for several sections (Siver & Wolfe 2005, 2016; Siver & Lott 2012; Siver *et al.* 2015; Siver 2015, 2018a, b): *Planae* (*M. media* Siver & Lott, *Mallomonas schumachii* Siver, *M. elephantus* Siver, *M. porifera* Siver & Wolfe, *Mallomonas* sp. GP 13), *Heterospinae* (*M. skogstadii* Siver), *Leboimianae* (*M. aperturata* Siver), and *Pseudocoronatae* (*M. giraffensis* Siver & Wolfe).

Large scales are also characteristic for modern tropical flora. Generally, these taxa have been found in two sections, *Planae* and *Quadratae*. Representatives of the first section are *Mallomonas matvienkoae* var. *grandis* (Dürschmidt & Cronberg 1989) and *M. lamii* (Gusev *et al.* 2019c), and from the second section, *M. bronchartiana* Compère (Compère 1974), *M. gusakovii* Gusev, Kapustin, Martynenko, Guseva et Kulikovskiy (Gusev *et al.* 2019b), *M. pseudobronchartiana* Gusev, Siver & Shin, and *M. velari* Gusev, Siver & Shin (Gusev *et al.* 2017b). With *M. vietnamica* Gusev, Kezlya & Tran, sp. nov., a member of section *Mallomonas* is added to this list of large-scaled and large-celled species. This raises the question of whether these taxa with large cell and scales are relict species, or if it is an ecological adaptation. For example, zooplankton taxa are generally smaller in the tropics than in either the north or south temperate zones (Fernando 1980); large cells with long bristles can prevent grazing from filtrators.

However, the discovery of *M. vietnamica* Gusev, Kezlya & Tran, sp. nov. and *M. neoampla*, which have ultrastructural elements similar to fossil scales, shows that tropical flora includes a number of relict taxa. Further studies of tropical areas, which can be refugia for ancient taxa, should lead to species findings that will help clarify the evolution of siliceous structures of synurophycean algae.

Acknowledgements

Authors are grateful to the staff of the Centre of Electron Microscopy of the Papanin Institute for Biology of Inland Waters, RAS, and staff of the Joint Russian-Vietnamese Tropical Research and Technological Centre for technical assistance. Financial support was provided by the Russian Science Foundation (20–14–00211) for the TEM and SEM studies, sample processing and by framework of the state assignment (theme AAAA–A19–119041190086–6) for finishing manuscript. We also thank two anonymous reviewers for helpful comments.

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*Submitted on 30 September 2020;
accepted on 16 December 2020;
published on 3 March 2021.*