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Mallomonas bronchartiana Compère revisited: Two new species described from Asia

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Abstract – Species from the *Mallomonas bronchartiana* complex were examined using material from Vietnam, the U.S.A. and South Korea. The original description of *Mallomonas bronchartiana* is expanded and descriptions of two new taxa, *M. pseudobronchartiana* and *M. velari*, are given. All taxa possess body scales that are large, broad, with an asymmetrically-placed posterior rim, an internal honeycomb reticulation, and are covered externally with a layer containing papillae. A distinct V-rib is lacking on *Mallomonas bronchartiana* scales. Scales of *M. pseudobronchartiana* have a thin V-shaped rib situated on the scale surface, while the V-rib of *M. velari* is formed from an upward folding of the surface of the scale. Scales of *Mallomonas velari* are also easily separated from the other species by the presence of a large forward projecting wing. The positions of all three taxa within the genus are discussed, and placement in section Quadratae is proposed. The distributions and habitat conditions of each species are summarized.

***Mallomonas* / Planae / Quadratae / new species / South Korea / synurophytes / USA / Vietnam / V-rib**

INTRODUCTION

The genus *Mallomonas* consists of flagellated, unicellular organisms covered with siliceous scales and bristles, and includes about 200 modern and fossil taxa in 19 sections (Kristiansen & Preisig, 2007; Siver *et al.*, 2015). Taxonomy of the genus is based largely on ultrastructural characters of the scales and bristles. The main diagnostic characters of scales used to separate species into sections are the presence/absence of a V-rib and dome, the nature of secondary siliceous structures such as papillae, ribs and reticulations, and the morphology and position of different scale types on the cell covering. Taxa with the simplest scales that lack a V-rib and a dome were placed in section Planae Momeu & L.S. Péterfi (Momeu & Péterfi, 1979; Kristiansen & Preisig, 2007). It was assumed, that these more simple scales lacking a V-rib and a dome represented a primitive state, and that derived scale types became more complex (Asmund & Kristiansen, 1986; Kristiansen & Preisig, 2007).

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In the first comprehensive multigene study, Jo *et al.* (2011) showed that the V-rib was an important event in the evolution of the genus, taxa with domes possibly evolved multiple times, and more complex scales and scale arrangements are not always derived characters. In the same study, Jo *et al.* (2011) demonstrated that the genus was divided into two well-supported clades, one possessing scales with a V-rib and the other lacking this structure, and that the section Heterospinae belonged with section Planae. Later, using an expanded dataset, Jo *et al.* (2013) showed that two strains of *Mallomonas bronchartiana* Compère, a species previously placed in section Planae, clustered with species within section Quadratae. Species in section Quadratae have scales that are thick, more or less rhombic in shape, lack a dome and V-rib, and with an internal secondary reticulation of ribs (Kristiansen & Preisig, 2007). However, based on molecular data, species from section Quadratae form a subclade within the V-rib clade despite having scales that lacked an obvious V-rib (Jo *et al.*, 2013; Škaloud *et al.*, 2013; Kim *et al.*, 2014; Siver *et al.*, 2015). In a more recent study, Siver *et al.* (2015) clearly showed that the two strains of *M. bronchartiana* included in the Jo *et al.* (2013) study had scales with a V-rib structure that were visible only with SEM and not TEM. The original description of *M. bronchartiana* based on light microscopy and TEM does not indicate the presence of a V-rib (Compère, 1974) due to the thickness and complexity of the scale. Since it was not clear if the strains studied by Jo *et al.* (2013) with a V-rib structure were indeed *M. bronchartiana*, Siver *et al.* (2015) referred to the two strains as *Mallomonas* sp. 1.

We have now been able to further examine specimens within the *Mallomonas bronchartiana* complex from Vietnam, the United States and South Korea, and have determined that the specimens represent three distinct species. The goals of this paper are to expand description of *M. bronchartiana*, describe the two new species, and summarize information on the distribution of all three taxa.

MATERIAL AND METHODS

Samples from 18 localities were included in this study, seven from Vietnam, four from the U.S.A., and two from South Korea (Table 1). Descriptions of sites from the Cat Tien National Park in Dong Nai Province are given in Gusev *et al.* (2016), sites in the Khanh Hoa Province by Gusev (2015) and Gusev & Siver (2017), sites in the U.S.A. by Siver (1991) and Siver & Lott (2006; 2012), and some of the sites in South Korea by Kim *et al.* (2009). The Mulyeongari, Sumang-ri, Namwon-eup, Seogwipo-si locality in South Korea is all part of a shallow lake system associated with an extinct volcano.

Anh Hai Lake in Vietnam is situated on Con Son (Côn Sơn) Island in Bà Rịa–Vũng Tàu Province in Con Dao National Park. The island is part of Con Dao (Côn Đảo) archipelago which is situated about 150 km to the east of the mainland. The type localities for the two new species are both located in Vietnam. One of the new species was found in Dau Ca Lake situated in Cat Tien National Park, Dong Nai (*Đồng Nai)* Province, in the Southeastern part of the country. This area has a tropical monsoon climate. The average annual temperature ranges from 25-28°C, relative humidity between 80-94%, annual precipitation between 1800-2100 mm, and the annual evaporation between 1000-1200 mm (Schmidt-Thomé *et al.*, 2015; Inland Water Ecology, 2014). The other new species was found in Dak Lua swamp, situated along the northeastern border of the Nam Cat Tien area near Dak Lua ranger station.

Table 1. Ecological conditions and distributional records for *Mallomonas bronchartiana*, *M. pseudobronchartiana* and *M. velari*. Code: na = not available; BD = below detection

Taxon/Site	*Coordinates*	*pH*	*Spec Cond* ($\mu S \cdot cm^{-1}$)	*Temperature*[1] (*°C*)	*Total P* ($\mu g \cdot L^{-1}$)	*Total N* ($\mu g \cdot L^{-1}$)	*Reference*
M. bronchartiana							
1. Dzua River, Khanh Hoa Province, Vietnam	12°15'1" N 109°09'5" E	6.6	246	30	na	na	This study
2. Sandpit in Cam Ranh Peninsula, Khanh Hoa Province, Vietnam	12° 04' 43"N 109° 11' 26"E	6.0	75	34	64	580[1]	This study
3. Ta Lai reservoir, Dong Nai Province, Vietnam	11°23'26" N 107°21'49" E	6.4	37	30	na	na	This study
4. Temporary forest pool, Dong Nai Province, Vietnam	11° 24' 26" N 107° 24' 49" E	6.1	29	31	na	na	This study
5. Anh Hai Lake (Con Dao Island), Baria-Vung Tau Province, Vietnam	8° 40' 40" N 106° 35' 52"E	na	na	na	na	na	This study
M. pseudobronchartiana							
1. Dau Ca Lake, Cat Tien National Park, Vietnam	11° 28' 26" N, 107° 20 ' 34" E	5.9	22	28	44	540[1]	This study
2. Mulyeongari, Sumang-ri, Namwon-eup, Seogwipo-si, South Korea	33° 22'10"N 126° 41'36"E	na	na	15-30	na	na	This study
3. Dongbaek-dongsan, Chocheon-eup, Jeju-si, South Korea	33°31'0"N 126°43'3"E	6.0	135	15-30	na	na	Kim *et al.* 2009
4. Bigelow Pond, CT, USA	41° 35' 24"N 72° 4' 12"W	6.1	37	20	37	267	Siver, 1991
5. Echo Lake, Ocala National Forest, Florida, USA	29° 3' 36"N 81° 23' 24"W	5.3	47	24	17	502	Siver & Lott, 2006
6. Dresdan Bog, Maine, USA	44° 3' 36"N 69° 24' 36"W	5.7	28	18	9	540	This study
7. Lower Hadlock, Maine, USA	69° 24' 36"N 68° 10' 12"W	6.3	51	16	BD	108	This study
M. velari							
1. Dak Lua swamp	11°30'43" N, 107°22'56" E	5.2	10	24	na	na	This study

[1] Total Kjeldahl Nitrogen

Samples were collected from surface waters using a plankton net with a mesh size of 10 μm or 20 μm. For electron microscopy studies, aliquots of each sample from the Vietnam sites were washed by repeated centrifugation in deionized water. Drops of the washed sample were either dried directly onto stubs or digested for 4-5 minutes in sulfuric acid with potassium dichromate. For observation with SEM, Vietnam samples were placed directly onto aluminum stubs. Aliquots of samples from the USA were dried directly onto heavy duty aluminum foil, trimmed, and mounted onto aluminum stubs with Apiezon wax. For the Korean samples, cells were filtered using nylon membrane filters (Whatman Ltd, Maidstone, UK), rinsed in distilled water, fixed in 1% OsO_4, dehydrated in an ethanol series, and dried in a HCP-2 critical point dryer (Hitachi Co., Tokyo, Japan). Samples were coated with either gold or platinum and observed with a JEOL 6510 LV SEM (Vietnam samples), a Leo 982 FE-SEM (U.S.A. samples), or a JEOL model JSM 7000F FE-SEM (South Korean samples). For TEM studies, aliquots of samples were mounted on Formvar-coated grids (EMS FF200-Cu-50, Electron Microscopy Sciences) and observed with a JEM-1011 TEM.

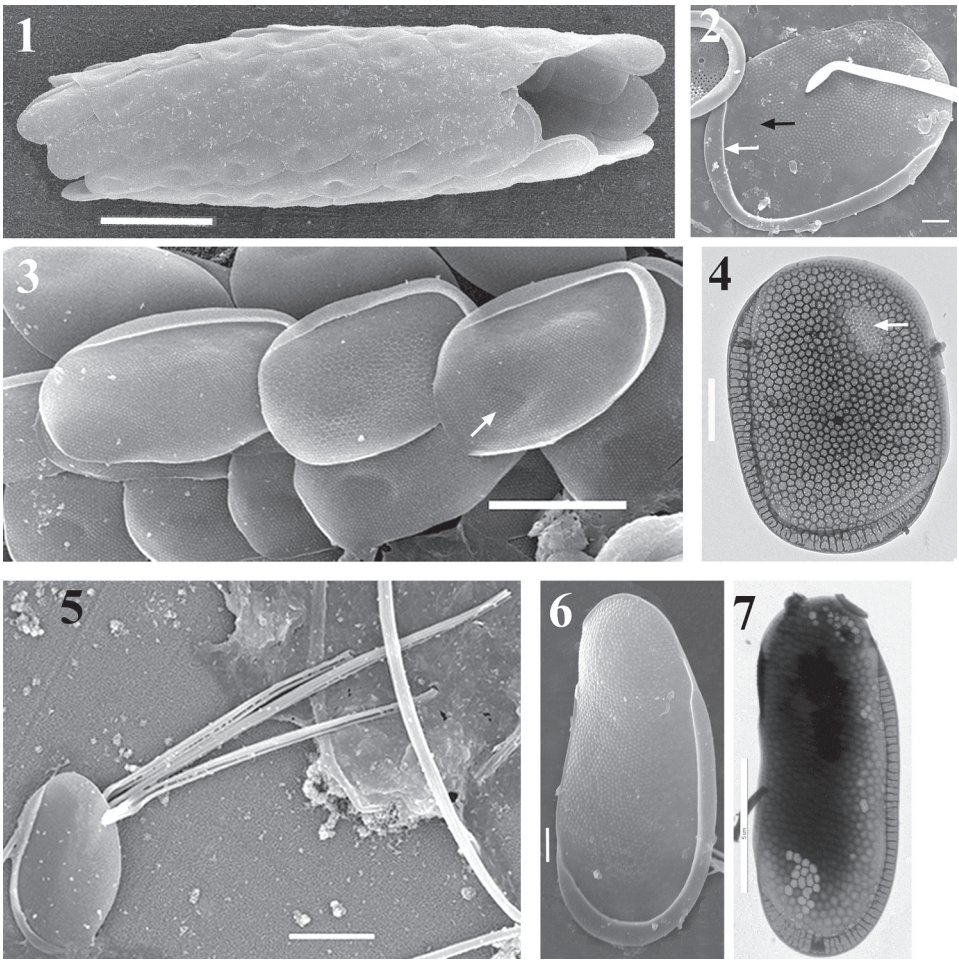
Specific conductance, pH and temperature measurements were made with either a Hanna Combo HI 98129 unit (Hanna Instruments, Inc., USA), or with a Hydrolab Datasonde 4a (Hach Hydromet, Loveland, CO, U.S.A.). Methods for measurement of total phosphorus and total nitrogen are given by Siver and Lott (2012).

RESULTS

Specimens of *Mallomonas* initially thought to belong to the relatively rare species *M. bronchartiana* were found in collections made in Vietnam, the U.S.A. and South Korea (Table 1). After close examination it was clear that in addition to *M. bronchartiana* the collections contained remains of two additional and undescribed species. Scales of all three taxa are large, robust, have an internal reticulation of meshes, and are covered externally with a thin siliceous layer bearing small papillae. However, scales of all species have unique sets of characteristics that can be used to distinguish between the taxa. Below we expand the original description of *M. bronchartiana* and formally describe the two new species.

Mallomonas bronchartiana Compère **Figs 1-7**

According to Kristiansen (2002), cells are elongate-ellipsoidal or elongate-obovoid, range in size from 50-60 × 13-25 μm, and with bristles found on both ends of the cell. Body scales lack bristles. Body scales are large, broadly oval with an upturned posterior rim, and with a secondary layer consisting of a honeycomb-like reticulation of ribs covered externally by a thin layer bearing papillae (Figs 2-4). The meshes comprising the internal reticulation range in outline from circular to various polygon shapes, range in diameter from 220 to 320 nm, and extend across most of the scale surface from just under the posterior rim to near the apical end of the scale (Fig. 4). Although evidence of the meshes can be seen with SEM, they are best observed with TEM. A set of thin parallel ribs underlie the posterior rim and connect to the honeycomb, or mesh, structure (Fig. 4). The external layer is covered with small, closely spaced, papillae that extend across the scale, but are lacking in a zone along the posterior rim, forming a hyaline region as observed with SEM (arrows,



Figs 1-7. SEM (1-3, 5-6) and TEM (4, 7) images of *Mallomonas bronchartiana*. **1.** Broken part of a whole cell. **2.** Body scale. Arrows indicated the region of the hyaline area between the posterior rim (black arrow) and area with papillae (white arrow). **3.** Group of body scales. Note the raised shield on the left scale and the depressions in distal portions of the scale overlying the region lacking the internal mesh reticulation (arrow). **4.** Body scale. Arrow indicates the area lacking the internal mesh reticulation in the distal portion of the scale. **5.** Scale with bristles. Note the open slit running the length of the shaft. **6-7.** Elongated posterior scales. Scale bars = 10 μm (Fig. 1); 5 μm (Figs 3, 5, 7); 2 μm (Fig. 4); 1 μm (Figs 2, 6).

Fig. 2). The internal mesh reticulation is usually lacking in a small region on the apical portion of the scale, yielding a clearer view of the surface papillae when viewed with TEM (arrow, Fig. 4). This region lacking the mesh reticulation appears slightly sunken when viewed with SEM (arrows, Fig. 3). The posterior rim encircles approximately half of the perimeter of the scale, but extends further along the right side resulting in an asymmetric shape (Fig. 3). Base plate pores are very small, inconspicuous, and scattered. Posterior scales are elongate-oval with the same basic

design as body scales except that the posterior rim is even more asymmetrically positioned (Figs 6-7). Body and posterior scales from the Vietnam population ranged in size from 8.0-10.0 × 5.0-7.5 μm and 10.2-13.5 × 4.6-6.3 μm, respectively. Bristles are straight, smooth, with a wide groove that runs the length of the shaft, a square-shaped apex with small teeth, and a flat foot extending at a 45 angle from the shaft (Figs 2, 5). Bristles ranged from 18 to 27 μm long in the Vietnam populations.

Mallomonas bronchartiana is reported here for the first time from Vietnam, where it is reported from five localities that represent a range of slightly acidic and dilute habitat types (Table 1).

***Mallomonas pseudobronchartiana* sp. nov. E. S. Gusev, P.A. Siver & W. Shin**
Figs 8-22

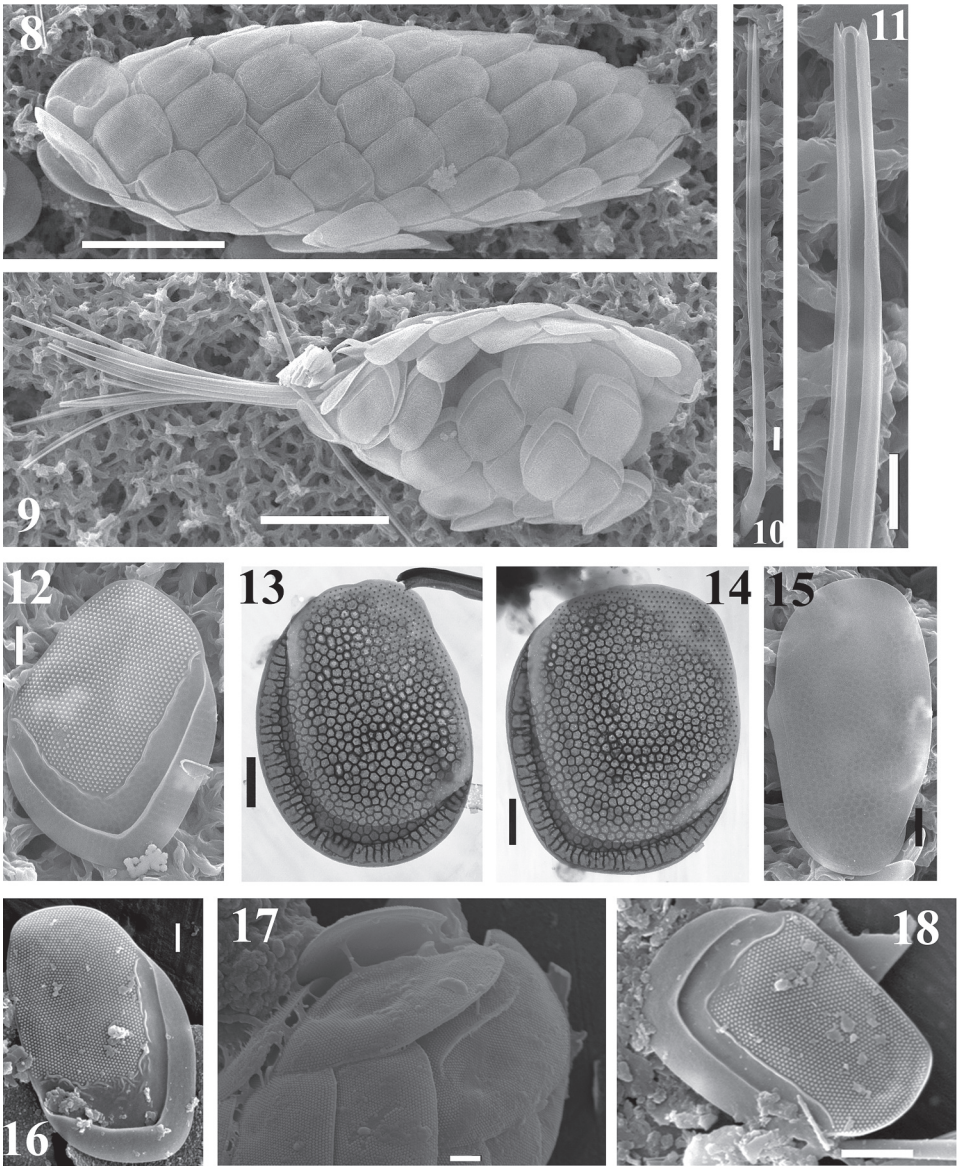
Description: Cells are ellipsoidal, range in size from 39-54 × 13-16 μm, and with bristles found on the ends of the cell. Body scales are large, broadly elliptic to broadly ovate, with an upturned posterior rim, a thin V-shaped rib situated on the scale surface, and a secondary layer consisting of a honeycomb-like reticulation of ribs covered externally by a thin layer bearing papillae (Figs 12-14; 18-20). The posterior rim is thick, encircles approximately half of the perimeter, and usually extends further along the right side of the scale resulting in an asymmetric shape (Fig. 12). The V-shaped rib is a continuous structure on most scales, but on some specimens it is composed of a series of smaller ribs that may not be fused together (Fig. 16). The honeycomb reticulation with irregular meshes, best observed with TEM, extends across most of the scale surface, but is lacking along the perimeter (Figs 13-14). Diameter of each mesh varies from 170 to 310 nm. A set of thin parallel ribs underlie the posterior rim and connect to the honeycomb structure. The external layer is covered with small, closely spaced papillae that extend from the base of the V-shaped rib to the scale perimeter, but are lacking in the posterior flange, forming a distinct hyaline zone. The hyaline zone is easily observed with SEM (Fig. 19), but not with TEM (Fig. 20). Caudal scales have the same basic design as body scales, but are more elongate and not as wide (Fig. 15). Apical scales surrounding the flagellar pore have a more circular shape. Base plate pores are very small, scattered, and visible only with TEM. Body and caudal scales range from 6.4-8.6 × 5.1-6.3 μm and 8.5-9.7 × 4.7-5.6, respectively. Small apical scales, adjacent to the flagellar pore, are 4.4-4.8 × 3.0 μm. Bristles are straight or slightly curved, smooth, with a wide groove that extends the length of the shaft, terminates with four small teeth, a flattened foot, and range in length from 20-25 μm (Figs 9-11).

Holotype specimen: Portion of a single gathering of cells on SEM stub No. CT13 deposited at the Herbarium of the I.D. Papanin Institute for Biology of Inland Waters RAS, Borok (IBIW). Material from Dau Ca Lake, Cat Tien National Park, Vietnam collected by E.S. Gusev on the 9th of October, 2010. Figure 18 is a representative scale from the specimen.

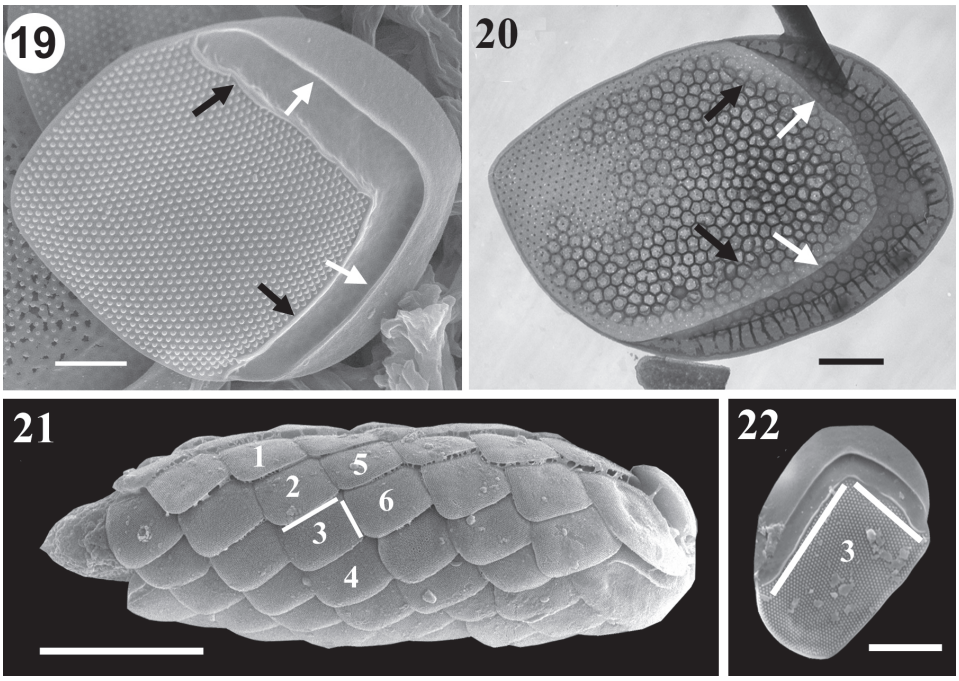
Type Locality: Dau Ca Lake, Cat Tien National Park, Vietnam. Latitude/Longitude: 11° 28’ 26’’ N, 107° 20 ’ 34’’ E.

Epithet: The species name is derived from the fact that its scales resemble, and could be confused with, those of *Mallomonas bronchartiana*.

Distribution: In addition to the type locality, Dau Ca Lake in Vietnam, this species has been observed in South Korea (Kim *et al.*, 2009; Siver *et al.*, 2015; this study), and multiple regions in the United States (Siver, 1991; Siver & Lott, 2006; 2012). In general, the waterbodies harboring *M. pseudobronchartiana* are acidic, poorly buffered, dilute, humic stained, and with low nutrient content (Table 1).



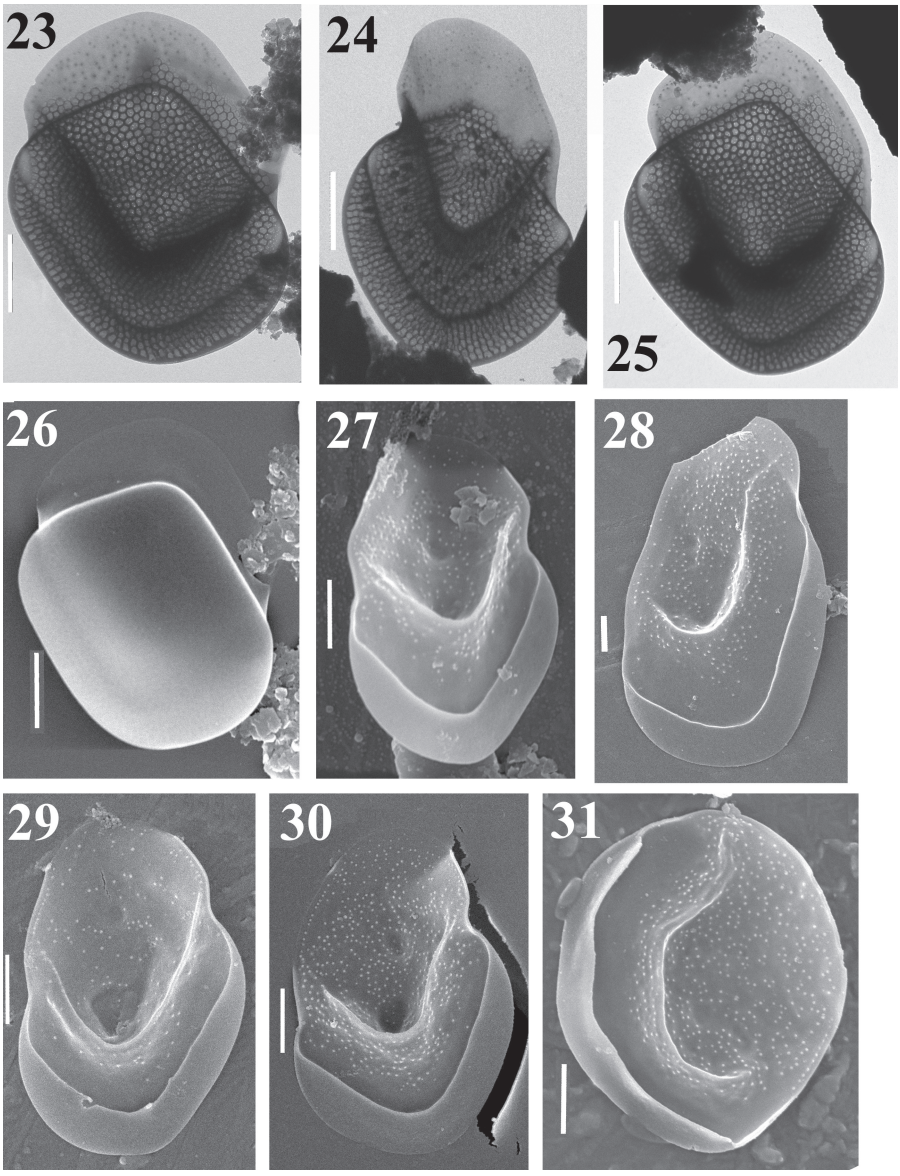
Figs 8-18. SEM (8-12, 15-18) and TEM (13-14) images of *Mallomonas pseudobronchartiana*. **8.** Whole cell denoting the pattern of overlap of the scales. The flagellated end is towards the left. **9.** Portion of a cell with bristles still attached. **10.** Isolated bristle. **11.** Distal end of a bristle denoting the wide slit running the length of the shaft, and the arrangement of small teeth on the apex. **12-14, 16, 18.** Body scales. Scale in Fig. 18 is from the Holotype specimen. **15.** Undersurface view of an elongated posterior scale. **17.** Group of scales from the anterior end of the cell. Scale bars = 10 µm (Figs 8-9); 2 µm (Fig. 18); 1 µm (Figs 10-17).



Figs 19-22. SEM (19, 21-22) and TEM (20) images of *Mallomonas pseudobronchartiana*. **19-20.** Black arrows indicated position of the V-rib structure and white arrows the posterior rim. **21.** Overlap of scales on the cell covering. The body scales labeled 1-4 are all positioned in the same spiral row and are overlapped by scales 5-6 in the spiral row above it, towards the anterior of the cell. The white bars on scale 3 denote the position of the asymmetric V-rib structure relative to the spacing of the surrounding scales. See text for details. **22.** Asymmetric body scale representative of scale 3 in Figure 21. Scale bars = 1 μm (Figs 19-20); 2 μm (Fig. 22); 10 μm (Fig. 21).

***Mallomonas velari* sp. nov. E. S. Gusev, P.A. Siver & W. Shin Figs 23-31**

Scales are of two types, body scales with forward projecting wings, and scales lacking wings. Body scales are large, robust, rhombic, slightly asymmetric, and range in size from 9.0-10.4 × 5.7-7.0 μm (including the wing) (Figs 23-30). Body scales possess a wide posterior rim, lateral incurvings, a well developed V-rib structure, a large forward projecting wing, an extensive internal secondary reticulation, and an external covering with papillae (Figs 23-30). The posterior rim extends further, and to various degrees, along the right perimeter of the scale, yielding the asymmetric outline. The V-rib is broad, thick, positioned just below the center of the scale, with arms that extend to and connect with the forward projecting wing. The V-rib is an upward fold of the scale surface that rests on top of the internal secondary reticulation and not directly on the base plate (Figs 23-25). The forward projecting wing is elevated above the base plate, and extends on both sides of the scale. On the more asymmetric-shaped scales, the V-rib and wing extend further on the right side of the scale (Fig. 28). The internal reticulation of enclosed meshes, observed best with TEM, extends over the scale surface from the posterior perimeter, across the posterior flange and shield, and onto the lower end of the wing. The



Figs 23-31. TEM (23-25) and SEM (26-31) images of *Mallomonas velari*. **23-30.** Body scales. Note the forward projecting wing, internal mesh reticulation (23-25), thick posterior rim, folded surface layer forming the V-rib, depressed areas on the shield, and scattered surface papillae. The specimen in Fig. 26 denotes the undersurface of the scale. The scale pictured in Fig. 27 is from the Holotype specimen, and the one in Fig. 28 represents an asymmetric scale. **31.** Circular scale believed to represent an apical scale surrounding the flagellar pore. Scale bars = 2 μm (Figs 23-27, 29-31); 1 μm (Fig. 28).

meshes often become aligned in rows under the posterior rim. The meshes range in diameter from 205 to 270 nm. The internal reticulation is covered with a siliceous layer. Small, widely and unevenly-spaced papillae are found on the V-rib, shield and wing. Body scales possess one, and usually two, wide depressions on the shield, easily observed with SEM, but not as obvious with TEM. One depression is situated within the angle of the V-rib, and the second one more towards the center of the shield. Based on observations with TEM, the internal mesh reticulation is not missing in the areas covered by the depressions (Figs 23-25). Posterior flange is broad, smooth and lacks papillae. Base plate pores are very small, widely distributed, and largely inconspicuous (Figs 23-25). The second scale type is more circular in outline, 10.1 × 8.5 μm, with the same basic structure as body scales, except for the lack of an anterior wing (Fig. 31). Whole cells and bristles were not observed.

Holotype specimen: Portion of a single gathering of cells on SEM stub number DL2/3 deposited at the Herbarium of the I.D. Papanin Institute for Biology of Inland Waters RAS, Borok (IBIW). Material from Dak Lua swamp in Cat Tien National Park, Vietnam. Sample collected 10th of March, 2014, by E.S. Gusev. Figure 27 is a representative scale from the specimen.

Type Locality: Dak Lua swamp in Cat Tien National Park, Vietnam, Latitude/ Longitude: 11°30'43" N, 107°22'56" E.

Epithet: The species name is derived from the Latin word for awning or covering in reference to the elevated wing.

Distribution: To date, *Mallomonas velari* has only been observed in Dak Lua swamp where it was rare. At the time of collection, the pH = 5.2, temperature = 24°C, and specific conductance = 10 μS cm^{-1} (Table 1).

DISCUSSION

Mallomonas pseudobronchartiana is closely aligned with *M. bronchartiana*, each species possessing body scales that are large, broad, with an asymmetrically-placed posterior rim that extends further along the right side of the scale, an internal honeycomb reticulation, and covered externally with a layer containing papillae. Both species have similar cell shape and size, and bristles restricted to the ends of the cell. Three features serve to distinguish scales of the two species. First, the most obvious is the presence of the thin V-shaped rib on the surface of scales of *M. pseudobronchartiana* that delineates the hyaline region from the portion of the scale covered with papillae. Second, scales of *M. bronchartiana* usually have a depression zone on the anterior end caused by the lack of the internal reticulation, a feature lacking on *M. pseudobronchartiana* scales. Third, the posterior rim is considerably thicker and extends further over the base plate on *M. pseudobronchartiana* scales as observed with both SEM and TEM. Siver *et al.* (2015) reported that *Mallomonas* sp.1 has the thick posterior rim but mislabeled it as the V-rib structure (see Fig. 11B). Although the V-shaped rib on *M. pseudobronchartiana* scales is obvious with SEM, scales of the two species can be hard to separate with TEM on the basis of this feature since it is difficult to delineate (compare Figs 19-20). Thus, care must be used to distinguish between the two species using TEM.

Like scales of *M. bronchartiana* and *M. pseudobronchartiana*, those of *M. velari* are also large, robust, with a wide and asymmetrically-placed posterior rim that extends further along the right side of the scale, an internal honeycomb

reticulation, and an external covering with papillae. However, scales of *M. velari* also have both the prominent wing and the thick folded V-rib structure, features that clearly separate it from the other two taxa. In addition, scales of *M. velari* are more rhombic-shaped, have deeper surface depressions, and less dense and more unevenly-spaced papillae. Although we did not observe whole cells, we believe that the circular scales lacking a wing (Fig. 31) surround the flagellar pore, and the more asymmetric scales (Fig. 28) are found on the caudal region of the cell. Except for the prominent wing, scales of *M. velari* share features with those of *M. adamas* Harris & Bradley, including an internal reticulation, surface papillae, a wide posterior rim, a V-shaped surface fold, and the presence of large surface depressions.

It is not clear if the thin linear V-shaped rib situated on the surface of *Mallomonas pseudobronchartiana* scales, or the upward folded surface on *M. velari* scales, are homologous to a true V-rib, or rather structures evolved to serve a similar function of properly spacing the scales on the cell surface (Siver & Glew, 1990; Siver *et al.*, 2015). Typically, the V-rib is usually anchored directly onto the base plate, is a solid siliceous structure, and generally represents a prominent feature of the scale. In the case of *M. pseudobronchartiana* the thin V-shaped rib structure is formed on the top of the scale, above the secondary layer that covers the scale surface. In other words, it is not directly attached to the base plate. Similarly, the structure found on *M. velari* scales represents a V-shaped fold of the top siliceous layer and is clearly underlain by the internal reticulation.

Despite whether the thin V-shaped rib structure on *M. pseudobronchartiana* is homologous with a true V-rib, it is clearly involved in spacing the scales as it is precisely aligned with the pattern by which the scales overlap to form the scale covering around the cell (Figs 21-22). As described by Siver & Glew (1990), and true for most species in the genus, scales of *M. pseudobronchartiana* are aligned in spiral rows such that each scale is partially overlapped by the scale situated behind it in the same row. For example, scales 1-4 in Figure 21 depict four scales within a spiral row. Scale #2 extends over the right arm of the posterior rim and abuts with the V-shaped structure on scale #3. Each scale is also partially overlapped along the left side by portions of two scales in the row situated above or towards the anterior of the cell. In this example, scales #5 and specially #6 overlap scale #3. The overlap on the left side extends less and aligns with the shorter side of the posterior rim (Fig. 22). There is often an irregularity on the scale at the point where the two overlapping scales within the more anterior row meet. We conclude that the asymmetry of the body scales is such as to accommodate the resulting pattern of alignment on the cell surface, and we further suspect that this may be the case for many, and perhaps all, of the species within the genus bearing asymmetric scales. Although we did not observe whole cells of *M. velari*, we believe that the folded V-shaped surface layer is also directly involved with spacing of the scales on the cell coat.

Using molecular gene sequences, Jo *et al.* (2011) and Siver *et al.* (2015) clearly showed that the genus *Mallomonas* diverged early in its evolutionary history into two major clades based on the presence or absence of a V-rib. One monophyletic subclade within the clade bearing the V-rib contained species classified in the section Quadratae, including *Mallomonas splendens* (West) Playfair em. Croome *et al.* and *M. adamas*. Siver *et al.* (2015) further showed that *M. pseudobronchartiana* (identified as *M.* sp. 1 in that work) was aligned with the species in the section Quadratae. The placement of *M. pseudobronchartiana* within section Quadratae is not surprising since taxa within this section share numerous characteristics with it, including a) thick scales with an internal reticulation, a broad posterior rim, and

surface papillae; b) bristles restricted to one or both ends of the cell; c) scales in the middle of the cell positioned within spiral rows with their longitudinal axes perpendicular to the longitudinal axis of the cell and; d) similar bristle structure. In addition, scale alignment on species within section Quadratae is achieved by structures associated with the surface of the scale that are often V-shaped, but that likely represent modifications of a true V-rib (*i.e.* attached directly to the base plate). It is likewise possible that the ancestor of the section Quadratae subclade developed thick scales, lost the ability to form a V-rib anchored on the base plate, and subsequently evolved the suite of surface structures observed on extant species. Based on these hypotheses and similarities in scale morphology, we suspect that it will be shown that *M. bronchartiana* will be removed from section Planae where it is currently placed (Kristiansen, 2002), and moved to section Quadratae. Similarly, and for the same reasons, we suspect that *M. velari* also belongs in section Quadratae.

Mallomonas bronchartiana is a warm water organism largely distributed in tropical regions (Cronberg, 1989; Kristiansen, 2002). Originally described from Lake Chad in Africa (Compère, 1974), *M. bronchartiana* has also been reported from other tropical localities including Botswana (Cronberg, 1996), the Amazon tropics (Kristiansen & Menezes, 1998), Malaysia (Dürrschmidt & Croome, 1985), India (Saha & Wujek, 1990) and Indonesia (unpublished results), as well as subtropical sites in South America (Vigna, 1990) and the southeastern United States (Wujek & Bland, 1991; Wujek, 2000). Thus, it is not surprising that the species was also found in tropical Vietnam. However, some of the previous records for *M. bronchartiana* may actually represent ones for *M. pseudobronchartiana*, and others (e.g. Saha & Wujek, 1990; Hansen, 1996) are misidentifications and do not represent either species. In particular, scales illustrated by Cronberg (1989), Wujek & Bland (1991), Kristiansen & Menezes (1998) and Wujek (2000) likely belong to *M. pseudobronchartiana* given the width of the posterior rim and lack of an anterior depression. If these records indeed represent *M. pseudobronchartiana*, then it is possible that *M. bronchartiana* has not been reported from either South America or North America. Still, caution needs to be exercised regarding the previous records of *M. bronchartiana* since they are based on TEM images where some of the pertinent features can't be discerned.

Here we describe *M. pseudobronchartiana* from one site in tropical Vietnam, but recognize that this taxon has a more cosmopolitan range extending further into subtropical and temperate climates (Table 1). Siver & Lott (2006) reported *M. pseudobronchartiana* (as *M. bronchartiana*) from a humic-stained acidic site in subtropical Florida, and populations have also been found in temperate regions of North America (as *M.* cf. *bronchartiana* in Siver, 1991) and Asia (Kim *et al.* 2009; as *M.* sp.1 in Siver *et al.*, 2015; this study). Scales of *M. pseudobronchartiana* have also been observed in two poorly buffered, acidic ponds along coastal Maine in the USA, extending its range to 45º N (Table 1). Thus, although records of *M. bronchartiana* and *M. pseudobronchartiana* are still limited, it appears that the latitudinal range of the latter taxon is wider, extending well into temperate regions. At this time, *Mallomonas velari* is only known from Vietnam.

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