



## ***Mallomonas neoampla* sp. nov. from Vietnam, a new species that bridges the gap between fossil and modern taxa**

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With 21 figures

**Abstract:** *Mallomonas neoampla* is described from the Dzua River, located in Khanh Hoa Province, Central Vietnam, and represents the third taxon now known from the section *Multisetigerae*. *Mallomonas neoampla* shares some features of the scale morphology with the fossil species *M. ampla*, and others with the modern and widely distributed *M. multisetigera*. *Mallomonas neoampla* has large, oval, slightly asymmetrical scales with a highly variable-shaped V-rib, and a shield covered with papillae and small evenly-spaced pores. Papillae and base plate pores are largely lacking on the posterior flange. Bristles of *M. neoampla* have partially rolled edges forming a shaft that is U-shape in cross section, an expanded bifurcate tip, and a highly recurved and hook-like proximal foot. Based on scale size, the degree to which the dome is recessed, and the structure of the bristles, *M. neoampla* clearly represents the closest extant congener of the Eocene fossil species *M. ampla*.

**Key words:** Eocene, *Mallomonas*, *Multisetigerae*, new species, synurophytes, Vietnam

### **Introduction**

The section *Multisetigerae* of the genus *Mallomonas* was established by Asmund & Kristiansen (1986) to accommodate a single species, *Mallomonas multisetigera* Dürschmidt, with a unique suite of characters. Scales of *M. multisetigera* are tripartite with a well-defined V-rib where the arms curve and become continuous with the anterior submarginal ribs, a shallow and broad dome, and numerous small papillae covering the shield, dome and often anterior flanges (Siver 1991, Kristiansen 2002). Base plate pores

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are notably larger on the posterior flange than on the remainder of the scale, a feature rarely observed within the genus and useful for identifying the organism. Another rather unique feature of this species is that scales are usually associated with multiple bristles, and up to five bristles have been reported for anterior scales (Dürschmidt 1982). Originally described from Chile, *M. multisetigera* is now known from Europe, North America, South America, Africa and Australia, and is considered to be globally distributed (Kristiansen 2002, Kristiansen & Preisig 2007).

Recently, a second species assigned to section *Multisetigerae*, *Mallomonas ampla* Siver & Lott, was described from middle Eocene mudstones at the Giraffe locality situated near the Arctic Circle in northern Canada (Siver & Lott 2012). Scales of this fossil species differed from those of *M. multisetigera* by being significantly larger, having base plate pores with uniform diameter over the entire surface, and possessing a dome recessed from the anterior margin. In addition, the fossil species had bristles that were U-shaped in cross section over the entire shaft, and a distinctly recurved and hook-like proximal end (e.g. the foot). Given similarities in scale ultrastructure, especially in dome structure, between *M. ampla* and *M. multisetigera*, Siver and Lott (2012) argued that the lineage represented by section *Multisetigerae* is ancient, extending to at least the Eocene and likely considerably further back in geologic time.

The purpose of this study is to describe another modern species within the section *Multisetigerae* from two waterbodies in Vietnam that possesses morphological characters in between those of *M. multisetigera* and *M. ampla*, but closer to the latter taxon.

## Material and methods

Samples were collected from the Dzua (Dúra) River located near Nha Trang City (12°15.016'N, 109°09.083'E) and an unnamed sand pit with standing water situated on the Cam Ranh Peninsula (12°04.824'N, 109°10.996'E) using a plankton net with a mesh size of 20 µm. Both sites are located in Khanh Hoa Province, along the South Central Coast region of Vietnam. This region of the country has a tropical monsoon climate with a mean annual temperature ranging between 25–28°C, relative humidity between 80–94%, annual precipitation between 1800–2100 mm, and an annual evaporation of 1000–1200 mm (Schmidt-Thomé et al. 2015, Inland Water Ecology 2014).

For morphological studies we used fixed material from the Dzua River site and from a culture isolated from this location. Strain BOROK VN819 was isolated from the Dzua River site and is deposited in the collection BOROK WDCM602 (Papanin's Institute for Biology of Inland Waters). Clones were isolated by micropipette, put through a series of washings using Waris-H medium (McFadden & Melkonian 1986) and grown in plastic Petri dishes containing ca. 10 ml of the culture medium. The culture was kept at 20–22°C under natural light from a north-facing window. For electron microscopy studies, an aliquot of each sample was washed three times by repeated centrifugation with deionized water. Drops of each washed sample were dried directly onto stubs (SEM) or grids (TEM) in case of cultures, or first digested 4–5 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 conductivity, pH and temperature measurements were made with a Hanna Combo (HI 98129) device, Hanna Instruments, Inc., USA.

## Results

### *Mallomonas neoampla* Gusev & Siver, sp. nov.

Figs 1–17

DESCRIPTION: Cells ellipsoidal, approximately  $24\text{--}32 \times 15\text{--}18\text{ }\mu\text{m}$ , covered by scales (Fig. 13). Scales oval, slightly asymmetric,  $4.9\text{--}7.4 \times 2.5\text{--}3.9\text{ }\mu\text{m}$ . Shield with papillae. Base plate with numerous pores on the shield and few scattered pores on the posterior flange in SEM view. Based on TEM, large pores are evenly distributed on the scale surface, but a lot of pores are covered with a siliceous layer and do not penetrate the scale. The V-rib is conspicuous, hooded, acute, and the arms curve and become continuous with the anterior submarginal ribs, extending to the base of the dome. The dome is shallow, set back from the anterior margin, and is covered with papillae. The posterior rim is thin, encircles approximately half of the scale perimeter, and can be asymmetric, extending further along one side of the scale. The posterior flange can have a few papillae. The anterior flange is without papillae. Bristles are  $8\text{--}16\text{ }\mu\text{m}$ . The foot of the bristle is hooked, the distal tip is slightly bifurcated, and the margins of the bristles are rolled to form an open U-shaped groove. There are several pores on the bristles shaft. Cysts were not observed.

HOLOTYPE SPECIMEN: Portion of a single gathering of cells on SEM stub number VN 819 deposited at the Herbarium of the I.D. Papanin Institute for Biology of Inland Waters RAS, Borok (IBIW). Material is from culture BOROK VN 819 established from sample DKH made by E.S. Gusev on 25<sup>th</sup> of June 2012. Figure 5 is a representative scale from the specimen.

TYPE LOCALITY: Dzua (Dúra) River,  $12^{\circ}15'01''\text{N}$ ,  $109^{\circ}09'05''\text{E}$ , Khanh Hoa Province, Vietnam.

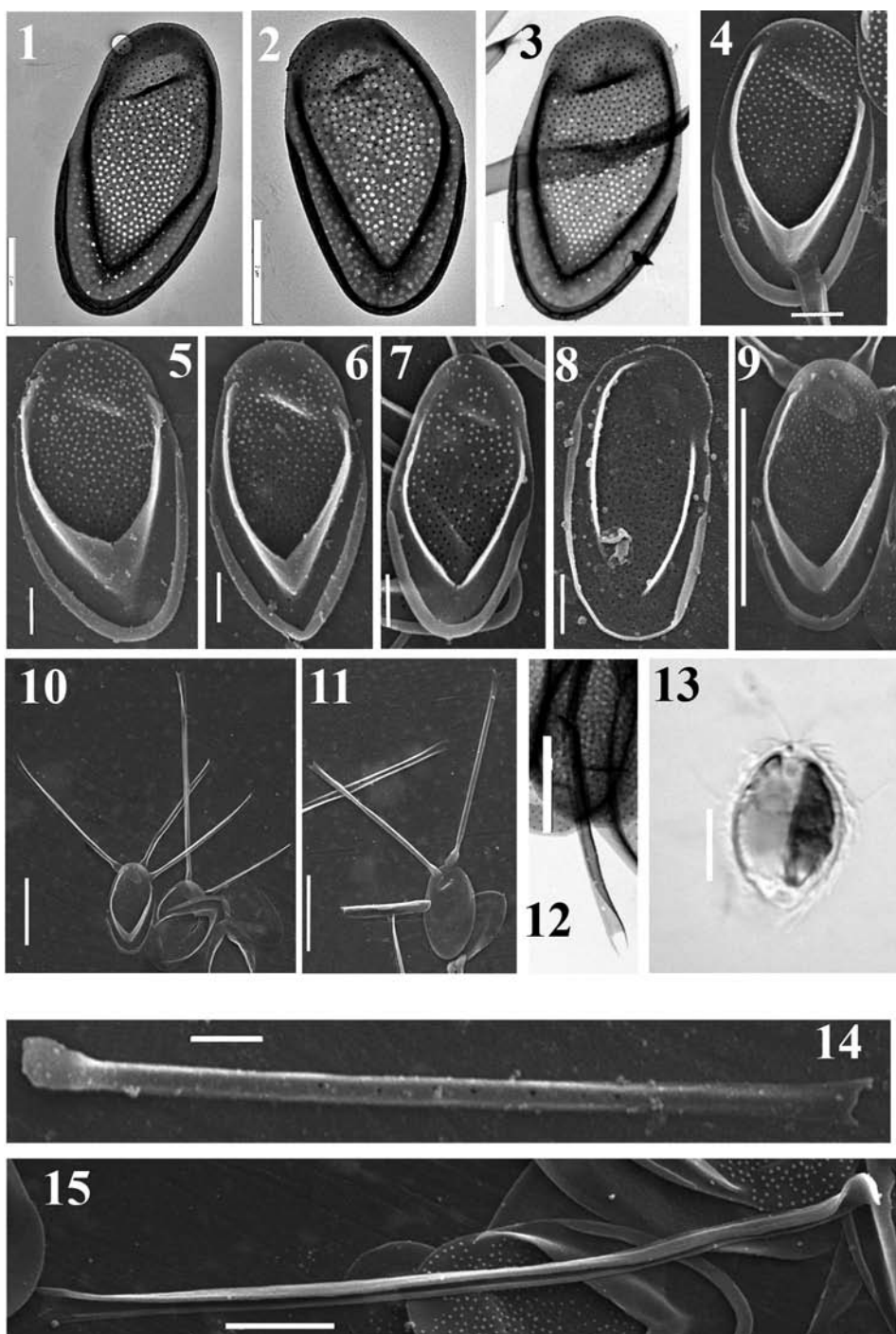
ETYMOLOGY: The epithet reflects the similarity with *M. ampla*.

DISTRIBUTION AND HABITAT: *Mallomonas neoampla* was found in the type locality (Dzua River,  $246\text{ }\mu\text{S cm}^{-1}$ , pH = 6.6, temperature  $33^{\circ}\text{C}$ ) and Cam Ranh Peninsula (unnamed sandpit,  $54\text{ }\mu\text{S cm}^{-1}$ , pH = 5.4, temperature  $33^{\circ}\text{C}$ ) in Vietnam. This species was also reported from Singapore by Neustupa & Řezáčová (2007) under the name "*Mallomonas multisetigera*" from a site with a pH = 6.8 and a temperature =  $31^{\circ}\text{C}$  at the time of collection.

**New Record for *Mallomonas multisetigera*:** A second species from the section *Multisetigerae*, *Mallomonas multisetigera*, is reported for the first time from Vietnam (Figs 18–19). This species was found in an unnamed pond located in Khanh Hoa Province ( $12^{\circ}37.722'\text{N}$ ,  $109^{\circ}06.669'\text{E}$ ) with a pH = 6.3, specific conductivity =  $54\text{ }\mu\text{S cm}^{-1}$ , and a temperature =  $32^{\circ}\text{C}$ .

## Discussion

With the discovery of *Mallomonas neoampla*, there are now three species within the section *Multisetigerae*, two contemporary taxa (including *M. multisetigera*) and one fossil taxon (*M. ampla*). From a morphological point of view, *Mallomonas neoampla* shares some features with *M. ampla* (Figs 20–21) and others with the widely distributed *M. multisetigera* (Figs 18–19). Scales of *M. neoampla* ( $4.9\text{--}7.4 \times 2.5\text{--}4.1$ ) and *M. ampla* ( $4.7\text{--}6.7 \times 3.1\text{--}4.1$ ) are of similar size, and both significantly larger than those



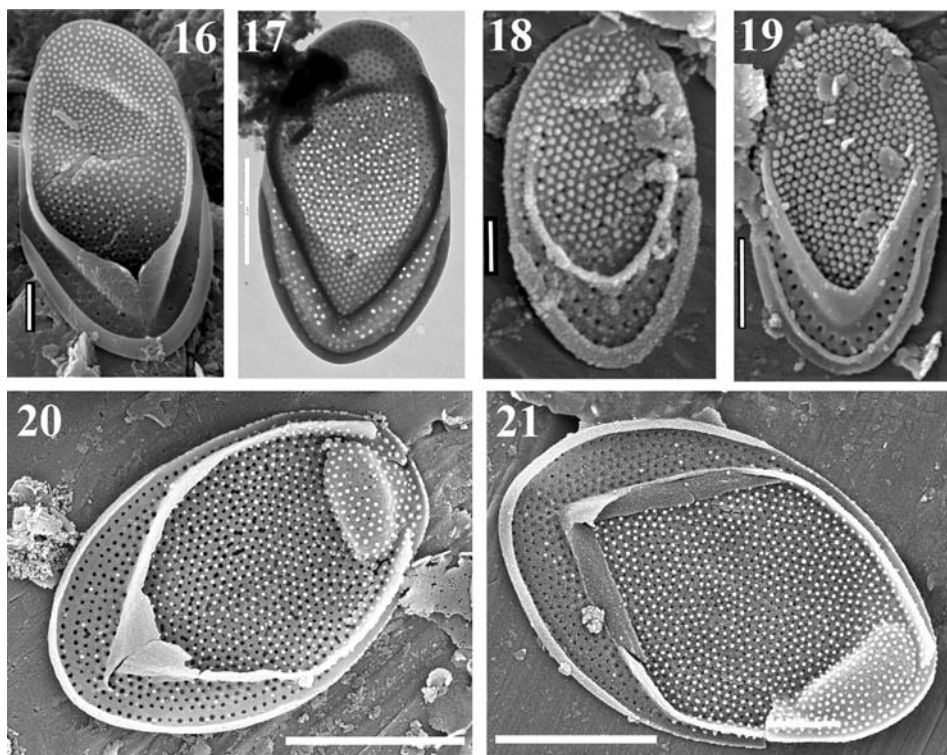
of *M. multisetigera* ( $2.5\text{--}5.0 \times 1.8\text{--}2.5$ ). Scales of *M. ampla* are oval and symmetrical compared with the other two species, which often have scales with asymmetrically placed domes, V-rib arms, and posterior rims. In addition, the widest positions on *M. neoampla* and *M. multisetigera* scales are often closer to the anterior end than found on *M. ampla*, yielding an asymmetrical scale outline, compared with the oval and largely symmetrical scales found on *M. ampla*. Scale asymmetry may reflect differences in how the scales are positioned on the cell covering, with a greater degree of asymmetry associated with scales oriented at angles with the longitudinal cell axis (Siver & Glew 1990, Siver 1991). The degree to which the dome is recessed from the anterior margin is greatest in *M. ampla*, less on *M. neoampla* scales, and the least on *M. multisetigera*.

Likewise, the size and arrangement of base plate pores differs on all three species, especially regarding the posterior flange, and is a useful character for distinguishing between the taxa. *Mallomonas ampla* has small pores (30–55 nm) of similar diameter distributed more or less evenly across the entire scale, including up to six rows on the posterior flange (Siver & Lott 2012). Scales of *M. neoampla* also have small pores (30–40 nm) evenly distributed on the scale shield, but they are largely lacking on the posterior flange. The base plate pores on *M. multisetigera* scales are of two distinct sizes, with notably larger pores (90–110 nm) on the posterior flange that are usually arranged in two concentric rows (Figs 18–19, Dürschmidt 1982). Interestingly, based on TEM observations, scales of *M. neoampla* have circular patterns with less dense silica within the posterior flange that are of similar size to the large pores observed on *M. multisetigera*, but arranged as the pores found on *M. ampla*. However, as observed with SEM, these less dense structures are clearly covered with silica on both sides of the scale. This pattern is not a consequence of culture conditions as it is a feature of both populations from Vietnam (Figs 16–17).

There are also differences in the V-rib, the distribution of papillae on the scales, and the structure of the bristles between the three species. The arms of the V-rib on *M. neoampla* and *M. multisetigera* scales extends to the dome or along the side of the dome, whereas on the fossil taxon they extend further and terminate near the anterior end of the scale. Papillae are distributed on the shields and domes of all three taxa, although they are denser on *M. ampla*. Scattered papillae can also be found on the posterior flange of *M. ampla*, less so on *M. neoampla*, and they are lacking on *M. multisetigera*. Bristles of *M. neoampla* are very similar to those of the fossil species in having partially rolled edges forming a shaft that is U-shape in cross section, an expanded bifurcate tip, and a highly recurved and hook-like proximal foot. Although bristles of *M. multisetigera* also have a bifurcate tip, they are not U-shaped in cross section and lack a recurved proximal end. Siver & Lott (2012) hypothesized that the

Figs 1–15. Images of *Mallomonas neoampla* sp. nov. taken from culture strain VN819. Figs 1–3. TEM view. Scale bar = 2  $\mu\text{m}$ . Figs. 4–7. SEM view. Scale bar = 1  $\mu\text{m}$ . Fig. 8. SEM, undeveloped scale. Scale bar = 1  $\mu\text{m}$ . Fig. 9. Scale with 3 attached bristles. Scale bar = 5  $\mu\text{m}$ . Fig. 10. Scales with bristles. Scale bar = 5  $\mu\text{m}$ . Fig. 11. Bottom view of scale with two bristles. Scale bar = 5  $\mu\text{m}$ . Fig. 12. TEM view of bristle. Scale bar = 2  $\mu\text{m}$ . Fig. 13. Whole cell with light microscopy. Scale bar = 10  $\mu\text{m}$ . Fig. 14. SEM view of bristle, "dorsal" side. Scale bar = 1  $\mu\text{m}$ . Fig. 15. SEM view of "ventral" side of the bristle. Note the hooked foot. Scale bar = 2  $\mu\text{m}$ .





Figs 16–21. Images of *Mallomonas neoampla* sp. nov. (Figs 16–17), *M. multisetigera* (Figs 18–19) and *M. ampla* (Figs 20–21). Fig. 16. SEM, fixed sample, natural population, Dzua River. Scale bar = 1  $\mu$ m. Fig. 17. TEM, fixed sample, natural population, Dzua River. Scale bar = 2  $\mu$ m. Fig. 18. SEM, *M. multisetigera*, Vietnam, unnamed pond. Scale bar = 1  $\mu$ m. Fig. 19. SEM, *M. multisetigera*, Vietnam, unnamed pond. Scale bar = 0.5  $\mu$ m. Figs 20–21. SEM images of body scales depicting the V-rib with arms extending to near the front of the dome, laterally expanded and recessed dome, and distribution of papillae and base plate pores. Note the multiple rows of small-diameter base plate pores on the posterior flange. Scale bars = 2  $\mu$ m. Figs 20–21 are published with permission from Nova Hedwigia.

recurved foot found on *M. ampla* probably aids attachment of the bristle to the shallow and recessed dome. Further, as is the case for both modern species, the broad and expanded dome of *M. ampla* probably secured multiple numbers of bristles.

To date, *M. neoampla* has been reported from three localities, all in southeast Asia. As part of this study, it was found in a river and a small pool in the same province. In addition, a scale believed to belong to this species was also reported from Singapore (Fig. 9, Neustupa & Řezáčová 2007). Collectively, these sites are slightly acidic with a range in pH from 5.4 to 6.8, relatively low in dissolved salts with specific conductivity ranging from 54 to 246  $\mu$ S  $\text{cm}^{-1}$ , and the specimens were collected under very warm conditions with water temperatures above 30°C. Interestingly, even though the fossil

*M. ampla* was described from the lower Arctic, it lived under greenhouse conditions where ice in the northern hemisphere was lacking, winter temperatures were above freezing, and the mean annual temperature was significantly warmer than today (Zachos et al. 2008). In fact, the mean annual temperature of the Giraffe locality is estimated to be 17°C warmer than today (Wolfe et al. submitted). Thus, *M. ampla* also likely grew best under warm conditions.

In conclusion, *M. neoampla* shares features with both *M. multisetigera* and *M. ampla*, and the three species are undoubtedly closely related. Based on scale size, the degree to which the dome is recessed, and the structure of the bristles, *M. neoampla* clearly represents the closest extant congener of the Eocene fossil species *M. ampla*.

### Acknowledgements

This work was funded, in part, with support to E.S.G. by RFBR project 15-04-04181 a, and to PAS from the U.S. National Science Foundation (DEB-0716606 and DEB-1144098). The authors are grateful to the staff of the Russian-Vietnam Tropical Centre and the staff of the Centre of Electron Microscopy of the Papanin's Institute for Biology of Inland Waters, RAS, for technical assistance. We also thank two anonymous reviewers for helpful comments.

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Manuscript submitted August 10, 2016; accepted September 24, 2016.