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Kulikovskiyia gen. nov. (Bacillariophyceae) from the lateritic rock pools of the Western Ghats, India and from Hainan Province, China

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ABSTRACT

A new triundulate naviculoid diatom genus is described from the Western Ghats of Peninsular India and Hainan Province, China. The new taxon, *Kulikvoskiyia* gen. nov. has robust conical spines along its margin and at the apices and the external valve face has longitudinally-oriented siliceous slat system extending the length of the valve. The external distal raphe ends bifurcate and terminates on the valve face. There appear to be superficial similarities between this Asian genus and species and *Playaensis*, a genus comprised of two species found only in the western USA. The systematic position of *Kulikoskiyia* is discussed, and other than noting its similarities to other biraphid naviculoid diatoms due to symmetry features and the position of the raphe, we are uncertain about its systematic placement at finer levels of classification.

Key words: ephemeral pools, freshwater, *Kulikvoskiyia triundulata* gen. et sp. nov., naviculoid diatom, river, taxonomy.

INTRODUCTION

In the last two decades, there have been significant changes in the recognition of genera amongst the naviculoid diatoms, those diatoms with a raphe placed at or near the median line and with valves symmetrical about both the apical and transapical axes. These changes in recognition include resurrection of previously described genera, review and analysis of previously-described species leading to the description of new genera and discovery of new taxa. Examples of discovery of new genera include *Playaensis* Spaulding & Kociolek from the southwest of the United States (Spaulding *et al.* 2002), many genera from Lake Baikal (Kulikovskiy *et al.* 2012).

Review and documentation of freshwater diatoms from Asia is receiving renewed attention. The basis of these studies, like many parts of the world, begins with Ehrenberg's (1854) *magnum opus* Mikrogeologie, and there have been some prolific workers in the region previously, for instance B.V. Skvortzow and H.P. Gandhi. Gololobova (2012) presents a vivid account of Skvortzow's work mainly from China, Japan and Mongolia and Karthick (2009) documented the new taxa (around 300) described by Gandhi across the Western India. For about 40 years (1970 to 2010) work in the region was modest (some exceptions include Zhu & Chen's 2000 work on the diatoms from Tibet). More recently, there have been the description of new genera of centric (*Edtheriotia* Kociolek, Q.You, Stepanek, R.L.Lowe & Q-X.Wang in Kociolek *et al.* 2016b), araphid (*Tibetiella* Y.L.Li, D.M.Williams & Metzeltin in Li *et al.* 2010) and raphid diatoms. Expeditions in India and across China have yielded many new taxa already (Li *et al.* 2010, Karthick *et al.* 2011, 2015, 2017, Gong & Li 2012, Karthick & Kociolek 2012a, b, Hu *et al.* 2013, Alakananda *et al.* 2015, Liu *et al.* 2014, Kociolek *et al.* 2016a, Zhang e*t al.* 2016, You *et al.* 2017, Yu *et al.* 2017, Cheng *et al.* 2018, Jiang *et al.* 2018) and documentation of taxa from those studies will continue for some time. In this paper we report a new genus of naviculoid diatom from collections originated from India and China. We present observations made with light and scanning electron microscopy to formally describe a new genus and species and compare it with other naviculoid diatoms.

MATERIAL AND METHODS

The samples used for the description of the new genus and species originates from India. The diatoms attached to the *Eriocaulon* sp. were collected from the trench along the side of the road on the lateritic rocky plateau near Padel village (16.49511111N, 73.40943889E) in Deogad Taluka, in the Sindhudurg District of Maharashtra, India. The plateaus are made up of ferricretes of laterite and characteristic of the northern Western Ghats (Watve 2013) and the geology of this region is volcanic in origin (Valdiya 2015). The sample originates from the trench made along the sides of the road to avoid the plateau water entering into the road. The trench is around 1m wide and 1 m deep (see Fig. S1 in Supporting Information) with supporting luxuriant amount of submerged and emergent aquatic vegetation. The sample used in the present study originates from the squeezing of emergent plant (*Eriocaulon* sp.) growing inside the trench (see Fig. S2 in Supporting Information). The resulting diatom sample was stored in Whirl-Pak® (Nasco, USA) sampling bag. Water quality variable such as pH, water temperature, electrical conductivity were measured using HQ40D portable multi-parameter meter (Hach, Loveland, Colorado, USA) . Nitrate and phosphate were measured using a portable spectrophotometer DR1900 (Hach, Loveland, Colorado, USA) using Hach made chemicals (NitraVer® 3 Nitrate and NitraVer® 6; PhosVer® 3 Phosphate). Geographical coordinates and elevation readings were taken using eTrex® 30x (Garmin®, Kansas, US) instrument.

Specimens in the Chinese material are very rare and only one external valve was observed. Here we are presenting the ultrastructural details of the taxon from China merely to confirm the wide distribution covering Hainan Province of China to the Western India. In China, a sample of epiphytic algae was collected from near the bottom of an unnamed river in Hainan Province (Figs S3, S4 in the Supporting Information) (18.76050000N, 109.64361111E; elevation 455 meter a m.s.l.).

In the lab the samples were cleaned by boiling in nitric acid, then washed and centrifuged by R-8C BL (Remi, Mumbai, India) centrifuge machine for five times with distilled water at 1730 g (relative centrifugal force) to remove all organic matter until neutral. Cleaned sample was air-dried on to cover slips and then mounted on permanent slides with Naphrax® (Brunel Microscopes Ltd, Wiltshire, UK) for light microscopy (LM). For LM, slides were examined with BX53 and BX51 (Olympus, Tokyo, Japan) light microscope equipped with differential interference contrast (DIC). The images were recorded using Olympus cellSens standard 1.16 imaging software.

For scanning electron microscopy (SEM), cleaned material was air-dried on to a small cover slip and placed on aluminium stubs with carbon tape and coated with gold using Emitech K575X (Quorum Technologies, Lewes, UK) sputter coater. Our initial observations resulted in only documenting the external valve views of the taxon of interest and it looks like that the frustule is firmly attached and we never observed the internal valves. Hence, the cells were isolated using a glass pasteur pipette (Andersen 2005), under the CKX 53 (Olympus, Tokyo, Japan) inverted microscope at 400 magnification and stored in a 1.5 mL microcentrifuge tube with little amount of distilled water. The entire content of the microcentrifuge tube was sonicated bydigital ultrasonic water bath (Wensar®, Chennai, India) in 25 kilohertz (kHz) wave frequency for 2, 30, 60, 100 and 120 minutes and the wave output power was 400 Watt (W). A part of the resulting suspension was used for preparation of stubs. Observations were made on a EVO® MA 15 (Zeiss, Oberkochen, Germany) SEM at an operating voltage (OV) of 20 kV and working distance (WD) of 6.0–7.0 mm and WD of 10.4 mm. SEM observations were made on Chinese specimens on a S-4800 (Hitachi, Tokyo, Japan) field emission electron microscope with OV 15kv.

Diatom images were compiled with GIMP 2.10.2 (available from: http://www.gimp.org) and Inkscape 0.92.3 (available from: http://www.inkscape.org) software. Terminology of the diatom valves follows Ross *et al.* (1979) and Spaulding *et al.* (2002).

RESULTS

Order: Naviculales Bessey

Family: Naviculaceae incertae sedis

Kulikovskiyia S.Roy, Kociolek, Y.Liu et B.Karthick gen. nov.

Description. Cells bilaterally symmetrical. Valves are linear-lanceolate and tri-undulate. Valve apices cuneate. Spines and silica slats present over the external valve surface. Striae parallel or radiating from center and chambered. Central area almost absent to rounded. Raphe straight. Longitudinal lines can be visible in LM due to the presence of internal axial plate. External distal raphe ends are bifurcate and terminate on the valve face, proximal raphe ends slightly dilated towards the same side. Internal distal raphe ends meet with helictoglossae and proximal raphe ends strongly deflected to the same side. Chloroplast two in number and triundulated in shape.

Type species. *Kulikovskiyia triundulata* (see below).

Etymology. The genus is dedicated to our friend and colleague Dr. Maxim Kulikovskiy of Moscow, Russia, in recognition of his important contributions to the study of diatoms in general, and of the diatoms of Asia in particular.

Distribution. Lateritic rocky pools in the Western Ghats, India and in Hainan Province, China.

Kulikovskiyia triundulata S.Roy, Y.Liu, Kociolek, Lowe et B.Karthick sp. nov. (Figs 1–8)

Holotype. Circled specimen on microscope slide marked 25-031 from material #1216, illustrated in Fig. 2b,c. Deposited at the Agharkar Research Institute Herbarium (AHMA), Diatom Section, India.

Type locality. INDIA. Lateritic Rocky Plateau, near Padel village in Deogad Taluka in Sindhudurg district of Maharashtra state; 16.49511111N, 73.40943889E; elevation 51 meter a m.s.l.

Etymology. The specific epithet refers to the triundulate nature of valve margins.

Distribution. Other than the type locality this taxon was also found from two other lateritic rocky pools in the Western Ghats, India i.e. material AHMA #2123 from Nayne plateau in Mandangad taluka of Ratnagiri district of Maharashtra, India and material AHMA #2189 from Kivona plateau in Sanguem taluka of South Goa district of Goa, India. Currently known only from the rocky pools of the lateritic outcrops of the northern region of the Western Ghats.

Ecology. The epiphytic sample was collected from submerged *Eriocaulon* sp. growing inside the trench of the lateritic rocky plateau, near Padel village of northern Western Ghats where the water quality recorded for the sample was pH = 9.92, electrical conductivity (EC) = 50.40 µS/cm, water temperature = 27.5 °C, phosphate = 0.33 mg/L and nitrate = 0.8 mg/L. This new species is rare in the type material, co-occurred with *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg and two unnamed species of *Stauroneis* Ehrenberg, *Brachysira procera* Lange-Bertalot & Gerd Moser, *Frustulia crassinervia* (Brébisson ex W.Smith) Lange-

Bertalot & Krammer, *Gomphonema graciledictum* E.Reichardt, *Navicula notha* J.H.Wallace, two unnamed species of *Pinnularia* Ehrenberg, *Sellaphora pulchra* Enache & Potapova, and *Eunotia exigua* (Bréb. in Kütz.) Rabenh. Water quality parameters of material #2123 shows pH = 6.19, dissolved oxygen (DO) = 6.97, EC = 78.5 μS/cm, water temperature = 27.3°C and material #2189 shows pH = 5.72, DO = 2.18, EC = 99.2 μS/cm, water temperature = 30.9 °C. So water quality parameters showed slightly acidic to alkaline water with low conductivity, and oligotrophic condition. This taxon can tolerate low to moderate levels of DO.

LM description (Figs 1, 2). Cells solitary, isopolar, biraphid and exhibit bilateral plane of symmetry. Valve view of cells showing two triundulate shaped chloroplasts (Fig. 1a,c). Two big oil droplets are visible in both valve and girdle views (Fig. 1c,d). Girdle views show frustules to be elongated and rectangular (Fig. 1b,d). In valve view frustules are linear-lanceolate to lanceolate. Valves with tri-undulation and apices cuneate or acute. Valve length 19.69–22.77 µm, valve width 5.08–6.36 µm. Raphe straight and prominent with straight proximal ends, distal raphe ends becoming filiform. Central undulation slightly wider than other two distal undulations. Axial area narrow and straight, central area almost absent or slightly circular. Striae parallel throughout but becoming slightly radiate towards the apices from where the distal undulations start, 23–24 in 10 µm. Internal axial plate giving the appearance of a longitudinal line in different focal plane (Fig. 2f,g).

SEM description (Figs 3–7). In external view (Figs 3–5), the cells show a prominent raphe system along with oppositely orientated conical spines (arrowheads) (Fig. 3a,b). Only the raphe and central area are devoid of the siliceous slat system that runs across the valve along the apical axis. Spines are made up by these siliceous slat system and around the periphery of the valves (Fig. 3a,b). In girdle view marginal spines are evident with robust apical spines which are longer than marginal ones (Fig. 3c). The raphe branches have slightly expanded or dilated central endings which bend in the same direction (Fig. 3a,b; Fig. 4a). Distal raphe ends slightly undulate to one side of the valve and bifurcated to form “Y”-shaped ends that terminate on the valve face (Fig. 4b,c). One branch of the distal raphe end fork is slightly more elongate than the other (Fig. 4b,c). In detailed girdle views (Fig. 4d–f), marginal spines evident (Fig. 4d–f) and asymmetrical silica ornamentation are noticed beneath the marginal spines all over the girdle (Fig. 4d–f). Apical spines has some kind of small side branch (arrowhead) (Fig. 3c; Fig. 4e,f). Only one robust terminal spine is present at the valve terminus, just in front of each “Y” -shaped distal raphe end (Fig. 4e,f; Fig. 5a). Marginal spines are more hooked towards the apices (Fig. 4e,f; Fig. 5b). Marginal spines are absent on the cuneate valve apices (Fig. 4b,c; Fig. 5a). This siliceous slats are more or less parallel to each other and comprised of different lengths (Fig.). Striae are covered by a thin siliceous covering (Fig. 3a,b; Fig. 5c). Broken valves show the raphe fissure with simple slit (arrowhead in Fig. 5d,e).

Internally (Figs 6, 7), the raphe is straight (Fig. 6a,b). The central raphe ends are strongly deflected in the same direction (Fig. 6c). Small helictoglossae are present on the distal raphe ends (Fig. 6d,e). An axial plate dominates the entire internal valve (Fig. 6) and this axial plate allows only a small area of the striae to be evident (Fig. 6a–c). The inner mantle area is devoid of any striation or slats (Fig. 6b–e). Broken chambered striae (arrowheads in Fig. 7a,b) and simple raphe slit structure internally are shown in Fig. 7a,b (arrow). Apical portion of a broken valve shows open chambers (arrowheads) (Fig. 7c–f).

Specimens in the Chinese material are very rare and only one external valve was observed. Here we are presenting the ultrastructural details of the taxon from China merely to confirm the wide distribution covering Hainan Province of China to the Western India.

Kulikovskiyia sp. (Fig. 8)

In external valve view, frustule exhibits solitary, isopolar, biraphid and bilaterally symmetrical nature. Valve is linear-lanceolate and tri-undulate in shape, apices cuneate and exhibits straight and prominent raphe system (Fig. 8a). Valve length 32.07 µm, valve width 8.70 µm. Axial area slightly broad and straight and forming ovoid central area (Fig. 8a,b). Striae almost parallel at the poles but radiating from center. Striae 16 in 10 µm. Central area with peculiar proximal raphe ends that dilated on the same direction and unidirectionally hooked (Fig. 8b). Apical portion of the valve shows conical spines along with the marginal spines (Fig. 8c,d). Fig. 8e disclosing the robustness of conical spine. Marginal spines present in the centre (Fig. 8f) are smaller than those situated near the apices (Fig. 8c).

Since we do not have enough LM and SEM observations, we are not pretending to determine the taxonomic position of this taxon. But the different striation pattern and striation density shows that it should be a another species. However, it is too early to come to conclusion on the taxonomic position of this taxon with just one valve.

Ecology. The sample was collected from a epiphytic algae from near the bottom of an unnamed river in Hainan Province, China. The water quality recorded for the sampling site was pH = 7.62, electrical conductivity (EC) = 60 µS/cm, water temperature = 26.2 °C. This species is very rare in this material. The sample only contains few species, including a putative new genus co-occurred with some 2-3 species of *Pinnularia* Ehrenberg and 2 species of *Gomphonema* Ehrenberg.

Original material, cleaned sample and permanent holotypic slides were deposited at diatom collection at the Agharkar Research Institute, Pune, India (AHMA). Cleaned material and slides from China are deposited at the Hydrobiological lab in Harbin Normal University (HNU) and Kociolek Collection at the University of Colorado, Boulder (COLO).

DISCUSSION

The remarkable morphology of *Kulikovskiyia* appears most similar to the genus *Playaensis* Spaulding & Kociolek in Spaulding *et al.* (2002), described from shallow playa lakes in the southwestern region of the United States, and later reported from other parts of the American West (Bahls 2014). The two species of *Playaensis*, like *Kulikovskiyia*, have marginal spines and the internal proximal raphe ends that are curved in the same direction. In *P. furtiva* Spaulding & Kociolek in Spaulding *et al.* (2002) showed that there are well-developed slats of silica present near the apices and along the raphe sternum, resembling the longitudinal slats in *K. trindulata*. Both species of *Playaensis* are triundulate, the same shape as the only described species of *Kulikovskiyia*. And the shallow environments in which *Playaensis* is found are somewhat similar to the shallow habitats in which our new genus and species were discovered.

There are, however, many differences between the two genera. *Kulikovskiyia* has an internal plate of silica extending from the raphe sternum towards the margin of the valve forming a chamber; no plate or resulting chamber is present in either *Playaensis* species. In

Kulikovskiyia the bifurcate, external distal raphe ends terminate on the valve face while in *Playaensis* the distal raphe ends are not bifurcated and extend onto the mantle. The small, numerous siliceous nodules covering the valve exterior of *Playaensis* species are lacking in *Kulikovskiyia*. The spines along the margin and at the apices of *K. triundulata* are distinctly conical, while the spines of the two *Playaensis* species are spathulate in shape. Striae are more or less parallel in *K. triundulata*, while in *Playaensis* the species have characteristically strongly radiate striae around the center of the valve. Finally, the longitudinal slats of *Kulikovskiyia* run the entire length of the valve, and across the entire valve face, while they are only found along the raphe sternum and at the apices of *P. furtiva*; such longitudinally-oriented slats are lacking in *P. circumfimbriata* Spaulding & Kociolek in Spaulding *et al.* (2002), the other species of *Playaensis*.

Possession of an axial plate in *Kulikovskiyia* might suggest some relationship with members of the Pinnulariaceae, especially some species of the genus *Caloneis* Cleve (Krammer & Lange-Bertalot 1986, Mann 2001) which, like *K. triundulata*, are also triundulate in outline. Differences in the structure of the striae (multiseriate in the Pinnulariaceae, Edgar 1980; Krammer 2000; uniseriate in *Kulikovskiyia*) preclude placement of *K. triundulata* in *Caloneis*. Additional work will be necessary to determine if *Kulikovskiyia* might be placed in the Pinnulariaceae. Comparison of morphological characteristics of *Kulikovskiyia* gen. nov., *Playaensis* and *Caloneis* is presented in the Table 1. Presence of similar internal laminae in other groups, such as *Gomphoneis* Cleve (Kociolek & Stoermer 1988), but assigning this new genus within the Gomphonemataceae does not seem warranted based on the morphological data presented herein. Likewise, the presence of siliceous slats on the valve face of

Kulikovskiyia appear similar to those found in the genera *Craticula* Grunow (Mann & Stickle 1991, Lange-Bertalot 2001) and *Haslea* Simonsen (Round *et al.* 1990, Witkowski *et al.* 2000) but there is little additional morphological evidence to suggest a relationship between *Kulikovskiyia* and these genera.

This newly-described monotypic genus is currently known only from Asia, extending from peninsular India to Hainan Province, China. In comparison to other continents, Asia has nearly twice the number of endemic genera of freshwater diatoms (fossil and extant) than all other continents combined (Kociolek, Accepted). Given that so little of the Asian continent has been studied in detail with regard to freshwater diatom biodiversity, and that there are important biodiversity hotspots across the continent (Myers *et al.* 2000, Noss *et al.* 2015), it seems likely that we would expect additional reports of new genera and species from the Asia.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figs S1–S4. Photographs of sampling sites including the holotypic locality

TABLE 1. Comparison of morphological characteristics of *Kulikovskiyia* gen. nov., *Playaensis* and *Caloneis*

Characters	*Playaensis*	*Caloneis*	*Kulikovskiyia*
Valve shape	Triundulate	Sometimes with undulate margins	Triundulate
Silica ornamentation in outer valve	Present	Absent	Present
Valve external siliceous nodules	Present	Absent	Present
Marginal spines	Present	Absent	Present
Terminal spines	Absent	Absent	Present

Shape of spines	Spathulate	Absent	Conical
Striae	Uniseriate, punctate and strongly radiate around the center of the valve	Multiseriate, chambered and parallel to radiate	Uniseriate, chambered and more or less parallel
External distal raphe ends	Not bifurcated and extend onto the mantle	Not bifurcated, curved and extend onto the mantle	Bifurcated and terminate on the valve face
Internal proximal raphe ends	Curved in the same direction	?	Curved in the same direction
Internal axial plate	Absent	Present	Present
Source	Spaulding *et al.* (2002)	Spaulding & Edlund (2009)	Present study

LEGEND TO THE FIGURES

Figs S1–S4: Photographs of sampling sites including the holotypic locality. S1. The photograph of the holotypic locality showing the trench made in the lateritic plateau near Padel village of the Western Ghats, India. S2. Photograph showing the growth of emergent plant (*Eriocaulon* sp.) growing inside the trench and the sample used in the study originates from the squeezing of *Eriocaulon* sp. S3,S4. Photographs showing the sampling site from a river in Hainan Province, China.

Fig. 1. Live cells with chloroplast structure of *Kulikovskiyia triundulata* gen. et sp. nov. (a,b) Girdle view and valve view of the same cell. (c,d) Girdle view and valve view of the same cell. (Scale bar represents 10 μm).

Fig. 2. Light microscopy of *K. triundulata* gen. et sp. nov., from the type population; valve views showing the size diminution series. Holotype depicted in b,c. (b,c) Frustules in valve view with different focal plain showing distinct raphe and central area of both valves. (f,g) Longitudinal lines due to the internal axial plate under different focus. (Scale bar represents 10 μm). Specimens from the type locality, India.

Fig. 3. SEM of *K. triundulata* gen. et sp. nov., external valve views. (a,b) Valve views showing peripheral and apical spines, siliceous slats and tri-undulate valve shape, arrowhead showing opposite orientation of conical spines. (c) External girdle views, apical spines with small side branch (arrowhead). (All scale bars represents 5 μm). Specimens from the type locality, India.

Fig. 4. *K. triundulata* gen. et sp. nov., SEM external views. (a) Middle portion of the valve showing proximal raphe ends. (b,c) Details of marginal and apical poles of a valve and “Y” -shaped distal raphe end. (d) Middle part of the girdle view showing marginal spines and asymmetrical silica ornamentation. (e,f) Apical part of the girdle view showing apical spines with small side branch (arrowhead) and asymmetrical silica ornamentation. (All scale bars represents 1 μm). Specimens from the type locality, India.

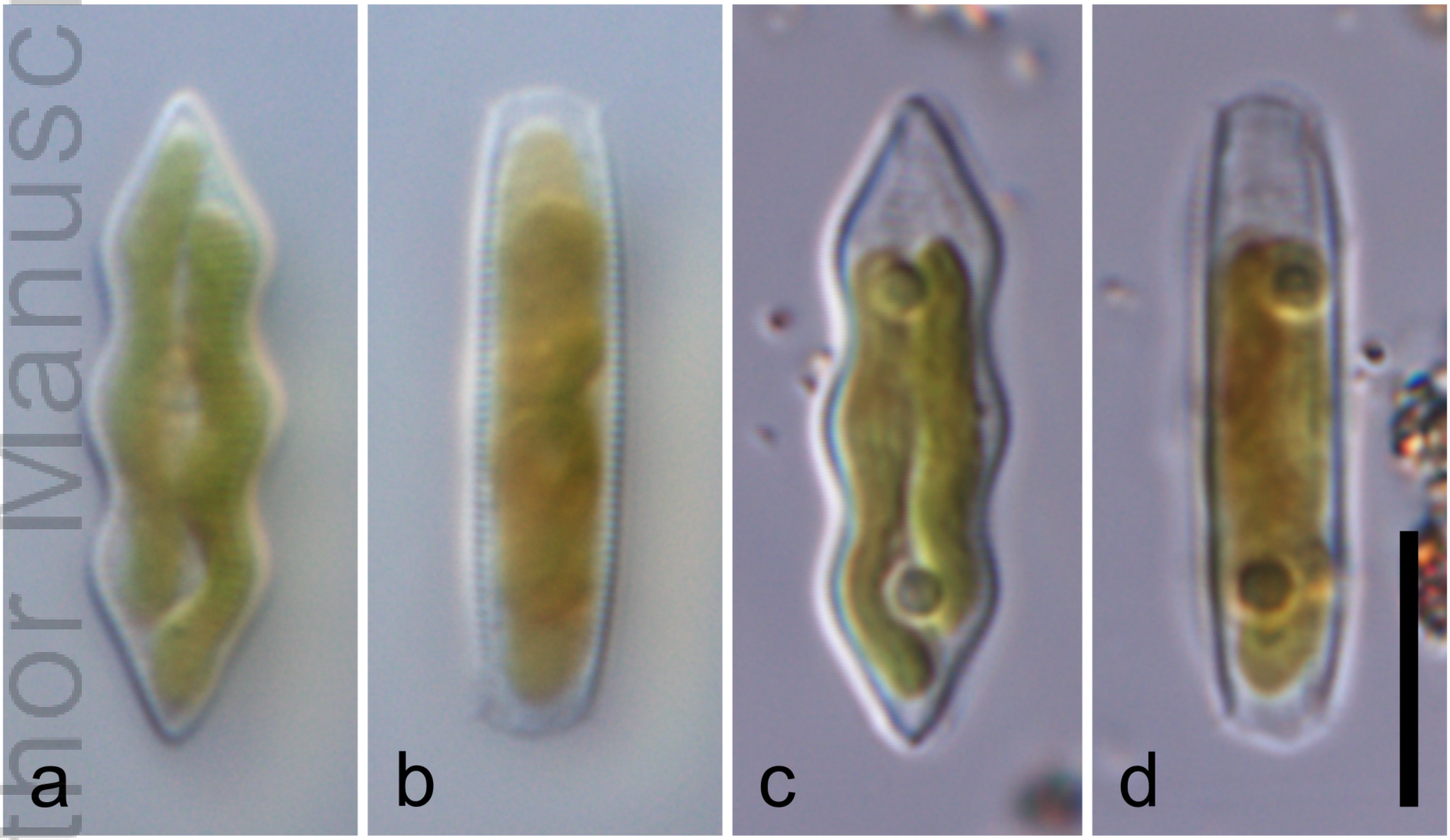
Fig. 5. SEM images of *K. triundulata* gen. et sp. nov. (a) Robust terminal spine with marginal spines. (b) Magnified view of marginal spines. (c) Parallel siliceous slats are evident with occluded striae. (d,e) External broken valves showing raphe fissure (arrowhead). (All scale bars represents 1 μm). Specimens from the type locality, India.

Fig. 6. *K. triundulata* gen. et sp. nov., SEM internal views. (a,b) Whole valve view, with axial plate evident as well as distinct helictoglossae at the poles. (c) Central area, showing proximal raphe ends strongly deflected in same direction. (d,e) Valve apices, with small, elongated helictoglossa. The axial plate extends at the centre and narrows near the apices, note the siliceous slat system (arrowheads). Scale bars = 5 μm (a,b); 1 μm (c–e). Specimens from the type locality, India.

Fig. 7. *K. triundulata* gen. et sp. nov., SEM internal views of broken valves. (a,b) Showing broken chambered striae (arrowheads) and raphe fissure (arrow). (c) Apical portion showing open chambers (arrowheads) and Internal strut (fibulae-like) structures (arrows). (d) One broken chambered stria (arrowhead) with siliceous slats bearing inner side of valve outer cover. (e) Broken apical part showing chambered stria (arrowhead) beneath the axial plate

and raphe fissure (arrow). (f) Chambered stria (arrowhead), raphe fissure (arrow) and helictoglossa fissure (round pointer). (All scale bars represents 1 µm). Specimens from the type locality, India.

Fig. 8. *Kulikovskiyia* sp. from the Chinese material. SEM of the external part from one single frustule. (a) Whole valve view. (b) Close up view of the valve center with proximal raphe ends. (c,d) Distal raphe endings with apical and marginal spines. (e) Close up view of conical apical spine. (f) Close up view of marginal spines. (Scale bars: a = 10 µm; b = 5 µm; c = 3 µm; d = 2 µm; e,f = 1 µm).



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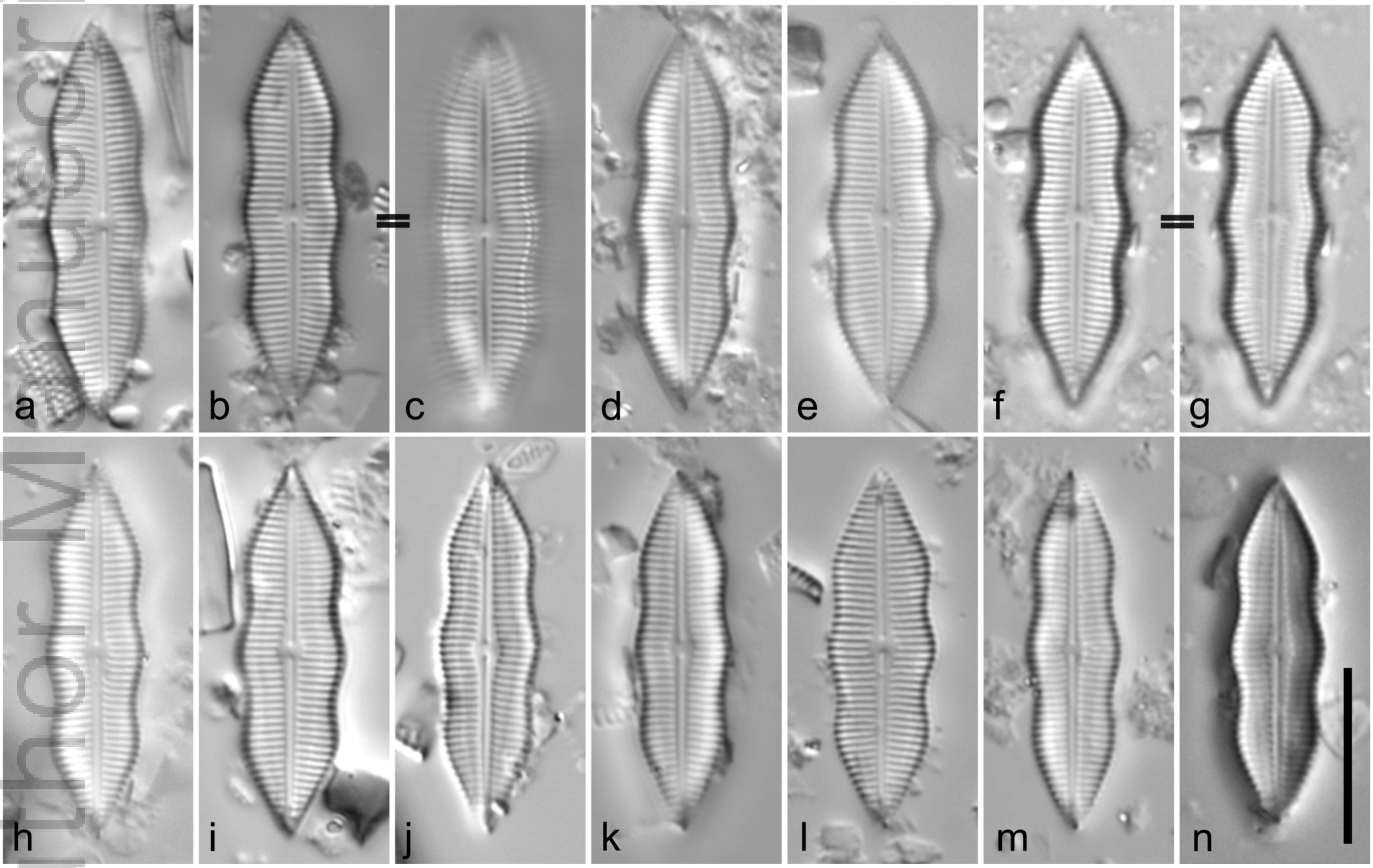

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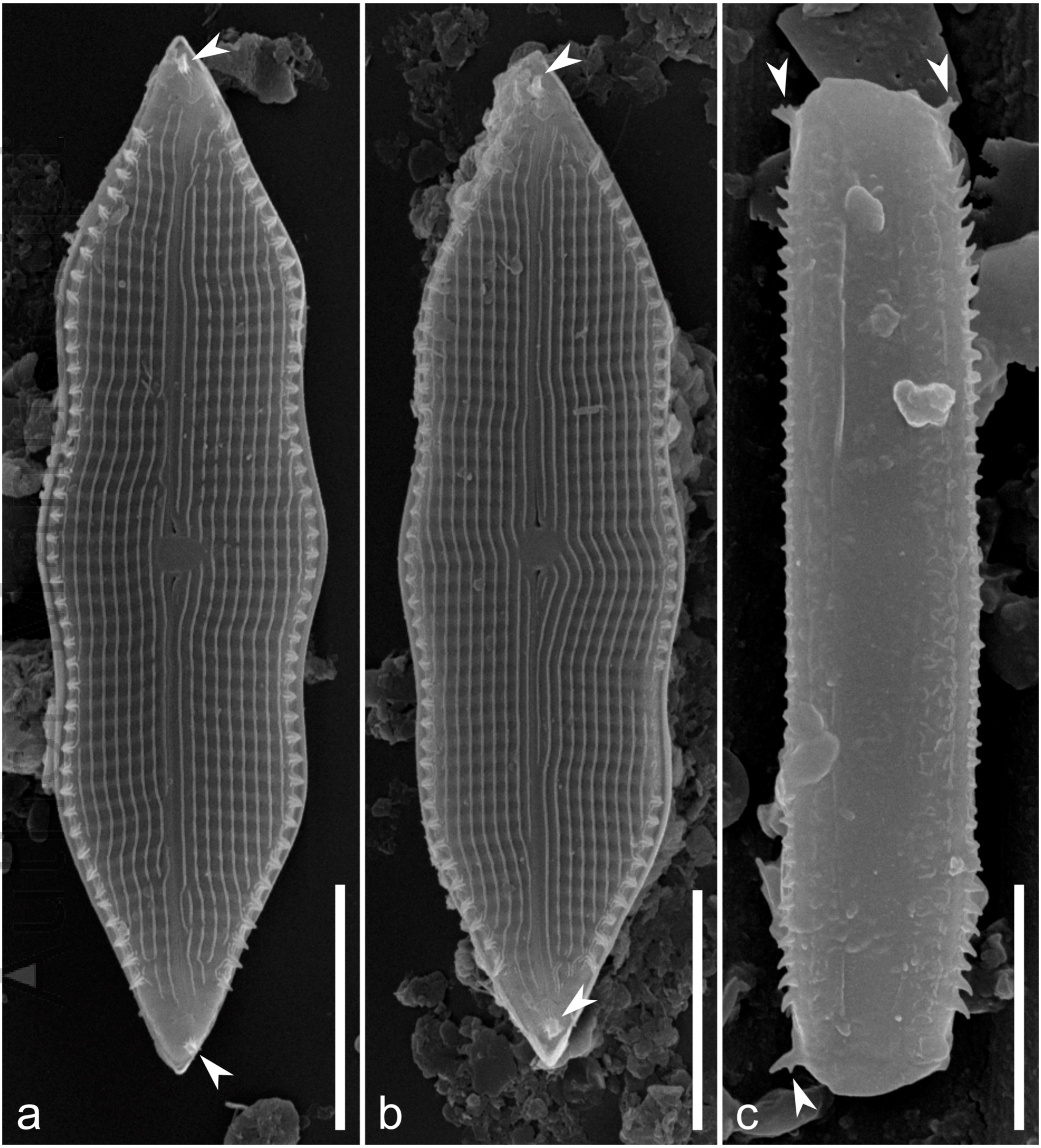

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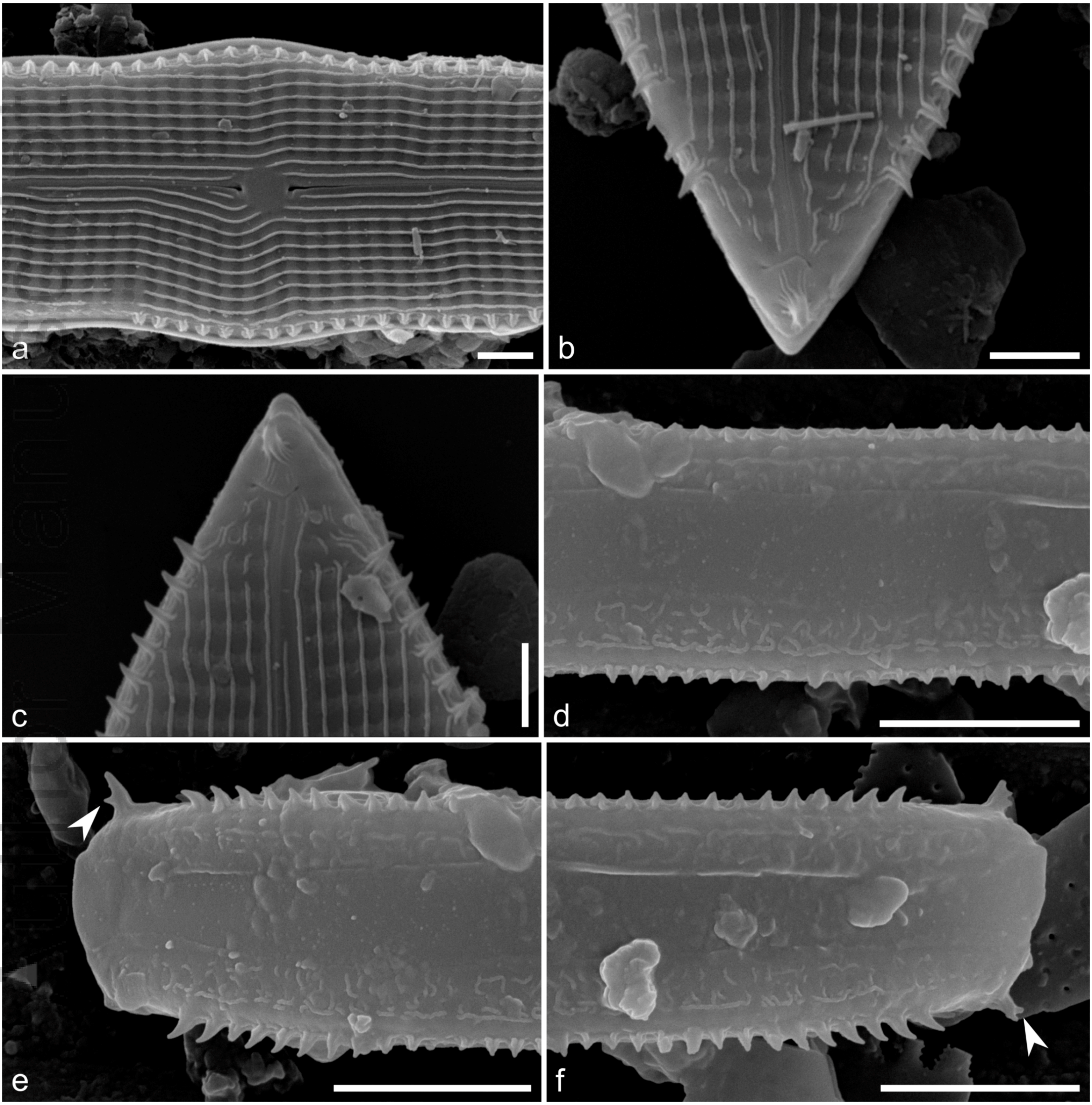

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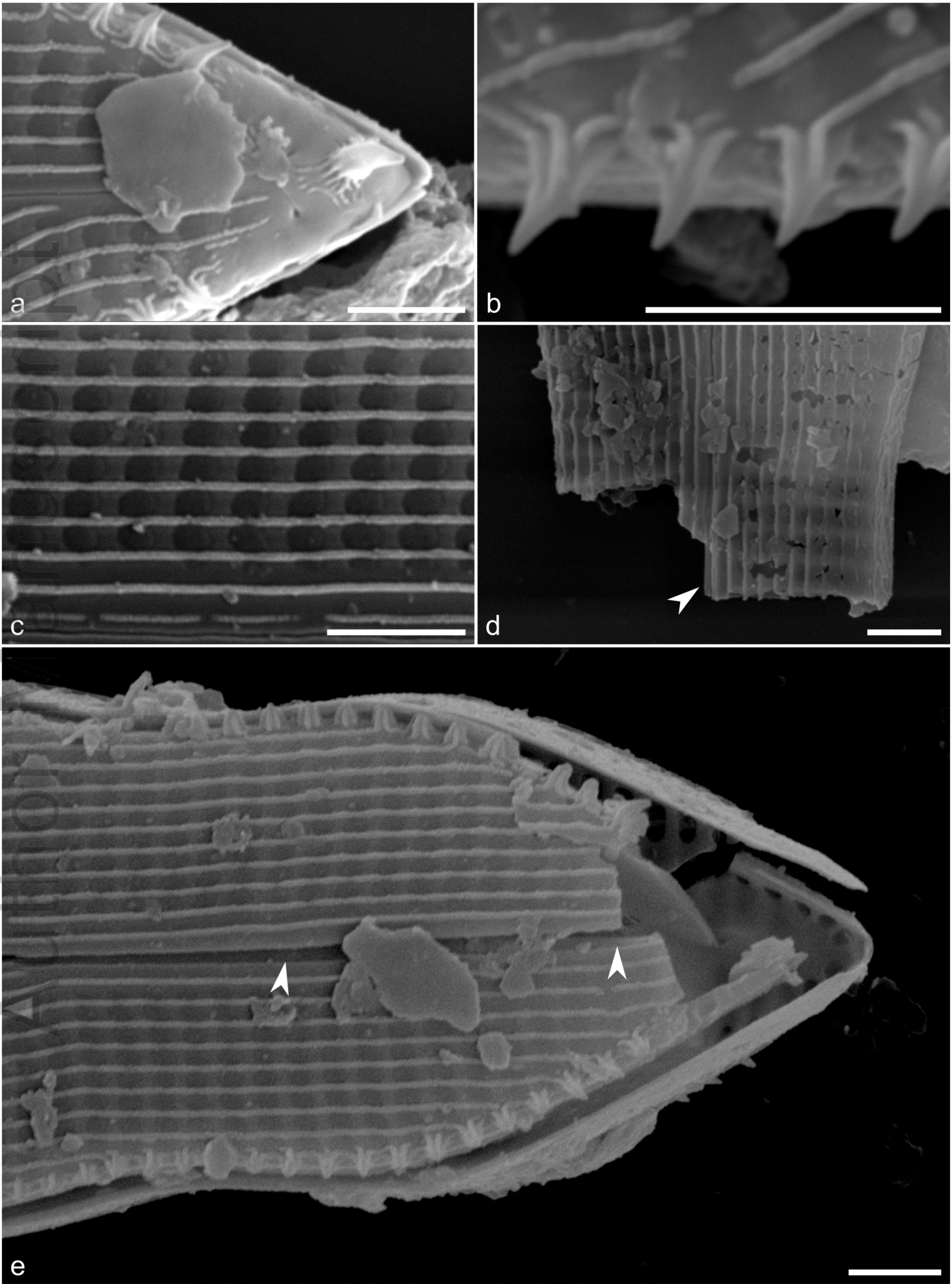

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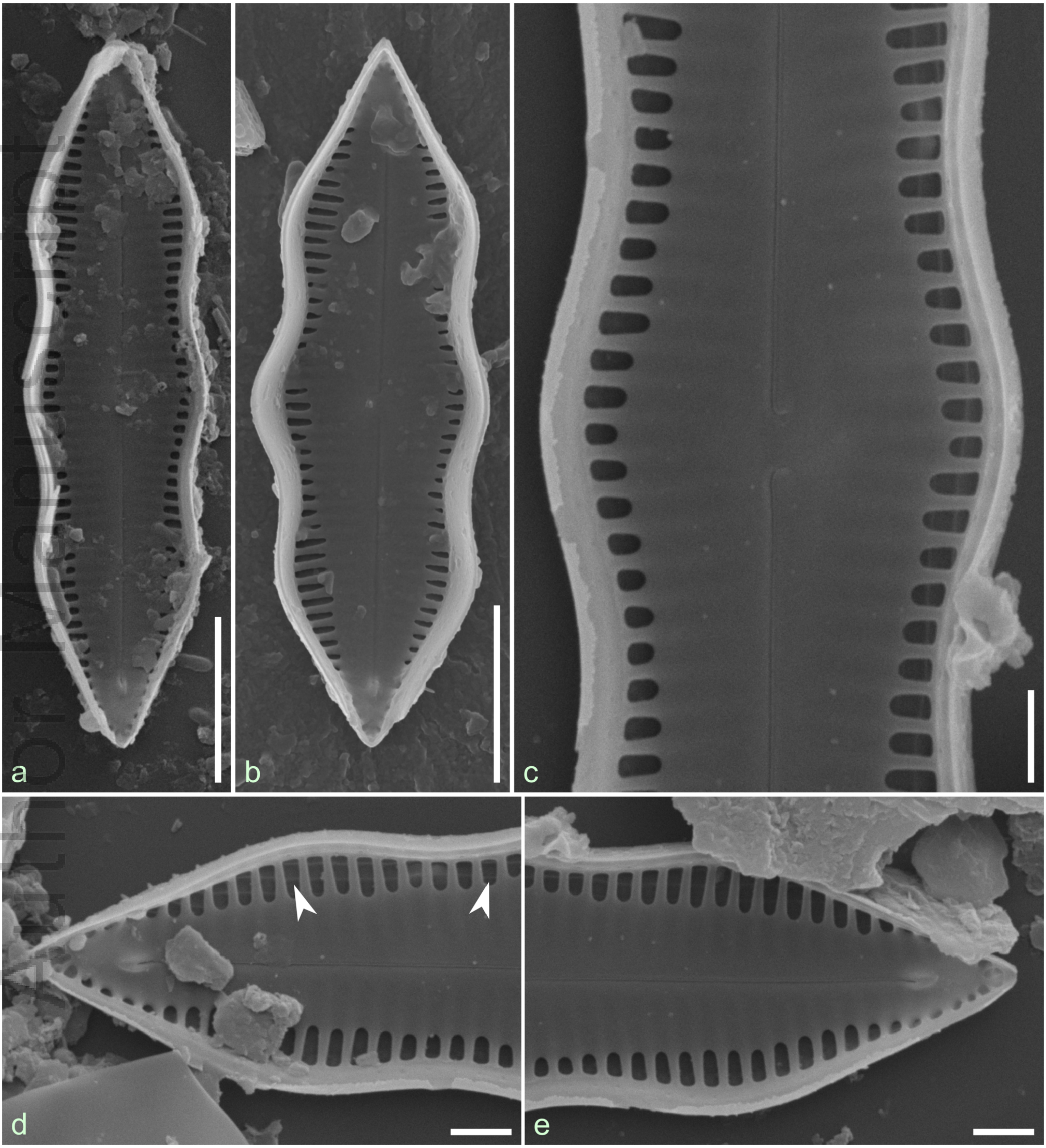

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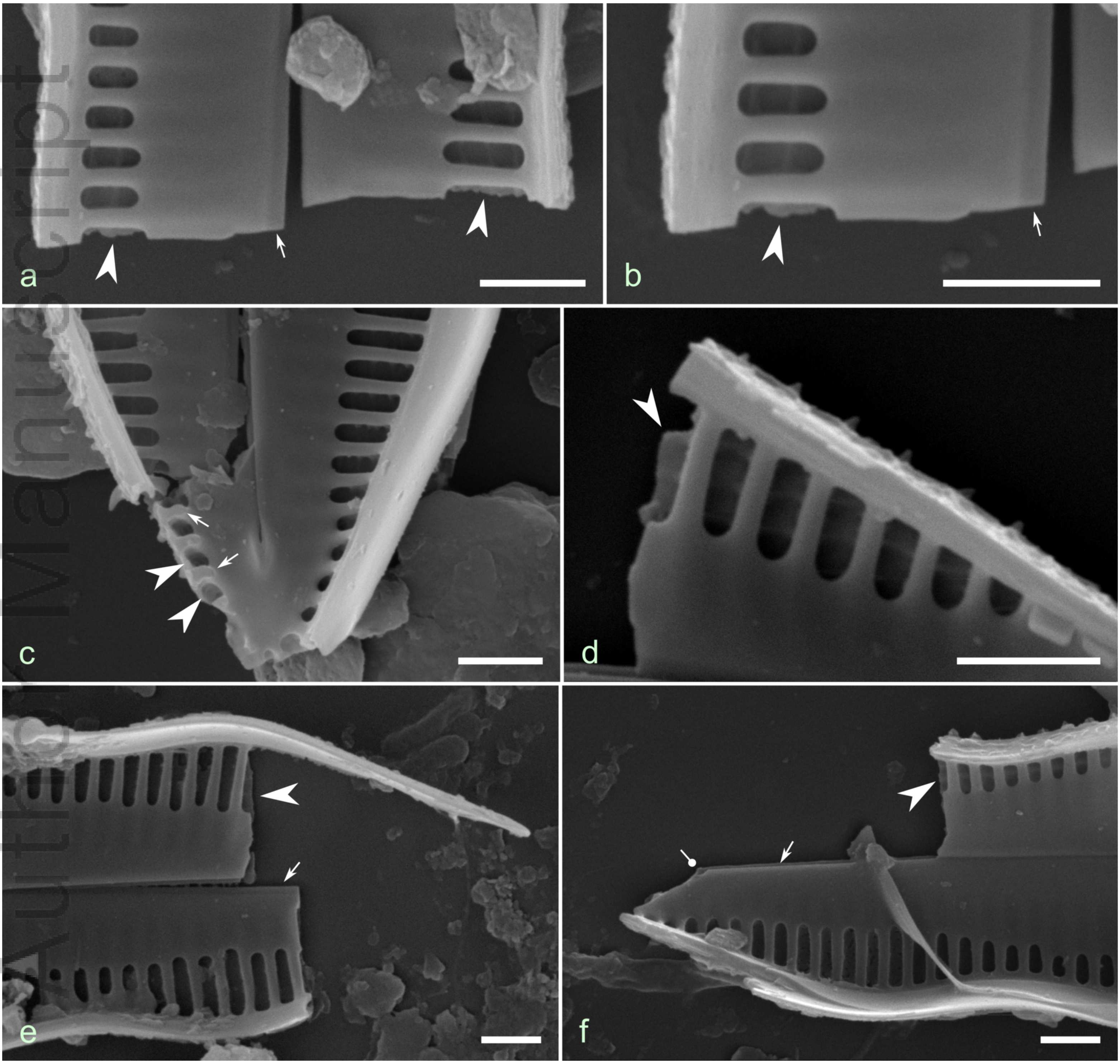

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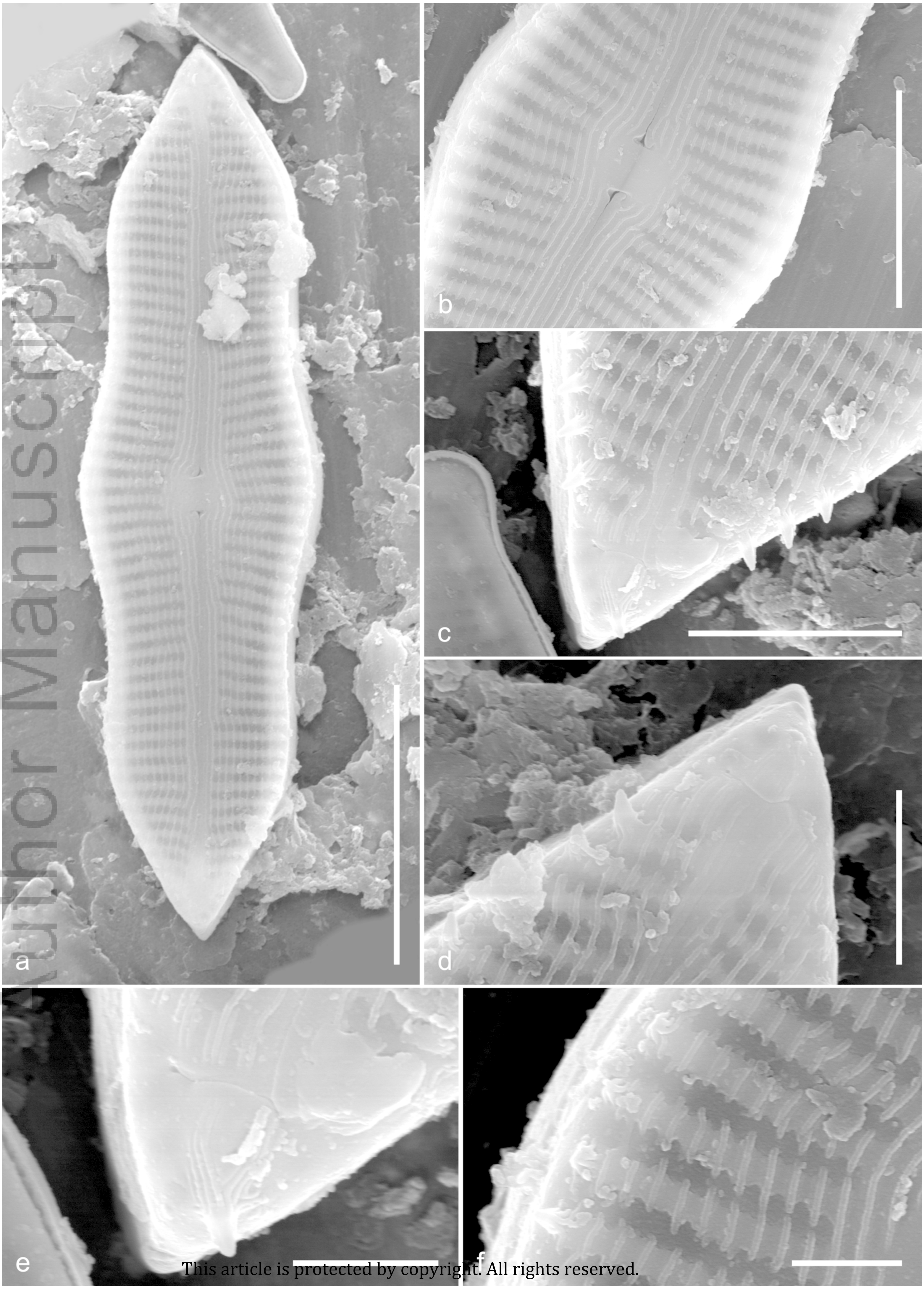
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