Proceedings of 9th Socratic Lectures **2024**

Repository

Scanning electron microscope images of isolates of small cellular particles *Phaeodactylum tricornutum* grown in media supplemented with Guillard's (F/2) marine water enrichment solution, BG11 broth and Lennox LB broth

Bedina Zavec Apolonija[1] , Božič Darja[2,3], Hočevar Matej[4], Iglič Aleš[3,5], Jeran Marko[2,3], Kralj-Iglič Veronika[2,*], Romolo Anna[2]

[1]National Institute of Chemistry, University of Ljubljana, [2]University of Ljubljana, Faculty of Health Sciences, Laboratory of Clinical Biophysics, [3]University of Ljubljana, Faculty of Electrical Engineering, Laboratory of Physics, [4]Institute of Metals and Technology, [5]University of Ljubljana, Faculty of Medicine, Laboratory of Clinical Biophysics, Ljubljana, Slovenia
* Correspondence to Veronika Kralj-Iglič, veronika.kralj-iglic@zf.uni-lj.si

Citation: Bedina Zavec A, Božič D, Iglič A, Jeran M, Kisovec M, Kralj-Iglič V, Romolo A. Scanning electron microscope images of isolates of small cellular particles Phaeodactylum tricornutum grown in media supplemented with Guillard's (F/2) marine water enrichment solution, BG11 broth and Lennox LB broth Proceedings of Socratic Lectures. **2024**, 9, 328-350.
https://doi.org/10.55295/PSL.2024.D13

Publisher's Note: UL ZF stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2024 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC0) license

Abstract: Scanning electron microscope images of small cellular particles isolated from conditioned media of microalgae *Phaeodactylum tricornutun* are presented. Each image is supplemented by description of the preparation of the sample and the data on the imaging technique and equipment.

The data curators of the repository are Veronika Kralj-Iglič and Anna Romolo. More data on experiments with microalgae small cellular particles can be found in (Adamo et al., 2021), (Picciotto et al., 2021) and (Božič et al., 2022).

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 801338 and ARRS projects P1-0391, P2-0232, P3-0388, J2-4447, J2-4427, L3-2621, J3-3066, IO-0006 (A) and National Research, Development and Innovation Office (Hungary), grant number SNN 138407.

Keywords: Extracellular vesicles, Extracellular particles, Nanoalgosomes, Exosomes

References

1. Adamo G, Fierli D, Romancino DP, et al. Nanoalgosomes: Introducing extracellular vesicles produced by microalgae. *J Extracell Vesicles*. 2021;10(6):e12081. doi:10.1002/jev2.12081
2. Picciotto S, Barone ME, Fierli D, et al. Isolation of extracellular vesicles from microalgae: towards the production of sustainable and natural nanocarriers of bioactive compounds. *Biomater Sci*. 2021;9(8):2917-2930. doi:10.1039/d0bm01696a
3. Božič D, Hočevar M, Jeran M et al. Ultrastructure and stability of cellular nanoparticles isolated from *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* conditioned media. *Open Res Europe* 2022, 2:121 (https://doi.org/10.12688/openreseurope.14896.1)

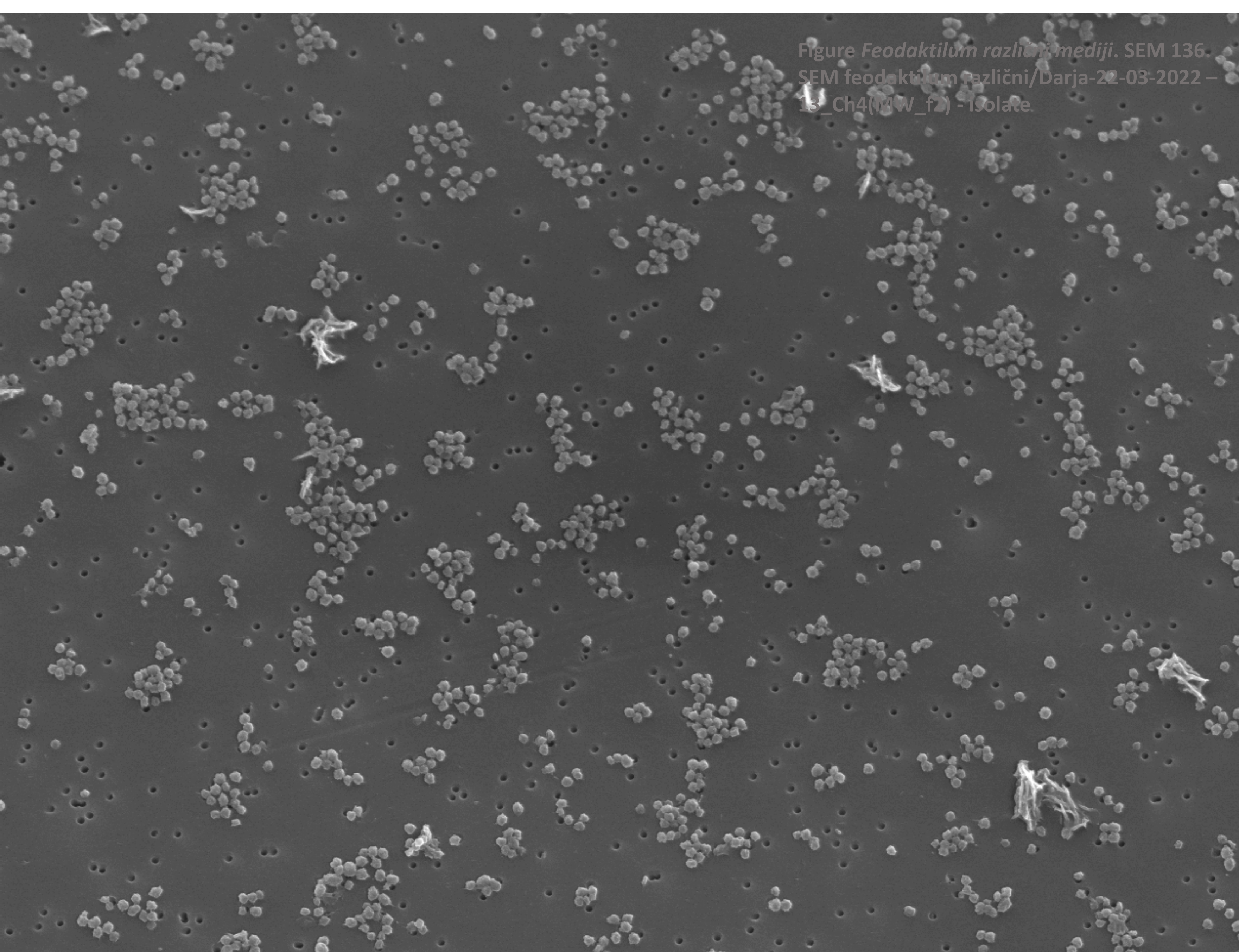

Figure *Phaeodactylum tricornutum* isolate F2 SEM 1.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Guillard's (F/2)** Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)) using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan). From: https://zenodo.org/record/6908895.
Image:10

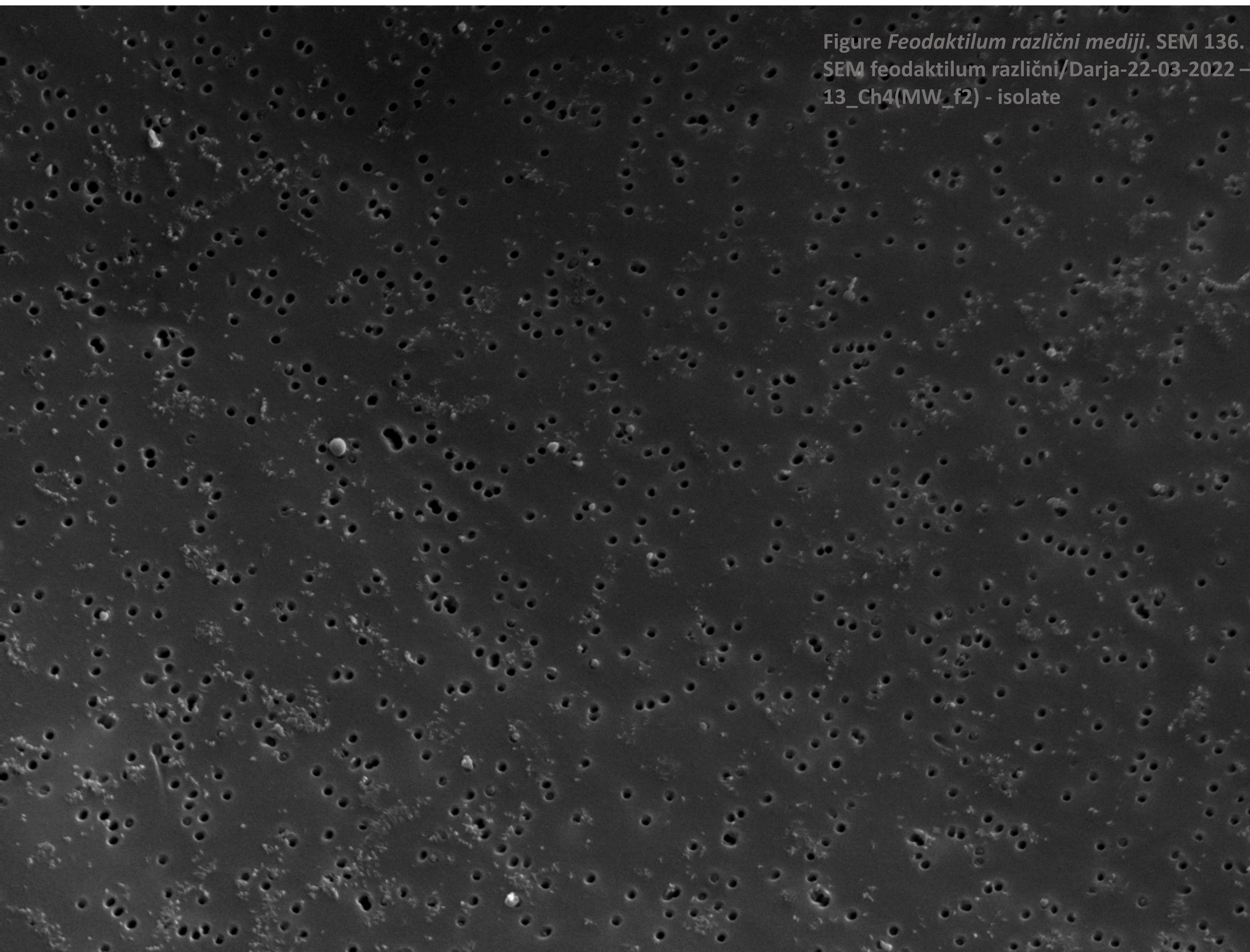

Figure *Phaeodactylum tricornutum* isolate F2 SEM 2.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore^{TM}, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

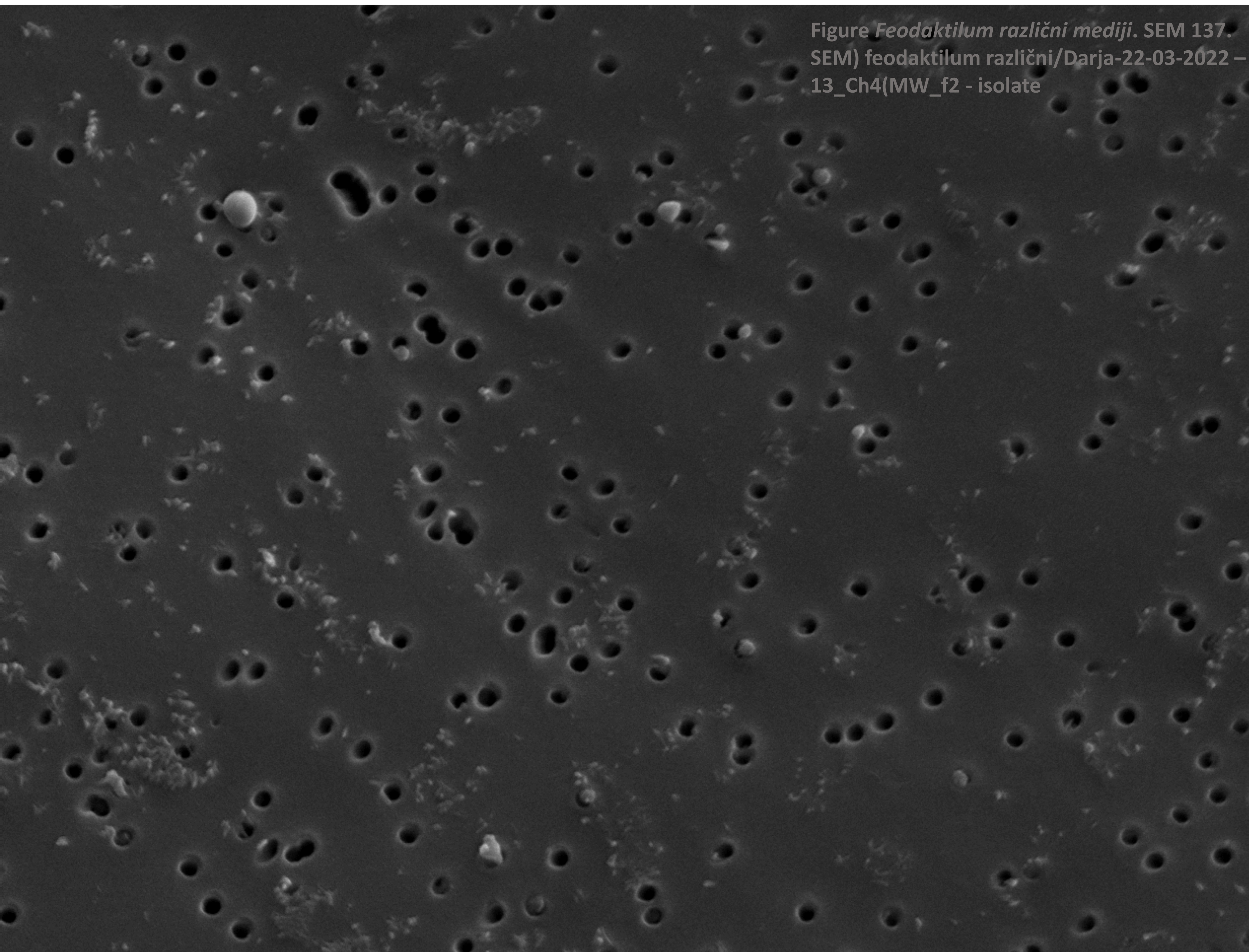

Figure *Phaeodactylum tricornutum* isolate F2 SEM 3.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with Guillard's (F/2) Marine Water Enrichment Solution (ref. G0154, Sigma Aldrich, USA)17. Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of small cellular particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

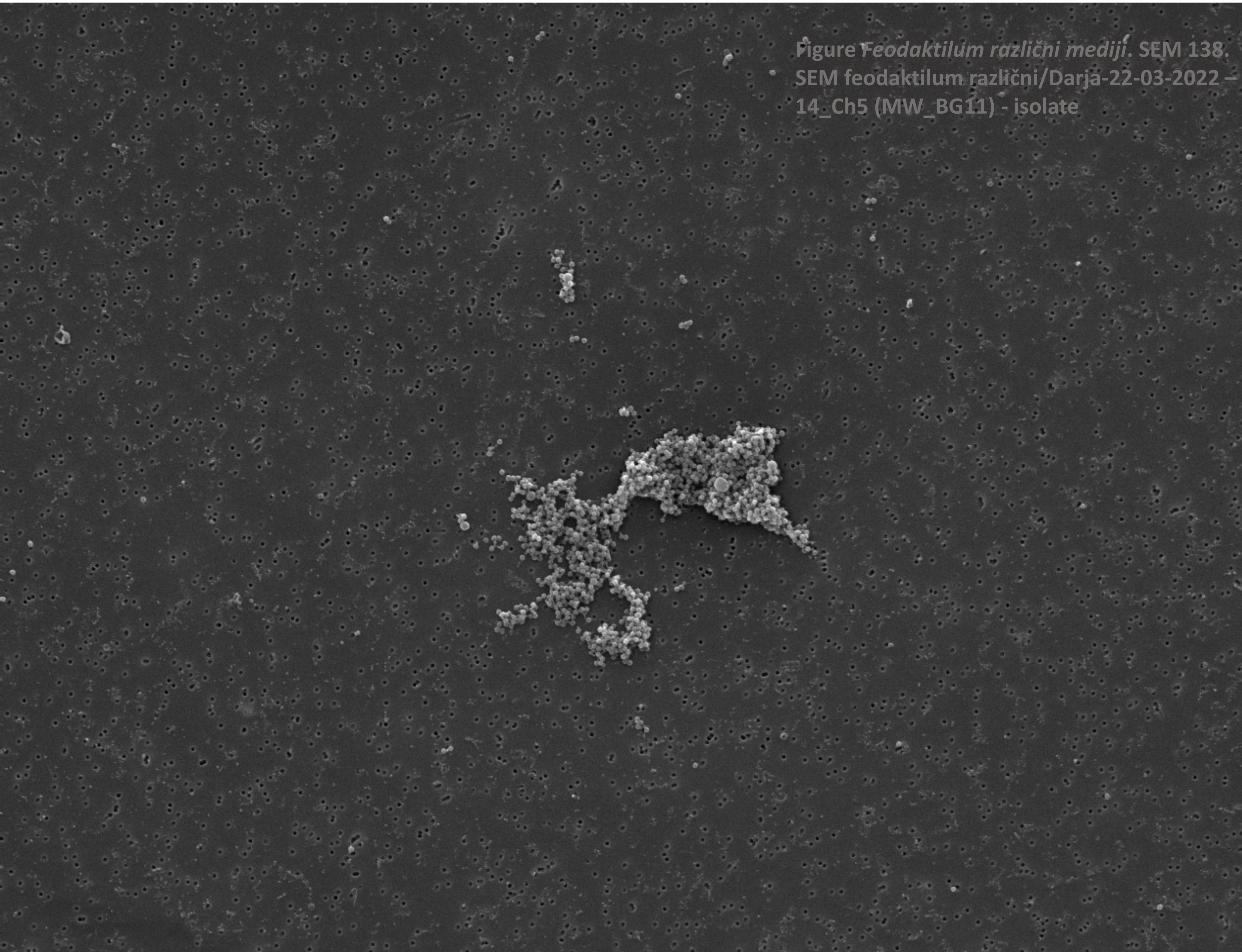

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 4.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore^{TM}, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

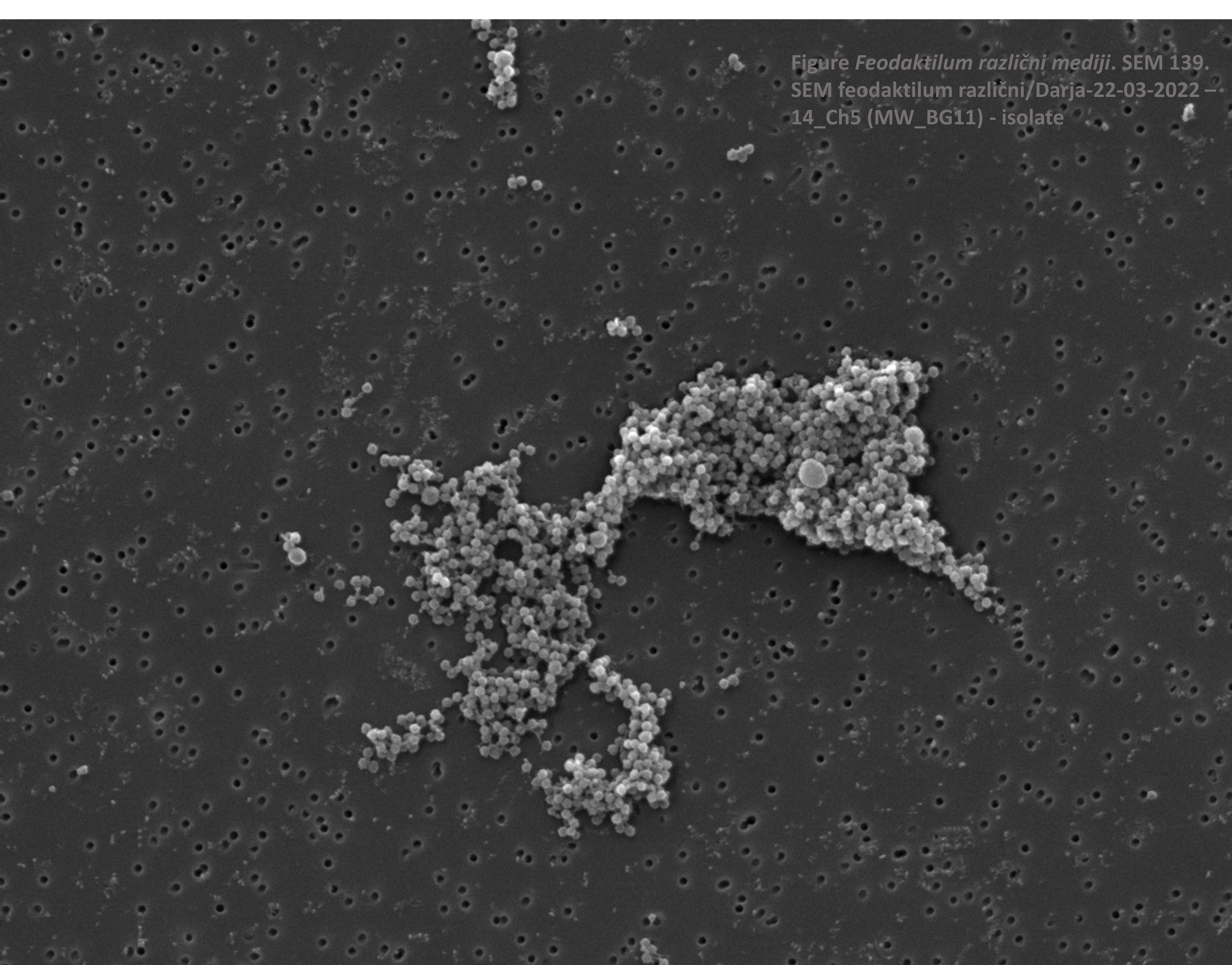

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 5.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

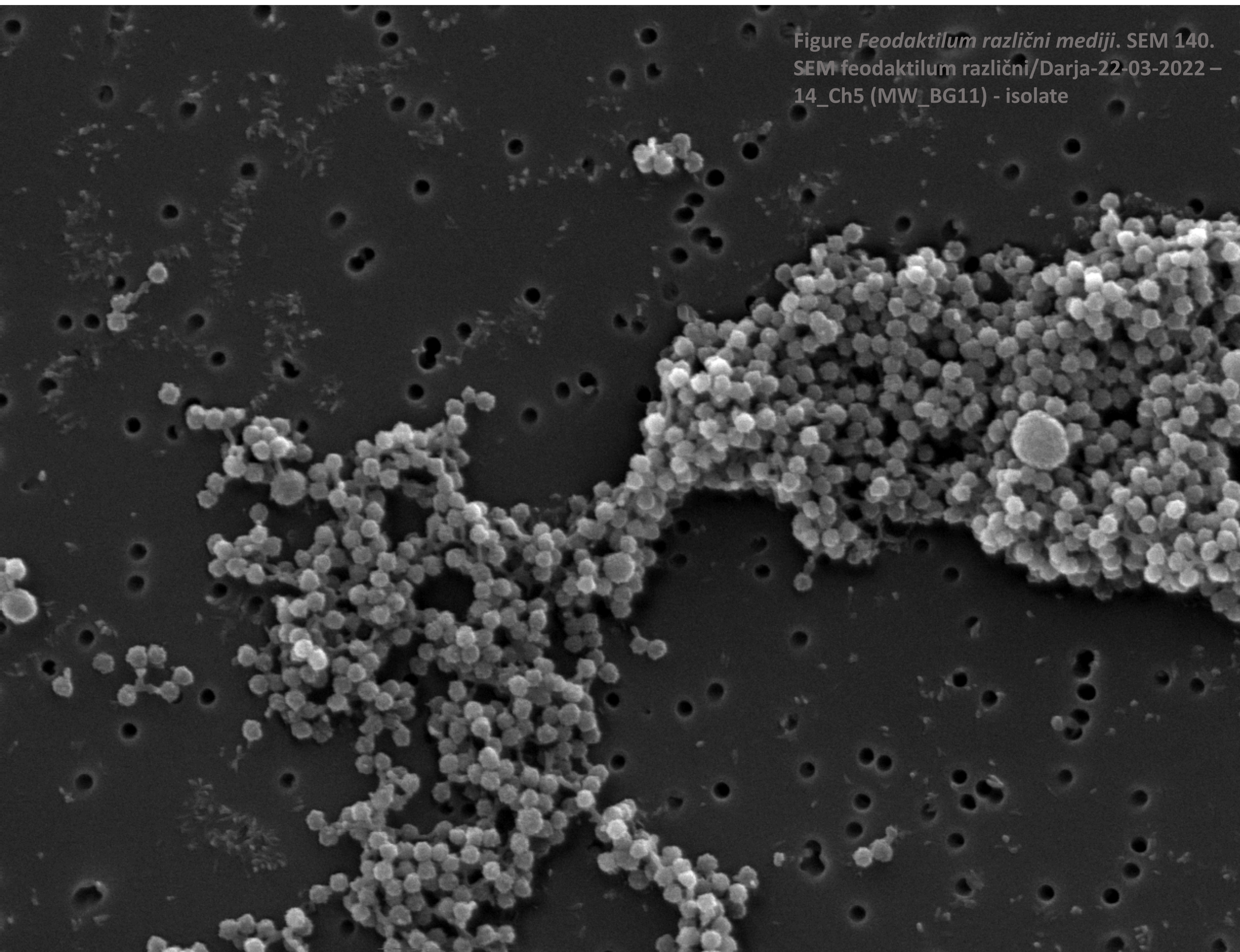

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 6.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **BG11 Broth** (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore^{TM}, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

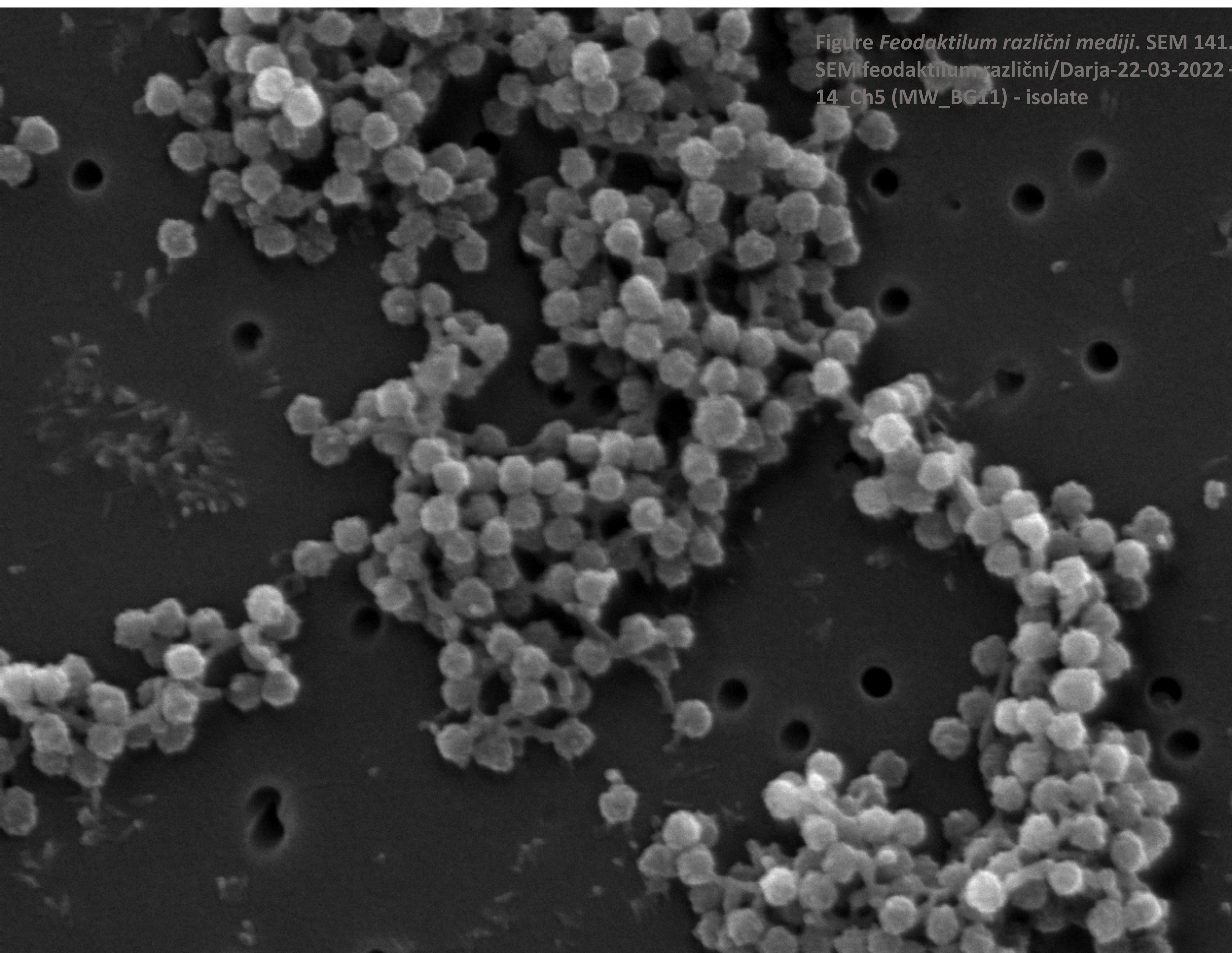

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 7.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

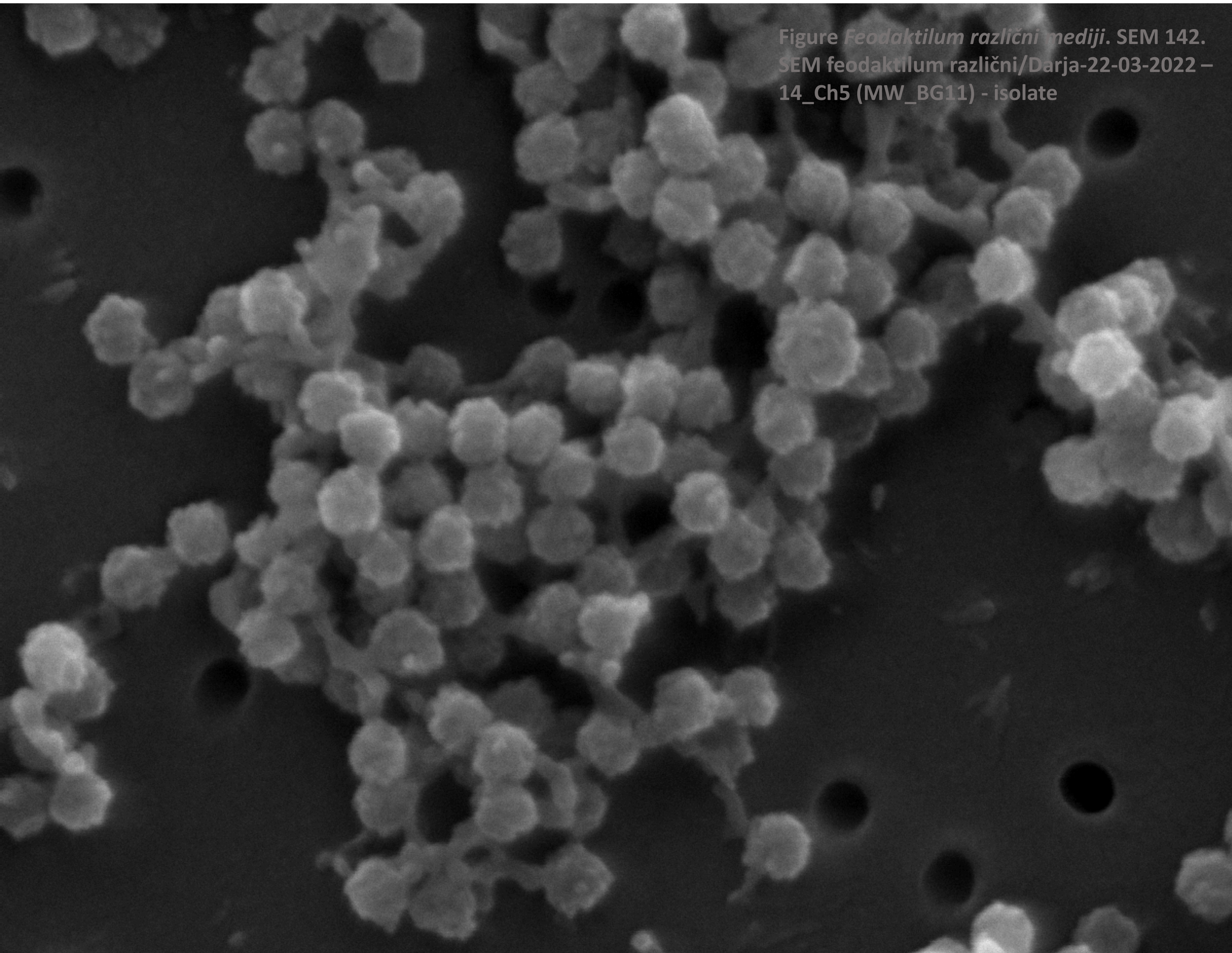

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 8.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

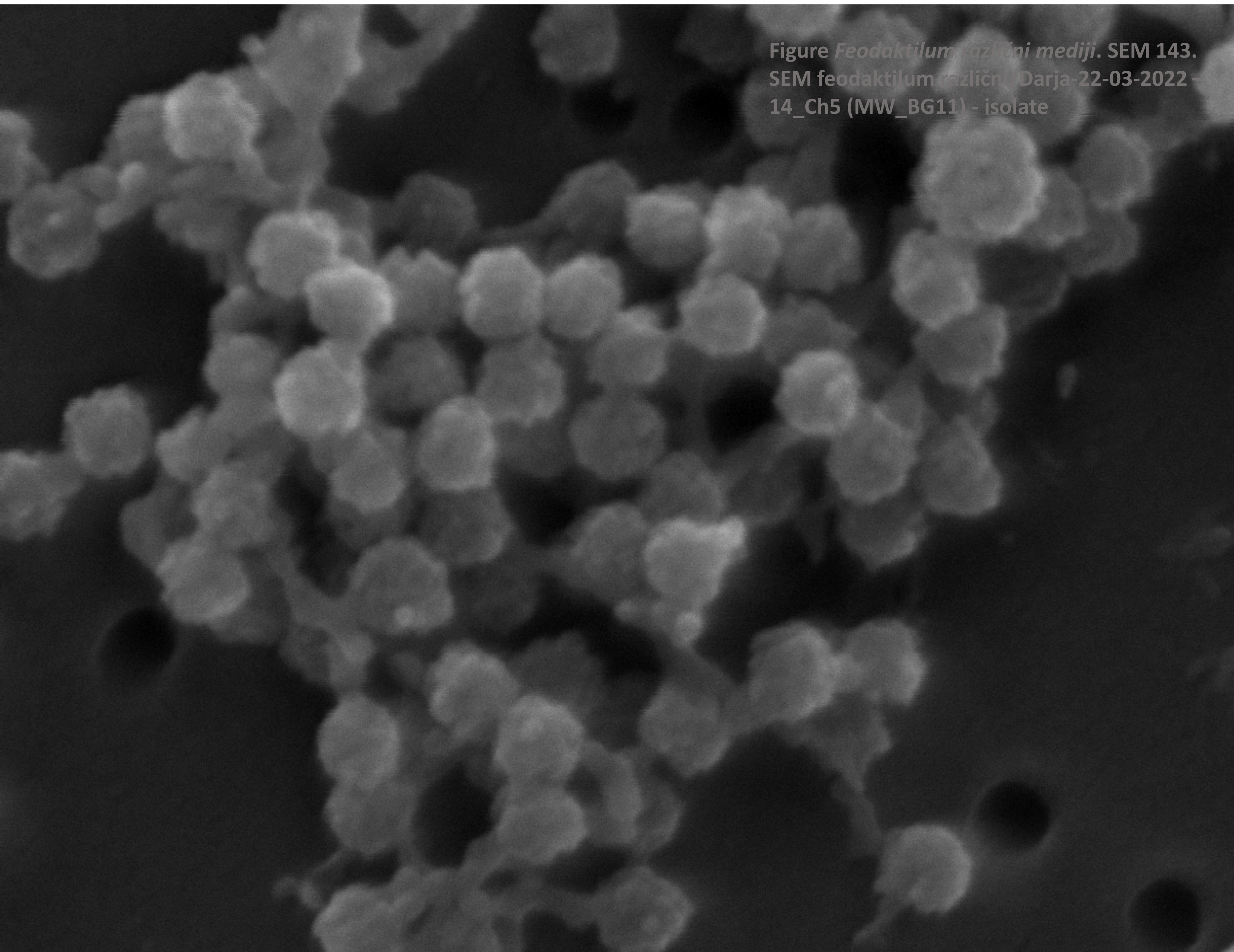

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 9.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 µmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

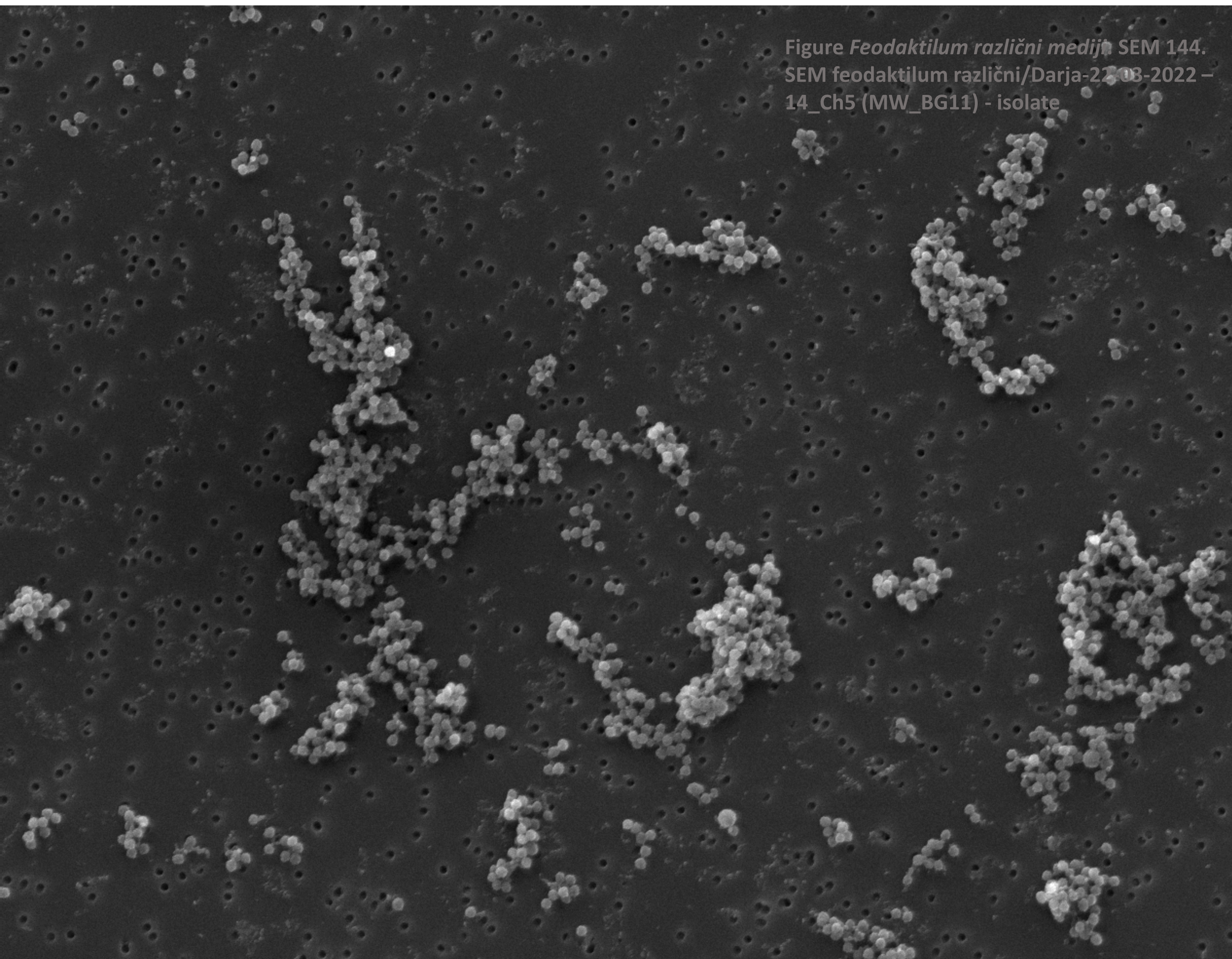

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 10.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore^{TM}, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

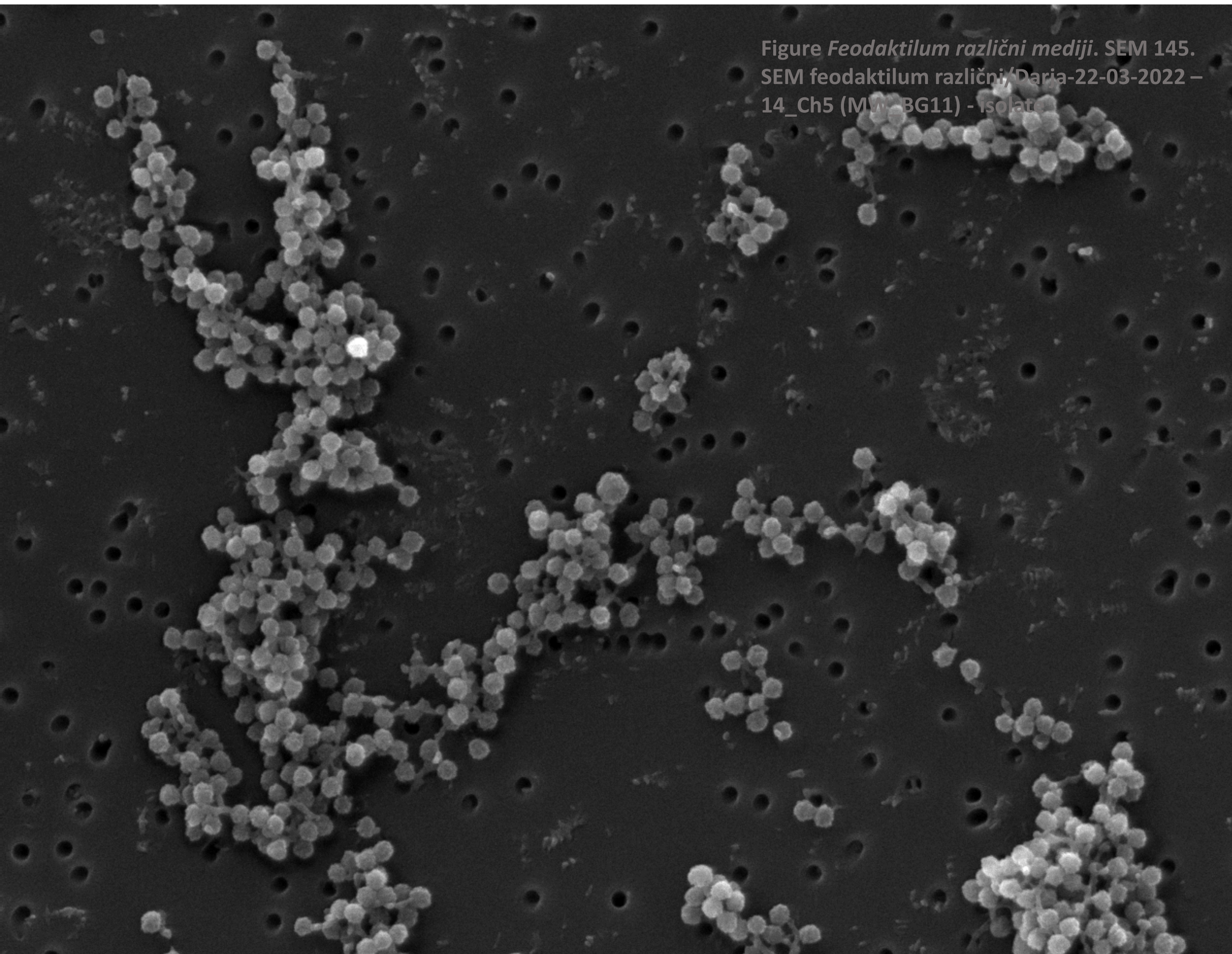

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 11.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

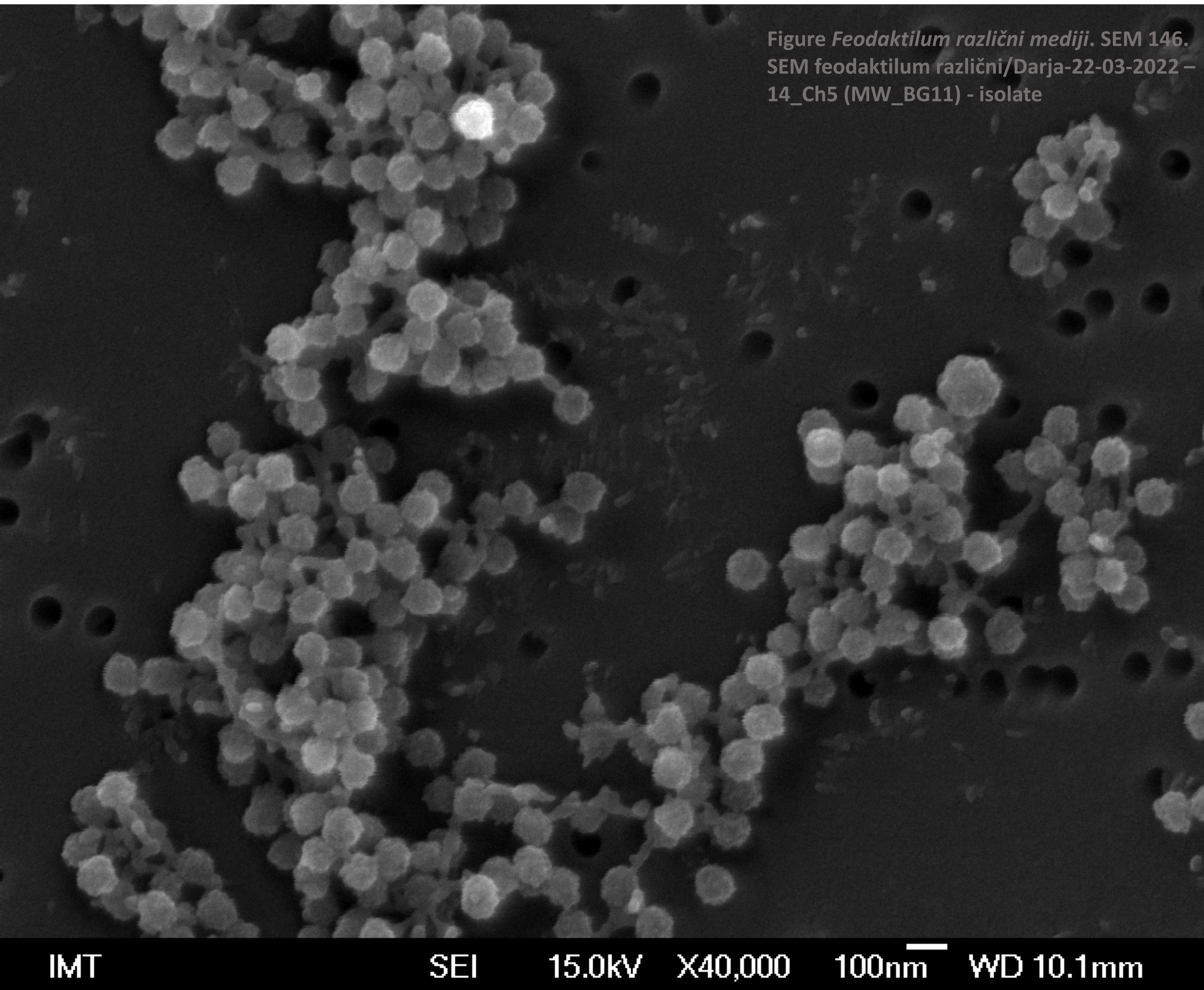

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 12.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

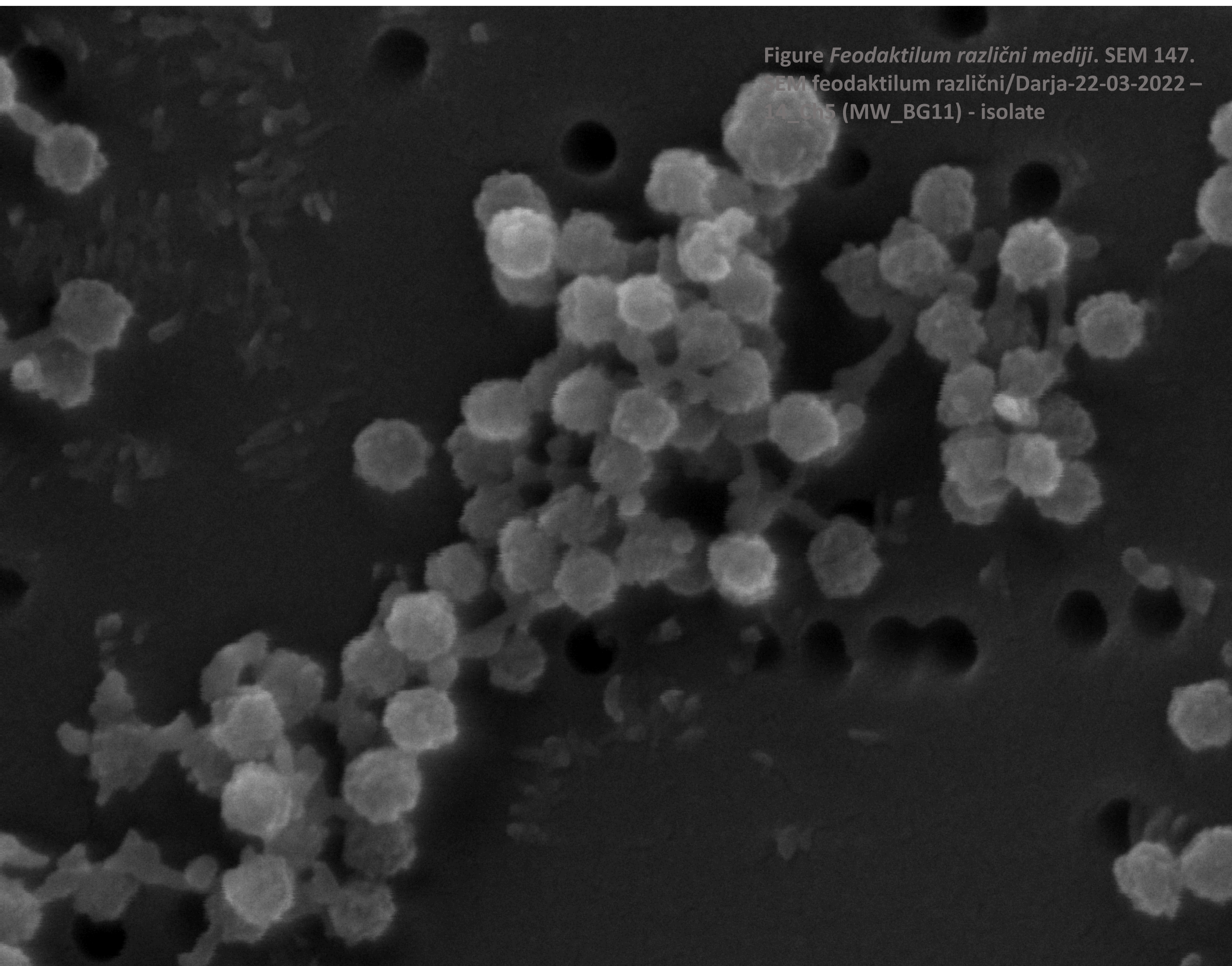

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 13.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isola)b Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

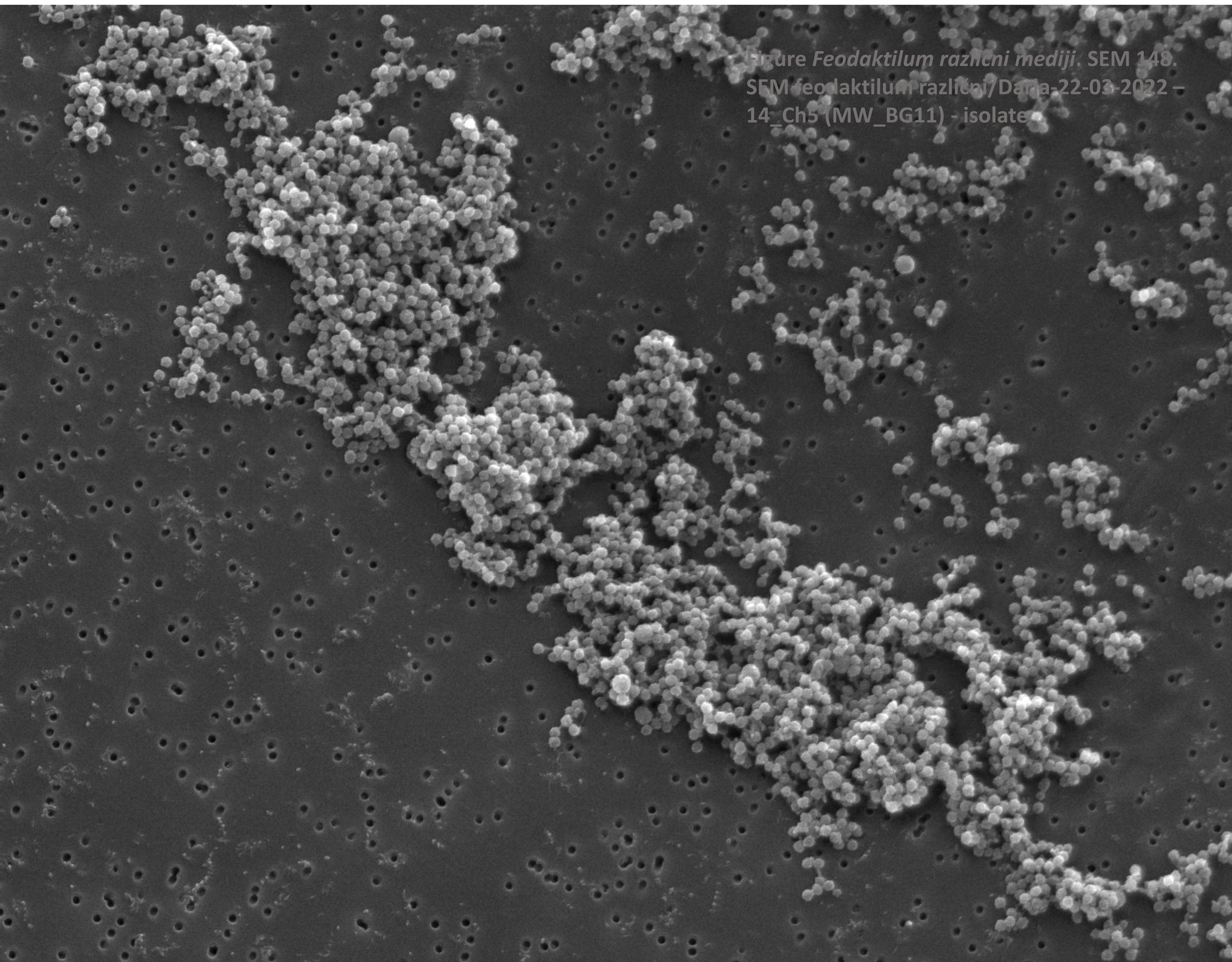

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 14.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

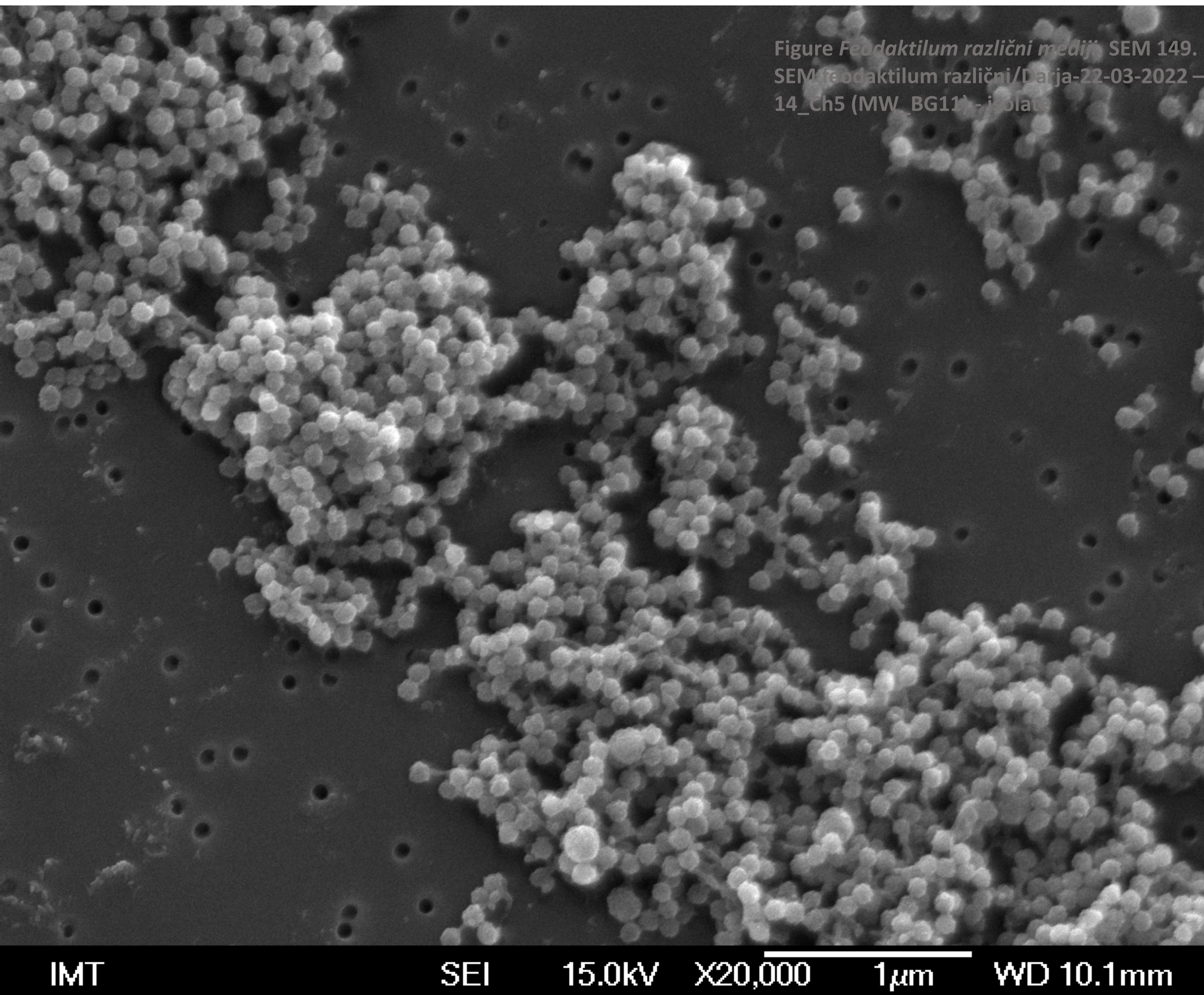

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 15.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore^{TM}, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

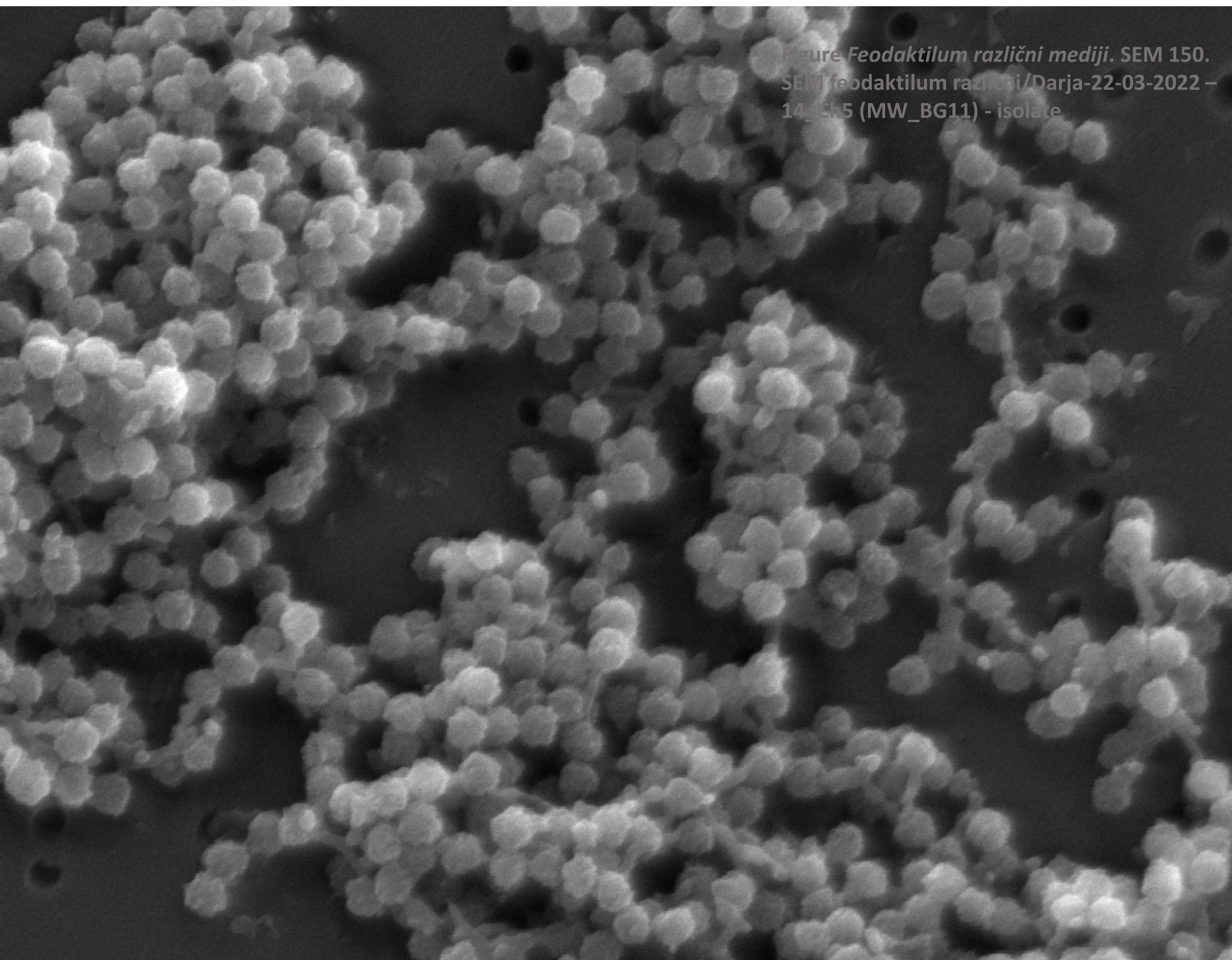

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 16.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

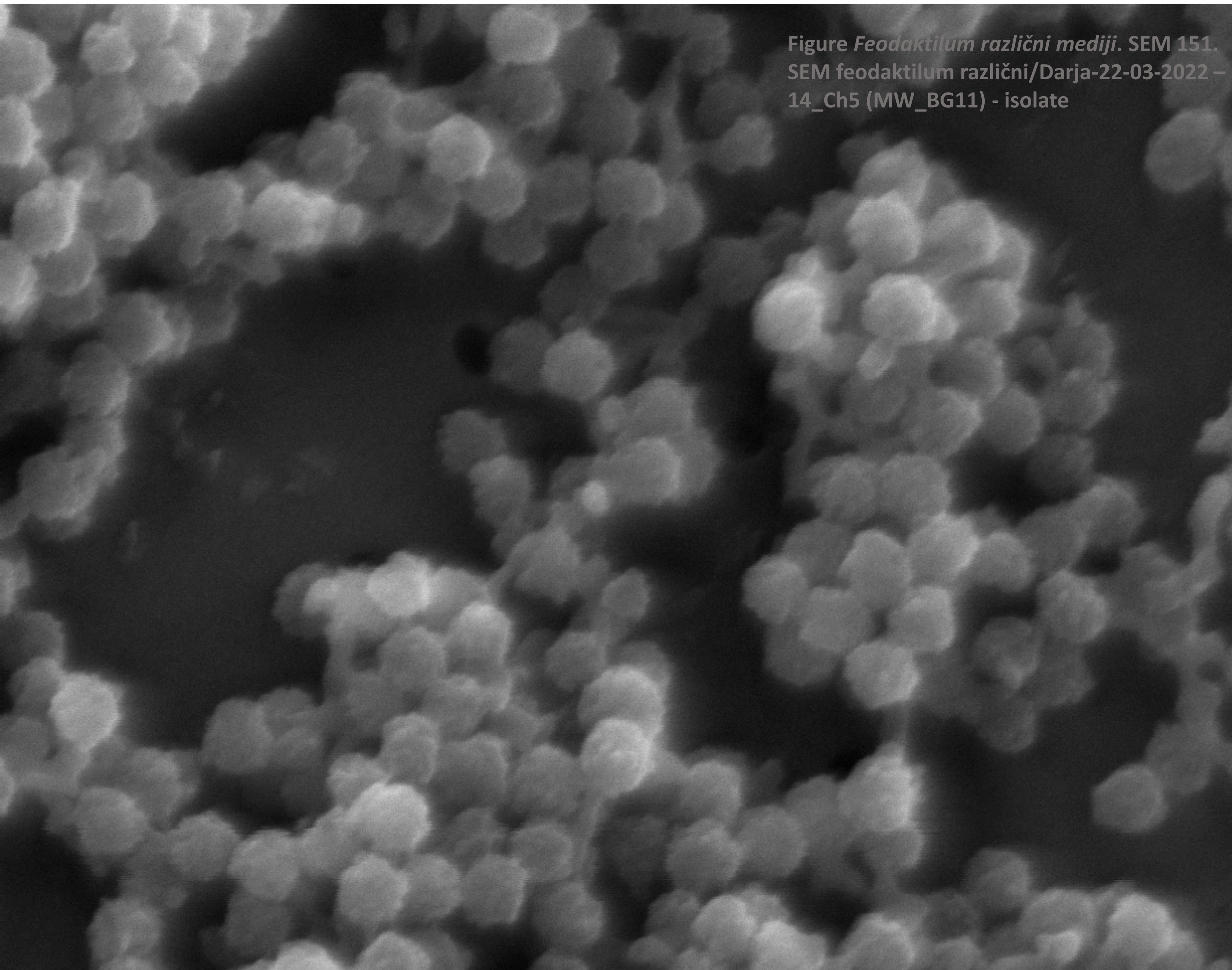

Figure *Phaeodactylum tricornutum* isolate BG11 SEM 17.

Cultivation of algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with BG11 Broth (ref. 73816, Sigma Aldrich, Switzerland). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore^{TM}, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

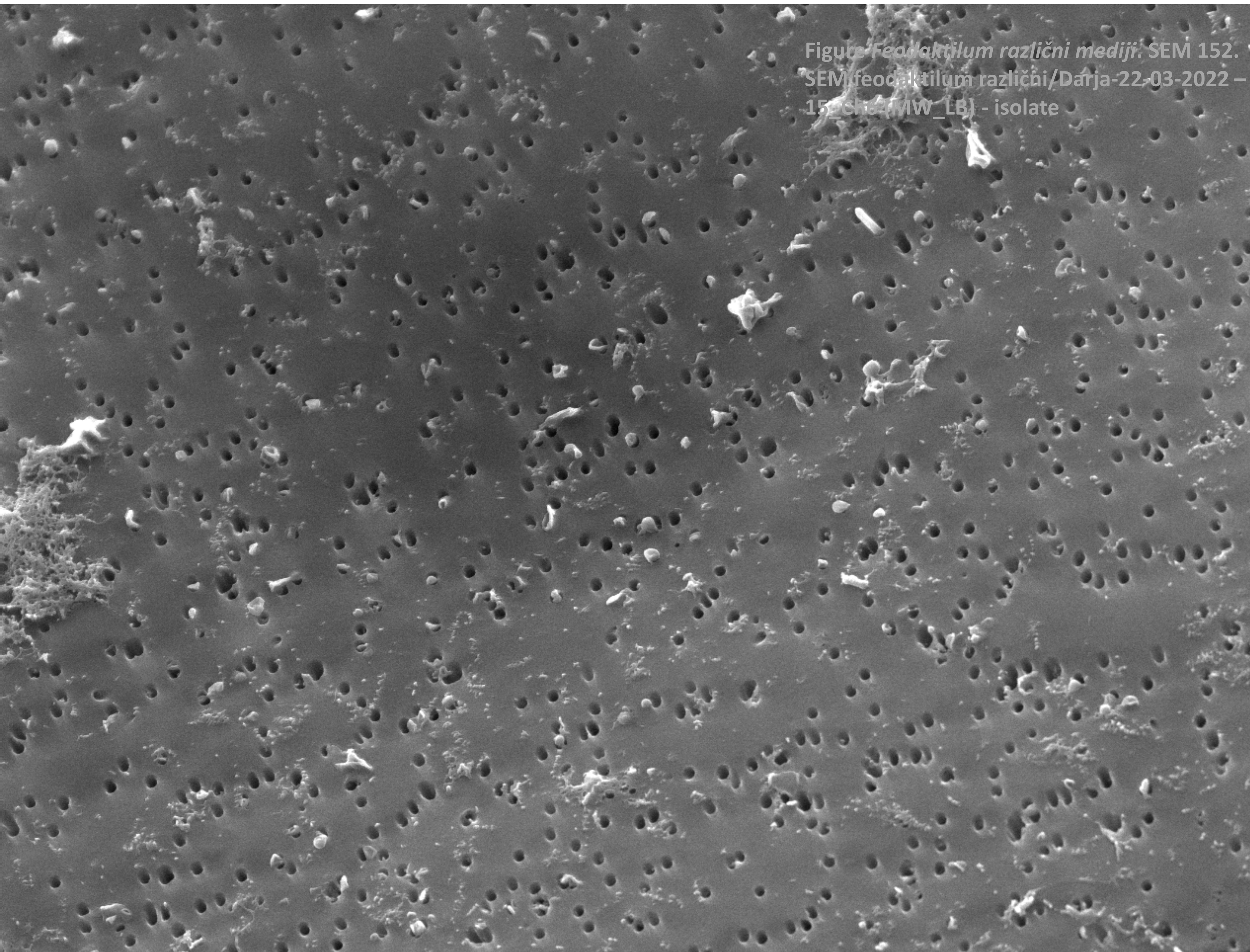

Figure *Phaeodactylum tricornutum* isolate LB SEM 18.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (app. 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore^{TM}, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

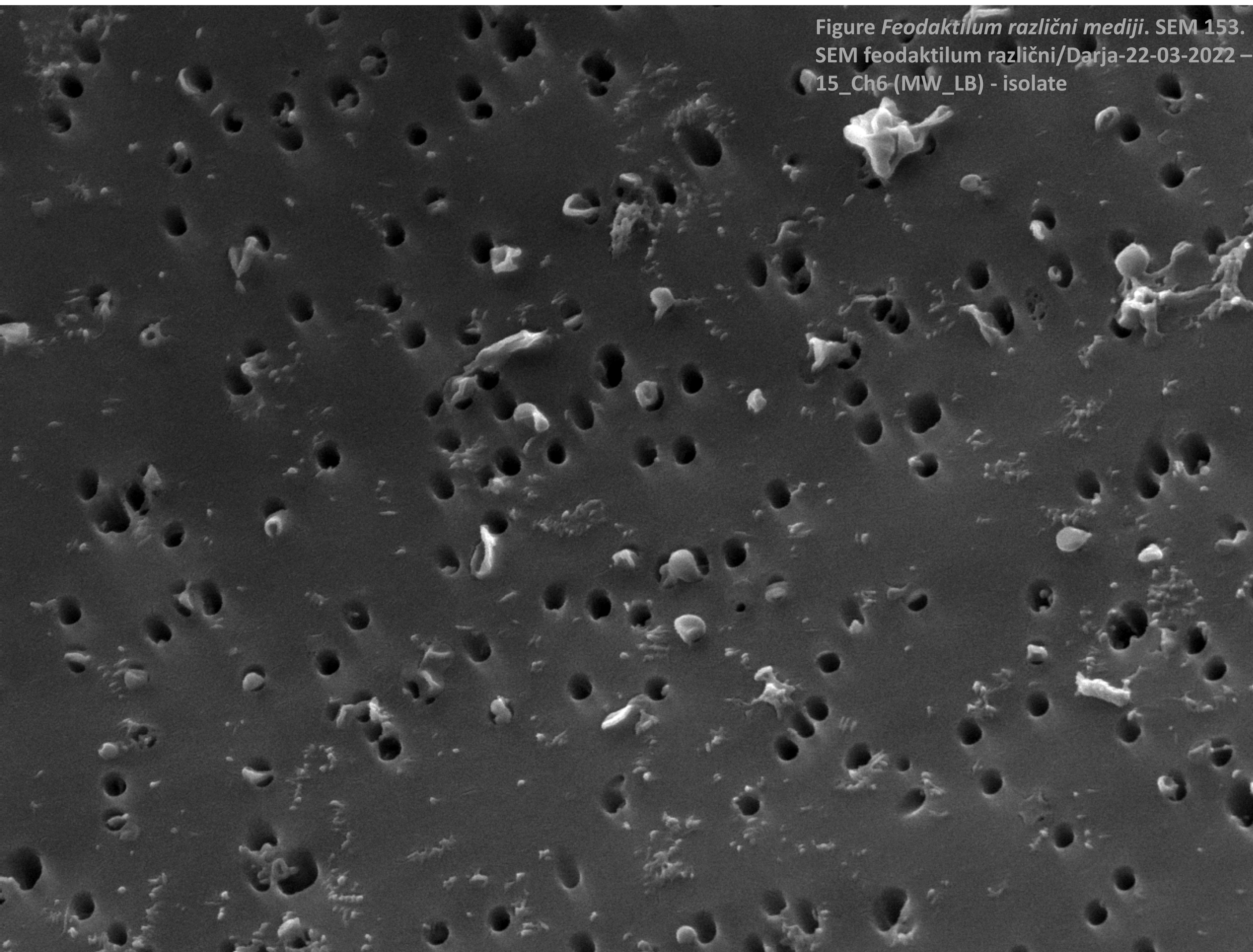

Figure *Phaeodactylum tricornutum* isolate LB SEM 19.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

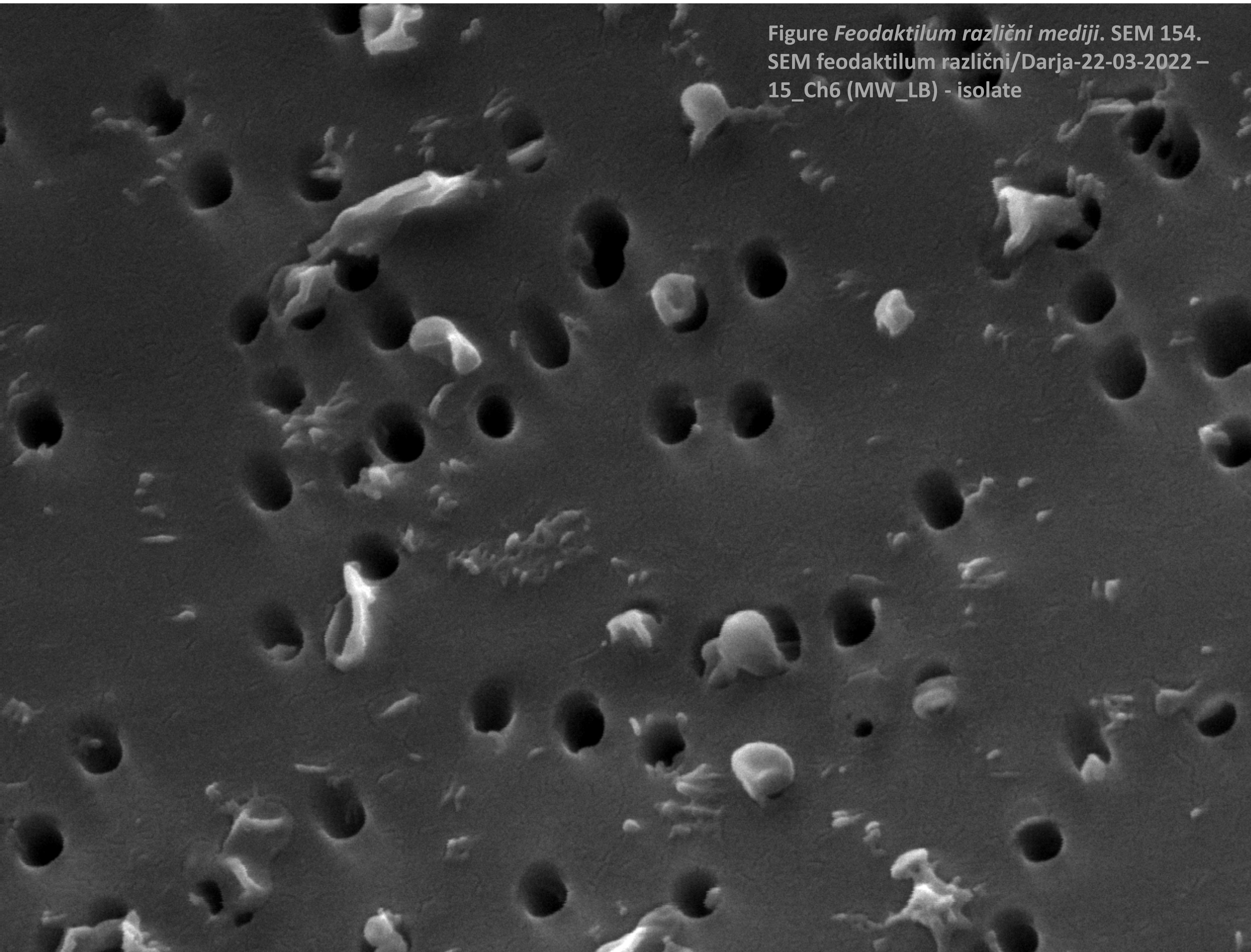

Figure *Phaeodactylum tricornutum* isolate LB SEM 20.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore^{TM}, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

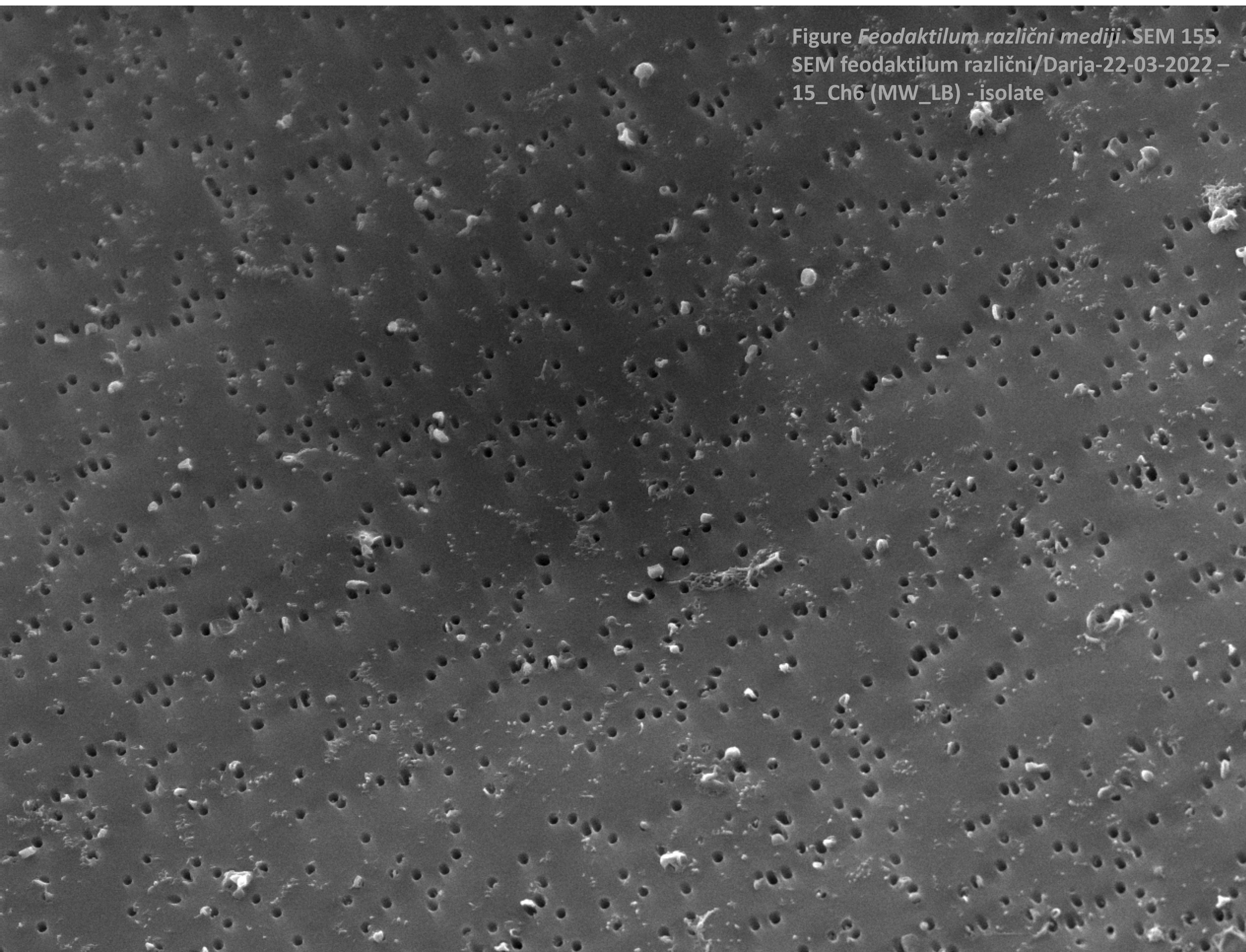

Figure *Phaeodactylum tricornutum* isolate LB SEM 21.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with LB Lennox LB broth (ref. L3022, Sigma Aldrich, USA). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

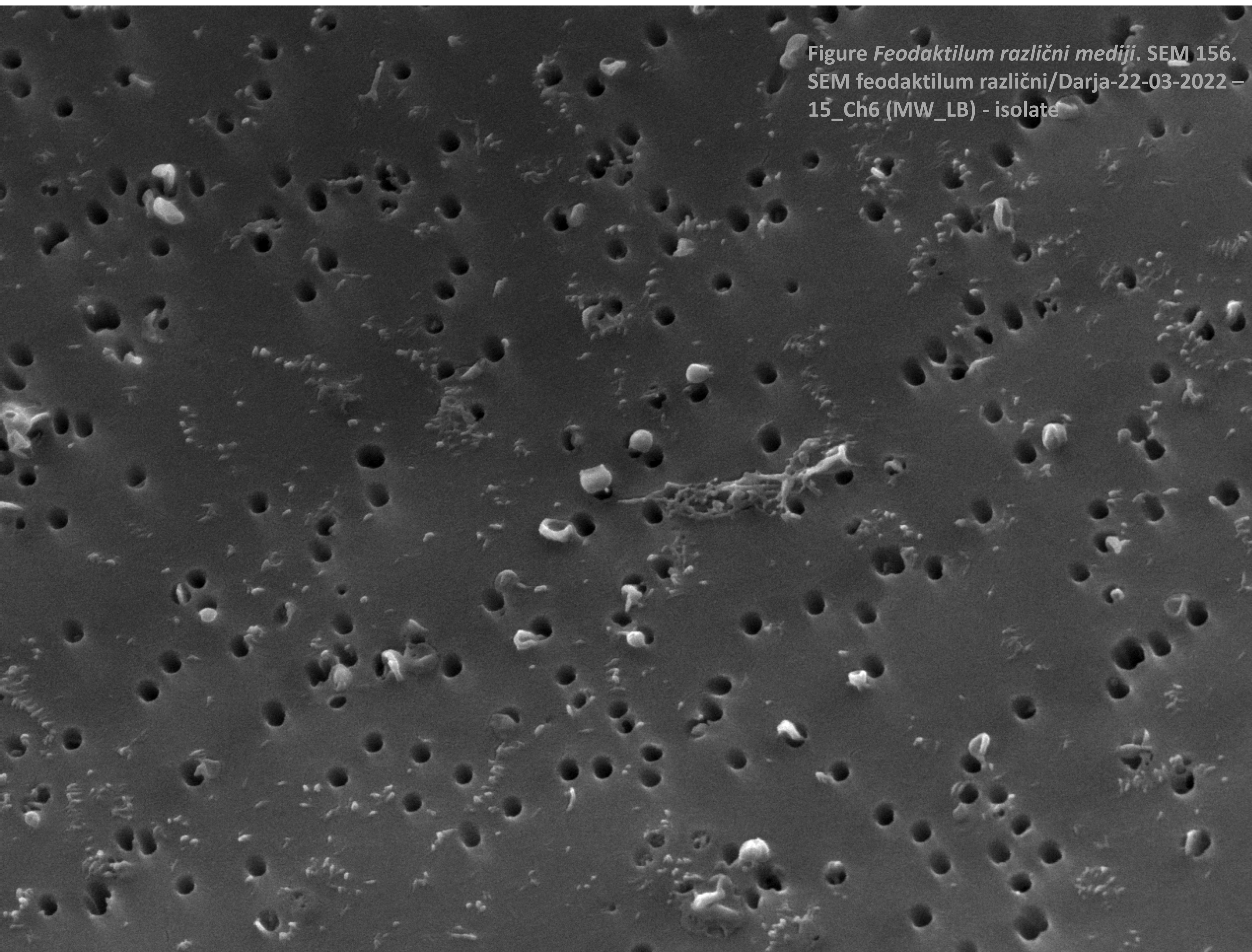

Figure *Phaeodactylum tricornutum* isolate LB SEM 22.

Cultivation of the algae

Culture of *Phaeodactylum tricornutum* CCAP 1052/1A from the Culture Collection of Algae and Protozoa (CCAP) of SAMS (Oban, Scotland) was grown in artificial seawater (Reef Crystals, Aquarium Systems, France). 22 g of salt was dissolved in one litre of distilled water, sterile filtered (0.2-micron cellulose filters, ref. 11107-47-CAN, Sartorius Stedim Biotech GmbH, Germany), autoclaved, and supplemented with **Lennox LB broth** (ref. L3022, Sigma Aldrich, USA). Culture was grown in a respirometer (Echo, Slovenia) in 0.5-L borosilicate bottles, at 20 °C and 20 % illumination (approximately 250 μmol/m2s) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

Isolation of Small Cellular Particles (SCPs)

SCPs were isolated by differential centrifugation. The cells from algal culture were removed by low-speed centrifugation (300 g, 10 min, 4°C, centrifuge Centric 260R with rotor RA 6/50 (Domel, Slovenia)), using 50 mL conical centrifuge tubes (ref. S.078.02.008.050, Isolab Laborgeräte GmbH, Germany); and 2000 g, 10 min, 4°C (Centric 400R centrifuge with rotor RS4/100 (Domel, Slovenia)), using 15 mL conical centrifuge tubes (ref. S.078.02.001.050, Isolab Laborgeräte GmbH, Germany). Each step was repeated twice. Then, the cell-depleted medium was centrifuged twice at 10 000 g and 4°C for 30 min (Beckman L8-70M ultracentrifuge, rotor SW55Ti (Beckman Coulter, USA)), using thin-wall polypropylene centrifuge tubes (ref. 326819, Beckman Coulter, USA) to remove larger cell debris. Finally, NPs were pelleted by ultracentrifugation at 118 000 g and 4°C, for 70 min in the same type of ultracentrifuge and ultracentrifuge tubes.

Scanning Electron Microscopy (SEM)

Samples were loaded onto 0.05-micron MCE filters (MF-Millipore™, ref. VMWP01300) and incubated in 2% OsO_4 for two hours. Then the osmium was removed, and the filter was taken out from the holder and further treated in a 24-well plate by changing the bath solution. After washing three times in distilled water, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, absolute), treated with hexamethyldisilazane (30%, 50% mixtures with absolute ethanol, followed by pure hexamethyldisilazane), and air-dried. Samples were sputtered with Au/Pd (PECS Gatan 682) and examined with a JSM-6500F Field Emission Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).