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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

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Abstract: Scanning electron microscope images of small cellular particles isolated from conditioned media of microalgae *Phaeodactylum tricornutum* 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-Igljč 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).

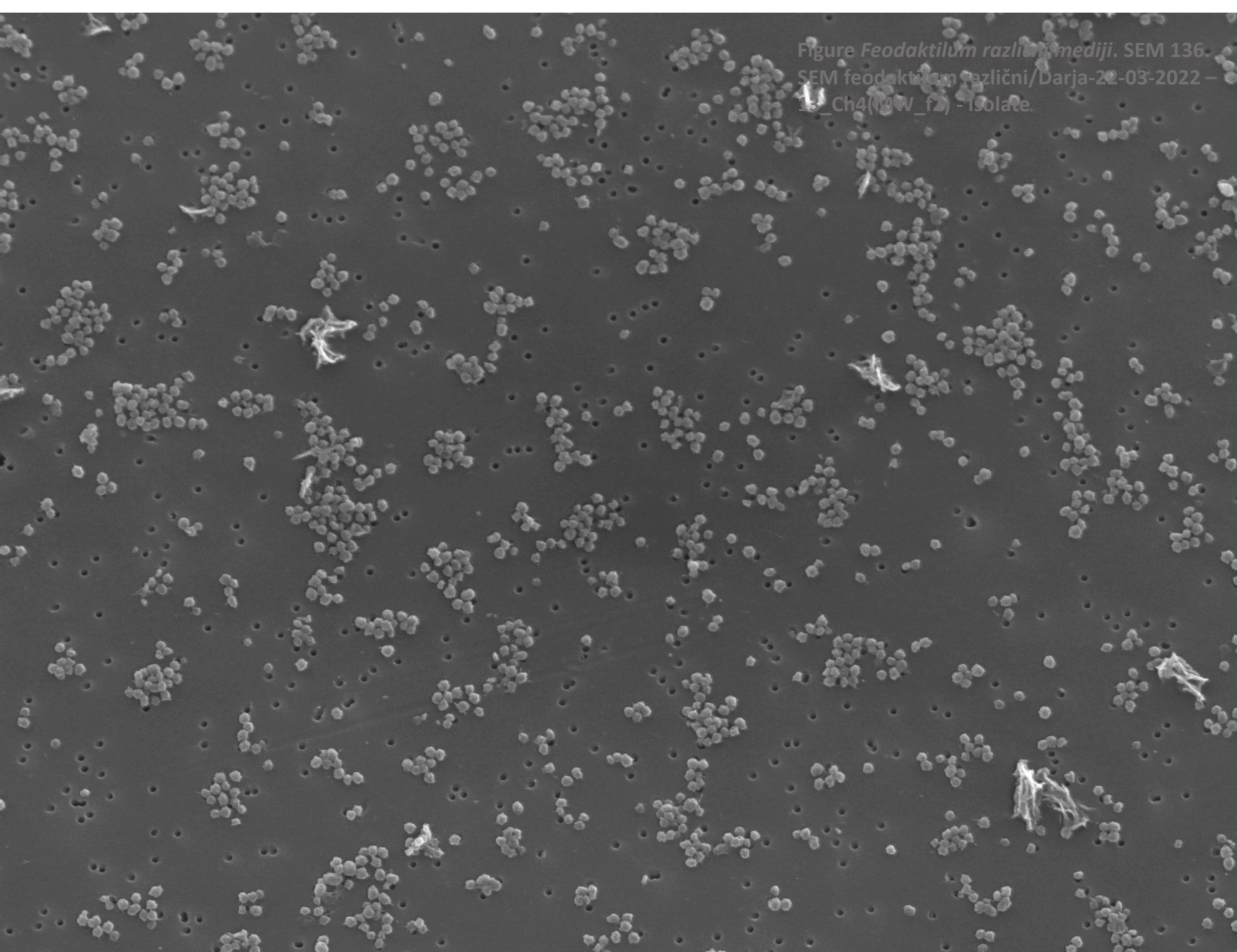
Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 801338 and ARRS projects P1-0391, P2-0232, P3-0388, J2-4447, J2-4427, L3-2621, J3-3066, IO-0006 (A) and National Research, Development and Innovation Office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

Keywords: Extracellular vesicles, Extracellular particles, Nanoalgosomes, Exosomes

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3. Božič D, Hočvar 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 *Feodactylum različní mediji*. SEM 136.
SEM feodaktulum različni/Darja-22-03-2022 –
15_Ch4(10kV_f2) - Isolate.



IMT SEI 15.0kV X10,000 1µm WD 10.2mm

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% OsO₄ 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 *Feodaktulum* različni mediji. SEM 136.
SEM feodaktulum različni/Darja-22-03-2022 –
13_Ch4(MW_f2) - isolate



IMT SEI 15.0kV X10,000 1µm WD 10.1mm

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.

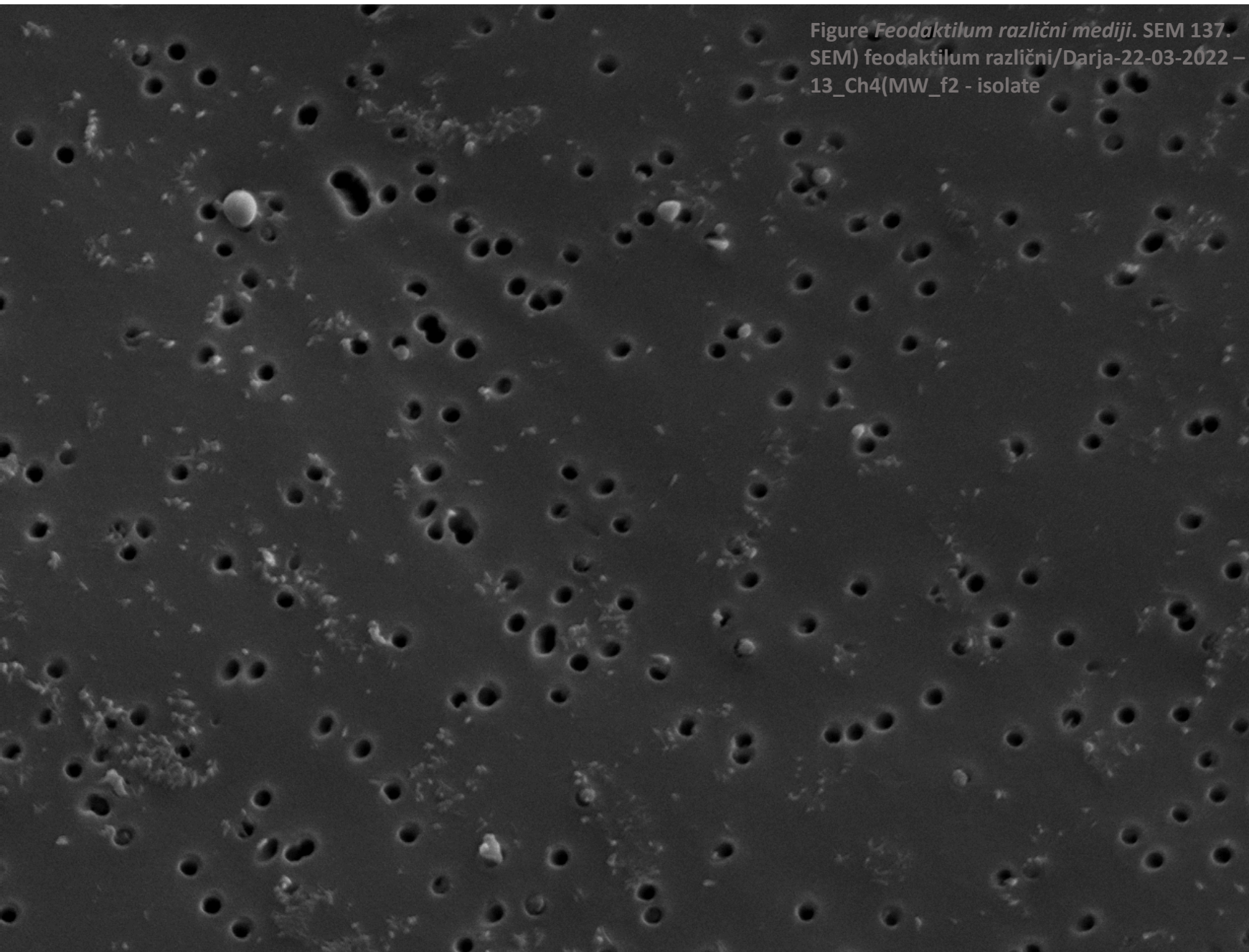
Isolation of small cellular particles (SCPs)

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™, ref. VMWP01300) and incubated in 2% OsO₄ 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 *Feodaktikum različni mediji*. SEM 137.
SEM) feodaktikum različni/Darja-22-03-2022 –
13_Ch4(MW_f2 - isolate



IMT SEI 15.0kV X20,000 1μm WD 10.1mm

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.

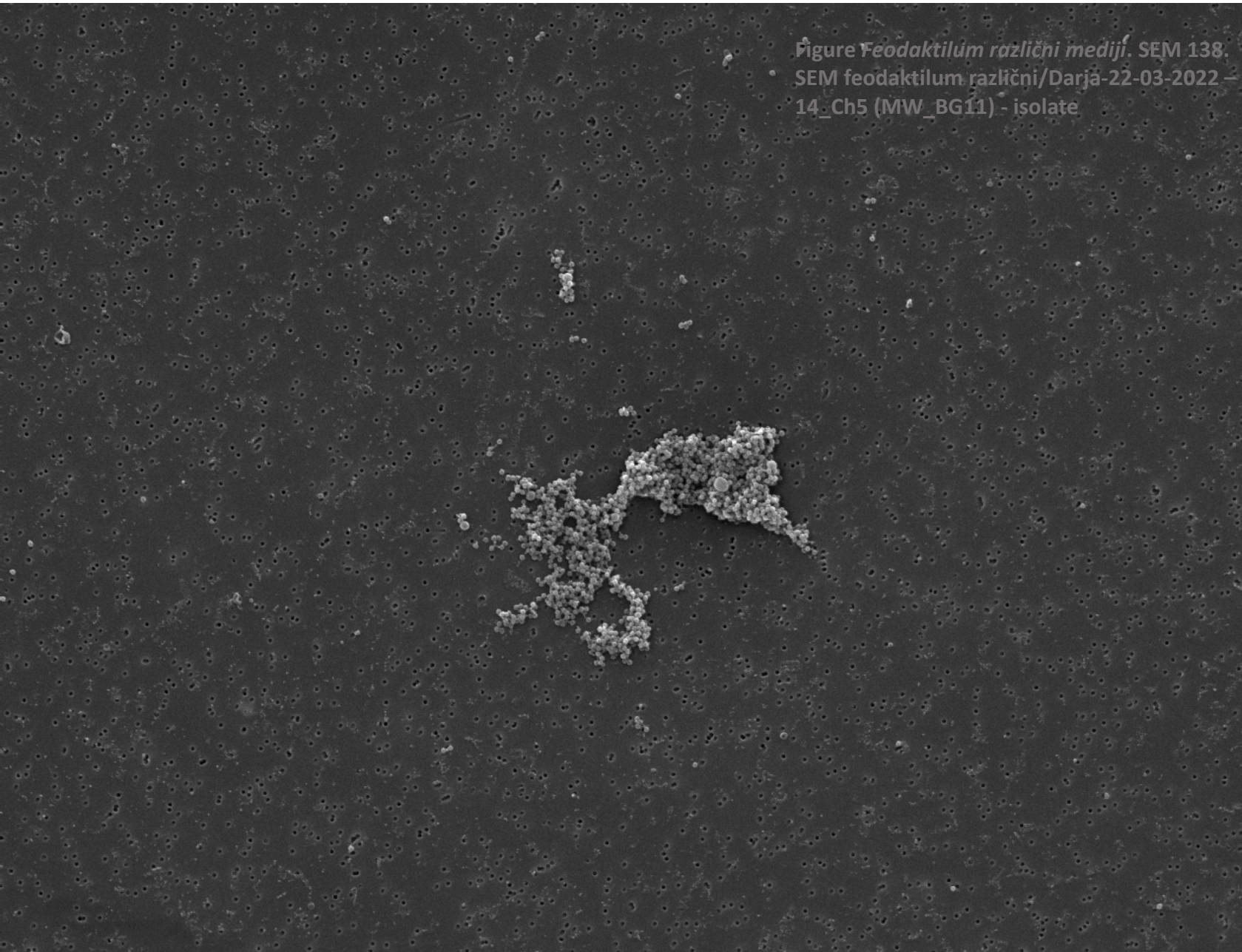
Isolation of small cellular particles (SCPs)

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% OsO₄ 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 Feodaktilum različni mediji. SEM 138.
SEM feodaktilum različni/Darja-22-03-2022 -
14_Ch5 (MW_BG11) - isolate



IMT SEI 15.0kV X5,000 1µm WD 10.1mm

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.

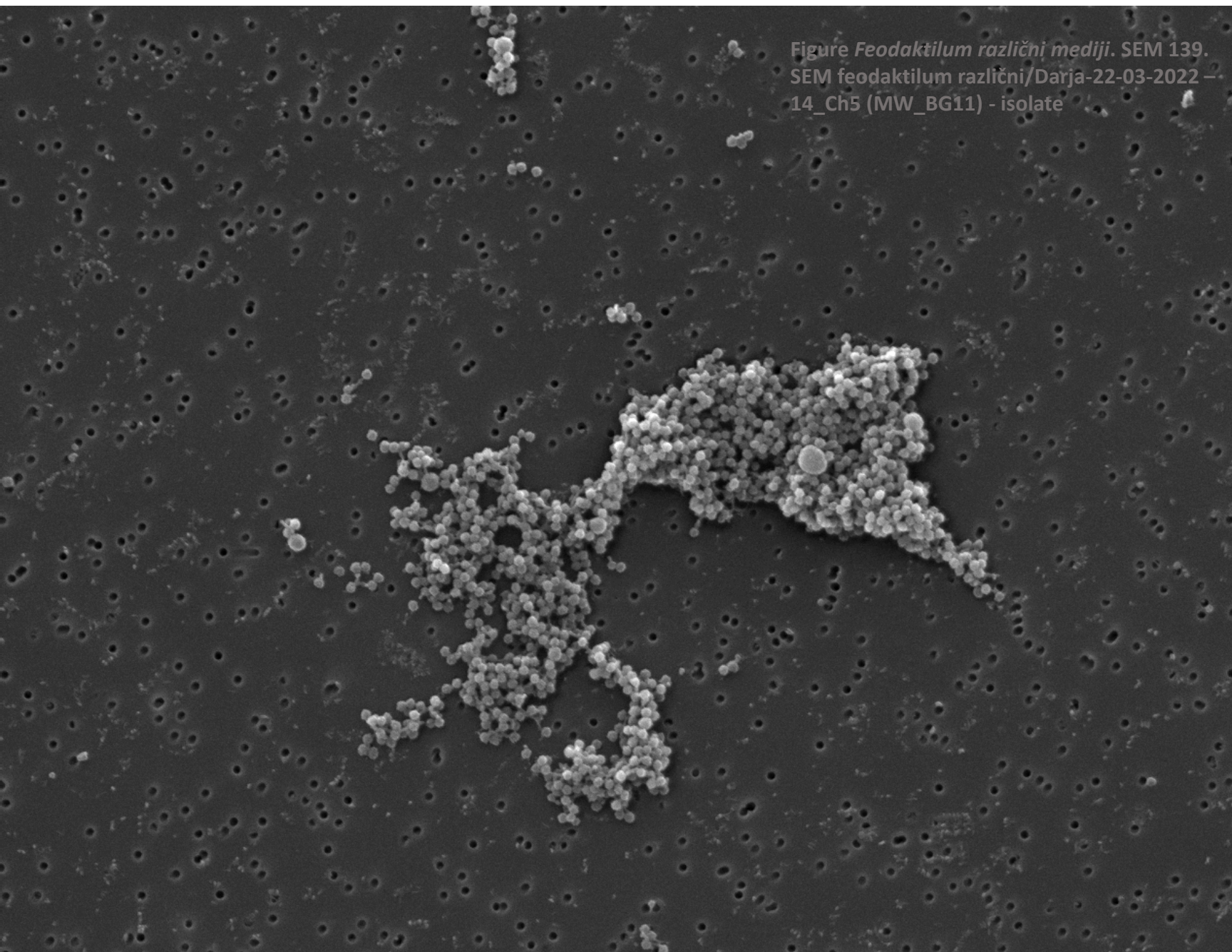
Isolation of Small Cellular Particles (SCPs)

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™, ref. VMWP01300) and incubated in 2% OsO₄ 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 *Feodaktikum različni mediji*. SEM 139.
SEM feodaktikum različni/Darja-22-03-2022 --
14_Ch5 (MW_BG11) - isolate



IMT SEI 15.0kV X10,000 1µm WD 10.1mm

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.

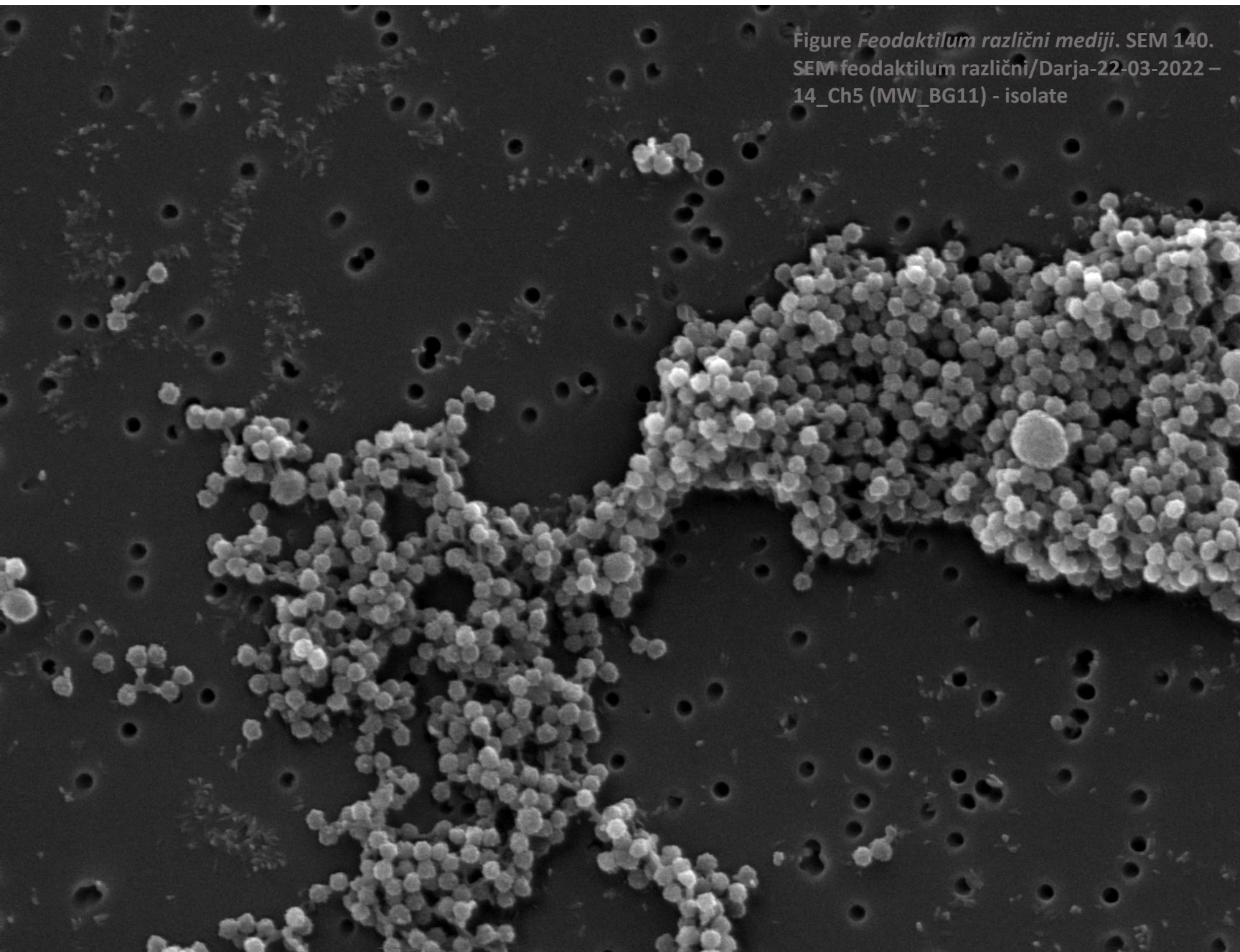
Isolation of Small Cellular Particles (SCPs)

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% OsO₄ 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 *Feodaktilum* različni mediji. SEM 140.
SEM feodaktilum različni/Darja-22-03-2022 –
14_Ch5 (MW_BG11) - isolate



IMT SEI 15.0kV X20,000 1 μm WD 10.1mm

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™, ref. VMWP01300) and incubated in 2% OsO₄ 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).

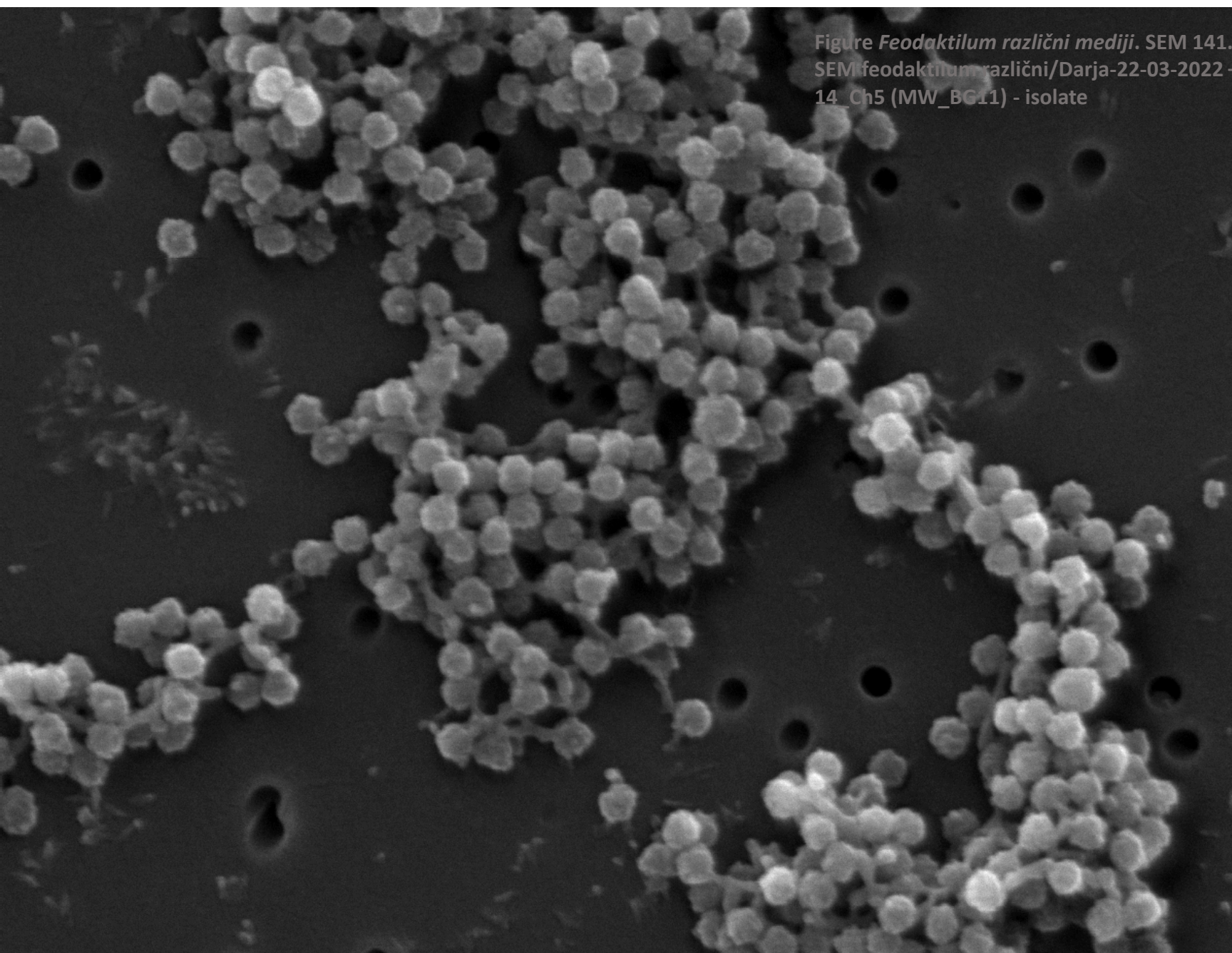


Figure *Feodactylum različni mediji*. SEM 141.
SEM feodaktinam različni/Darja-22-03-2022 –
14_Ch5 (MW_BG11) - isolate

**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 $\mu\text{mol}/\text{m}^2\text{s}$) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

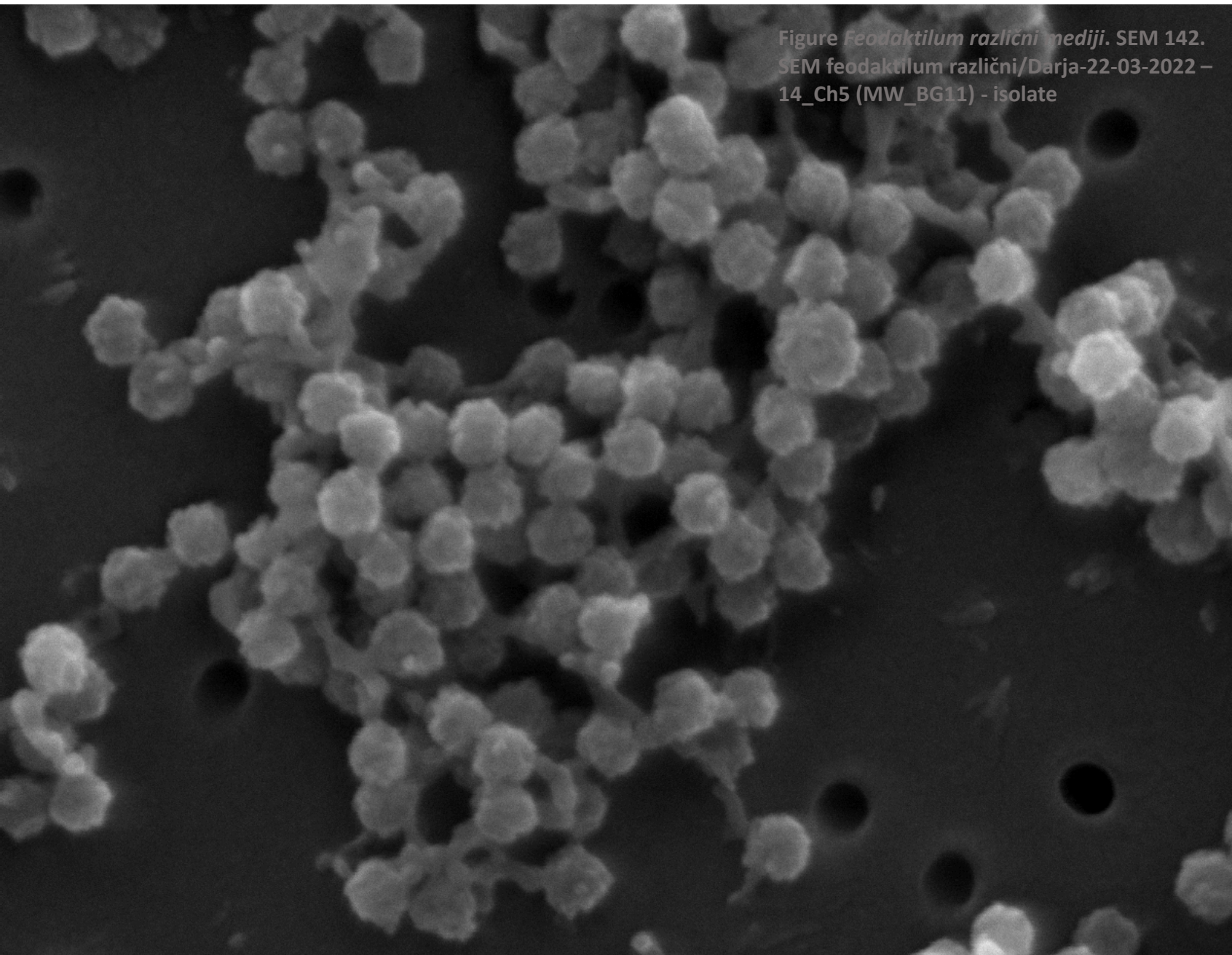
Isolation of Small Cellular Particles (SCPs)

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 *Feodaktikum različni mediji*. SEM 142.
SEM feodaktikum različni/Darja-22-03-2022 –
14_Ch5 (MW_BG11) - isolate



IMT SEI 15.0kV X70,000 100nm WD 10.1mm

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 $\mu\text{mol}/\text{m}^2\text{s}$) 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% OsO₄ 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).

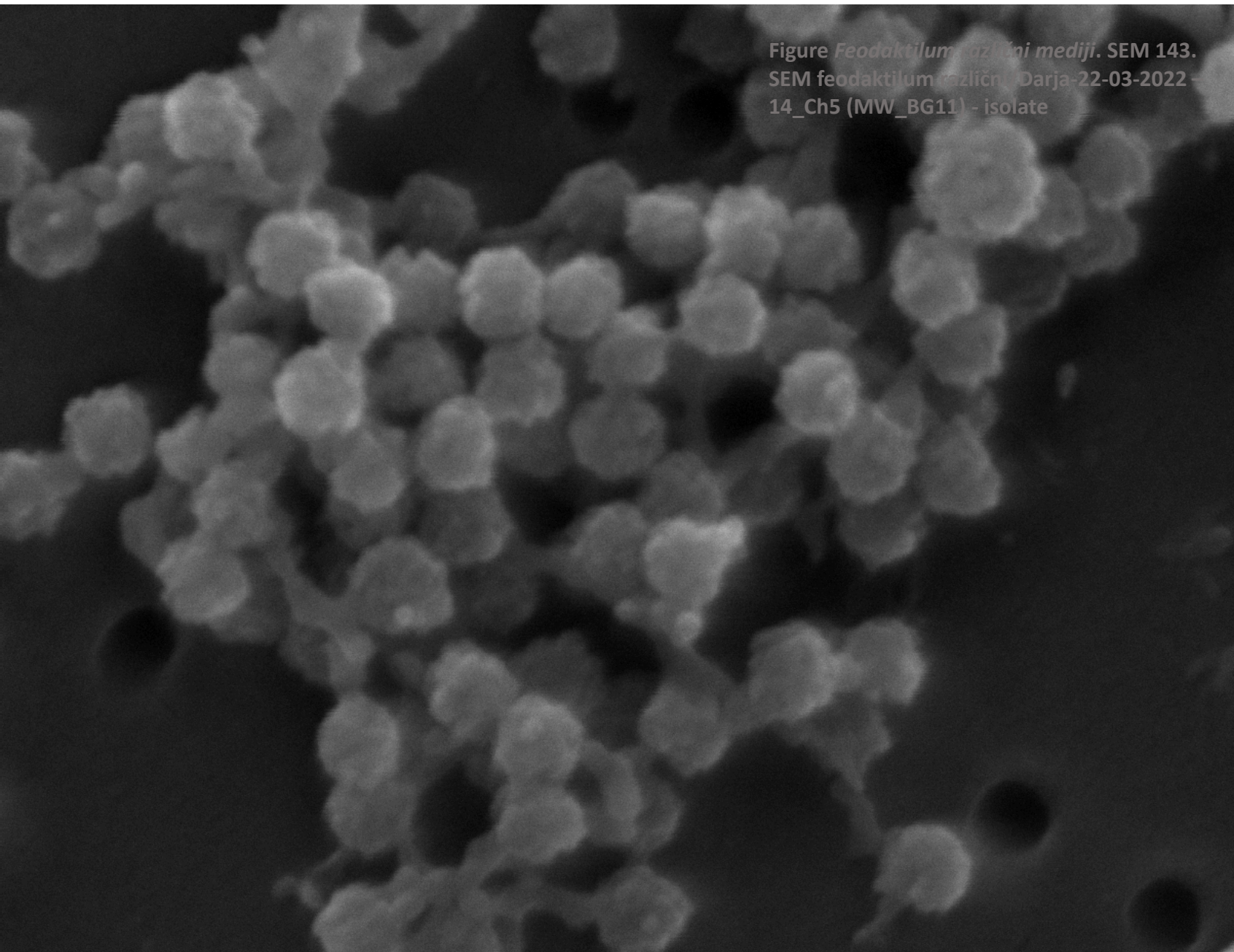


Figure *Feodaktilum aziliceni mediji*. SEM 143.
SEM feodaktilum aziliceni mediji. Darja-22-03-2022 -
14_Ch5 (MW_BG11) - isolate

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 $\mu\text{mol}/\text{m}^2\text{s}$) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

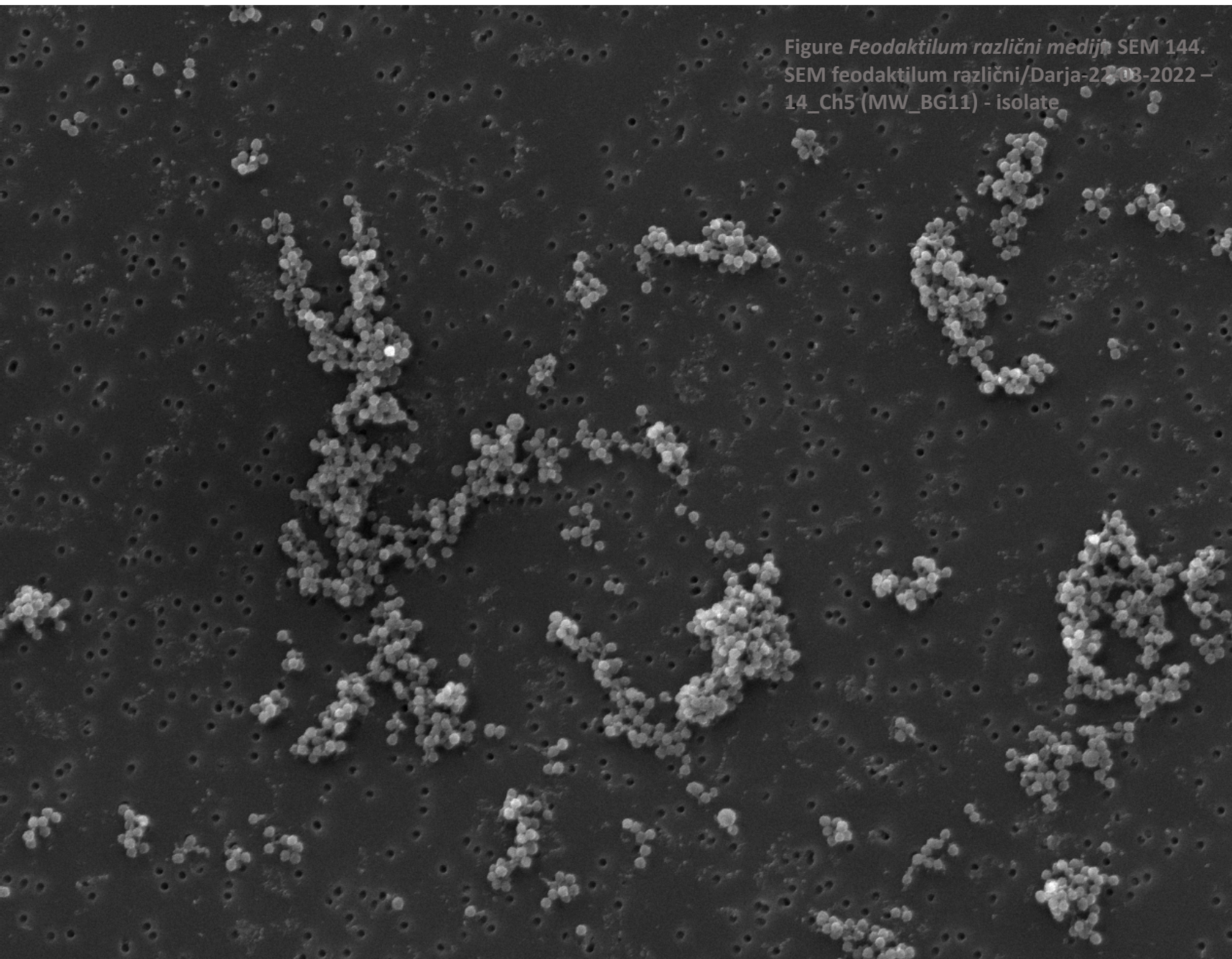
Isolation of Small Cellular Particles (SCPs)

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 *Feodaktulum različni mediji* SEM 144.
SEM feodaktulum različni/Darja-22.03.2022 –
14_Ch5 (MW_BG11) - isolate



IMT SEI 15.0kV X10,000 1µm WD 10.0mm

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™, ref. VMWP01300) and incubated in 2% OsO₄ 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).

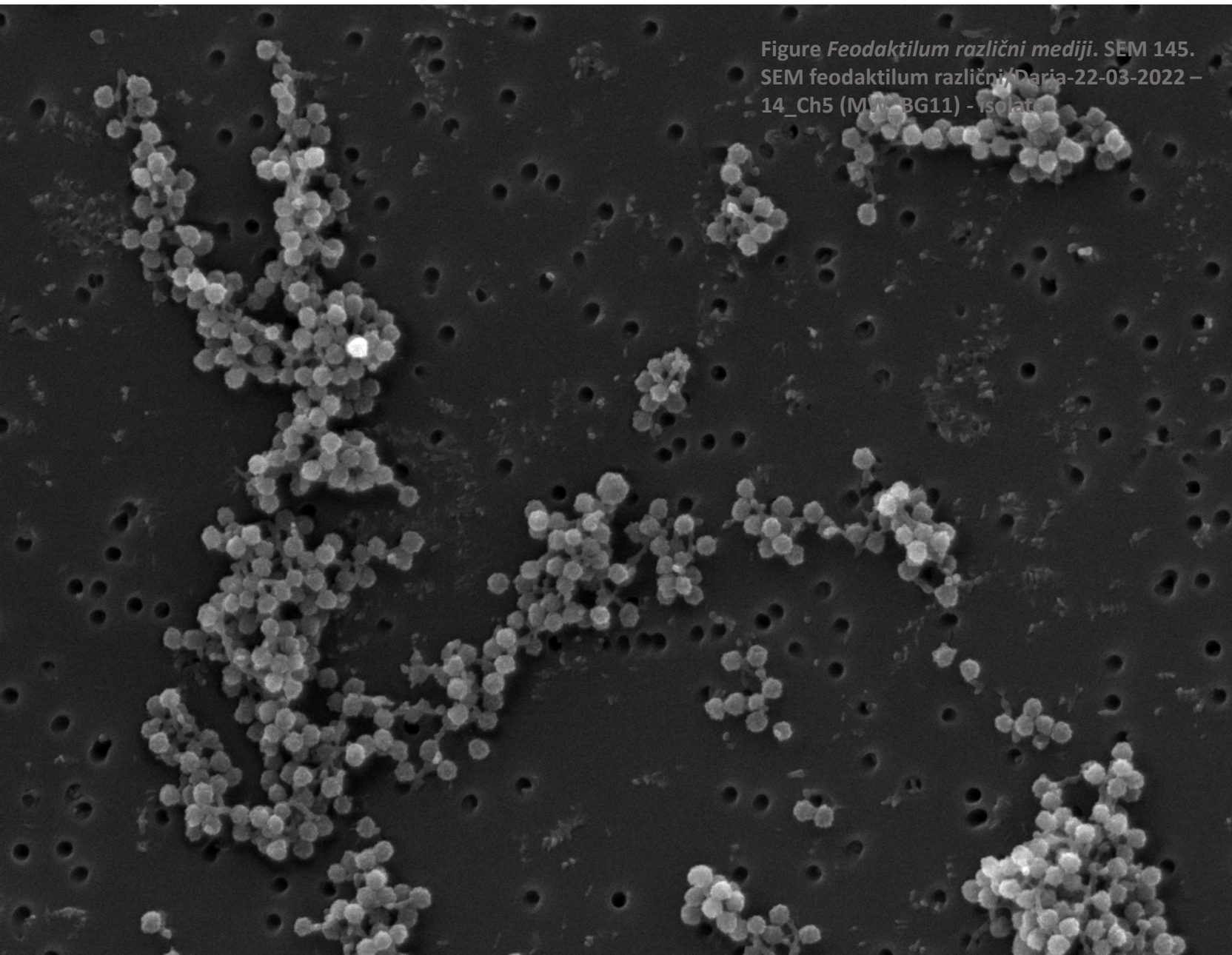


Figure *Feodactylum* različni mediji. SEM 145.
SEM feodaktilum različni/Daria-22-03-2022 –
14_Ch5 (M) - BG11) - Isolat

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 $\mu\text{mol}/\text{m}^2\text{s}$) 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).

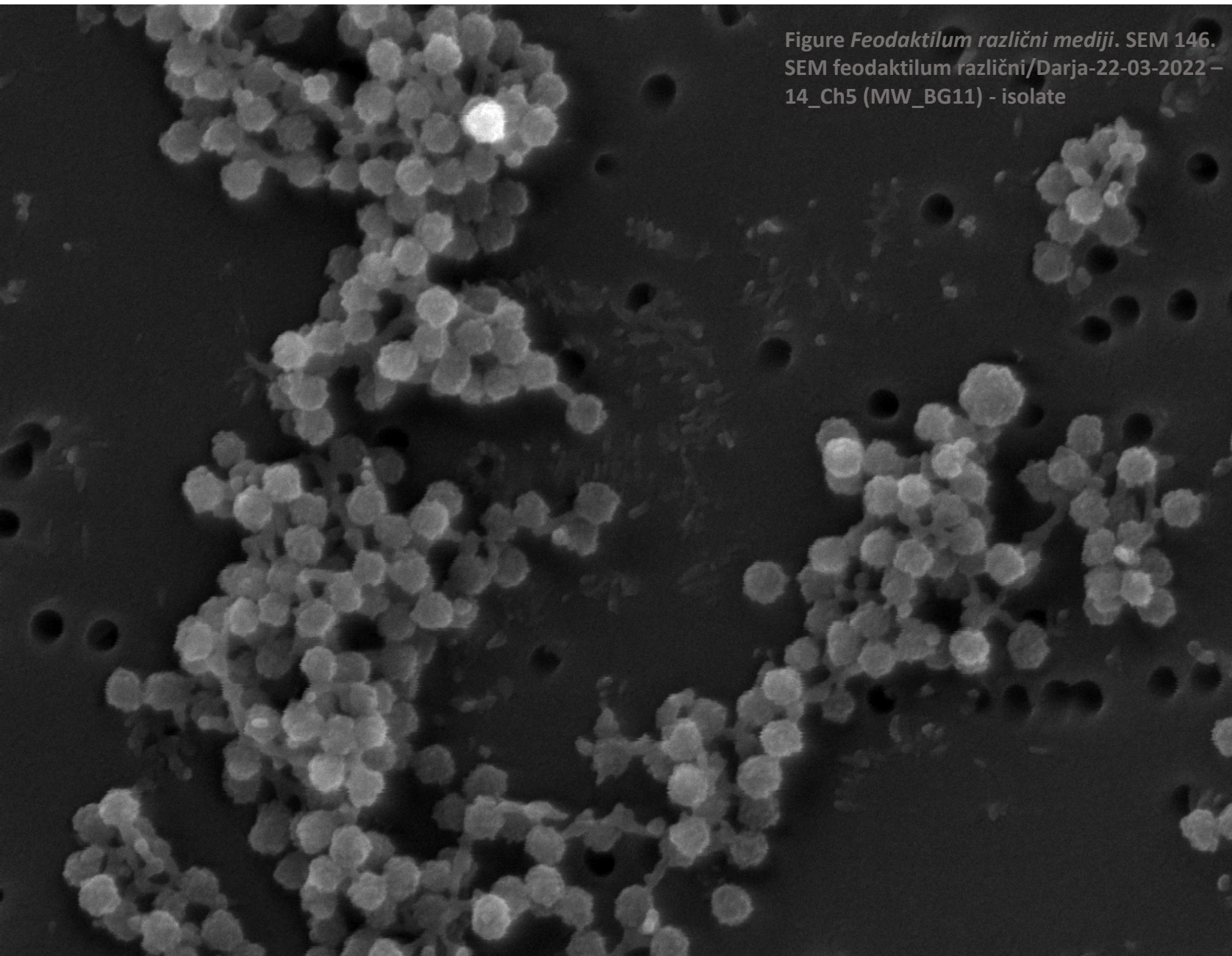


Figure *Feodactylum* različni mediji. SEM 146.
SEM feodaktikum različni/Darja-22-03-2022 –
14_Ch5 (MW_BG11) - isolate

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 $\mu\text{mol}/\text{m}^2\text{s}$) 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% OsO₄ 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).

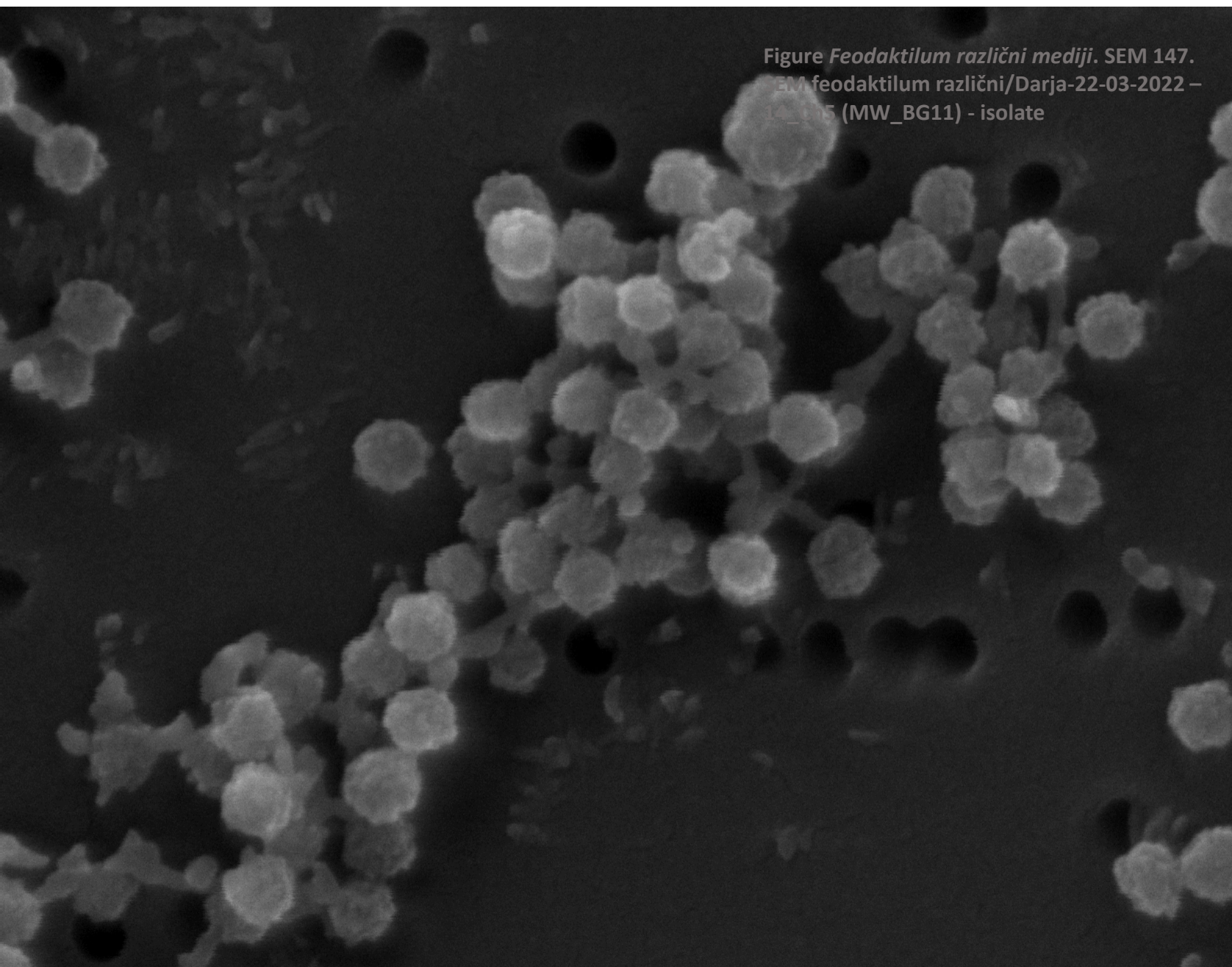


Figure *Feodaktilum* različni mediji. SEM 147.
SEM feodaktilum različni/Darja-22-03-2022 –
147_05 (MW_BG11) - isolate

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 $\mu\text{mol}/\text{m}^2\text{s}$) 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).

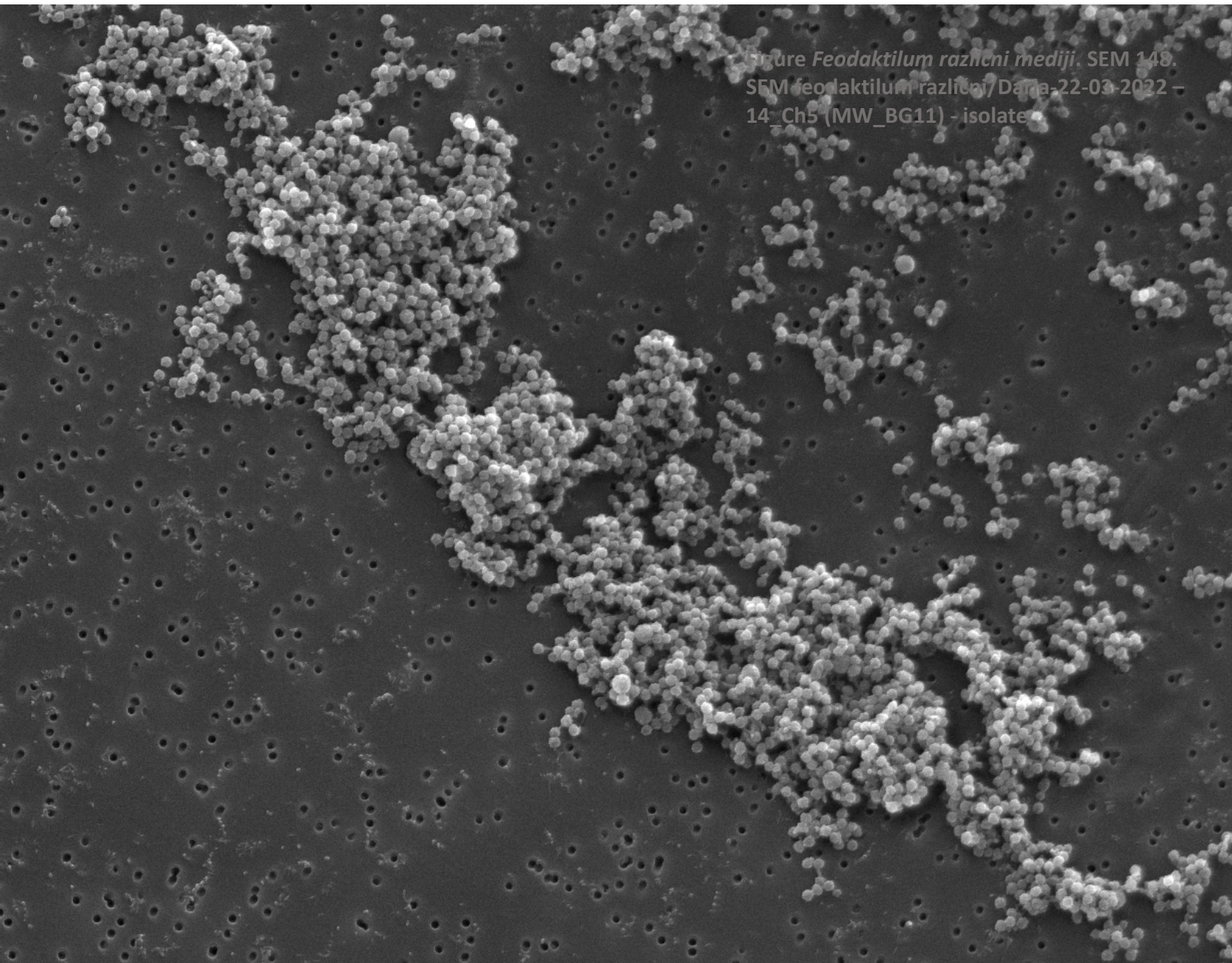


Figure *Feodaktikum različni mediji*. SEM 148.
SEM feodaktikum različni/Dana-22-03-2022 -
14_Ch5 (MW_BG11) - isolate

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 $\mu\text{mol}/\text{m}^2\text{s}$) 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% OsO₄ 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).

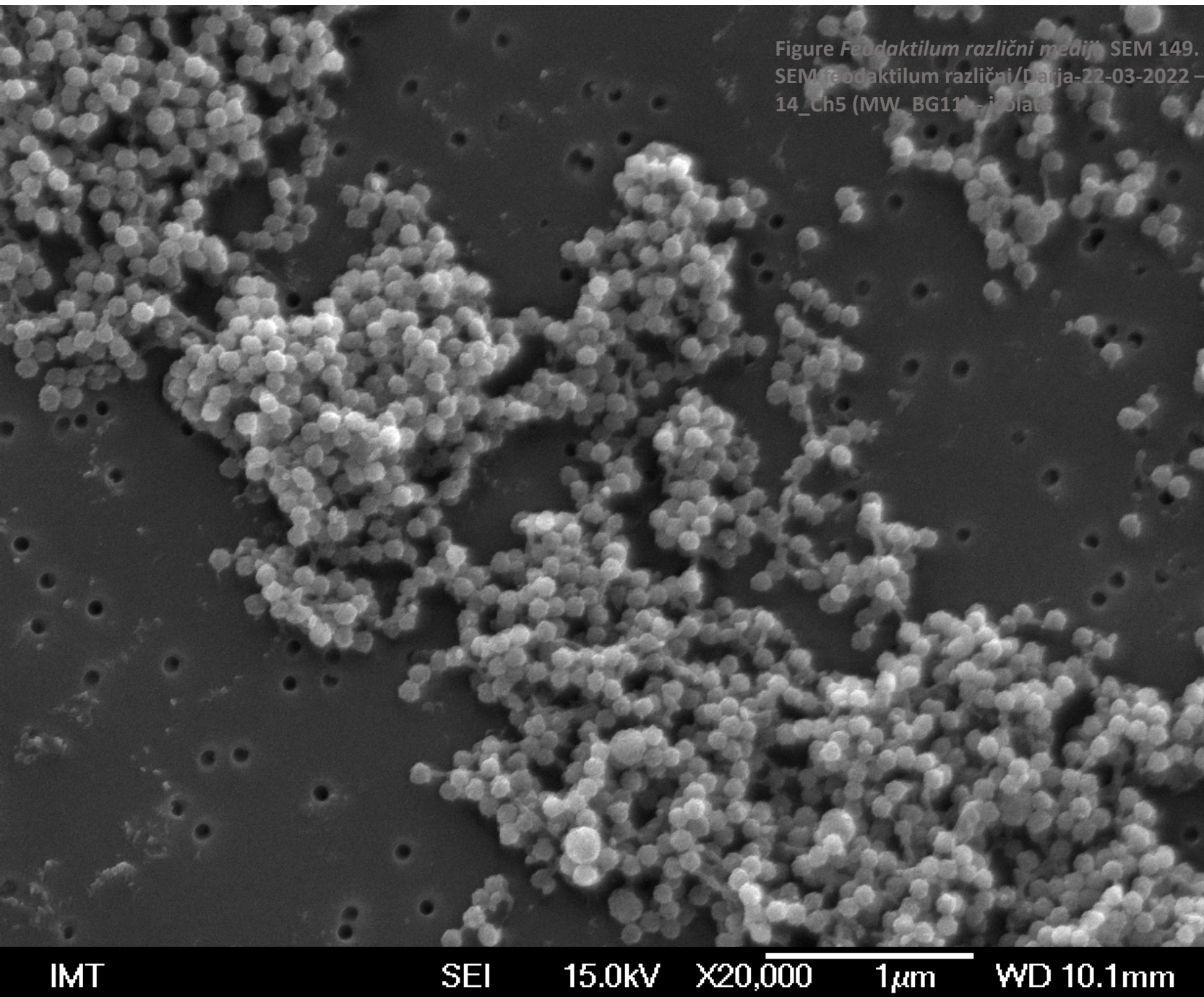


Figure *Phaeodactylum tricornutum* različni medij SEM 149.
SEM *Phaeodactylum tricornutum* različni/Darja-22-03-2022 –
14_Ch5 (MW_BG11) - isolat

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 $\mu\text{mol}/\text{m}^2\text{s}$) 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).

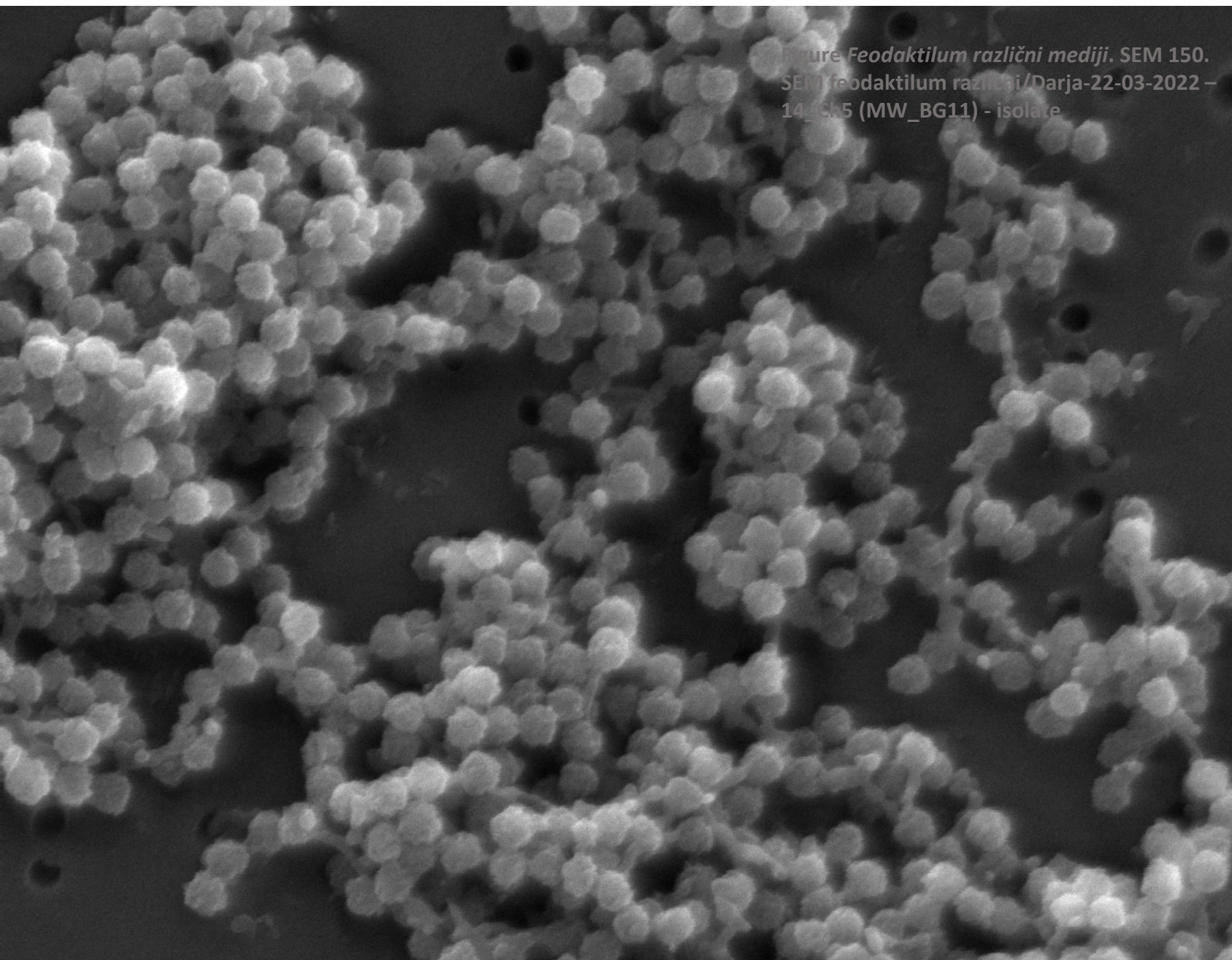


Figure *Feodaktilum različni mediji*. SEM 150.
SEM feodaktilum različni/Darja-22-03-2022 –
14_Ch5 (MW_BG11) - isolate

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 $\mu\text{mol}/\text{m}^2\text{s}$) with a 14-hour light / 10-hour dark cycle, with aeration of 0.2 L/min.

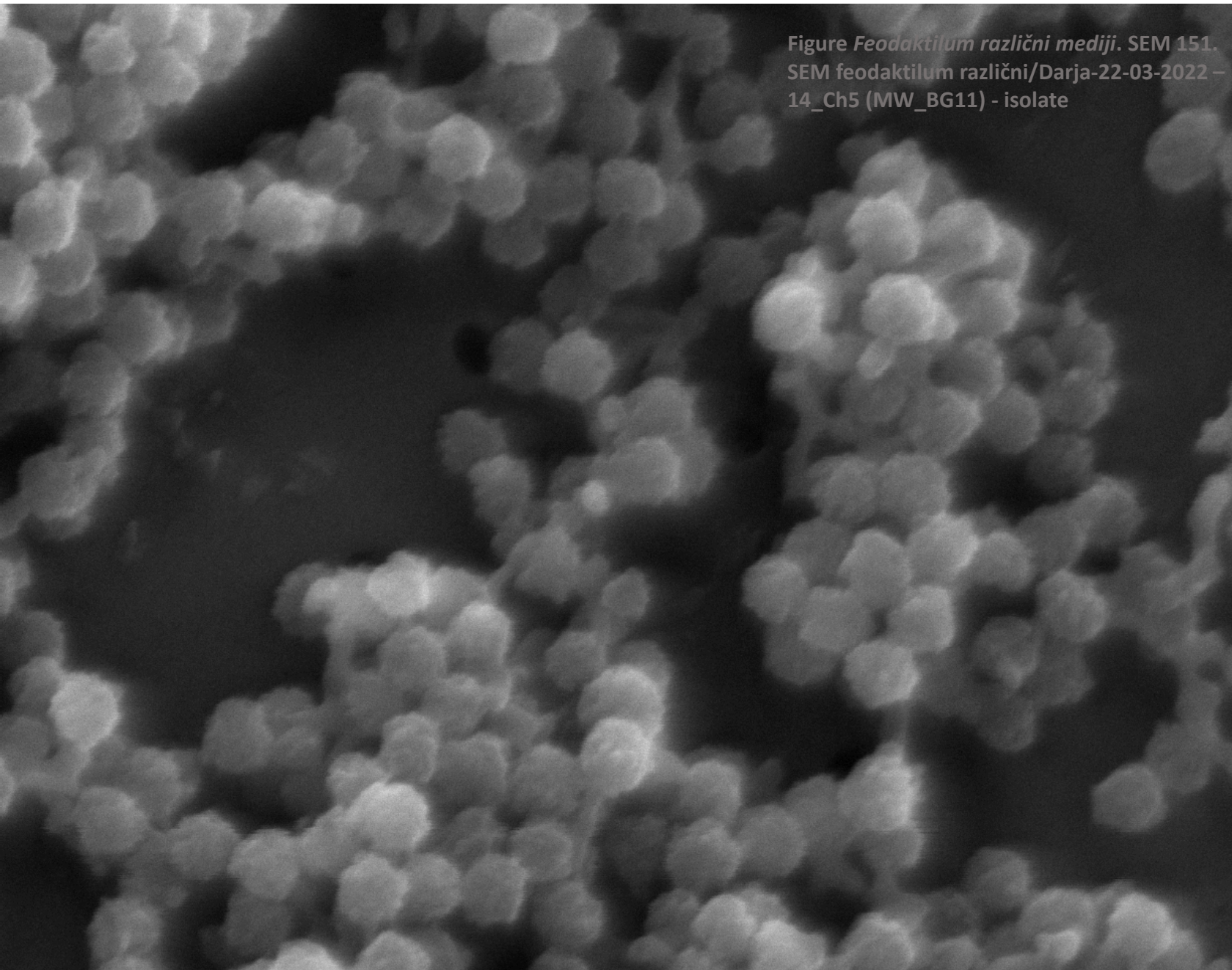
Isolation of Small Cellular Particles (SCPs)

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% OsO₄ 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 *Feodactylum* različni mediji. SEM 151.
SEM feodactylum različni/Darja-22-03-2022 –
14_Ch5 (MW_BG11) - isolate



IMT SEI 15.0kV X70,000 100nm WD 10.1mm

Figure *Phaeodactylum tricornerutum* isolate BG11 SEM 17.

Cultivation of algae

Culture of *Phaeodactylum tricornerutum* 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 $\mu\text{mol}/\text{m}^2\text{s}$) 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% OsO₄ 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).

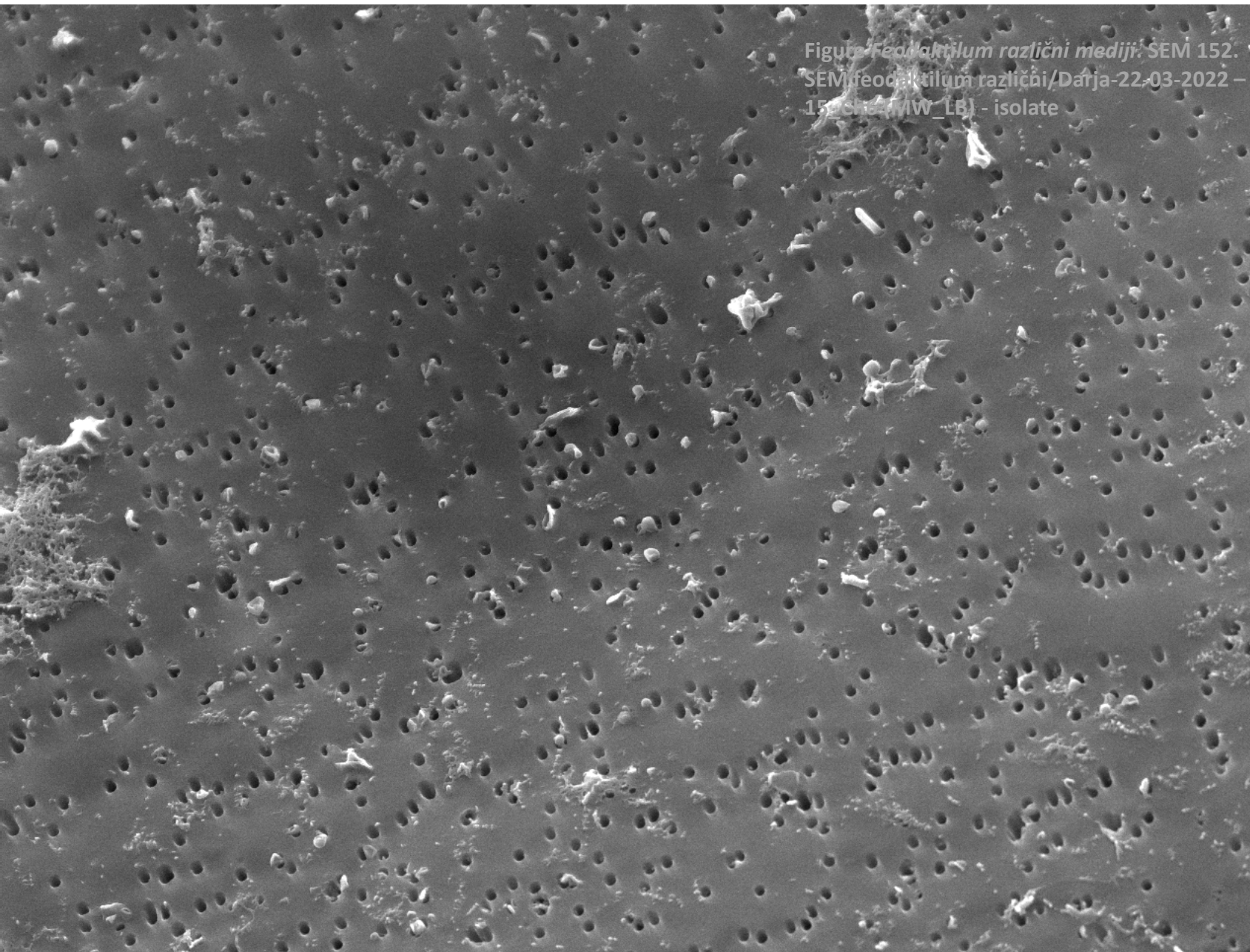


Figure *Phaeodactylum* različni mediji: SEM 152.
SEM: *Phaeodactylum* različni/Darja-22-03-2022 –
15_03_2022 (MW_LB) - isolate

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 $\mu\text{mol}/\text{m}^2\text{s}$) 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% OsO₄ 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).

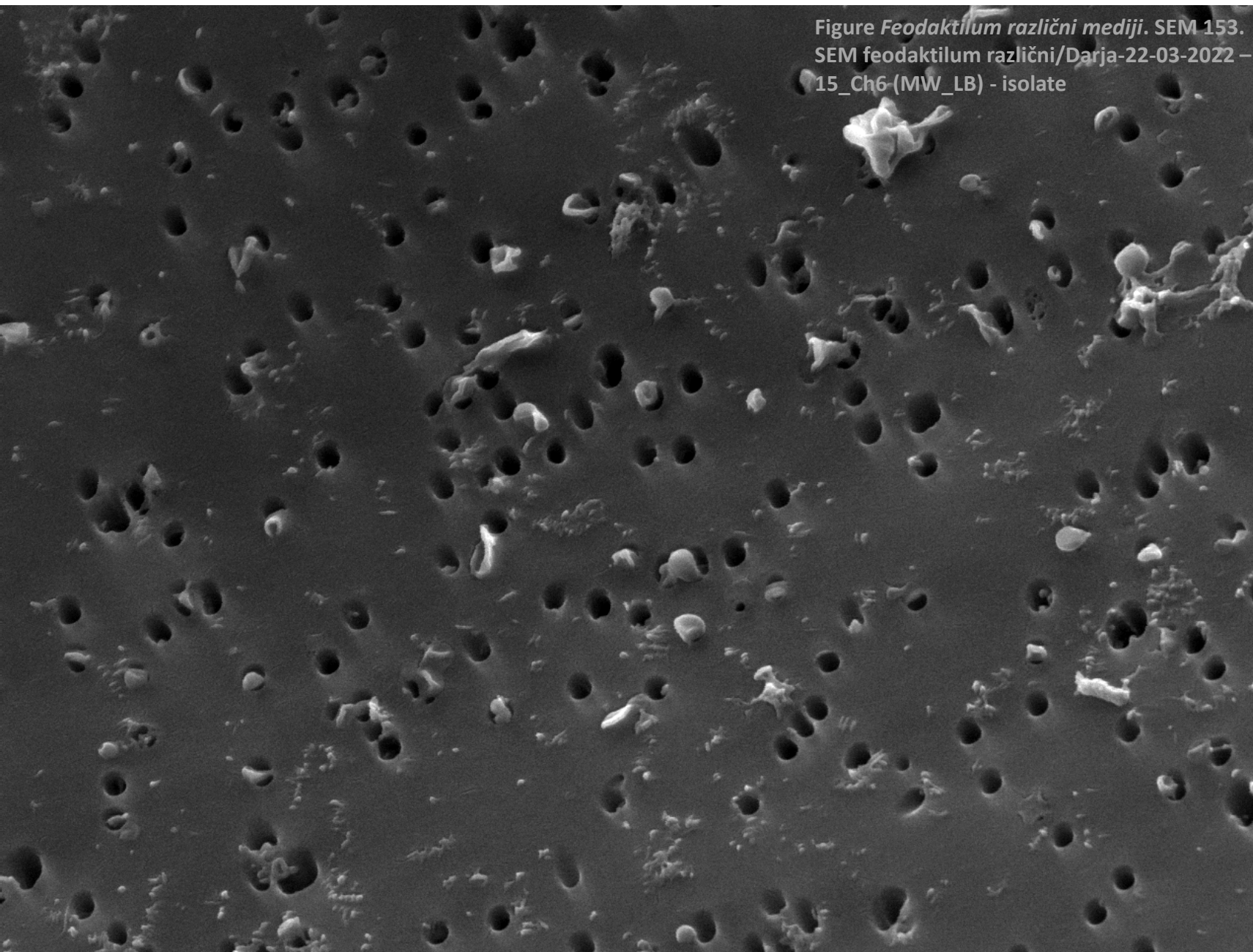


Figure *Feodactylum različni mediji*. SEM 153.
SEM feodaktikum različni/Darja-22-03-2022 –
15_Ch6-(MW_LB) - isolate

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 $\mu\text{mol}/\text{m}^2\text{s}$) 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).

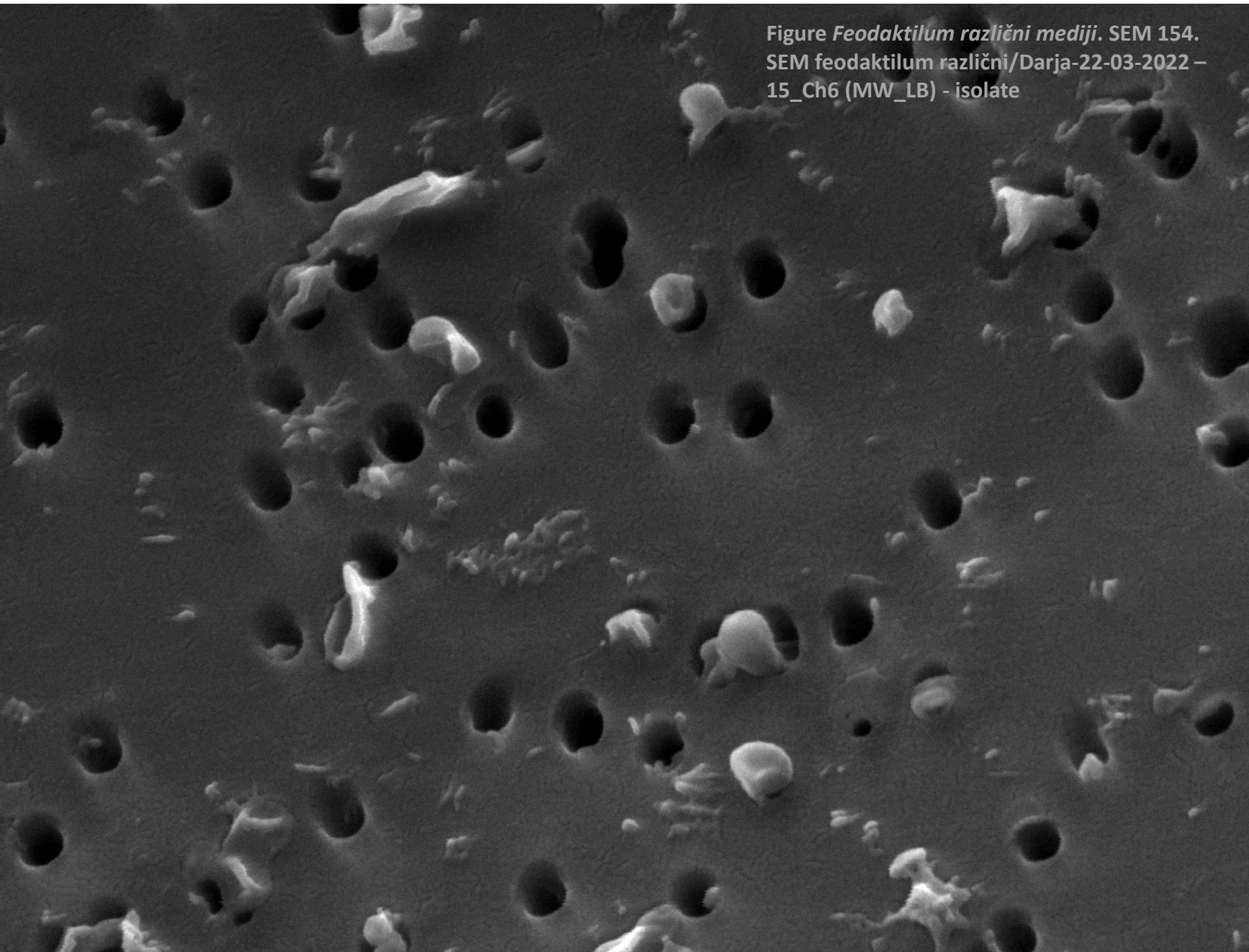


Figure *Feodactylum* različni mediji. SEM 154.
SEM feodaktilum različni/Darja-22-03-2022 –
15_Ch6 (MW_LB) - isolate

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 $\mu\text{mol}/\text{m}^2\text{s}$) 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).

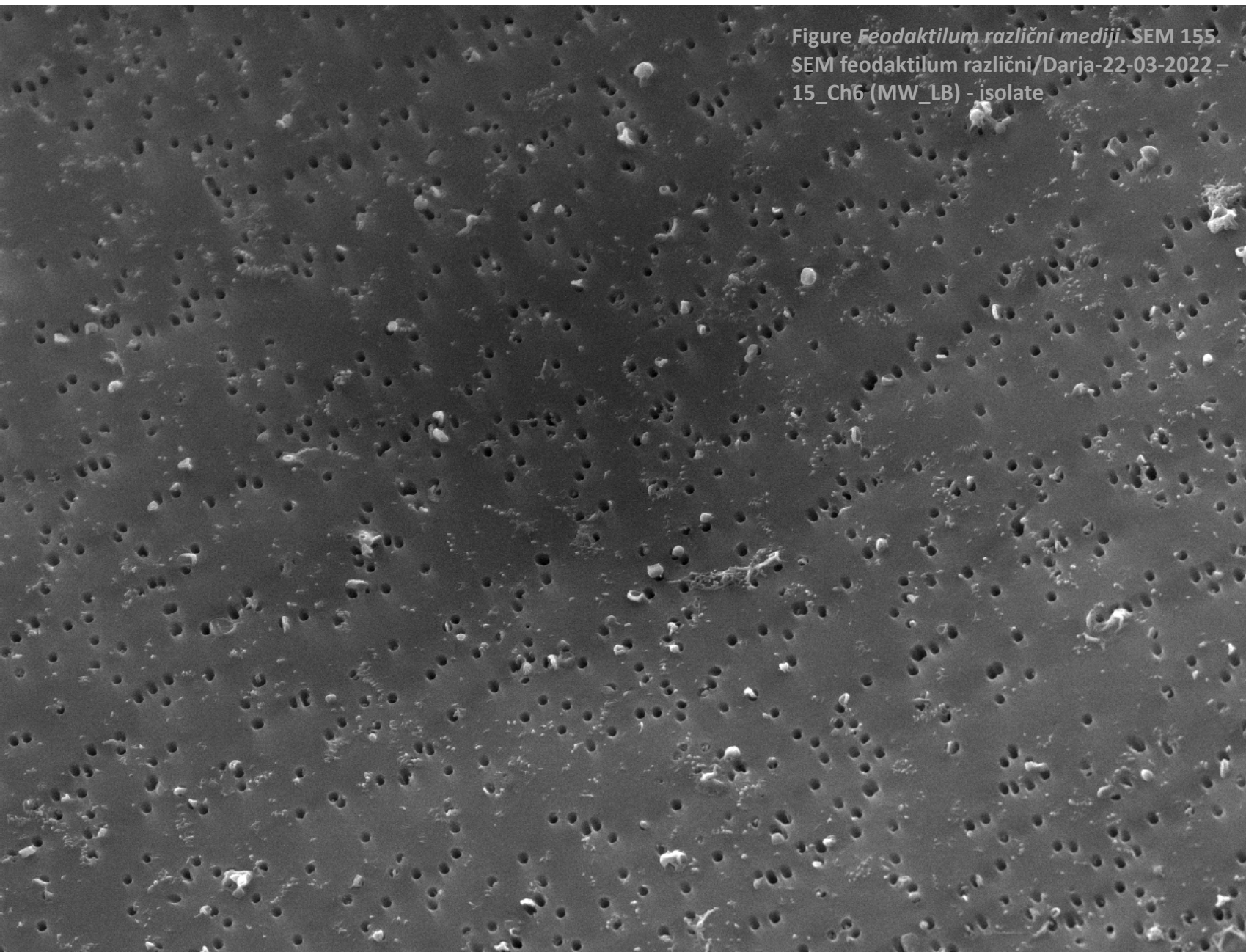


Figure *Feodaktulum različni mediji*. SEM 155.
SEM feodaktulum različni/Darja-22-03-2022 –
15_Ch6 (MW_LB) - isolate

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 $\mu\text{mol}/\text{m}^2\text{s}$) 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% OsO₄ 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).

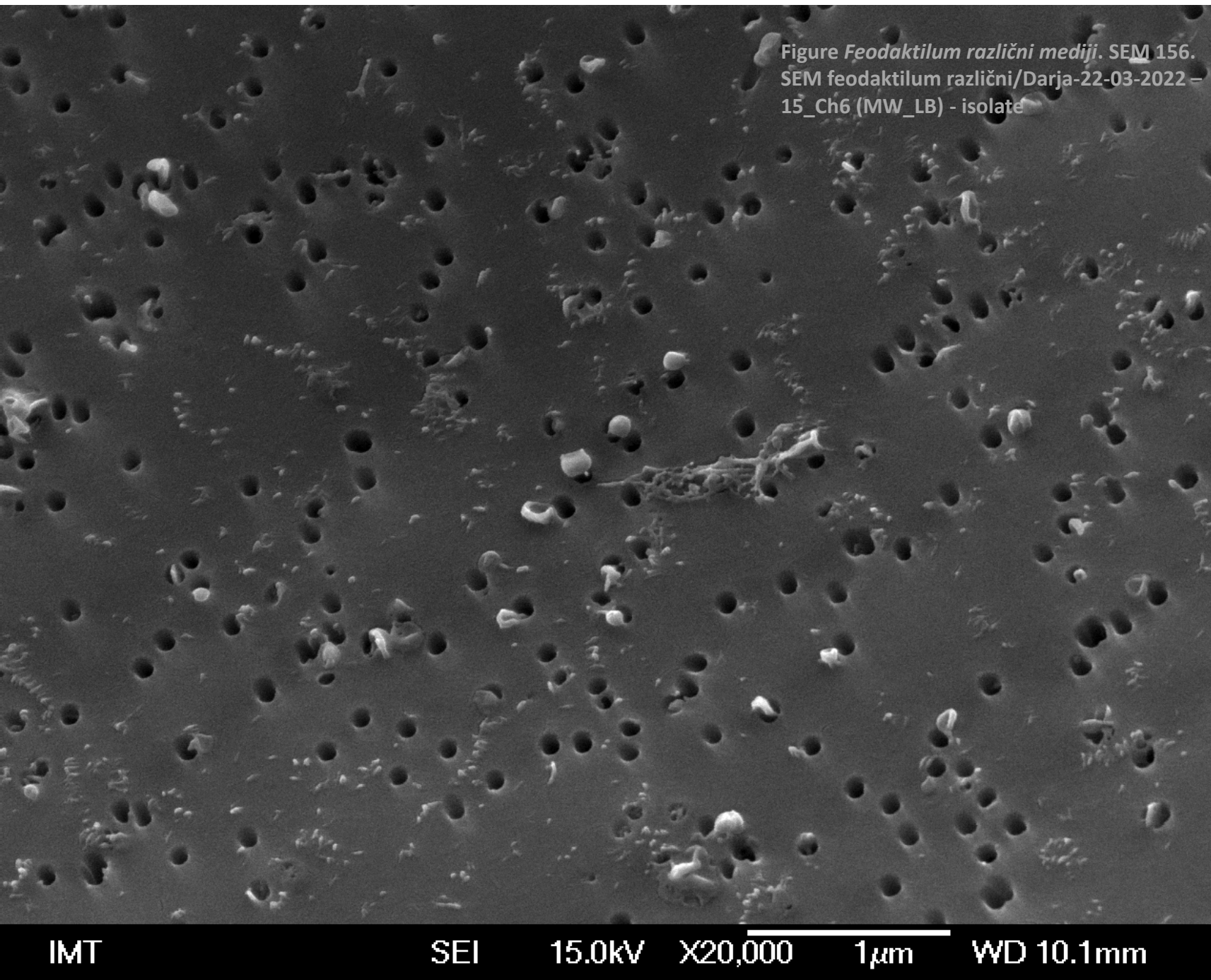


Figure *Feodaktylum različni mediji*. SEM 156.
SEM feodaktylum različni/Darja-22-03-2022 –
15_Ch6 (MW_LB) - isolate

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 $\mu\text{mol}/\text{m}^2\text{s}$) 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).