**Supplementary material**

**Plastics everywhere: first evidence of polystyrene fragments inside the common Antarctic collembolan *Cryptopygus antarcticus***

Elisa Bergami1**\***, Emilia Rota1, Tancredi Caruso2, Giovanni Birarda3, Lisa Vaccari3, Ilaria Corsi1

1 Department of Physical, Earth and Environmental Sciences, University of Siena, Siena 53100, Italy   
2 School of Biology & Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland

3 SISSI-Chemical and Life Science branch, Elettra–Sincrotrone Trieste, S.S. 14 Km 163.5, 34149 Basovizza, Trieste (Italy)

**\*Corresponding author:** Elisa Bergami; elisa.bergami@unisi.it; +390577232170; ORCID: https://orcid.org/0000-0001-8149-9584

**Materials and Methods**

**Sampling and description of the study area**

The field survey was conducted in the study area during an international expedition aimed at studying plastic pollution in Antarctica, in the framework of the “Plastic in the Antarctic environment” project, funded by the Italian National Antarctic Program (PNRA–14\_00090). The large piece of PS foam (34 x 31 x 5 cm) covered by microalgae, moss (predominantly *Sanionia uncinata*), lichens and microfauna was found in a fellfield (62°11′53.5″ S, 58°56′29.6″ W) along the shores of Maxwell Bay near the eastern end of the Fildes Peninsula, approximately 900 m from to the Antarctic station “Bellingshausen” (Russia). The PS item was placed in a paper bag and transported to the laboratory facilities of the research station “Profesor Julio Escudero” (Chile), where collembolans were collected.

The study area is at an ice-free area location of the Fildes Peninsula (King George Island, South Shetland Islands), approximately 120 km west of the Antarctic Peninsula. In the last decades the region is experiencing the highest warming conditions with respect to the Antarctic continent, which determines evident changes in the terrestrial biosphere, towards Antarctic greening [1]. In the study area, the local increase in temperature and precipitations during the summer periods has favoured the growth of moss banks in the fellfields, particularly on the soil sheltered by rocks (Figure 1), where microclimatic conditions and surface microtopography likely ensured protection for the inhabiting organisms from a variety of stresses, including limited water availability, cryoturbation and high solar radiation [2]. Along with the rapid climate change, the region is characterized by a high anthropic pressure [3], related to the activities of the several base stations present in King George Island, especially in the ice-free areas of the Fildes Peninsula [4], and some of the most accessible tourism landing sites in Antarctica [5].

**Species identification and sample treatment**

Collembolans were imaged under an optical microscope (Olympus BX51), equipped with a DP50 camera using Olympus DP-software. Body length was measured using the ImageJ software (Version 1.49, Wayne Rasband, National Institutes of Health, USA) in 18 specimens, as the distance from the last abdominal segment to the anterior margin of the head, without considering the antennae, according to [6].

In order to assess the plastic ingestion, specimens of the Antarctic collembolan (*C. antarcticus*) were extensively washed in ultrapure water and transferred (final volume of 20 µl) to glass vials (Phenomenex, lot: G107-218, 9 mm), with 225 µl of 5 mM Calcium chloride (CaCl2) solution (pH 8.0) and 5 µl of Proteinase K (Sigma P4850 from *Tritirachium album*, final concentration of 170 µg/ml). The samples were left in gentle shaking (30 rpm) in a water bath incubator at 37°C, within the optimal temperature range for the enzyme activity, for 2 days. The 230 µl solution was gently removed from the vials and the same volume of 3% hydrogen peroxide (H2O2) solution was added. The samples were kept in a water bath incubator at 65°C for another 2 days.

**Immagine che contiene animale, antropode, insetto

Descrizione generata automaticamente**

**Figure S1.** Antarctic collembolans (*Cryptopygus antarcticus*) before (left) and after (right) the sample treatment using Proteinase K and H2O2 to remove organic matter. Scale bar: 100 µm.

**Characterization by µ-FTIR**

µ-FTIR measurements were performed at the Synchrotron Infrared Source for Spectroscopy and Imaging (SISSI) beamline, Chemical and Life Sciences branch, Elettra Synchrotron, Trieste (Italy). Each sample was positioned onto a CaF2 optical window. The samples were imaged using a 64 x 64 pixel focal plane array detector (FPA) through a x15 cassegrain objective in transmission mode, granting a magnified pixel size of 2.62 x 2.62 µm. Images were acquired averaging 64 scans for each FPA tile (170 x 170 µm) with 8 cm-1 spectral resolution. Spectra were compensated for water vapour and CO2 using OPUS (Bruker Optik GmbH, Ettlingen). The integral bands for proteins (1700–1500 cm-1) and lipids (3000–2800 cm-1) were used for the visualization of the organism, while to visualize the digested PS debris we used the sharp signal at 1490 cm-1, that can be assigned to the aromatic rings of the polymer. Transmission single point measurements on isolated PS pieces (positive control), retrieved in the same sampling site as the Antarctic collembolans, were carried out with the same optical setup, using a MCT (mercury cadmium telluride) detector and a diamond compression cell (Diamond EXPress Compression Cell, ST-Japan), averaging 128 scans at 4 cm-1 spectral resolution. RGB images were obtained by assigning to a specific channel the values of the above mentioned band integrals.

**Quality control**

Sample preservation in ethanol allows for a long storage under cool conditions, without significant effects on microplastic properties or numbers [7]. For the sample treatment, all the solutions were pre-filtered at 0.20 µm and stored in glass containers, previously rinsed with filtered ultrapure water. Negative and positive controls were also included as reported in the Methods section. When possible, collembolans were transferred using glass Pasteur pipettes. Sample handling was conducted in a small laboratory under chemical fume hood and all laboratory surfaces were previously cleaned with ultrapure water and 70% ethanol. Laboratory coats (100% cotton) and gloves were worn during sample handling at the laboratory facilities of the Antarctic station “Profesor Julio Escudero” (Chile) and during all the steps of sample treatment.

**Results**

Immagine che contiene fotografia, mostrando, diverso

Descrizione generata automaticamente

**Figure S2.** Multi-panel image presenting the other springtails analysed by Fourier Transform InfraRed (µ-FTIR) microscopy. (a, c, e) optical images of the three springtails analysed and (b, d, f) the corresponding RGB composite images obtained by the integration of the IR bands: the green channel represents the protein distribution, the blue channel is used to show the lipids distribution, and in red thee areas where ingested m-PS are detected. Scale bars: 100 µm.

**References**

1. Amesbury MJ, Roland TP, Royles J, Hodgson DA, Convey P, Griffiths H, Charman DJ. 2017 Widespread biological response to rapid warming on the Antarctic Peninsula. *Curr. Biol.* **27**, 1616–1622. (doi:10.1016/j.cub.2017.04.034)

2. Block W, Lewis Smith RI, Kennedy AD. 2009 Strategies of survival and resource exploitation in the Antarctic fellfield ecosystem. *Biol. Rev.* **84**, 449–484. (doi:10.1111/j.1469-185X.2009.00084.x)

3. Braun C, Mustafa O, Nordt A, Pfeiffer S, Peter HU. 2012 Environmental monitoring and management proposals for the Fildes Region, King George Island, Antarctica. *Polar Res.* **31**, 18206. (doi:10.3402/polar.v31i0.18206)

4. Padeiro A *et al.* 2016 Trace element contamination and availability in the Fildes Peninsula, King George Island, Antarctica. *Environ. Sci. Process. Impacts* **18**, 648–657. (doi:10.1039/c6em00052e)

5. Bender NA, Crosbie K, Lynch HJ. 2016 Patterns of tourism in the Antarctic Peninsula region: a 20-year analysis. *Antarct. Sci.* **28**, 194–203. (doi:10.1017/S0954102016000031)

6. Folker-Hansen P, Krogh PH, Holmstrup M. 1996 Effect of dimethoate on body growth of representatives of the soil living mesofauna. *Ecotoxicol. Environ. Saf.* **33**, 207–216. (doi:10.1006/eesa.1996.0027)

7. Courtene-Jones W, Quinn B, Murphy F, Gary SF, Narayanaswamy BE. 2017 Optimisation of enzymatic digestion and validation of specimen preservation methods for the analysis of ingested microplastics. *Anal. Methods* **9**, 1437–1445. (doi:10.1039/c6ay02343f)