

## Research



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### Author for correspondence:

Elisa Bergami  
e-mail: [elisa.bergami@unisi.it](mailto:elisa.bergami@unisi.it)

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## Global change biology

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

Elisa Bergami<sup>1</sup>, Emilia Rota<sup>1</sup>, Tancredi Caruso<sup>2</sup>, Giovanni Birarda<sup>3</sup>, Lisa Vaccari<sup>3</sup> and Ilaria Corsi<sup>1</sup>

<sup>1</sup>Department of Physical, Earth and Environmental Sciences, University of Siena, Siena 53100, Italy

<sup>2</sup>School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland

<sup>3</sup>SISSI-Chemical and Life Science branch, Elettra-Sincrotrone Trieste, S.S. 14 Km 163.5, 34149 Basovizza, Trieste, Italy

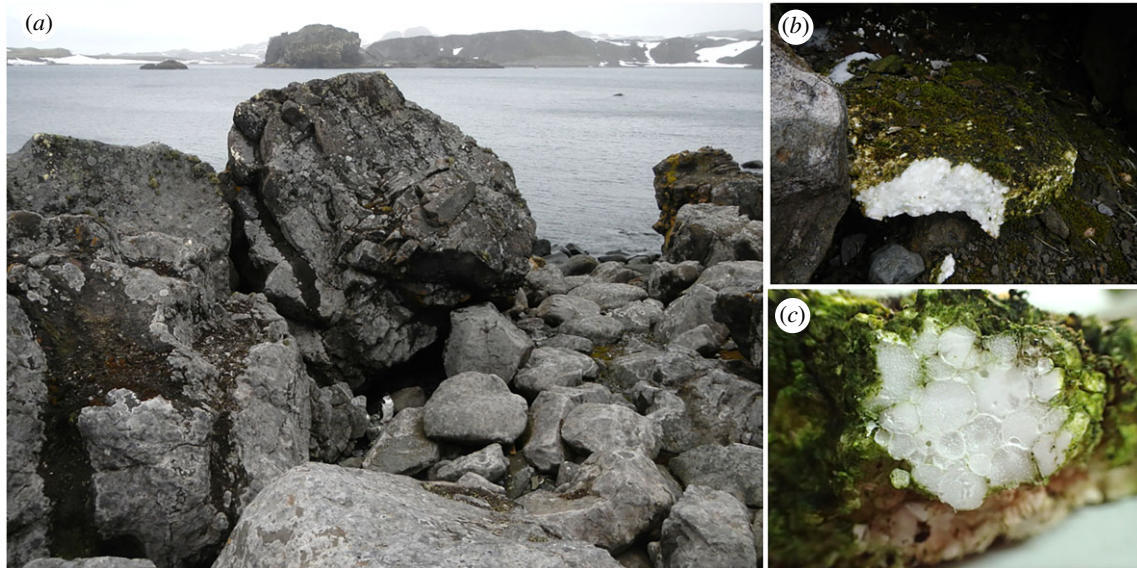
EB, 0000-0001-8149-9584; ER, 0000-0002-8235-6419; TC, 0000-0002-3607-9609; GB, 0000-0003-2418-058X; LV, 0000-0003-2355-114X; IC, 0000-0002-1811-3041

There is evidence and serious concern that microplastics have reached the most remote regions of the planet, but how far have they travelled in terrestrial ecosystems? This study presents the first field-based evidence of plastic ingestion by a common and central component of Antarctic terrestrial food webs, the collembolan *Cryptopygus antarcticus*. A large piece of polystyrene (PS) foam (34 × 31 × 5 cm) covered by microalgae, moss, lichens and microfauna was found in a fellfield along the shores of the Fildes Peninsula (King George Island). The application of an improved enzymatic digestion coupled with Fourier transform infrared microscopy ( $\mu$ -FTIR), unequivocally detected traces of PS (less than 100  $\mu$ m) in the gut of the collembolans associated with the PS foam and documented their ability to ingest plastic. Plastics are thus entering the short Antarctic terrestrial food webs and represent a new potential stressor to polar ecosystems already facing climate change and increasing human activities. Future research should explore the effects of plastics on the composition, structure and functions of polar terrestrial biota.

## 1. Introduction

Plastic pollution has become an overwhelming environmental issue on a global scale [1,2]. Small plastic fragments have been documented in virtually every ecosystem. However, most research has focused on aquatic systems, especially the marine ones, while contamination on land has been largely overlooked [3–5]. There are methodological challenges in detecting microplastics in soil and its biota, which are mainly owing to the carbonaceous nature of microplastics hampering their detection in complex organic samples [6]. Scientists have only recently been approaching the issue of plastic debris in soils [7,8] and in terrestrial food webs, mostly through bench-scale experiments [9]. The potential negative and direct effects of soil microplastics on human and environmental health remain unclear [10,11].

Plastics, as well as many globally distributed pollutants, have finally reached Antarctica. Early observations of floating or stranded macroplastics (larger than 1 cm) date back to the 1980s [12], and more recently, meso- and microplastics (1–10 mm and 1–1000  $\mu$ m, respectively; [13]) have been found in surface waters and sediments below 60° South [14–16]. Documented impacts of plastic debris on Antarctic biota mainly include entanglement [17] and ingestion by marine mammals and seabirds at sub-Antarctic and Antarctic islands [18–20]. The spreading of multiple antibiotic resistance associated with stranded plastics in the maritime



**Figure 1.** Plastic pollution in the Antarctic terrestrial environment. (a) Coastal fellfield at King George Island where the PS foam item ( $34 \times 31 \times 5$  cm) was collected. (b,c) close-ups of the PS surface, overgrown with microalgae, moss and lichens.

Antarctic has also been reported [21]. However, whether plastics are able to enter the Antarctic terrestrial food webs is still unknown.

Only a little less than 1% of Antarctica is ice-free [22], with small and isolated terrestrial and freshwater systems dominated by microbial communities, moss banks and very few species of invertebrates [23,24]. Soil microarthropods, such as Antarctic mites and collembolans, have adapted to extreme but stable environmental conditions, at least since before the Last Glacial Maximum, if not since a multi-million year age [25], and, together with microorganisms and nematodes, form simple but functional food webs [26–29].

In this paper, the presence of micro-sized polystyrene fragments (m-PS) in the gut of specimens of the common Antarctic collembolan *Cryptopygus antarcticus* is documented for the first time, the fragments originating from a large item of PS foam found stranded on King George Island (South Shetland Islands). We combined an optimized digestion method with Fourier transform infrared microscopy ( $\mu$ -FTIR) analysis, which has proved to be successful for the detection of trace amounts of plastic ingested in soil microarthropods. Plastics have therefore entered even some of the most remote soil food webs on the planet, with potential risks for the whole biota and ecosystems.

## 2. Material and methods

### (a) Sampling

The study area (figure 1a, at  $62^{\circ}11'53.5''$  S,  $58^{\circ}56'29.6''$  W) is located on the Fildes Peninsula of King George Island (South Shetland Islands, Antarctica), where a large piece of PS foam ( $34 \times 31 \times 5$  cm) covered by microalgae, moss, lichens (figure 1b,c) and microfauna was collected in February 2016 (see the electronic supplementary material for more details regarding the study area). Collembolans found on the PS foam item were carefully transferred to 70% ethanol. Likewise, millimetric foam fragments from the same PS item were preserved in 70% ethanol for characterization.

### (b) Species identification and sample treatment

Collembolans were identified to species level using the taxonomic key in [30–32]. To determine the plastic ingestion,

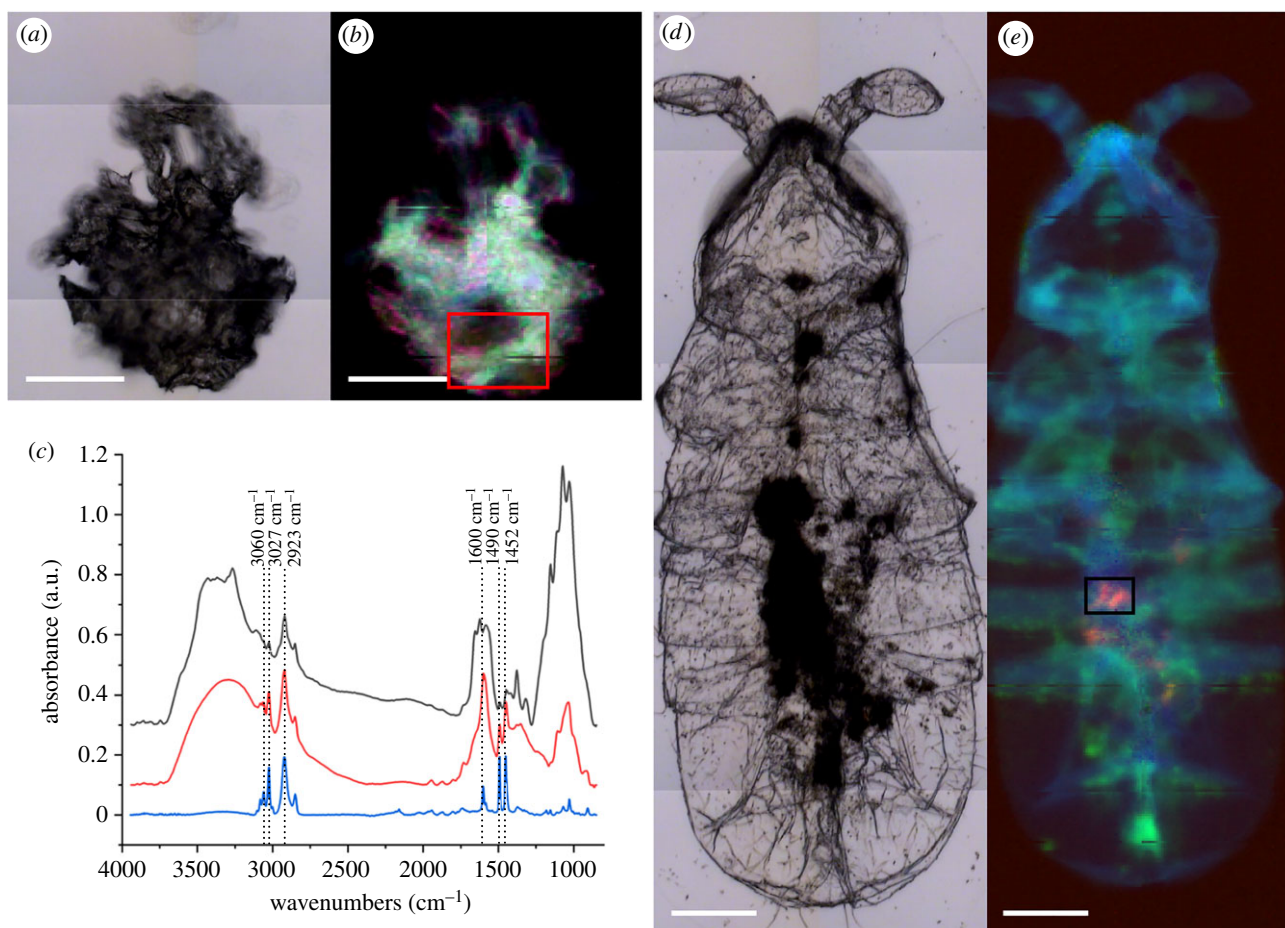
the specimens ( $n = 18$ , average body size of  $839 \pm 24 \mu\text{m}$ ) underwent enzymatic digestion followed by hydrogen peroxide treatment (see electronic supplementary material). By this treatment, most of the organic matter content was removed from the collembolan's body (electronic supplementary material, figure S1), thus reducing its optical density for infrared (IR) transmission measurements that would otherwise be characterized by strong IR absorption bands of the organic matrix [33]. Collembolans were then washed with ultrapure water and stored in clean glass vials at  $+4^{\circ}\text{C}$  for characterization by  $\mu$ -FTIR. Procedural blanks (treatment solutions only) were run as negative control of the sample treatment, while millimetric PS fragments, from the same PS item as the collembolans, were processed as positive control to determine whether the sample treatment could affect PS structure and interfere with its chemical signature. Every six samples, one negative control and one positive control were run.

### (c) Characterization by Fourier transform infrared microscopy

All processed collembolans, PS pieces from positive controls and procedural blanks were inspected under a LEICA-EZ4-W microscope at  $\times 10$  or  $\times 40$  magnification. No visible debris was found in the negative controls. Four specimens of Antarctic collembolans and two pieces of PS from the positive controls were randomly selected for  $\mu$ -FTIR transmission, performed using the Hyperion 3000 microscope coupled with VERTEX 70v interferometer (Bruker Optik GmbH, Ettlingen) (see electronic supplementary material for details). FTIR spectra were compared to the reference PS spectrum [34], showing the characteristic PS stretching mode of the C–H of the aromatic rings at  $3081\text{--}3000\text{ cm}^{-1}$ , the asymmetric and symmetric stretchings of the  $\text{CH}_2$  moieties at  $2923\text{ cm}^{-1}$  and  $2850\text{ cm}^{-1}$ , respectively, the weak overtones of the mono-substitute aromatic ring at  $1945\text{--}1725\text{ cm}^{-1}$ , and the  $1490\text{ cm}^{-1}$  and  $1452\text{ cm}^{-1}$  peaks of the C=C stretching and  $\text{CH}_2$  bending. Quality control was conducted to minimize contamination by airborne plastics (see electronic supplementary material).

## 3. Results

All sampled specimens of Antarctic collembolans associated with PS foam from King George Island (figure 1) belonged to the species *Cryptopygus antarcticus* Willem, 1901 (Isotomidae).



**Figure 2.** Detection of PS traces in Antarctic collembolans. (a) PS fragment from positive control measured by  $\mu$ -FTIR. (b) RGB image showing spectral regions of lipids (blue, 3000–2800  $\text{cm}^{-1}$ ), proteins (green, 1700–1500  $\text{cm}^{-1}$ ) and PS (red, peak at 1490  $\text{cm}^{-1}$ ). The red square indicates the pixels averaged to obtain the red spectrum in (c). (c) Comparison between PS average spectra inside the collembolan (black line), hydrated PS from the positive control (red line) and PS fragment after drying (blue line). The vertical lines identify the characteristic peaks of PS. Spectra are offset vertically for clarity. (d) Collembolan analysed by  $\mu$ -FTIR. (e) RGB image showing spectral regions of lipids (blue), proteins (green) and PS (red). The black rectangle indicates the pixels averaged to obtain the black spectrum in (c). Scale bars: 100  $\mu\text{m}$ .

The  $\mu$ -FTIR measurements on the sampled PS fragments (positive control) (figure 2*a,b*) were compared with one from literature [34], allowing to confirm the presence in our samples of the characteristic PS peaks (see spectrum in figure 2*c*, red; vertical dotted lines). The RGB image in figure 2*b* and the average spectrum in figure 2*c* (red line) show that the PS material in the positive control is not homogenous and it is embedded in a mixture of lipid (blue channel) and proteinaceous material (green channel). After pressing the same small piece in the compression cell, entrapped material is released and the PS spectrum results are cleaner (figure 2*c*, blue line).

Although collembolans were previously digested, they still presented some organic residues along the digestive tract, which hindered the detection of PS using the C–H stretching signals. Thus, the intensity of PS peak at 1490  $\text{cm}^{-1}$ , which better characterizes the plastic in the biological samples, was used to locate the m-PS inside the collembolans. By integrating this peak area, inner traces of m-PS were detected inside all the analysed specimens and in different regions of the gastrointestinal canal (fore-, mid- and hindgut). The optical and RGB composite images reported in figure 2*d* and *e*, respectively, depict one of the inspected animals, with the areas in red, orange or violet (figure 2*e*) representing PS traces. The m-PS, sized in the range of a few to tenths of microns, are located all over the length of the sample and intertwined with organic matter

residues. Optical and chemical images of other collembolans are reported in electronic supplementary material, figure S2.

From a comparison between the average PS spectrum from the positive control, obtained from a selection of red pixels of m-PS inside the organisms, and a reference PS spectrum (figure 2*c*), no peak shifts or variations in peak ratio emerge, indicating that neither the ingestion by the collembolans nor our digestion procedure altered the chemical signature of PS.

## 4. Discussion

This study presents the first clear evidence of the ingestion of plastic material by a common Antarctic collembolan, going beyond previous findings and analytical constraints on this insect group and, more generally, soil-dwelling invertebrates [35,36].

The collembolans that internalized m-PS were retrieved from a PS foam stranded along the shores of King George Island (maritime Antarctica). This substrate was largely colonized by algae, moss and lichens. We thus hypothesize that, while grazing their usual food (microalgae and lichens) [37,38], collembolans were also ingesting plastic fragments. *C. antarcticus* is an entomobryomorph collembolan ubiquitous in the maritime Antarctic [39] and often the dominant species in recently deglaciated areas, near penguin rookeries and seal

wallows [40], like the coastal fellfield sampled in our study. This hardy species evidently finds a suitable micro-environment in the PS foam overgrown with microbial mat and vegetation, as found at King George Island. *C. antarcticus* has mouthparts apt to cutting and grinding, and an overall length of the toothed region of the mandibles reaching 100 µm in the largest specimens [41]. These morphological traits and the size range of the m-PS detected inside the gut suggest a comminuting activity by the collembolans, with consequent bioerosion of the PS foam. Compared to other anthropogenic substrates, styrofoam has a highly porous structure characterized by hydrophobic cells separated by tiny channels filled with air, with the potential of absorbing a large quantity of water [42]. This may enhance the biofilm formation onto the PS surface, thus creating the conditions for grazing and ingestion. Antarctic collembolans could also have been exposed directly to m-PS released from the bulk material through weathering processes triggered by high levels of solar radiation, low temperature and interaction with ice [43].

The lipid and proteinaceous residues found on PS fragments both inside the collembolans and in field samples indicate that this polymer entered the collembolan gut along with other food material, and without alterations in the polymer, as expected. In all previous studies, the lack of evidence of plastic ingestion suggested that any negative impact on collembolans from their exposure to polymers should arise from indirect effects. In a bench-scale study, exposure of *Folsomia candida* to polyvinyl chloride microplastics for 56 days resulted in significant alterations of isotopic incorporation, body growth and reproduction as well as in gut microbiota composition. This was interpreted as owing to changes in the collembolans' micro-environment, feeding behaviour and nutrient uptake [36]. In a subsequent study [44], a strong avoidance response by the same species towards soil contaminated with polyethylene microplastics was interpreted again as owing to deteriorated soil quality, affecting in turn the gut microbiota and the fitness and activity of collembolans. However, although *F. candida* is a model organism largely used in ecotoxicology, these findings cannot be generalized to the animal group as a whole.

Further research will be needed to establish whether the ingestion of m-PS may have *per se* negative effects on *C. antarcticus*. PS can carry pathogens [45–47] and antibiotic-resistant genes [21] as well as hydrophobic contaminants [48,49], which may become bioavailable and harmful for the collembolans and related food web, once plastics have entered their alimentary tract. The analysis of collembolans collected at increasing distances from the plastic source (e.g. PS item) will help to elucidate the potential bioaccumulation in this species and the extent of the plastic contamination in Antarctic terrestrial communities.

Considering the wide occurrence of *C. antarcticus* in the Antarctic terrestrial environment, the ingestion of PS could contribute to the spreading of micro- (and likely sub-micron) PS along the food web. Different mechanisms

can be hypothesized: (i) ingestion/egestion in different locations, by comminuting and releasing chitin-encased particles, surrounded by a peritrophic membrane that slows down their degradation (i.e. zoological retarding) [50]; (ii) passive bioturbating activities, by moving and distributing microplastics attached on setae and body cuticle (including during moulting) or as an effect of the collembolan movement over the fragments [35], as already observed for fungal propagules and natural carbon-rich particles, such as biochar and charcoal [51]; and (iii) transfer along the Antarctic terrestrial trophic web, for example when *C. antarcticus* is eaten by its common predator, the Antarctic mite *Gamasellus racovitzai*, which also occurs abundantly in the studied area [52].

Our study reveals a potential risk associated with the occurrence of PS debris in Antarctic terrestrial ecosystems. The study area is located in one of the most contaminated regions of Antarctica, owing to the widespread anthropic activities related to scientific research stations, logistics (including the airport facilities and military stations) and even tourism [53]. Together with several other stressors, including chemical pollutants [53–57], plastic waste constitutes a further threat for the fragile Antarctic terrestrial communities and the recovery of a large PS item with associated soil organisms is a warning for monitoring programs in remote regions.

The fact that one of the most abundant collembolans in remote Antarctic soils is ingesting microplastics implies that these anthropogenic materials have deeply entered the soil food web, will be redistributed through the soil profile and may or have already become an integral part of the biogeochemical cycles in soils. Future research should explore the ecosystem level consequences of this additional significant global change factor that humans have imposed on natural ecosystems.

**Ethics.** The sampling of plastic debris and associated microbiota has been conducted in the framework of the 'Plastic in the Antarctic environment' project (PNRA-14\_00090), with the required authorization from the Italian National Antarctic Program.

**Data accessibility.** Further data are supplied as electronic supplemental information (one file, 'electronic supplementary material').

**Authors' contributions.** E.B. and I.C. coordinated the study and obtained financial support. E.B. conducted the sampling activities and sample treatment. E.R. and T.C. identified the collembolan species and reviewed its biology. G.B. and L.V. performed the FTIR analysis. All authors provided a significant input for the interpretation of the results obtained, drafted and revised the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

**Competing interests.** We declare we have no competing interests.

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