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Baseline

Tardigrades in the marine plastisphere: New hitchhikers surfing plastics

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SUMMARY

Tardigrades are remarkable microorganisms known for their extraordinary resilience in diverse environments, including extreme conditions such as outer space. They are known for their interactions with natural substrates in terrestrial and aquatic systems, but have remained largely unexplored in relation to marine plastics. This study aims to investigate the colonization of plastics, ranging from fossil fuel-based to bioplastics, in the coastal zones of four countries (Brazil, Ireland, France and Italy). Here, we report the first documented occurrence of tardigrades colonizing plastic substrates. We identified five amplicon sequence variants (ASVs) belonging to the Tardigrada phylum, specifically in a post-consumer polypropylene, in the coastal zone of Galway, Ireland. This discovery raises questions about the characteristics of different plastics influencing on tardigrades' adhesion. Tardigrades hitchhiking on plastics in the oceans could expand their habitat range, possibly displacing native species and altering trophic interactions, with potential consequences for the overall biodiversity.

1. Introduction

Tardigrades, or 'water bears', have been reported worldwide in the most diverse environments, including terrestrial and aquatic ecosystems (Nelson, 2002), being even able to survive in space (Jönsson et al., 2008; Rebecchi et al., 2009). Tardigrades originated in the marine environment, and present adaptability to resist extreme conditions (Kinchin, 1994; Richaud et al., 2020), which allow them to be distributed across all ocean basins, from intertidal zones to abyssal depths, inhabiting a variety of substrates (Mobjerg et al., 2011). To survive, these organisms require only a layer of water surrounding them. Tardigrades are resilient to dissection, microgravity, and radiation, being able to enter a reversible ametabolic state known as cryptobiosis (Horikawa et al., 2008; Rebecchi et al., 2009; Richaud et al., 2020). Increasing scientific interest in tardigrades over the last decade, enhanced by the recent genomic sequences available, has deepened our understanding of their diversity and remarkable survival mechanisms (Hashimoto et al., 2016; Koutsovoulos et al., 2016). Despite the research carried thus far, marine tardigrades remain largely unexplored (Mobjerg et al., 2011) and their interactions with plastics are virtually unknown.

Plastics in aquatic systems act as rich substrates covered by epiplastic organisms referred to as the "plastisphere" (Zettler et al., 2013). The marine plastisphere can harbor a wide range of taxa including microalgae, bacteria, fungi, and various invertebrates (Amaral-Zettler et al., 2020). Known for its ubiquitous distribution, from the depths of the oceans to the mountain tops (Nelson, 2002), tardigrade records were absent from plastic substrates. Moreover, only a few studies have addressed the interactions between plastics and tardigrades. Notably, Daghighi et al. (2023) recently conducted a comprehensive review on the impacts of microplastics in soil mesofauna, highlighting the limited uptake of plastic microfibers by tardigrades. In addition, tardigrades were absent from sediments treated with the addition of a microplastic mixture (Corinaldesi et al., 2022). However, the wider consequences of plastics harboring these resistant organisms and may acting on their transport across marine environments remain a mystery.

Plastics in the marine environment can potentially be a transport vector of Invasive and Alien Species (IAS). A recent report by the Intergovernmental Science-Policy Platform for Biodiversity and Ecosystem Services revealed that IAS are responsible for 60 % of recorded plant and animal extinctions (IBPES, 2023), which impact ecosystem services and might pose a threat to environmental and human health, and ultimately, the global economy. Since the first record of epiplastic organisms made by Carpenter and Smith in 1972 (Carpenter and Smith, 1972), and particularly the subsequent research conducted since the early 2000's on plastic pollution, scientists have been raising awareness and alerting to the transport of non-native species attached to the plastisphere, with its potential disruption of natural ecological boundaries. Biosecurity implications associated with drifting plastic debris reveal that these materials can not only act as a vector for invasive species, but can also be facilitators of secondary dispersal (Mghili et al., 2023) and ultimately, carry pathogens (Audrézet et al., 2021).

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2. Methods

To ensure synchronization, both setup and sampling procedures occurred simultaneously at all sites. This involved deploying stainless steel cages (81.3 L × 27.9 W x 33H cm) as anchoring structures submerged at sea (1 m depth) to house plastic containers. Each container, drilled for optimal water circulation, held plastic squares of nine different polymers (including fossil fuel-based and bioplastics), across four countries (Brazil, Ireland, France and Italy) (Fig. 1). These experiments were part of a larger project (JPI-Oceans MicroplastiX), which aimed at assessing weathering, degradation and fragmentation of microplastics in environmental conditions. Each of the nine containers had 100 1cm^2 plastic pieces, of which 196 (only from 3 polymers) were used for the analysis in this study. After a 3 months submersion period, the containers were retrieved and replaced with new ones containing pristine polymer pieces, for another trimester. This process was repeated four times over a year to ensure consistency across different seasons. In this study, the three polymer types analyzed were Low-density Polyethylene – LDPE, Post-consumer Polypropylene – PP-PC, and Polylactic Acid – PLA, sampled in triplicates at intervals of 7, 30 and 90 days within each season. The choice of polymers was based on their very different characteristics, as we aimed to compare virgin and recycled fossil fuel-based polymers (LDPE vs. PP-PC) with biopolymers (PLA).

Following sampling, plastics were preserved at −80 °C until DNA extraction. The plastic squares were rinsed in sterile 0.2 μm-filtered seawater to remove weakly associated organisms (organisms co-occurring with plastics during sampling), before DNA extraction. The total biofilm DNA of each individual plastic item was extracted using a PUREGENE kit (Qiagen), with an optimized protocol (Supplementary Material). Succinctly: 1 mL of Lysozyme (1000 U/μL) was added in the first step to increase the breakdown of cell walls, and 1 mL of glycogen on step 12 was added to facilitate the DNA precipitation; in the last step, the DNA was eluted in a lower buffer volume (20–30 μL) to increase its yield and concentration.

The extracted DNA was sent to Integrated Microbiome Resource (IMR), in Canada, for Polymerase Chain Reaction (PCR) and Next-Generation Sequencing (NGS). The rRNA 18S gene (V4 region) was amplified to assess the eukaryotic diversity. Details on library preparation and NGS can be found at the IMR's website (https://imr.bio/protocols.html). The raw sequence reads were trimmed to remove primer DNA sequences. Quality filtering, denoising, merging pair-end sequences, and calling amplicon sequence variants (ASVs) were performed using the DADA2 package version 1.8 (Callahan et al., 2016), implemented on R environment (R studio 4.2). Singleton ASVs were removed. Samples that yielded an extremely low post-filtering number of reads (< 420 reads) were removed from further analysis.

We cross-referenced the 18S amplicon dataset with the PR^2 database (Guillou et al., 2013). Detailed taxonomy was confirmed by "Basic Local Alignment Search Tool (BLAST)" against the full National Center for Biotechnology Information (NCBI/Genbank) database. A phylogenetic tree was constructed on MEGA11: Molecular Evolutionary Genetics Analysis version 11 (Tamura et al., 2021), using the method Maximum Likelihood based on the Tamura-Nei model, with the DNA sequences obtained on this study and tardigrade DNA sequences retrieved from Genbank.

3. Results and discussion

We identified five amplicon sequence variants (ASV) belonging to the Tardigrada phylum. Our results show the presence of tardigrades colonizing one PP-PC sample, collected after a 3-month incubation period during the summer season in Galway city, Ireland. Although the presence of tardigrades has been documented in other substrates, such as sediments, from all our evaluated sites (Miller and Perry, 2016; DeMilio et al., 2016; Grimaldi and D'Addabbo, 2001), it has not been possible to find a conclusive explanation for their absence in the plastisphere in sites apart from Galway in our experiment, despite the consistent application of identical sampling techniques in these locations. All ASVs, except one (ASV 5), were classified as *Isohypsibius* sp. according to PR^2 (Table 1).

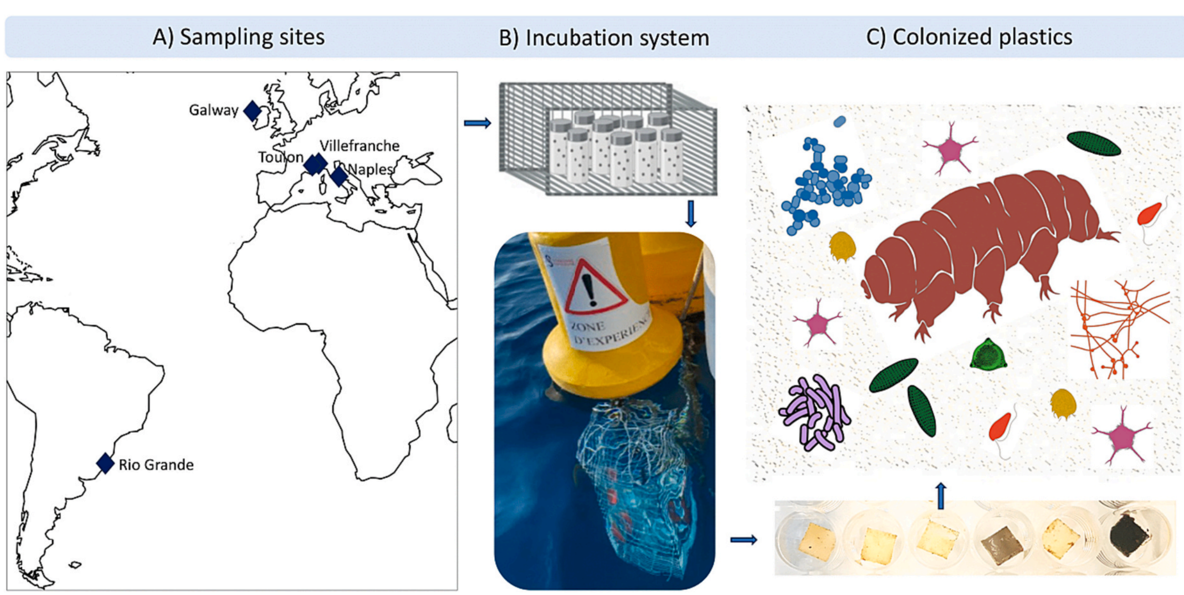

Fig. 1. A) Sampling sites of cage in situ deployment across the Mediterranean Sea (Villefranche-sur-mer and Toulon, France; Naples, Italy) and the Atlantic Ocean (Galway, Ireland; Rio Grande, Brazil). B) Incubation system with plastic containers (top), and cage deployed at sea in Villefranche-sur-mer (bottom). C) Illustration of a tardigrade and other species commonly found in our samples (top) - in blue and purple: bacteria; in pink: cercozoa; in orange: fungi; in green: diatoms; in yellow: ciliophora; in red: dinoflagellates; plastic squares after 3 months of incubation in summer season (bottom). No size scale applied. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Map created on free online map maker Canva. Plastisphere image created by the authors using source images from https://www.phylopic.org/. Silhouette images colored on PixLr Editor at https://pixlr.com/e/. Credit for silhouette images to Levi Simons, Emily Jane McTavish, Sarah Frail, Matthew Crook, Malio Kodis, and Michael Keesey.

Table 1
Comparative analysis of tardigrade Amplicon Sequence Variants (ASVs) classified based on the PR^2 database and Genbank, derived from 18S—V4 amplicon libraries of the plastisphere from the coastal region of Galway city, Ireland.

ASV number	PR^2 Taxonomy	Genbank Taxonomy	Genbank Accession	Identity (%)
ASV1	*Isohypsibius* sp.	*Eremobiotus* sp. n. 1	MK675928.1	98.18 %
		Ursulinius silvicola	MK675935.1	97.95 %
ASV2	*Isohypsibius* sp.	*Eremobiotus* sp. n. 1	MK675928.1	98.19 %
		Ursulinius silvicola	MK675935.1	97.96 %
ASV3	*Isohypsibius* sp.	*Eremobiotus* sp. n. 1	MK675928.1	98.18 %
		Ursulinius silvicola	MK675935.1	97.95 %
ASV4	*Isohypsibius* sp.	*Eremobiotus* sp. n. 1	MK675928.1	97.73 %
		Ursulinius silvicola	MK675935.1	97.50 %
ASV5	Not assigned	*Eremobiotus* sp. n. 1	MK675928.1	98.17 %
		Ursulinius silvicola	MK675935.1	97.95 %

When comparing our tardigrade ASVs against the NCBI/Genbank database, the 18S—V4 rRNA sequences we found exhibit homologies and are attributed to the Eutardigrade class. This taxonomic group encompasses marine, terrestrial, and freshwater tardigrades characterized by a cosmopolitan distribution (Nelson et al., 2018). Specifically, the highest matches were either with *Ursulinius silvicola* (Gąsiorek et al., 2019) or *Eremobiotus* sp. (Table 1), which are classified as limno-terrestrial tardigrades (Camarda et al., 2022; Pilato et al., 2011). However, a recent phylogenetic study (Mioduchowska et al., 2021) has raised concerns about the accuracy of this specific deposited sequence of *Eremobiotus* sp. (Genbank reference number MK675928.1) and other *Eremobiotus* spp. on Genbank, indicating a potential misidentification.

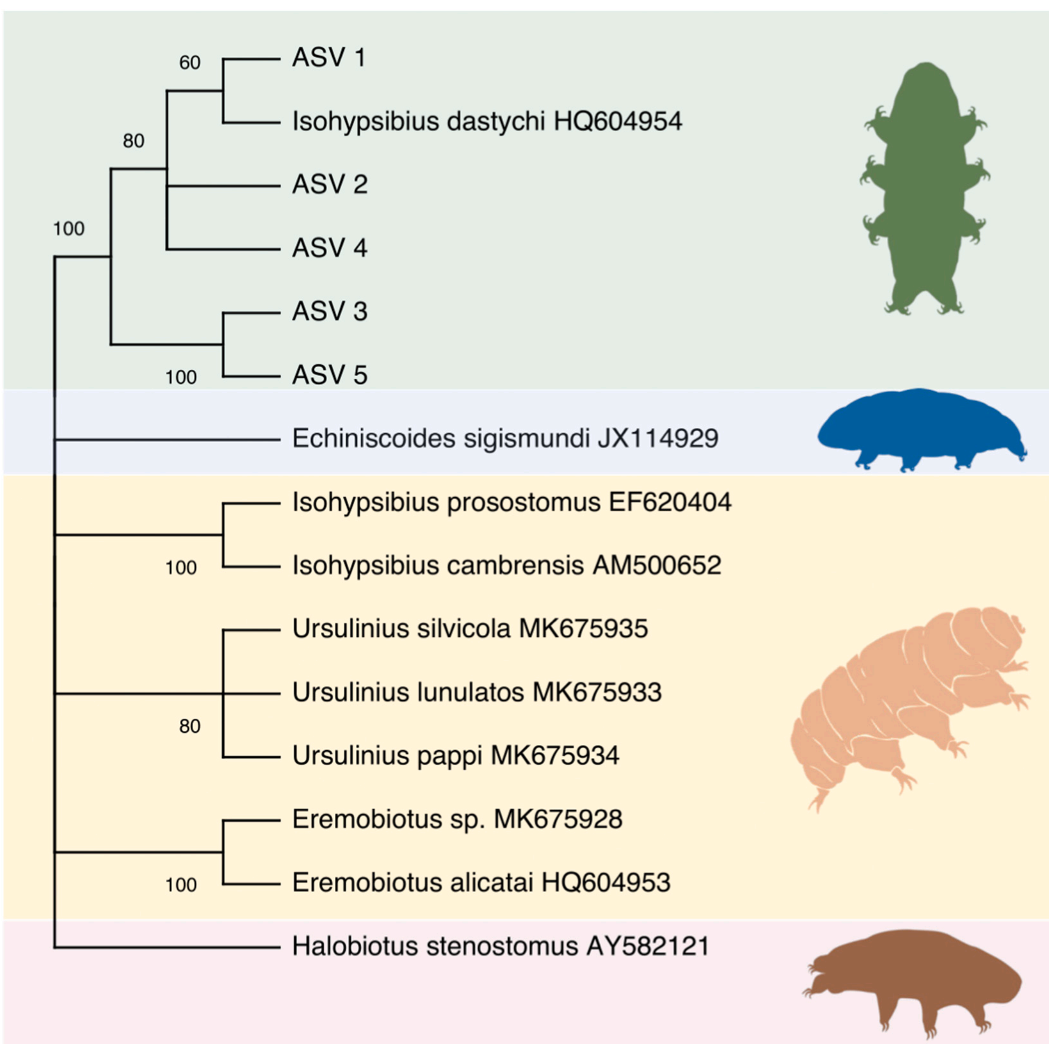

Fig. 2. Maximum-likelihood phylogenetic tree of Tardigrada Amplicon Sequence Variants (ASV 1–5) found in this study. Tardigrade species belonging to *Isohypsibius, Ursulinius, Eremobiotus, and Halobiotus genera* represent sequences retrieved from Genbank (access numbers available). *Echiniscoides sigismundi*, an Heterodardigrade, was used as an outgroup (Genbank access number available).
Image created by the authors using source images from https://www.phylopic.org/. Credit for silhouette images to Levi Simons, Michelle Site, Guillaume Dera, and Malio Kodis. Silhouette images colored on PixLr Editor (https://pixlr.com/e/).

Additionally, the taxonomy of some *Ursulinius* species, formerly classified as Isohypsibius, remains complex, considering this genus is polyphyletic, with species exhibiting diverse evolutionary lineages within the Isohypsibiidae family (Gąsiorek et al., 2019).

The phylogenetic tree (Fig. 2) shows a cluster of the tardigrade ASVs obtained in this study (ASV 1–5), with a sub-cluster of ASV 3 and ASV 5, and another cluster with ASV 1, ASV 2 and ASV 4 with *Isohypsibius dastychi* (HQ604954), a tardigrade sequence retrieved from the Genbank database. *Eremobiotus, Ursulinius* and other *Isohypsibius* species, as well as *Halobiotus stenostomus* were grouped in different clusters. The Heterotardigrade *Echiniscoides sigismundi* (JX114929) was used as an outgroup.

Our findings are a noteworthy discovery due to the fact that we found tardigrades colonizing plastics for the first time, and also for their association with recycled polymers, which are still rare in the ocean. Considering the well-documented dietary preference of tardigrades for microbial and detrital particles (Nelson, 2002), which constitute a significant portion of the marine plastisphere (Amaral-Zettler et al., 2020), it is plausible to assume that such nutritional sources facilitate their establishment within epiplastic communities in any polymer type. However, this study was carried in multiple locations and with multiple polymers, with only PP-PC from one site, in a particular season, presenting tardigrades.

As we detected five tardigrade ASVs in only one sample of the dataset, it remains uncertain whether it was an active population. Nevertheless, reporting on the occurrence of this taxon on plastics is noteworthy to see if such a resilient group can colonize the marine plastisphere. This raises the possibility that these organisms could be transported by floating plastics to locations far from their natural habitats. It is imperative to further investigate whether distinctive characteristics of various polymer types, such as virgin versus recycled, or fossil fuel-based vs. bioplastics, have any influence in the formation of biofilm, and ultimately on the adhesion of tardigrades to plastic surfaces.

Tardigrades can live associated with substrates in marine systems, where some species are semi-benthic, while others are epibenthic on subtidal algae, coral, barnacles and other organisms (Nelson, 2002). In Ireland and Northern Ireland, over fifty tardigrade species have been reported, particularly in beach sediments, in seaweed from intertidal shores, as well as in moss and lichen samples (DeMilio et al., 2016). Many species commonly found in the plastisphere usually live in close association with tardigrades, therefore it is surprising that this study is the first record of tardigrades in the marine plastisphere. Considering the wide distribution of tardigrades in various aquatic substrates, we would expect them to be more frequently found in plastic substrates in the oceans.

The lack of clarity on tardigrades taxonomic classification may have resulted in the apparent affinity of our tardigrade ASVs with limno-terrestrial groups. Should this be inaccurate and the tardigrades we identified indeed belong to terrestrial groups, possibly transported to the ocean through mechanisms such as winds and avian-mediated transport (Mogle et al., 2018), the situation becomes even more noteworthy. The communities established on plastic substrates can exhibit similarities to mosses and lichens, offering tardigrades both food and protection from grazing (Đatkauskienë, 2012). Furthermore, the degradation of plastics in marine environments, influenced by UV radiation and other bio-physical-chemical processes (Andrady, 2011), induces the development of surface irregularities on plastics. These irregularities, manifesting as cracks and holes, can serve as micro-habitats for invertebrates, including tardigrades. In this sense, further research into the presence and dispersal of tardigrades hitchhiking on plastics in the oceans is recommended.

As plastic production is expected to triple by 2050 (Geyer, 2020), the ecological implications of the plastisphere colonization by unexpected marine organisms, including tardigrades and pathogens, might represent a concern in terms of spread and distribution of invasive species. While hitchhiking on floating artificial substrates, tardigrades, which comprise cosmopolitan but also rare or endemic species (Nelson, 2002), could colonize areas outside of their natural habitat range. In light of the remarkable resilience exhibited by tardigrades to extreme environmental conditions, it is conceivable that they may successfully colonize diverse ecological niches. Such a scenario could lead to significant ecological changes, potentially characterized by the displacement of indigenous species and alterations to existing trophic interactions, affecting the overall biodiversity.

Ethics declarations

The authors declare no competing interests.

CRediT authorship contribution statement

Ana Luzia Lacerda: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **João Frias:** Funding acquisition, Investigation, Methodology, Project administration, Writing – review & editing. **Maria Luiza Pedrotti:** Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

DNA sequences for Amplicon Sequence Variants are available in the Supplementary Material.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.116071.

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