Biodiversity and evolution of italian tardigrades in terrestrial and freshwater environments integrating morphological and molecular studies

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Project background. There are still significant gaps in scientific knowledge about the ecological status and distribution of many species, especially the smaller ones, leading to a lack of conservation strategy for most of Earth's biodiversity. In Italy the biogeography of tardigrades is poorly understood at present. Some tardigrades species are known from many different parts of the world, while others are known from just one or few localities, but it is not clear whether this represents a true reflection of these distributions or merely results from insufficient collected material, in almost every case, the latter is most likely. At present, Integrative Taxonomy is the most widely used method for species description, which is based on molecular (DNA barcoding method), morphological (LM and SEM) and morphometrical analysis. However, it has limitations such as sampling difficulties and time-consuming processes for extracting and managing the animals. On the other hand, eDNA metabarcoding could be the winning strategy for studies concerning the biodiversity of tardigrades because it seems a very sensitive method that combines the identification power of a short DNA fragment with a good taxonomic resolution, with the help of innovative technologies (e.g., NGS) to identify taxa. The distribution pattern and dispersal of tardigrade species could be affected by abiotic (humidity, temperature, type of substrate, altitude, wind, chemical compounds, food availability) and biotic (life cycle, reproductive strategies, competition, predation) factors but still little is known because ecological studies at small scales are very limited. For this reason, deepening what abiotic and biotic factors allow cosmopolite species to survive in different environments than local ones, would be the key to deepen the biogeography of tardigrades.

Objectives. The objectives of my PhD project are:
i) To describe **new species** using **Integrative Taxonomy**.
ii) To investigate whether **eDNA metabarcoding** successfully captures **tardigrade biodiversity**. The captured diversity from both methods (Integrative Taxonomy and eDNA metabarcoding) will be directly compared.
iii) To investigate what **abiotic factors** (humidity, temperature, type of substrate, altitude, wind, chemical compounds) should affect most the distribution of species.
iv) To describe and compare **reproductive strategies** (physiological, morphological and behavioral adaptations) among species.

Materials and methods. *i), ii), iii)* Samples (mainly litter and freshwater sediment) will be collected from sites with different environmental characteristics (humidity, temperature, type of substrate, altitude, wind, chemical compounds). Each sample will be collected using a non-random sampling, but by identifying the areas where tardigrades are most likely to be found. Each sample will be homogenized and divided into three equal parts: one will be used for tardigrade species identification with the help of Integrative Taxonomy, the second for the eDNA metabarcoding experiment, while the third will be kept as backup. *iv)* Reproductive strategies (physiological, morphological and behavioral adaptations) will be studied through several approaches: breeding, karyotype analysis, measuring reproductive success (by the number of eggs and hatching rates), and video analysis.