Darwin’s expedition revisited to reveal the evolution of plant–microbe interactions on Galápagos

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The Galápagos archipelago is situated approximately 1,000 km from the coast of Ecuador and consists of 13 main islands and numerous smaller islets in the Pacific Ocean. The islands arose from repeated volcanic activity and are relatively young in geological terms, ranging from around 0.5 to 4 million years old. The heterogeneity of ecosystems within and across these islands, as well as their remote geographic isolation, led to the evolution of unique endemic fauna and flora. The biodiversity of the Galápagos islands showcases classic examples of adaptive radiation mechanisms, where a single ancestral species diversified into a variety of forms to survive in different ecological niches. For this reason, the Galápagos and other oceanic islands are referred to as natural laboratories of evolution.

During his visit to the Galápagos in 1835, Charles Darwin was inspired by the phenotypic fauna and flora diversity. Although Darwin is most recognized for his work on animal species, which contributed to his theory of evolution by natural selection[1], a large part of his scientific investigations included plants[2]. To date, few studies have reported the diversity of microorganisms associated with terrestrial and marine species such as iguanas, coral reef fishes, turtles and penguins[3] living on Galápagos. Studies on microorganisms associated with the endemic plants of Galápagos, such as the genus *Scalesia* (Asteraceae, Asterales and Magnoliidae) — the focus of our research — are lacking.

The giant daisy *Scalesia* comprises 15 different species and is the most iconic plant genus of the Galápagos. It grows as easily recognizable, evergreen shrubs or trees on 11 islands (Fig. 1), and it originally drew Darwin's attention due to its variation in sizes, leaf shapes and floral characteristics. These diverse leaf and flower morphologies allowed the adaptation of *Scalesia* plants to the contrasting environments of dry lowlands and humid highlands, and to different pollinators[2,4]. In this context, *Scalesia* plants have been referred to as the 'Darwin's finches' of the plant world.

The genus *Scalesia* is currently the only plant group on Galápagos that has a resolved phylogeny[4]. These analyses confirmed patterns of common ancestry and rapid speciation across the islands. Their unique evolution in harsh habitats throughout the archipelago represents a remarkable example of adaptive radiation with differentiation into various species occupying different ecological niches. We now know that 8 of the 15 species of *Scalesia* are 'island endemics', meaning they grow on only one of the Galápagos islands, possibly due to their seeds lacking a pappus (a structural adaptation to long-distance dissemination). Although several plant and animal lineages on the Galápagos and other islands (Hawaii and the Canary Islands) follow the 'progression rule' hypothesis[5], in which earlier diverging lineages are expected to occupy older islands, some radiating lineages such as Darwin's finches and *Scalesia* do not follow this rule. Altogether, this makes *Scalesia* a good model for studying the evolution of plant–microbe interactions.

The future existence of *Scalesia* species is threatened by human activities that led to the introduction of different invasive plant species that out-compete *Scalesia*. Hence, immediate action is needed to preserve and restore *Scalesia* and other endemic plant populations under threat. Plant-associated microorganisms present a novel potential strategy to improve seed germination, plant growth and health via enhanced nutrient acquisition, plant immunity and tolerance to biotic and abiotic stresses. Understanding the diversity and functional traits of the *Scalesia* microbiome could inform the design of microbiome-based strategies that can complement existing approaches developed in ongoing restoration programmes of this endangered plant species.

The *Scalesia* Microbiome Project

The 'Barcode Galápagos' project was established as a citizen science model in 2021 to characterize the genetics of all organisms on Galápagos[6]. It provided infrastructure and research training for local citizens, as well as economic and social resilience. As part of the Barcode Galápagos project, we established the '*Scalesia* Microbiome Project' (SMP). The SMP aims to characterize the microbiome (diversity, assembly, functions and evolution) associated with the roots and leaves of *Scalesia* using non-invasive sampling techniques to answer several research questions:

- Do the Galápagos soil and plant microbiomes follow specific evolutionary trajectories like their host, the *Scalesia* species?
- Are there island-specific soil and plant microbiomes?
- Are there undiscovered microbial taxa, microbial genes and functions?
- To what extent do microorganisms contribute to the seed germination, growth and stress resilience of the *Scalesia* species?
- Can soil and plant-associated microbiomes be implemented in ecological restoration programs?

The expedition

In the spring of 2023, our team of six scientists, a Galápagos National Park ranger, a film producer and documentarian, and four crew members embarked on a two-week expedition following in Darwin's footsteps. The international scientific team included microbial ecologists and plant ecologists with extensive knowledge of soil and plant microbiomes, molecular microbiology, bioinformatics, forest ecology and conservation.

We observed *Scalesia* plants, as well as other plant species, growing in volcanic soils in between lava rocks under extreme and harsh environmental conditions (for example, high temperatures and low humidity). Previous data on the geographic distribution of the different *Scalesia* species were crucial to accomplish the sampling, however, the GPS coordinates date back to studies published in the 1980s and 1990s and so many did not present the accurate position of individual plants located

Fig. 1 | Expedition journey to characterize the *Scalesia* plant microbiome across the Galápagos islands. Schematic map depicting the different Galápagos islands and distribution of the *Scalesia* species. *S. baurii* is also present on Wolf Island (not shown). Credit: Carlos Gavela (Archipiélago Films) and Mateo Davila (USFQ). A video of the expedition can be found on YouTube.

by previous researchers. This issue prevented us from sampling individual plants present in remote sites and scattered over extensive areas, such as *Scalesia microcephala* on the volcanic top of one of the younger islands, Fernandina.

Nonetheless, we successfully collected 12 *Scalesia* species (*S. affinis*, *S. aspera*, *S. atractyloides*, *S. baurii*, *S. stewarti*, *S. divisa*, *S. helleri*, *S. incisa*, *S. pedunculata*, *S. retroflexa*, *S. crockeri* and *S. villosa*) across 8 islands (Floreana, Fernandina, Isabela, Pinzón, San Cristóbal, Santa Cruz, Santa Fé and Santiago) (Fig. 1). We were also successful in sampling individuals of the closest *Scalesia* relative, *Pappobolus* spp., on the mainland of Ecuador during a separate field effort in the same period. From each site, we sampled bulk soils for physicochemical and microbiome analyses, plus leaves and roots of the 12 *Scalesia* species for metagenomic analysis and microbial culturing to establish the 'Galápagos Microbial Repository'.

Scalesia microbiomes as a component of ecological restoration

Human activities that introduce new species pose a serious threat to endemic species such as *Scalesia*. Several ongoing initiatives by the Galápagos Science Center, Galápagos Conservancy, Charles Darwin Foundation and the Galápagos National Park Directorate focus on understanding the impacts of introduced organisms, and controlling and eradicating invasive plant species to conserve *Scalesia* diversity[7,8]. However, restoring the *Scalesia* forests is challenging as invasive species, such as raspberry (*Rubus niveus*) and orange cestrum (*Cestrum auriculatum*), have high growth rates and large seed banks in the soil, which can survive for long periods[9].

Recent scientific investigations revealed that *Scalesia*, specifically *S. pedunculata*, is highly threatened by another invasive tree, *Cedrela odorata*. This introduced tree from the mainland employs allelopathy, the release of toxic chemical compounds into the soil, to compete with *Scalesia* for resources, thereby inhibiting its germination and growth[10]. We hypothesize that the soil invaded by *C. odorata*, where *S. pedunculata* used to grow, has altered its microbiome composition, which in turn may have contributed to the disappearance of this *Scalesia* species. Understanding the taxonomic and functional diversity of microorganisms associated with soils and *Scalesia* plants growing in undisturbed and disturbed environments (that is, threatened by invasive species) will be instrumental for the design of microbiome-based strategies to prevent establishment and growth of invasive species while promoting recolonization by *Scalesia* species, which could be a future conservation project focused on endangered plant species. For example, transplantation of soil harbouring beneficial microorganisms associated with healthy *Scalesia* plants may improve seed germination and enhance seedling growth and survival rates. Culture collections established by the SMP will serve as microbial repositories, helping to document and conserve microbial biodiversity of the Galápagos, and could also be used to inform microbiome-based restoration strategies. This is particularly relevant for *Scalesia gordilloi* or *S. retroflexa*, which exhibit very low abundances across the islands, ranging from a hundred to less than twenty individuals, respectively.

Conservation of *Scalesia* species and restoration of *Scalesia* forests, as found in the highlands of Santa Cruz, is crucial for ecosystems associated with these plants, such as endemic invertebrate and bird species. Thus, we expect these initial efforts to profile the taxonomic and functional diversity of the microbiome of *Scalesia*, the Darwin's finch of the plant world, will generate insights into the evolution of microbiomes of endemic plant species beyond *Scalesia*. Furthermore, our study will reveal whether these microbiomes follow similar patterns of adaptive radiation and whether they diversified taxonomically and/or functionally into a composition that benefits, at least in part, the survival of the corresponding host plant species in their distinct ecological niches. Revisiting Darwin's expedition may also lead to the identification of undiscovered microbial taxa and the evolution of functional genes across islands ranging from only 0.5 to 4 million years of age. Importantly, we hope that studying the plant microbiome in this unique environment will provide fundamental insights into the biology, ecology and evolution of this symbiosis.

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Competing interests

The authors declare no competing interests.