Seed dispersal by vertebrate frugivores is a mutualistic interaction in which the animals benefit from eating the edible pulp of the fleshy fruits while the plants benefit from the movement of their seeds. Frugivores ingest fruits, transport seeds in their guts, and regurgitate or defecate them in conditions that may be suitable for germination.

This mutualism involves, for example, up to 98% of tropical rainforest or 60% of temperate woody plant species. Understanding the dispersal vector species for different plant species is crucial to assess the consequences of species extinctions and to foresee the potential collapse of forest regeneration due to loss of key dispersal services.

A methodological issue that has limited advances in our understanding of seed dissemination by frugivorous animals and its consequences is identifying the frugivore species that dispersed the seeds sampled in field studies.

On one hand, obtaining direct observations of frugivores feeding on fruits and using different landscape sectors is often complicated owing to the elusive character of the species or to the presence of dense vegetation. On the other hand, visually identifying frugivore species from faecal remains is impossible whenever they belong to closely related or similar-sized species.

Specific identification is a crucial aspect to determine which frugivore species contribute to critical dispersal events (e.g., long-distance dispersal) that allow the connectivity of plant populations and the colonization of non-occupied habitats. Ultimately, specific identification is necessary to understand the particular role of different frugivores in the dynamics and regeneration of the vegetation.

“Now, DNA barcoding opens new research avenues for frugivory and seed dispersal studies...”
We have described DNA barcoding protocols (GuSCN-silica based, for degraded DNA) successfully applied to bird-dispersed seeds sampled in the field. During the winter of 2013–2014, we periodically sampled seeds of fleshy-fruited plants in seed traps that were placed across different microhabitats of Mediterranean woodland. Avian DNA was extracted from the surface of defecated or regurgitated seeds (i.e., remains of avian digestive tissue), allowing the identification of the frugivore species that contributed each seed dispersal event. Disperser species identification was based on a 464-bp mitochondrial DNA region (COI: cytochrome c oxidase subunit I). The obtained sequences were identified using the BOLD identification engine.

Now, DNA barcoding opens new research avenues for frugivory and seed dispersal studies as it provides a non-invasive technique that allows quantifying frugivory and seed dispersal mutualistic networks, assessing the contribution of each frugivore species to seed rain in different microhabitats, and testing whether different frugivore species select different fruit/seed sizes. DNA barcoding is thus applied to identify interactions, rather than species.

Given that seeds are sampled at the end of the dispersal process, this method enables linking the identity of the disperser species responsible for each dispersal event to plant traits (e.g. fruit/seed size) and environmental features (e.g. habitat/microhabitat of destination). It is precisely this bridge between frugivory and seed deposition patterns at the individual seed level which was unavailable to traditional field studies.

In conclusion, DNA barcoding can be used for characterizing the functional value of specific frugivore species within diverse mutualistic assemblages, opening new avenues to identify critically central interactions sustaining the web of mutual interdependencies between animals and plants.

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German Barcode of Life: Detailing Achievements in Taxonomic Coverage and Data Accessibility