

DNA BARCODING OF WILD ITALIAN DENDROFLORA: FIRST EVIDENCES OF APPLICATION IN *OLEACEAE*, *FAGACEAE* AND *ACERACEAE*

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DNA barcodes have been proposed as a molecular tool that would provide species identifications and as a way to accelerate the discovery of new species. Barcodes are short segments of DNA (<800 bp) that can be used to uniquely assign an unknown specimen to a species, particularly when diagnostic morphological features are absent, or insufficient. The advantage of this approach might be particularly relevant to cope with the fundamental crisis facing biodiversity, as a standardized, species-level identification tool for biodiversity assessment, life history and ecological studies.

In order to be useful, a DNA barcode sequence must not only easily produce PCR amplifications with universal reaction conditions and primers, but also contain enough variation to generate unique identifiers at either the species or population levels. Zoologists selected a mitochondrial gene (CO1) as the standard barcode for animals, but the slow evolutionary rate of plant mitochondrial DNA required an alternative solution. The plastid genome shares many of the desirable attributes, however, the rate of evolution is generally slow, and to find a plastid region that is sufficiently variable for DNA barcoding is a non-trivial problem.

A number of different chloroplast regions have been proposed, but at present there is no standard protocol for DNA barcoding of land plants. Different plant barcoding regions have been suggested.

Aim of our research is to identify the DNA barcode of wild Italian dendroflora. We selected two options among the ones proposed: rpoC1+matK+trnH-psbA and rbcL+trnH-psbA. We have started to test their applicability in 3 different plant groups of the Italian wild dendroflora: *Oleaceae*, *Aceraceae* and *Quercus* spp. (*Fagaceae*). These plants are largely widespread in the Italian peninsula and together collect about 30 (well-)acknowledged tree species, each of which plays an important role in the conservation of numerous land ecosystems, in the wood trade, and in the definition of biogeographic processes in the Mediterranean area. Nevertheless, tree species identification may pose serious difficulties, due to the high individual morphological plasticity and to the high levels of inter-specific crossability, which make hard to discriminate closely related taxa, contributing to the production of ambiguous rankings, and giving rise to multiple, often contrasting, taxonomies.

Ease of amplification with universal primers and reaction conditions were determined for each of the proposed markers in the three plant groups. New primer pairs were developed where needed. Generated sequences were compared and used to discriminate taxa. To this purpose, one to five individuals per 28 species were analysed. To evaluate the extent of intra-specific variation, samples from biogeographic regions other than the Italian peninsula were evaluated also. The level of sequence divergence, analyzed for each locus, confirmed the non-coding trnH-psbA intergenic spacer as the most variable region and highlighted the potential discriminatory power at level of species discrimination. However, the three plant groups responded differently to the analyses; some species of the quercus group were invariant at each of the five loci tested. The possible explanations for the lack of sequence variation are several (very low rates of sequence evolution, taxonomic misidentification, recent divergence or hybridization), and probably a nuclear marker will be needed to correct identification and description of the species considered in the present study.