

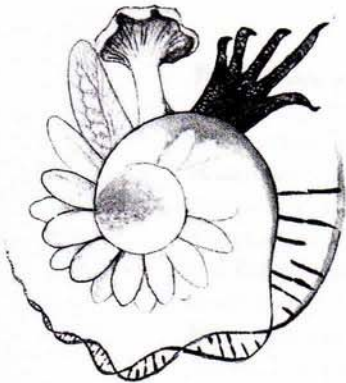
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**EMBO** Workshop



ACCADEMIA NAZIONALE DEI LINCEI

CENTRO LINCEO INTERDISCIPLINARE  
"BENIAMINO SEGRE"



# Molecular Biodiversity and DNA Barcode

**Accademia Nazionale dei Lincei**

**Rome, 17-19 May, 2007**

*Final programme - Abstracts book*

## • DNA barcoding in truffles: from taxonomy to an oligonucleotide barcode

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Truffles are fungi which accomplish their life cycle living in symbiosis with the roots of some plants. Some truffles have a remarkable commercial value, like *Tuber melanosporum* from Perigord and *T. magnatum* from Alba: due to its taste and fragrance, *T. magnatum* is one of the most expensive delicacies of the market (300-400 /100g -winter 2006). The name "truffle" is a broad term that comprises species of the genus *Tuber* belonging to the Ascomycota, order Pezizales and family Tuberaceae. The Index Fungorum recognizes 227 species, subspecies and varieties of truffles, however the *Tuber* classification based on the morphological features of ascomata and spores has led to many controversy. Currently, only 70-75 species have been validated in the world, 32 out of them in Europe. In the frame of a long-standing project, we investigated the phylogeny of the truffle species with a particular interest on Chinese species and set up an oligonucleotide DNA barcoding for their identification.

In the first part of the project we investigated the phylogeny of *Tuber* sp by sequencing several genes such as internal transcribed spacer (ITS) of the rDNA repeat, beta-tubulin, elongation factor 1 alfa, protein kinase C. Our analysis of a total of 32 species coming from Europe, Asia and North-America allowed us to identify 8 groups of species. Using the molecular clock theory we estimated the common ancestor for truffle around 250-300 Mya.

In a second step, we identified several species-specific oligonucleotides (or motifs), located within the ITS. The barcodes has been developed for five *Tuber* spp. (*T. magnatum*, *T. melanosporum*, *T. aestivum*, *T. indicum* and *T. mesentericum*) on the bases of 526 ITS sequences available in GeneBank and allowed a reliable in silico identification of these *Tuber* spp. The last step of the project involves the development of a phyloarray, which is currently under construction. Thanks to this array, where species-specific motifs are spotted, multiple *Tuber* species will be detected at the same time. This new identification tool will allow to identify truffles in complex matrix such as mycorrhizal roots and soil.