Recording and mapping the Mediterranean mycota: the case of Sicily (S Italy)

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Abstract. The assessment of fungal diversity in the Mediterranean area is considered by mycologists as a priority target. In recent times a number of associations, commissions and international networks recognized fungi as important tools to be included in research projects. The newly established Mediterranean Mycology Working Group with the European Mycological Association could be the focal point for increasing mycological research in the Mediterranean area. According to its strategic position in the Mediterranean Basin, Sicily could be considered a centre of diversity and a reference area. The evaluation and exploitation of fungal biodiversity in Sicily is illustrated here.

Key words: mapping, Mediterranean fungi, recording, Sicily

Introduction

In the last 15 years a number of scientific institutions, mycological societies and mycological amateur groups have paid more attention to recording, mapping and conservation of fungi. Presently the scientific community is engaged in recognizing the important role of fungi and in emphasizing that fungi are important organisms to be preserved from decline. At the fourth conference in Valencia (Spain, 2004), the Planta Europa Network undertook a review of the European Plant Conservation Strategy and Critical Targets. Target 6 for 2007 was the development of a provisional European Plants and Fungi Red List, depending on the performance of the IUCN European Plant Specialist Group.

At European level, some relevant activities were carried out by the newly set European Mycological Association and the European Council for the Conservation of Fungi.

The conservation of fungi is a very important tool and conservation strategies must be based on knowledge of the taxonomy of fungi, distribution patterns and field ecology. Inventories provide an idea of fungal diversity, mapping programmes should yield useful information about the rarity of species, their eventual decline and their heritage value. Inventories and mapping form the basis for publication of the Red Data Lists (Courtecuisse 2001).

In Italy the efforts of the Working Group for Mycology of the Italian Botanical Society were mainly addressed to the publication of the Checklist of Italian *Basidiomycetes*. The checklist is the result of an agreement between the Italian Ministry of Environment and the University of Tuscia in Viterbo, largely implemented and revised within the framework of programs launched by the Italian Ministry of Education, Universities and Research. The huge number of data to be managed to compile a list of the entire Italian mycobiota opted for a start only with the *Basidiomycota*. In fact macrofungi *Basidiomycetes* are more easily recordable and are usually collected and identified by a large number of mycologists.

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In the above-mentioned checklist, most of the records are reported at the level of species, only few as subspecies and many as varieties and forms. Each specific and infraspecific name is reported as binomial or trinomial, followed by the author's name and synonyms.

The most important nomenclature sources were the Dutch Checklist of Arnolds & al. (1995) and the CABI Bioscience Database of Fungal Names (http://www.indexfungorum.org/Names/Names.asp).

In the Checklist of Italian Fungi, the term Rare used for 295 taxa was not based on IUCN categories, since long-term observations are lacking. The distribution in each region is also reported, along with legislative data, if available, and 4296 taxa are listed on the basis of 22757 records (Onofri 2005). In terms of the Italian regions and the corresponding number of recorded fungi, Sicily makes stands out as one of the richest regions of Italy.

According to *Flora Europaea*, Sicily, the small surrounding islets and the Malta Archipelago are considered as a distinct floristic district and areas of high phytogeographic interest in the Mediterranean region. The vascular and cryptogamic floras of Sicily result from the convergence of different floristic elements which may coexist in a diversity of environments.

The vascular flora of Sicily had been well investigated from the 17th century onwards. The number of native species in the vascular flora amounts to ca. 2700 taxa, belonging to 137 families and 800 genera. Many authors also pointed out the correspondence between Sicilian, North African and Middle East floras.

The vegetation types of Sicily belong to the "Mediterranean zone", with six recognizable different vegetation belts. The vegetation belts are characterized by different forest ecosystems such as Mediterranean maquis, mixed oak woods and woods of *Quercus ilex* L., *Q. suber* L., *Castanea sativa* Mill., *Fraxinus oxycarpa* M. Bieb., and *F. ornus* L. From 1200 to 2000 m a.s.l. *Q. petraea* (Matt.) Liebl. and *Ilex aquifolium* L., *Fagus sylvatica* L., *Betula aetnensis* Raf., and *Pinus laricio* Poir. woods are present on the Madonie, Nebrodi, Peloritani Mts, and on the Etna volcano. A wide part of the area is covered by mixed afforested woodlands, with *Pinus*, *Eucalyptus* and *Cupressus* species.

Woody areas cover ca 10 % of the territory and are mainly concentrated in the hinterland and on the mountains.

In contrast with the interest evinced in the vascular flora and, consequently, the huge number of related scientific papers, cryptogams in general and fungi in particular were for a long time ignored by the researches. The starting point of modern mycological investigation in Sicily was the publication of the *Checklist of Sicilian Fungi* (Venturella 1991) based on literature and field data.

In 1991, the mycological flora of Sicily was quantitatively represented by 1532 species and 32 varieties, both of macromycetes and micromycetes, referred to 189 families and 496 genera. These data were recorded, together with 248 scientific papers published between 1814 and 1990.

At that time Mount Etna and the northern mountain chains of the Caronie and the Madonie Mts were moderately explored. Thanks to the studies of a mycological amateur Catanzaro, under the supervision of Prof. Ceruti at Torino University, the islet of Pantelleria was the most investigated area, along with the neighbourhood of the small town of Mazara del Vallo in the Trapani province.

Until 1991 the most investigated provinces were Catania, Palermo, Trapani and Messina, while the number of fungi recorded during the investigations in the other provinces was negligible.

The increase of mycological investigations in the last ten years permitted the evaluation of the number of fungi in different forest ecosystems and substrata and the biodiversity of Sicilian fungi within the whole territory. A huge number of taxa were recorded in oak woods, beech woods and artificial forests, such as conifer and chestnut woods.

In Sicily, the influence of climate on fructification of basidiomata and ascomata is evident and sometimes the emergence of species considered rare at local and European level, or related strictly to the Mediterranean area, corresponds to the unusual climatic conditions.

In such cases application of the term Rare is incorrect and we prefer to use the term Infrequent.

The finding of *Entoloma plebeioides* (Schulz.) Noordel., *Amanita tarda* (Trimbach) Contu, *Amanita pseudolactea* Contu, and *Colus hirundinosus* Cav. & Séch. set a good example of the influence of climatic conditions on the growth of infrequent species. The climatic trend of the year 2001 in Palermo has been characterized by a long drought period from June to mid-November. Afterwards temperatures drastical-

ly dropped down, followed by an unusual number of rainy days. Such unexpected climatic variation, very unusual for a region like Sicily characterized by a thermo-Mediterranean climate, could have probably brought about *E. plebeioides*, a species never collected before and considered rare and restricted to North Europe only (Venturella 2002).

The Laboratory of Mycology in Palermo started another investigation into the neglected group of lignicolous fungi, both *Aphyllophorales* and *Corticiaceae*. More than 250 taxa have been recorded so far in different forest ecosystems and on a huge number of woody substrata (Saitta & al. 2004).

In 1997 the Working Group for Mycology of the Italian Botanical Society proposed a preliminary list of 23 species of macrofungi, believed to be threatened in Italy. These taxa were referred to the IUCN category K, a category not yet considered in the last edition of the IUCN criteria (Venturella & al. 1997).

According to the preliminary red list and the investigations carried out in Sicily, 32 taxa should be considered Infrequent in Sicily and only two taxa as Rare: *Rhodotus palmatus* (Bull.) Maire and *Pleurotus nebrodensis* (Inzenga) Quélet.

Recent additions to the number of Sicilian fungi are represented by 232 taxa in the province of Trapani, 185 taxa in the province of Agrigento and 83 taxa in the province of Caltanissetta. Furthermore, over 1000 taxa were recently reported for the province of Palermo (Venturella & al. 2005). The mycological investigations of the eastern sector of Sicily are still in progress.

The methodology of mapping is based on the sheets of the Italian Official Map System, at a scale of 1:50000. Each sheet records the name of the more representative locality and a three-digit code and is divided into 64 subunits. The methodology adopted permits the publication of distribution maps for each recorded species.

Case studies

1. *Genus Daldinia* Ces. & De Not.

The taxonomy of *D. concentrica* s.l. has to be re-evaluated although such species have been known for a long time and is regarded as common in its area of distribution. It is now generally accepted that *D. concentrica* occurs primarily in West and North Europe, in temperate regions, and its stomata are preferentially encountered on *Fraxinus* species. Reports on its occurrence in Sicily and the Canary Islands gave impetus to evaluate these specimens, resulting in the recognition of *D. macaronesica* and *D. martinii*, respectively (Stadler & al. 2004).

The current results indicate that diversity within *Daldinia* is probably much higher than ever anticipated.

Daldinia martinii was found in Sicily to colonize fallen branches of *Quercus suber*, a Mediterranean oak species, as well as the trunks of pecan trees (*Carya olivaeformis* Nutt.) of American origin at the Botanical Garden in Palermo. Hence, it is difficult to decide whether the species was eventually introduced to Sicily from outside Europe, or if it had invaded the garden from Italian hosts. In the former case it might well be more widely distributed in the oak forests and plantations of Mediterranean Europe; if introduced along with an alien host plants, this species might be encountered among specimens previously characterized as other taxa of *Daldinia* from outside Europe.

Daldinia raimundi was originally reported on *Quercus ilex* in Sicily as *D. concentrica pro parte*. This report gave impetus to study materials from Sicily in comparison with the authentic materials of *D. concentrica*. One of those indeed turned out to be completely in agreement with *D. concentrica* with regard to both its anamorphic and teleomorphic features. *D. raimundi* was found to differ from other specimens and other records of *D. concentrica* in its teleomorphic and anamorphic morphology and in the more conspicuous ornamentation of its ascospore perispore by SEM.

2. *Pleurotus* on Umbelliferous plants

There are various species of *Pleurotus* in Sicily, with good organoleptic qualities, which grow as saprobes on the root residues of various Umbelliferous plants. The *P. eryngii* species complex includes populations of choice edible mushrooms, growing in the Mediterranean area in close association with different genera of plants of the family *Apiaceae*.

The study on *Pleurotus* growing on Umbelliferous plants is oriented towards the analysis of isolates samples, so as to define groups within the population, to delimit species, to look for phylogenetic relationships among the taxa, to exploit the biotechnological poten-

tial of selected fungi, and to promote ecosystem conservation and sustainability of natural resources.

Five taxa with different host plants were described: *Pleurotus eryngii* (DC. : Fr.) Quélet var. *eryngii* on *Eryngium campestre* L., *P. eryngii* var. *elaeoselini* Venturella, Zervakis & La Rocca on *Elaeoselinum asclepium* (L.) Bertol. subsp. *asclepium*, *P. eryngii* var. *ferulae* Lanzi on *Ferula communis* L. subsp. *communis*, *P. eryngii* var. *thapsiae* Venturella, Zervakis & Saitta on *Thapsia garganica* L., and *P. nebrodensis* (Inzenga) Quélet on *Cachrys ferulacea* (L.) Calest.

Each taxon is characterized by different habitat specialization and allochrony. Among the recorded taxa *P. nebrodensis* on *Cachrys ferulacea* is the only taxon that grows at an altitude higher than 1400 m and with a fructification period concentrated within approximately 40 days between the end of April and the first decade of June.

Research is carried out on the elucidation of taxonomy within the *P. eryngii* group, aiming to obtain biological material ready to be exploited for breeding purposes. Field surveys of the *Pleurotus* taxa growing on Umbelliferous plants were carried out in Sicily and each taxon was evaluated in respect to its taxonomical, morphological, anatomical, distributional, and ecological characteristics.

Pleurotus nebrodensis, sub *Agaricus nebrodensis*, was described by Giuseppe Inzenga in 1863. After that publication the author's suggestion of *A. nebrodensis* as a distinct species was confirmed by Elias Fries. Subsequently, many authors demoted it to synonymy, or reduced it to an infraspecific rank, but this interpretation was not confirmed by Zervakis & Venturella (1998).

Pleurotus nebrodensis has been known since ancient times, and the present-day natural product, somewhat difficult to find, is sold on the Madonie territory (northern Sicily) at a price between Euro 50 to 70 per kilogram. Since it is scarce, the natural product has no real market, but is used only by a few restaurants and is offered in many traditional recipes. The increasing number of mushroom gatherers, both professional and amateur, who are encouraged by the high prices fetched by these products, puts this mushroom at the risk of extinction in the near future. According to IUCN criteria, there have been moves to assign to *P. nebrodensis* the status of *Critically Endangered* (CR) species.

Forty-six dikaryotic strains belonging to the *P. eryngii* species complex, isolated from five different host-plant genera, were examined with the RAPD-PCR and isozyme techniques and evaluated in combination with ecological and morphological observations of basidomata.

The methodology adopted in collaboration with the Institute of Kalamata (Greece) consisted in the establishment of dikaryons in pure culture, enzyme extraction, starch-gel electrophoresis, and analysis of the isozyme data. Furthermore, DNA extraction, *Pleurotus* RAPD-PCR analysis and statistical analysis were also carried out.

The use of RAPD-PCR and isozyme analyses permitted grouping of the *P. eryngii* complex isolates into five main clusters, in accordance with the separation of individual populations by host specialization. *Pleurotus* isolates growing on *Cachrys ferulacea* formed a distinct group, with relatively high statistical support.

Therefore, their separation from the rest of the population examined and their classification within a distinct taxonomic entity at the species level, i.e. *P. nebrodensis*, seems well justified and confirms reports based on morphology (Venturella & al. 2000).

All other strains were positioned within the larger *P. eryngii* group, which was further divided into four main clusters, corresponding to *Eryngium*, *Ferula*, *Elaeoselinum,* and *Thapsia* hosts.

Since fungi have been recently considered by the Specialist Group for Fungi inside the Species Survival Commission (SSC) of the IUCN-The World Conservation Union as organisms to be listed for identification of threatened fungi, to be taken into account for conservation strategies, to promote actual conservation drives for the threatened fungi, and for testing and improving the Red List criteria and categories, a specific dossier for *P. nebrodensis* was prepared and submitted to IUCN. In 2005, *P. nebrodensis* was classified as CR, according to IUCN criteria. In fact, such mushrooms show a high level of exploitation, occur in areas less than 100 km^2, their population is characterized by a low number of mature individuals, and the population size is less than 250 mature individuals.

More recently, IUCN/SSC Mediterranean Island Plants Specialist Group recognized *P. nebrodensis* as a valuable species to be included among the TOP 50 Mediterranean Island Plants (de Montmollin & Strahm 2005).

Courtecuisse (2001) recognized different conservation strategies for fungi:

- *In situ conservation*: **a)** conservation of natural habitats especially valuable for fungi (mycological reserves); **b)** biological corridors with positive effects on the survival and restoration of various plants and animals. It is quite likely that they may also have positive effect on fungal populations.
- *Ex situ conservation*: among the proposed strategies of fungi conservation at European level the cultivation of saprotrophic species at risk of extinction and/or confined to very limited or threatened habitats is a valuable option.

Cultivation of *P. nebrodensis* is carried out in tunnels made of metal arches and varying in length from 20 to 30 metres, covered by a black net that provides 90 % shade.

Two or three mushroom beds are made inside the tunnel, each one meter long and separated by a path of about 50 cm. The beds may be below ground level, in a hole in the ground 20 to 25 cm deep, or elevated above ground and placed on tables or some other facilities, so as to create a containing wall 25 cm high.

The tunnel must be equipped with a humidifier, with irrigation tubes and nozzles arranged above the planting area, or by a simple manual watering device.

The beds are arranged with bags of incubated compost, placed next to each other, and only the top part of the plastic container should be carefully removed. These bags contain the mycelium of mushrooms gathered from the areas where they grow naturally. When the sacks are arranged on the mushroom bed and opened at the top, the surface of the compost is then covered with a 1–2 cm thick layer of soil, taking care to fill first the empty spaces between the bags.

The mycelium is isolated and left to develop in bottles containing seeds of cereal grains. The compost is composed of straw and remains from processing of other cereal plants, which are humidified and properly mixed. The compost is then closed in temperature-resistant sacks of polypropylene weighing 4 kg, placed in special baskets, and put into a sterile environment, where they are inoculated with special syringe with the previously obtained mycelium, and incubated at 25 °C until the mycelium is fully developed.

Eight to ten days after planting, under optimal climatic conditions, the mushrooms begin to appear in the beds, and the first harvest can begin after 18–20 days. After approximately a week, other mushrooms sprout, ready to be harvested in about 10 days.

Mushroom production happens in two rounds, the first of which yields approximately 70–80 % of the total production. Under optimal conditions production levels are on par with approximately 28–30 % of the weight of the compost. The yields amount approximately to 15–18 kg of mushrooms per square meter, and vary from 0.5 to 1.5 kg per bag of compost.

It is hoped that with intensive cultivation, the decline of this important genetic resource can be slowed down or halted and, along with this, prices can be lowered so that the product can be offered to a wider range of consumers.

3. *Hypogeous fungi*

Starting from the year 2000, a research team accompanied by dogs trained in the search of hypogeous fungi was involved in field investigation in different forest ecosystems of Sicily, in order to widen the knowledge on hypogeous fungi, to confirm data reported in literature and, for the first time, to provide distribution maps of species recorded in the region.

A huge number of hypogeous and semi-hypogeous fungi were collected, belonging to the genera *Balsamia* Vittad., *Elaphomyces* Nees, *Endogone* Link, *Fischerula* Mattir., *Genea* Vittad., *Geopora* Harkn., *Hydnangium* Wallr., *Hydnocystis* Tul., *Hymenogaster* Vittad., *Hysterangium* Vittad., *Labyrinthomyces* Boedijn, *Leucogaster* R. Hesse, *Melanogaster* Corda, *Pyrenogaster* Malençon & Riousset, *Radiigera* Zeller, *Rhizopogon* Fr. & Nordholm, *Setchelliogaster* Pouzar, *Stephensia* Tul., and *Tuber* F. H. Wigg.

Truffles are economically important and legally commercialized in Italy, which has laid down the foundations for their cultivation projects in Sicily.

Discussion

There are still some unsolved problems in the Mediterranean region, such as absence of an adequate number of mycologists, absence of pertinent mycological studies at the universities and research institutions, problems in the collaboration between scientists and mycological amateur associations, difficulties in alerting the broader scientific community to the importance of fungi, and difficulties in persuading the policy-mak-

ers and funding organizations of the need to support fungal diversity studies.

Such problems were discussed during a Forum of Mediterranean Mycology organized in Palermo in 2004. During the Forum, all participants agreed with the need to establish a Working Group of Mediterranean Mycology as a branch of the European Mycological Association (EMA) and to harmonize the actions of different mycological institutions in Europe.

The proposal to establish a Working Group of Mediterranean Mycology (MMWG) was accepted by EMA Governing Committee in Prague. It included representatives from around the Mediterranean and from other European countries with a Mediterranean climate.

The objectives of the MMWG are:

1. To promote mycological research, recording, mapping, and conservation of fungi;
2. To develop ethical and scientific methodological standards;
3. To encourage scientific debates among the Mediterranean mycologists;
4. To collect information on literature data, checklists, Red Lists and mapping projects in the Mediterranean countries;
5. To encourage the mycological amateur associations to collaborate with universities;
6. To establish an essential link between EMA and OPTIMA, Planta Europa, ECCF and other national, or international mycological institutions;

This group is open to all types of contribution and cooperation from other mycologists.

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