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Commentary

Monitoring fungal biodiversity – towards an integrated approach

SUMMARY

Biodiversity information databases and platforms have seen considerable progress in recent years. They have a high potential in conservation science in general, but may be even more revolutionary in relation to poorly known species groups such as fungi, whose practical conservation work has been jeopardised by scattered and poorly controlled information. We review the tradition of collecting information on species occurrences in mycology and discuss the characteristics of the present fungal biodiversity information databases. With a special focus on population trend monitoring of fruit body producing macrofungi, we emphasise several unrealised opportunities of these databases and point out some relevant future directions for them. As especially important, we see the more effective utilisation of citizen science effort and combining the traditional database information with the one derived with modern molecular methods. Also, we emphasise the importance of information on collection effort, including the use of GPS based tracking data, along with the observations.

Introduction

Practical conservation of biodiversity depends on reliable information on what kind, how much and where the diversity is. In many cases the basic level of biodiversity information is observations on species occurrences over time and space. Such data form the basis in many conservation priority approaches, e.g. in reserve selection procedures (Pressey *et al.* 1993) and red listing (Dahlberg & Mueller 2011), but is also crucial in more basic research on macroecological patterns and global change.

Unfortunately, data on species occurrences is not always accurate, and this may seriously undermine conservation priorities and scientific conclusions (Molina *et al.* 2011; Jetz *et al.* 2012). Accurate recording of species occurrence over large geographical areas is time consuming and therefore costly if performed by professional specialists. In practice funding for surveying species is very unequally distributed, and targeted towards organism groups that are generally considered spectacular, cute or intelligent. This means that fungi are highly underrepresented in the conservation literature (Heilmann-Clausen & Vesterholt 2008).

Within macrofungi (i.e. the fungi with visible fruit bodies), observations of species have traditionally been obtained from more or less random walks by experienced amateur mycologists. Less often they have been derived from targeted research or monitoring projects. These observations have improved our understanding of the ecology, distribution and phenology of fruiting in many fungal species, but the estimation of population sizes and trends based on traditional observation data is often difficult or impossible, as the information associated with the observations is not meant for this purpose (Pyke & Ehrlich 2010). For example, fungarium specimens and database records do not include information on the extent and nature of the collecting effort. Furthermore, they lack information on negative records (failures to detect a species in an attempt), which are as important as positive ones when evaluating population trends (Rhodes *et al.* 2006).

Many fungal species groups do not produce visible fruit bodies or other species-specific structures, or these structures are extremely rare, and cannot be detected in traditional surveys. These can now be studied using molecular methods (e.g. Jones *et al.* 2011; Liu *et al.* 2011). Even fruit body producing macrofungi are difficult to survey due to the unpredictable fruiting patterns (Halme & Kotiaho 2012; van der Linde *et al.* 2012). In many species fruit bodies can be found for only a short period of time, which may vary between years (Lagana *et al.* 2002). Furthermore, many species do not fruit annually (e.g. Straatsma *et al.* 2001). Molecular analysis of environmental samples might overcome these shortcomings and provide a more realistic or at least different insight into macrofungal community structure than offered by observations of fruit bodies (Porter *et al.* 2008; Geml *et al.* 2009). So far, molecular methods have, however, only been applied in scientific studies, and their potential in monitoring fungal biodiversity professionally or by the aid of amateurs is yet unexplored.

During the last decade numerous biodiversity information databases have been launched for data collecting. Considering the whole biota, there are more than 600 different databases worldwide (Borges *et al.* 2010). Most of these are taxonomically or regionally restricted. Moreover, the development of the

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databases has not been a coordinated effort. Even though the Biodiversity Information Standards initiative (www.tdwg.org) has tried to standardise the databases, there is a high variation in the database structure and usability even within a single species group or across taxonomical groups within a geographical area.

Within mycology, a recent rapid development of molecular databases has pushed the disorder even further. Presently the molecular information is spread over dozens of databases with different levels of accuracy in the nomenclature and quality of the included sequences (see e.g. Abarenkov *et al.* 2010; Öpik *et al.* 2010; Benson *et al.* 2011).

We believe that value and usability of databases can be increased by rethinking data collection, database structure and organisation. Against this background we review the way fungal records have been obtained and documented in the past, and investigate how molecular methods and online database platforms may boost fungal monitoring in the years to come. Based on this we discuss the potential to improve the quality of fungal recording databases so that they can be used to draw reliable conclusions about fungal biodiversity and its trends. Our focus is on species, because species are the units most often treated in biodiversity conservation work among all biota. Moreover, our focus is on macrofungi, since fungal conservation so far has focused on fruit body producing macrofungi (Dahlberg & Mueller 2011). We admit, however, that there is also a need for a more holistic view of fungal conservation, as recently proposed by Griffith (2012). We hope our recommendations will be inspirational in the attempts to answer these needs.

Recording macrofungi

Fungal foraying

The classical way to obtain records or specimens of macrofungi is fungal foraying. Foraying can be described as more or less random walks focussing on and recording species of interest to the forayer. The resulting data are highly unstructured and reflect the skills/interest of the forayer as well as the actual conditions for fungal fruiting on the actual day of the foray. One of the main qualities of opportunistic foraying is that it is often the best way to record rarely fruiting species that may be missed using more structured sampling methods (Mueller *et al.* 2004). Despite their lack of structure, heterogeneous foray data have been utilised to estimate population trends in several studies in the Netherlands (e.g. Arnolds 1988; Arnolds & Jansen 1992; see also; Barron 2011). To standardise for inequality in the foray activity over time these authors used the "relative foray frequency", i.e. the percentage of the records of a species on foray lists related to the total number of forays in the period (Arnolds & Jansen 1992). Recently, similar data have even been utilised to explore changes in fungal fruiting phenology and host selection in response to climate change in the UK and Norway (Gange *et al.* 2007, 2011; Kauserud *et al.* 2008, 2010).

While it is easy to standardise for changes in foray activity over time, it is far more challenging to standardise for changes in the quality or focus of forays over time or between people. For example in Finland, it is well known among mycologists that many nature conservationists mainly report their records of red-listed species, because it is considered the best way to promote fungal conservation. Thus, a change in the species' red-list status may result in changed number of records without any connection to true population trends of the species. Failure to account for changes in the quality or focus of forays over time may result in dubious conclusions (Heilmann-Clausen & Læssøe 2012).

Structured monitoring and research based on fruit bodies

More structured data on fungal records can be derived from professional field studies. Ideally, such studies are based on a well-described design, aimed at answering specific research or management questions. Unfortunately, little research has been carried out to optimise sampling designs (O'Dell *et al.* 2004; but see; Keizer & Arnolds 1990; Halme & Kotiaho 2012), and in practice field methodologies and the overall sampling regime vary enormously among studies, even within comparable habitats. Some studies have used fixed sample plots or transects of very varied size and length, others have sampled a specified amount of substratum. Specific recommendations were made by Mueller *et al.* (2004), but it is uncertain if these will make a lasting footprint on actual research and monitoring activities in the field.

So far, most professional field studies have been designed to study changes over space or environmental gradients, e.g. related to landscape history (e.g. Penttilä *et al.* 2006), climate (e.g. Lindblad 2001; Kernaghan & Harper 2001), forest type (e.g. Tyler 1985; Såstad 1995) or the intensity of forest management (e.g. Sippola *et al.* 2001; Luoma *et al.* 2004). If the field methodology is well defined and adequately described such studies can be repeated over time, and hence they have the potential to produce high-quality structured data, suitable to document changes in fungal fruiting over time. This approach has been utilised to investigate changes in fungal fruiting in acidic oak forests and conifer plantations in the Netherlands (Arnolds 1988). We strongly encourage researchers to follow this track in future.

Recently, schemes have been set up specifically to monitor changes in fungal fruiting over time in seven European countries (Senn-Irlet *et al.* 2007). Again the Netherlands have taken the lead, surveying annually 110 macrofungal species in 600 permanent plots in a citizen science project, coordinated by two employed coordinators (Arnolds & Veerkamp 2011).

Fungal monitoring beyond fruit bodies

The scientific relevance of surveys or monitoring based on fruit bodies has long been questioned, as they do not record presence of macrofungi as vegetative mycelium (Allmér *et al.* 2006) resulting in an imperfect understanding of the community present (Geml *et al.* 2009). Alternative techniques to fruit body surveys, based on isolation of fungi present in environmental samples have long been utilised for litter, wood and soil fungi (e.g. Allmér *et al.* 2006), and since the 1980's molecular tools have been applied (Lindahl & Boberg 2008; Porter *et al.* 2008). Isolation and early molecular techniques were laborious and applied mainly in basic research on community composition and development in fungal communities on limited spatial and

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temporal scales. However, these methods have high potential when applied in a specific conservation context. For instance, Parfitt *et al.* (2005) developed specific primers to detect selected wood-inhabiting species present in vegetative stages in living and dead trees. By using these, they found that their target species were more long-living, frequent and abundant in their substrata as mycelia than was evident from the presence of fruit bodies.

Many recently developed molecular techniques have the potential to be much more widely utilised in large scale monitoring and surveying. High throughput sequencing methods offer a promising tool to study fungal distribution patterns and population trends. Unlike with traditional sequencing it is possible to process bulk environmental samples and produce thousands of DNA sequences in a single run (see e.g. Nilsson *et al.* 2010; Ovaskainen *et al.* 2010; Lentendu *et al.* 2011). However, at the moment the high one-off cost for the sequencing combined with rarity of high throughput sequencing platforms and their location in academic or medical institutions, make this approach practically usable only for researchers. Further, some serious scientific challenges need to be resolved, before this approach will be broadly applicable in monitoring of fungal biodiversity.

One serious constraint is the lack of comprehensive reference sequence libraries. Thus, high-quality reference molecular data are present for less than 1 % of the estimated number of fungal species (Abarenkov *et al.* 2010; Öpik *et al.* 2010; Blackwell 2011). A large proportion of fungal sequences found in environmental samples currently remain unidentified, when matched against sequences in GenBank (Benson *et al.* 2011) or curated fungal sequence databases like Unite or BOLD (Ratnasingham & Hebert 2007; Abarenkov *et al.* 2010). Such unidentified sequences are typically clustered into molecular operational taxonomic units (MOTU) (Blaxter *et al.* 2005), operational taxonomic units (OTU) (e.g. Vrålstad 2011) or virtual taxa (Öpik *et al.* 2010) and uploaded to public sequence databases. Comparison of MOTUs (or OTUS and virtual taxa) across studies is laborious and confusing, because these entities are currently labelled with several, e.g. GenBank, accession numbers. In this sense the suggestion by Hibbett *et al.* (2011) for formal classification of environmental sequences is essential and well justified, and would be of great help for following fungal population trends based on high throughput sequencing of environmental samples. It is, however, crucial that this approach is combined with a structured approach to fungal taxonomy and barcoding of species missing in reference sequence databases.

Another challenge is that the overwhelming fungal diversity may inhibit effective species identification. This is especially the case with respect to the identification of sparse sequences and their distinction from the noise caused by sequencing and PCR amplification errors. However, the progress in methodological and data analysis techniques has been rapid and these obstacles are being addressed (e.g. Huson *et al.* 2007; Quince *et al.* 2009, 2011; Schloss *et al.* 2009). Difficulties around the internal transcribed spacer (ITS) region located in multiple copies in ribosomal nuclear DNA are an issue itself, since the barcode region ITS (Schoch *et al.* 2012) is the primary target of many molecular databases. In some fungal groups ITS is not sufficiently variable and fails to detect true species (Gazis *et al.* 2011 and references therein). However, in some taxa the intragenomic or intraspecific variation of the ITS is so high that it cannot be distinguished from interspecific variation (Nilsson *et al.* 2008; Lindner & Banik 2011). This is a problem especially in high throughput sequencing applications, since in sequencing runs multiple copies of ITS behave individually and no consensus sequence is produced. Thus, molecular species diversity estimates will be more or less distorted, if based on ITS alone.

Finally, fungal high throughput sequencing studies to date have mostly been DNA-based and are hence unable to distinguish securely between dormant and active life stages and even dead hyphae still containing intact DNA. RNA-based approaches would probably be a solution to this problem, but may involve other constraints (Rajala *et al.* 2011).

Monitoring fruit bodies in the molecular era

High throughput sequencing methods are still in their infancy and currently they are not used in monitoring programmes, as far as we are aware. When the above issues have been addressed, their potential is enormous, and we are convinced that fungal population trends in the near future can be followed in a meaningful way by using standardised sampling methods based on environmental samples, e.g. drilled saw dust samples (wood-inhabiting fungi) or soil samples (mycorrhizal and decomposing soil fungi).

However, we are convinced that monitoring based on fruit bodies will have relevance even in the future. First, fungal fruit bodies are appealing to ordinary people, which facilitates the inclusion of fungi in citizen science based monitoring of biodiversity. Citizen science invokes a big potential for increasing the public knowledge of species and conservation needs, hand in hand with the production of scientifically valuable data (Bonney *et al.* 2009). Also for the really rare species, the search for fruit bodies by professional and interested amateurs might be the only cost-effective way to obtain records, as environmental sampling is difficult to target towards very low frequent mycelia hidden in soil or other substrata. Second, fruit body observation is relevant in its own right. As with bird monitoring schemes that are often highly concerned with breeding success rather than the number of breeding pairs (e.g. Martin & Geupel 1993), the emergence of fruit bodies may tell more about the success of e.g. decay fungus in a fallen tree, than the presence of mycelia. Finally, data on fruit bodies present a longer time frame than molecular data. It is only within the last 5 yr that molecular studies have begun to generate substantial datasets allowing for an emerging insight in large scale patterns in fungal communities and the frequency of individual species over space. In comparison, data on fruit bodies show a continuity of 50 yr or more in many European countries (e.g. Arnolds & Jansen 1992; Gange *et al.* 2011).

Documenting fungal records

Physical specimens

Classically dried specimens have been deposited in fungaria, providing collections of samples of taxonomic value, typically

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biased towards rare or otherwise notable species or difficult species complexes. Thus, the detection of, for example, an increasing population trend is difficult based on fungarium specimens. The strength of fungarium collections is that they provide solid proof that a species occurred in a specific site at a given date. Further they allow rechecking the species identity when more taxonomic and molecular knowledge becomes available. Related to fungarium collections are fungal culture collections, keeping live cultures of fungi, but usually restricted in the number of strains kept of each fungal taxa. Thus, culture collections are of limited interest for evaluating species frequencies and distribution patterns in time and space.

Paper lists and database records

The main traditional source of fungal records is compiled lists from fungal forays, sometimes backed by deposited specimens, which are numerous in some countries (cf. Arnolds & Jansen 1992; Gange *et al.* 2011). In the digital era, paper lists from forays have been replaced by database entries. This shift involves drawbacks as well as advantages; classical foray lists were usually compiled by trained amateurs or professionals, which guaranteed a fair level of reliability. Online platforms for data submission attract untrained amateurs alike. This may seriously undermine data quality, but on the other hand modern databases offer new possibilities for data validation. For instance, photos can be submitted as documentation, and interactive validation systems can be set up. For instance, in the Danish basidiomycete atlas all species have been coded with specific requirements for validation (e.g. photo, dried material or notes on colour change or smell) and operates a forum function, allowing a dialogue between experts and amateurs connected to every single record. Here even documentation photos and notes on, for example, microscopical characters can be entered and stored, even if specimens are not kept for the future. Further, online databases offer a good platform for storing supplementary information, e.g. on habitat association and on survey input, as discussed later.

Tracking survey input

The amount and quality of fungal recording is inconstant over time, and this seriously restricts the potential to track changes in population sizes for specific species. One way to address this problem is by recording the amount and quality of survey input, i.e. the effort allocated to searching in the field and the methods used. In research projects and professional monitoring programmes survey input is normally well described, but this information is rarely stored in a standardised way. In more unstructured foraying survey input is often not recorded at all or only as written reports in note books.

The value of fungal recording can be increased considerably if survey input is recorded in a standardised way. Survey data should include details on time spent searching, the type of sampling conducted (e.g. random walks, targeted walks, survey of fixed plots or transects, environmental sampling or fruit body survey) and targets for the survey. It is important to accept that even "random walks" often have a narrower target than "all macrofungi" because most mycologists are not able or willing to survey all macrofungi growing on various substrata. Data quality can be increased by specifying even vague targets (e.g. all fleshy fungi, red-listed wood-inhabiting fungi, edible fungi etc.) for random walks, to allow possibilities for comparisons over time. By entering such data in a common database, it could be stated that, for example, in 2008 behind the 18 deposited specimens of one species there were 153 hr of active searching in a given habitat. This could then be compared to figures from other years. Comparison would also be made of how the effort matches with the potential or proven fruiting seasons of different species.

GPS systems offer an excellent opportunity to record survey input. Currently these systems enable tracking the survey routes of a field mycologist almost to an accuracy of 1 m. In Finland the officials conducting governmental inventories of wood-decaying fungi have tracked their routes with a standard method (Fig 1). After the track is saved on a computer, geographic information programs can be used to calculate the covered area from the track. Moreover, after the survey each area is given "an index of field work intensity" to display how carefully the inventory was carried out and which substrata the inventory concentrated on. This enables standardising the survey input to surveyed area without being forced to allocate time in establishing sample plots.

Storing and sharing fungal records

Public databases containing fungal biodiversity data have developed enormously within the last decade. They can be divided into four main types, which we here denote as taxonomic databases, molecular reference databases, fungarium databases and recording databases.

The taxonomic databases are best represented by Index Fungorum and the associated MycoBank, which serve as a reference or backbone for everybody working with fungal species. MycoBank (www.mycobank.org) has developed immensely in recent years to become the standard reference for fungal taxonomy and nomenclature, providing original descriptions and expert views on the validity of published names (Crous *et al.* 2004). Molecular reference databases e.g. GenBank and UNITE, basically serve the same purpose, but are targeted to contain genetic reference information derived from scientific studies and/or barcoding projects.

Several fungaria are now entering the digital era by digitizing their collections. Most impressive is the mycological database of the University of Oslo, containing more than 200 000 digitized specimens. The development of fungaria databases are a big step forward in fungal taxonomy and biogeography. They fulfil the traditional requirement of having a fungarium sample for the validation of the record. An obvious drawback, however, is that the information shows mostly the collecting activity instead of actual biodiversity trends, and hence they are only of limited relevance in population studies.

In the last decade recording databases have been set up in many countries so that amateur mycologists can enter their field sightings of fungi (Dahlberg *et al.* 2010; Supplementary appendix 1). Some are general biodiversity databases designed to collect information on many taxonomic groups. These databases, for example Hatikka in Finland (www.fieldjournal.org), generally serve well when the surveyed organisms are well-known. Fungi are not, and thus for fungal observations more

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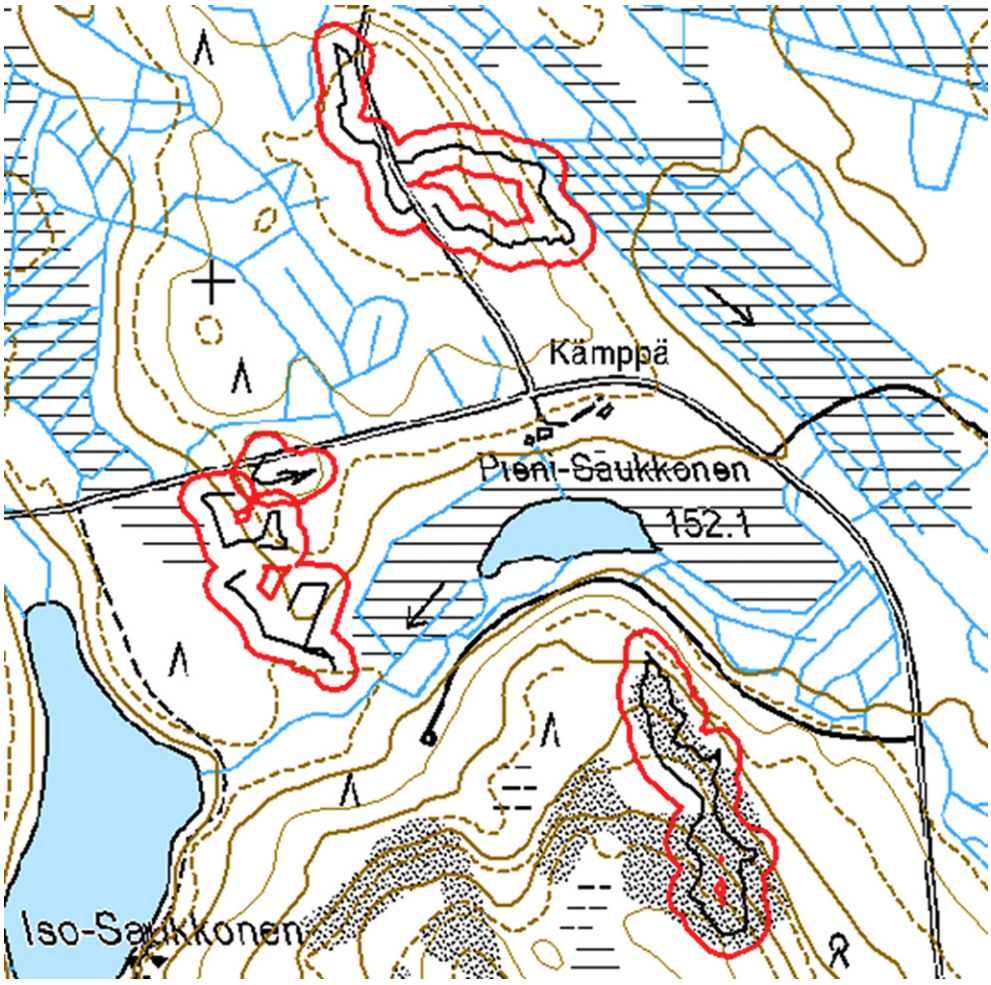

Fig 1 – Tracks produced by GPS tracking during an inventory focussing on wood-inhabiting fungi in Central Finland. The GPS was programmed to save a position after every 10 m. The track was drawn as a black line and the red line shows the estimated covered area (10 m in all directions from the true track). The breaks in the track are areas where the field worker has paused the tracking for example because of bypassing some uninteresting areas. (© National land survey of Finland, MML).

elaborate and dynamic tools and platforms are needed. Some platforms have been designed specifically for fungal biodiversity information and are, therefore, able to handle information on the validation of the observation. These recording platforms can generate substantial amounts of data. For instance the Danish basidiomycete atlas (www.svampeatles.dk) has received 50 000 fungal records per year since it started in 2009 (Læssøe *et al.* 2011). A majority of these records are not backed by fungarium specimens, and are hence "naked" and impossible to recheck in cases of altered taxonomy or uncertainty regarding the identification skills of the depositor.

Database accessibility ranges from easy to difficult and restricted in all the above mentioned categories. Language is often an obstacle and many recording databases can only be used with the language of their country of origin. The actual searches are open to all users in all the mentioned databases. Moreover, many of them are linked to the Global Biodiversity Information Facility (GBIF; available at www.gbif.org) which provides an information infrastructure, community-developed tools and capacity-building. Even more important, GBIF functions as a metadatabase, and enables free and open access to all input data. Thus, it provides the possibility of searching for the information saved in many databases (Telenius 2011).

Research and monitoring data

Data from scientific surveys and research constitute a special case. Firstly, researchers often design their own systems to handle their data. Secondly, researchers may be hesitant to deposit their data in metadatabases because they fear that other researchers may take advantage of their data, without proper acknowledgements. Several attempts to fight this phenomenon have been published, and recently some journals have been active in promoting the free distribution of published datasets (Whitlock 2011). However, it is still a huge paradox that in the world of increasing knowledge and possibilities for data distribution there are millions of records hidden in the hard drives of scientists' computers. It is crucial that such data are shared, before they are lost due to breakdown of hardware or simple neglect. Thus, we recommend all researchers to submit their fungal datasets to GBIF or other metadatabases as soon as the scientific highlights have been published.

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In fungal molecular research, it is a common practice to submit sequence data to GenBank or to similar databases, but the lack of data standards makes these data of little value in relation to meta-analysis of species trends, ecology and distribution patterns. We, therefore, highly welcome recent efforts to standardise data, and to include environmental information in data-submission from studies generating genetic data, e.g. from environmental samples (Yilmaz *et al.* 2011). Similarly it is promising that UNITE, which originally was developed as a reference database for identifying ITS sequences of ectomycorrhizal fungi, is now open to metadata relating sequences to specific sample plots and studies and providing details on soil type, host trees etc. (Abarenkov *et al.* 2010).

A catch all mycological database/platform

Many existing databases work well in their own context, but this is restricted. Valuable data are presently derived from unstructured and structured observations on fruit bodies as well as from molecular studies, but these different types of data are kept separately. Considering the advances both in molecular biology and information technology, as well as the need to collect fruiting data, we believe the way we collect and manage observation data on fungi needs rethinking. We see it as a crucial challenge to develop databases that enable incorporation of different types of information from various sources, yet retain a user-friendly interface.

An ideal scenario would be an open-access, user-friendly database that could be integrated to give information on any particular species, based on amateur sightings, fungarium samples, species lists saved by researchers and molecular records derived from high throughput sequencing. The platform should be interactive with several tools to explore the validity of the information, providing instant feedback and observation matrixes allowing evaluation of information based on the sampling effort utilised, personal knowledge of the collector, reliability of the molecular records and documentation such as photographs. The taxonomic stability should be supported as far as possible by linking to a universal taxonomic platform, as presently offered by MycoBank in collaboration with Index Fungorum. Ideally, this database would be a global platform, or global repository of national multifunction platforms, allowing world-wide comparison of

Table 1 – The minimum entry fields of a catch all database for documenting fungal observation data

Data level	Entry field	Possible content and variable stages
Taxon	Latin name	Unique name from www.mycobank.org or www.indexfungorum.org
	Taxon code (LSID)	Unique identifier from www.mycobank.org or www.indexfungorum.org
	Common name	Unique name (in different languages)
	Special status	E.g. red list status in different areas
	Validation requirements	E.g. need for photo documentation, specimen
Person	Name of person	Unique name
	education	E.g. biologist, forester, other academic
	Experience as mycologist	E.g. as time in years, or based on expert assessment
Recording event	Purpose	E.g. research, structured monitoring, expert foray, amateur foray
	Targets	E.g. edible species, all species, red-listed species, saproxylic species, ectomycorrhizal fungi
	Duration/extend	E.g. time in hr/size of sample, area covered
Record	Date of record	Entered by recorder
	Name of taxon	Entered by recorder, but linked to taxon base
	Name of recorder	Entered by recorder, but linked to person base
	Name of determinator	If different from recorder; linked to person base
	Name of confirmator	If different from determinator; linked to person base
	Life stage	E.g. fruit body, sterile macroscopic structure, mycorrhiza, spore, mycelium, unknown
	Abundance	E.g. missing but searched for (negative record), present (positive record), abundance
	Type of record	E.g. sighting, literature record, collection, environmental sample, sequence
	Type of documentation	E.g. none, notes on important characters, photo, dried specimen, stored sequence
	Validation status	E.g. none, expert validation, sequence match
	Precision of UTM coordinates	Free estimate or based on predefined categories
	Habitat type	Preferably from predefined list
	Substrate	Preferably from predefined list
	Host organism	Preferably from predefined list of host genera/species + free text
	Photo	As uploaded by recorder
	Sequence	E.g. ITS sequence
	Written documentation	E.g. field and lab notes documenting characters and the validation process

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population trends, species distributions and other interesting topics.

The emergence of such a database is probably several years away, but already it makes sense to standardise ways that data are collected across projects. In Table 1 we have provided our suggestions for minimum entries that a database of fungal records should contain to fulfil this goal.

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Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.funeco.2012.05.005.

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