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World biota conservation vs fungal conservation practice

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Abstract

The article analyzes the world experience of biodiversity protection with an emphasis on aspects of fungal protection. Information on environmental organizations, societies, unions, and documents developed by these organizations is presented in chronological order. The generally accepted principles of effective protection of fungi and methods that can direct the natural reproduction of rare species are considered. Our aim is to describe the situation with protection of fungal species (in-situ, ex-situ and re-situ) compared to the methods and practices for plants and animals, using the case studies in Hutsulshchyna Nature National Park, Ukraine.

Keywords – Conventions – ex-situ – in-situ – IUCN − Red list – re-situ

Introduction

Biodiversity protection and conservation are important components of scientific and practical activities of any developed country. This process is costly, time-consuming, procedurally lengthy and requires legal regulation. At the same time, keeping the species from extinction is an important prerequisite for maintaining ecological balance. In the long run, it is essential for the existence of mankind (Shaw 2021). Fungi are an important component of ecosystems; they are part of their species richness and significantly contribute to the conversion of substances and energy in wildlife. Interest in fungal distribution, ecology, population dynamics, taxonomy, phylogenetic relationships, interactions with other organisms, impact on ecosystems and the possibility of practical use in various fields is significant. Fungal researches are conducted in various areas such as biotechnology, traditional medicine, food, tinder production and entheogens, as well as for their dangers, such as toxicity or infection potential (Mueller et al. 2004, Spooner & Roberts 2005, Smith & Read 2008, Bandara et al. 2015, Fisher et al. 2020, Adeoye-Isijola et al. 2021). Even though fungi were isolated in a separate kingdom in the last century, the issue of their protection at the global level has long been ignored. However, there are no national lists of fungi in many countries, which complicates the analysis of world experience in protection of fungal biodiversity. Fungi are the objects of the wildlife that need of protection same way as plants or animals. Therefore, we analysed the chronological sequence of creation/adoption/implementation of biodiversity protection initiatives and identified how many of them relate to the conservation of fungi. The measures and steps for the protection of fungi are promising and the difficulties that arise in their implementation are discussed.

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At as the beginning of the twentieth century, it became clear that consumer attitudes towards nature lead to the extinction of many species of animals and plants. Therefore, the idea of active protection of certain species resulted in the creation of the first official international organization for nature protection – International Union for Conservation of Nature (IUCN). IUCN was aimed to provide scientific knowledge and tools to guide conservation action and encourage international cooperation. It brought together governments and civil society organizations with a shared goal to protect nature.

Today, IUCN counts more than 1,400 members – including States, government agencies, NGOs, and Indigenous Peoples' Organizations.

Representatives of China in IUCN are National non-governmental organization China Association for NGO Cooperation (CANGO), China Association of National Parks and Scenic Sites (CNPA), China Association of Traditional Chinese Medicine (CATCM), China Biodiversity Conservation and Green Development Foundation (CBCGDF), China Environmental Protection Foundation (CEPF), China Green Carbon Foundation (CGCF), China Green Foundation (GCF), China Mangrove Conservation Network, legal name: Putian Green Sprout Coastal Wetlands Research Center (CMCN/PGSCWRC), China Wild Plant Conservation Association (CWPCA), China Wildlife Conservation Association (CWCA), Chinese Society of Forestry (CSF).

Thanks to IUCN the Species Survival Commission (SSC) Fungal Conservation Committee FunCC (2021), The Global Fungal Red List (2014), The IUCN Red List of Threatened Species™ (1964) were founded. In fact, information about species of fungi is only in the mentioned documents, despite many International Conventions were adopted and World Wildlife Fund was founded since 1948 (Table 1).

Table 1 World experience of biodiversity protection.

Protection organization/ document/ initiative	**The year of adoption / foundation, place**	**Notes**	**The number of species of fungi as of February 2022**	**Links**
International Union for Conservation of Nature, IUCN	1948 Fontainebleau, France	IUCN aim was to encourage international cooperation and provide scientific knowledge and tools to guide conservation action. IUCN created IUCN SSC Fungal Conservation Committee FunCC (2021), The IUCN Red List of Threatened Species™ (1964), The Global Fungal Red List (2014).	----	https://www.iucn.org/
The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR)	1955 Vienna, Austria	Its mandate in the United Nations system is to assess and report levels and effects of exposure to ionizing radiation.	---	https://www.unscear.org/unscear/en/about_us.html
World wildlife Fund (WWF)	1961 Switzerland	One of the activities of the fund is preventing of the deforestation to avoid climate change and preserve animal biodiversity. Mushrooms as a separate category are not considered.	---	https://www.worldwildlife.org/

Table 1 Continued.

Protection organization/ document/ initiative	The year of adoption / foundation, place	Notes	The number of species of fungi as of February 2022	Links
The IUCN Red List of Threatened Species™ (1964)	1964	Established in 1964, the International Union for Conservation of Nature's Red List of Threatened Species has evolved to become the world's most comprehensive information source on the global extinction risk status of animal, fungal, and plant species.	639	https://www.iucnredlist.org/ https://www.iucnredlist.org/search?query=fungi&searchType=species
The Ramsar Convention on Wetlands	1971 Ramsar, Iran	Convention on Wetlands of International Importance, mainly as a Habitat for Waterfowl. The Convention is the intergovernmental treaty that provides the framework for the conservation and wise use of wetlands and their resources. Currently, the list of wetlands includes 2421 locations, which in total occupies 254589858 hectares. Does not apply to the fungi.	---	https://rsis.ramsar.org/
The Convention Concerning the Protection of the World Cultural and Natural Heritage	1972 Paris France	The developed from the merging of two separate movements: the first focusing on the preservation of cultural sites, and the other dealing with the conservation of nature. According to the Convention, Fungi are protected indirectly, for example in Bialowieza Forest, Surtsey, Gunung Mulu National Park and in 27 biosphere reserves in 131 countries, including 22 transboundary sites.	Objects and their territories are protected, but not specific species of fungi	http://whc.unesco.org/en/list/?search=Fungi&order=country http://whc.unesco.org/en/list/1133 https://en.unesco.org/biosphere
The United Nations Environment Programme (UNEP)	1972	UNEP's mission is to provide leadership and encourage partnership in caring for the environment by inspiring, informing, and enabling nations and peoples to improve their quality of life without compromising that of future generations. Some publications of UNEP are about fungi. For example,	---	https://www.unep.org/search/node?keys=fungi https://www.unep.org/about-un-environment

Table 1 Continued.

Protection organization/ document/ initiative	The year of adoption / foundation, place	Notes	The number of species of fungi as of February 2022	Links
		fungi are threatened by climate change, particularly temperature rises in high latitudes.		
The Convention on International Trade in Endangered Species of Wild Fauna and Flora, CITES	1973 Washington USA	Its aim is to ensure that international trade in specimens of wild animals and plants does not threaten the survival of the species. CITES has appendixes I, II, III counting 36,000 species of animals and plants. No fungal species is listed in the appendices.	---	https://cites.org/eng/app/appendices.php
The Convention on the Conservation of Migratory Species of Wild Animals	1979 Bonn, Germany	Does not apply to fungi. The Convention offers a platform for countries and partners to come together with concrete solutions to ensure the well-being of shared species, including whales, dolphins, sharks, antelopes, elephants, gorillas, and a wide variety of birds.	---	https://www.cms.int/en/about/cms-40
The Convention on the Conservation of European Wildlife and Natural Habitats	1979 Bern, Switzerland	It aims to conserve wild flora and fauna and their natural habitats and to promote European co-operation in that area. Bern Convention is a binding international legal instrument in the field of nature conservation, which covers the whole of the natural heritage of the European continent and extends to some African states. Bern Convention has I, II, III and IV appendixes. No fungal species is listed in the appendices.	---	https://www.coe.int/en/web/conventions/full-list?module=treaty-detail&treatynum=104
The Intergovernmental Panel on Climate Change (IPCC)	1988	IPCC was established by the United Nations Environment Program (UNEP) and the World Meteorological Organization (WMO).	----	https://www.ipcc.ch/about/history/ https://www.ipcc.ch/site/assets/uploads/2019/02/UNGA43-53.pdf
The Basel Convention on the Control of Transboundary	1989 Basel Switzerland	It is a global convention on the control of transboundary movements of hazardous wastes, drawing on the Cairo	---	http://www.basel.int/TheConvention/Overview/History/Overview/tabid/3405/Default.aspx

Table 1 Continued.

Protection organization/ document/ initiative	The year of adoption / foundation, place	Notes	The number of species of fungi as of February 2022	Links
Movements of Hazardous Wastes and their Disposal (Basel Convention).		Guidelines and the relevant national, regional, and international bodies. Does not apply to the fungi.		
European Red List of Globally Threatened Animals and Plants	1991	European Red List counts 15 060 species of animals and plants, no fungi. The Red List represents a review of the status of European species according to IUCN Regional Red List guidelines. It identifies those species that are threatened with extinction at the European level (Pan-Europe and the European Union) so that appropriate conservation action can be taken to improve their status.	---	https://ec.europa.eu/environment/nature/conservation/species/redlist/index_en.htm
The Convention on Biological Diversity	1992 Rio de Janeiro, Brazil	The objectives of this Convention are the conservation of biological diversity. It represents a dramatic step forward in the fair and equitable sharing of benefits arising from the use of genetic resources and conservation of biological diversity, the sustainable use of its components. A list of specific biodiversity species to be protected is not added. Important, the text of CBD has articles 8 and 9 about in-situ and ex-situ conservation.	---	https://www.cbd.int/
The Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade	1998 Rotterdam, Netherlands	The objectives of the Convention are: to promote shared responsibility and cooperative efforts among Parties in the international trade of certain hazardous chemicals in order to protect human health and the environment from potential harm; to contribute to the environmentally sound use of those hazardous chemicals, by facilitating information exchange about their characteristics, by providing	---	http://www.pic.int/TheConvention/Overview/tabid/1044/language/en-US/Default.aspx

Table 1 Continued.

Protection organization/ document/ initiative	The year of adoption / foundation, place	Notes	The number of species of fungi as of February 2022	Links
		for a national decision-making process on their import and export and by disseminating these decisions to Parties.		
The Stockholm Convention on Persistent Organic Pollutants (Stockholm Convention).	2001 Stockholm Sweden		---	http://www.pops.int/
Data Zone Bird Life International.	2002 Austria	LandSense project has received funding from the European Union's Horizon 2020 research. Doesn't apply for fungi.	---	http://datazone.birdlife.org/site/search
The Alliance for Zero Extinction (AZE)	2005	AZE was established to designate and effectively conserve the most important sites for global biodiversity AZE members have identified 853 AZE sites, which are the areas that hold the last-remaining populations of one or more species evaluated to be Endangered or Critically Endangered on the IUCN Red List. Currently, there are no data of AZE sites for fungi.	---	http://www.zeroextinction.org/search.cfm
The Vienna Convention for the Protection of the Ozone Layer.	2009 Vienna Austria		----	https://ozone.unep.org/treaties/vienna-convention
The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES)ю	2012 Panama City, Panama	IPBES is an independent intergovernmental body established by States to strengthen the science-policy interface for biodiversity and ecosystem services for the conservation and sustainable use of biodiversity, long-term human well-being, and sustainable development.	----	https://www.ipbes.net/about
The Minamata Convention on Mercury	2013 Geneva, Switzerland	The Minamata Convention on Mercury is a global treaty to protect human health and the environment from the adverse effects of mercury.	---	https://www.mercuryconvention.org/en/about

Table 1 Continued.

Protection organization/ document/ initiative	**The year of adoption / foundation, place**	**Notes**	**The number of species of fungi as of February 2022**	**Links**
World Database on Protected Areas	2014	The Protected Planet website can be explored for information about the World Database on Protected Areas (WDPA), World Database on OECMs, Global Database on Protected Area Management Effectiveness (GD-PAME), and a wealth of associated information. Website has information about 9 protected areas in China.	The protection of the territory in general is considered.	www.protectedplanet.net https://www.protectedplanet.net/en/search-areas?search_term=China&filters%5Bdb_type%5D%5B%5D=wdpa
The Global Fungal Red List	2014 Sweden	This is a crowdsourcing project, which is being carried out voluntarily. It counts total 1888 species by continent and fungal groups from 219 countries.	1888	http://iucn.ekoo.se/en/iucn/welcome http://iucn.ekoo.se/iucn/plan2015/
Key Biodiversity Areas – KBA	2018	KBA are the most important places in the world for species and their habitats. Faced with a global environmental crisis we need to focus our collective efforts on conserving the places that matter most. The KBA Program supports the identification, mapping, monitoring and conservation of KBAs to help safeguard the most critical sites for nature on our planet – from rainforests to reefs, mountains to marshes, deserts to grasslands and to the deepest parts of the oceans.	----	www.keybiodiversityareas.org/site/search https://www.keybiodiversityareas.org/
The IUCN SSC Fungal Conservation Committee (FunCC).	2021	The FunCC aims to raise awareness of the importance of fungi and the need of their conservation, enhance coordination among the fungal and the broader conservation communities, and foster action. Zhu L. Yang is a member of FunCC. He is a professor at Kunming Institute of Botany (KIB) of the Chinese Academy of Sciences (CAS).	Ongoing	https://www.iucn.org/news/species-survival-commission/202105/iucn-ssc-network-welcoming-fungal-conservation-committee https://www.iucn-fungi.org/

Despite considerable scientific, medicinal, nutritive interest in fungi, they are often insufficiently considered as objects of conservation. Fungi are not included in any international Conventions. Besides, the National Red Lists for fungi are not founded in many countries. According to the National Red List project started in 2012 (https://www.nationalredlist.org/home/about/), not all continents have available the Red Lists of fungi. South America, Australia, Africa (except Benin) haven't the National Fungal Red Lists.

These facts may be reasons of why fungi were poorly represented in the IUCN Red List of Threatened Species™ (Dahlberg & Croneborg 2003). People's Republic of China is marked in this map as a country without available National Red list of fungi. China started to use the IUCN Red List Criteria of Threatened Species to assess endangered species in the early 1980s (Zang et al. 2016). In Europe the first national Red List was formed in Germany only in 1982 (Dahlberg et al. 2010). In 1985 the European Council for the Conservation of Fungi (ECCF) was founded and almost all European countries were linked to ECCF (Anonymous 1998-2018). Therefore, protecting of fungi started in Europe and the National Red lists for fungi in Europe's countries started to populate. Since 1992 in EU fungi were protected indirectly by the Habitat Directive (Council 1992).

In 2008, the Ministry of Environmental Protection of the People's Republic of China and the Chinese Academy of Science started the project of the China Biodiversity Red List. According to Jiang et al. (2015), 73,255 biological species, including 29,088 species of animals, 1,653 species of protozoa, 36,333 species of plants, 3332 fungi, 463 bacteria, 1731 pigment, 655 virus's species were found and described in China.

The next step was the creation of the China Biodiversity Conservation Strategy and Action Plan (2011-2030) which considered and approved at the 126th executive meeting of the State Council (Xue 2011). In March 2015, the Central Committee of the Communist Party of China and the State Council released "Vision on the construction of ecological civilization", which puts the "Basic control of biodiversity loss and the stability of the national ecosystem will be significantly increased". Large biodiversity conservation projects will be implemented as one of the main goal processes, to take an active part in the negotiation and implementation of the International Convention on Biological Diversity (Zang et al. 2016).

Fungal conservation became global in 2019. The number of fungi in the Global Fungal Red List greatly increased due to the contributions of many mycologists from throughout the world. Contributors to website http://iucn.ekoo.se/iucn/summary/ was made for China thanks to scientists from China JIA Liu Kun, Kuan Zhao, Meixia Yang, Xinyu Wang, Yi-Jian Yao, Zhuliang Yang and Yajun Hou. In 2022 the Global Fungal Red List counts total 1888 species by continent and fungal groups from 219 countries (IUCN 2022a). In 2022 IUCN Red List of Threatened Species™ counts 639 species of fungi (IUCN 2022b). But in 2009 of the almost 45 000 globally red listed species, all but three are animals and plants. Only three fungi were listed in IUCN Red List of Threatened Species™, two lichens and one mushroom - the Sicilian endemic fungus *Pleurotus nebrodensis* (Inzenga) Quél. (Dahlberg et al. 2010). In 2021 only 453 species of fungi were noted in the IUCN Red List of Threatened Species™. Only in 2021 the Species Survival Commission of IUCN has created the IUCN SSC Fungal Conservation Committee (Table 1). This Committee includes members with different proficiency within this scientific community gathering expertise from all around the globe, covering all continents, except Antarctica.

Therefore, data about threatened species of fungi of the world are the youngest compared to plants and animals. In-situ conservation of fungi of the world is the youngest, too, because in-situ conservation focuses on conserving genes, species, and ecosystems in their natural surroundings, for example by establishing protected areas, rehabilitating degraded ecosystems, and adopting legislation to protect threatened species (Anonymous 2000).

While much progress has been made, the conservation status of many fungal species needs to be assessed. Participation by many is needed for the success to continue.

The next step of biodiversity conservation is ex-situ conservation (Kallow et al. 2020), also known as off-site, contrast to in-situ. Ex-situ conservation means the conservation of components of biological diversity outside or away from their natural habitats/location. Ex-situ conservation uses zoos, botanical gardens, aquariums, and gene banks to conserve species and their genetical material (Anonymous 2000, 2018).

The idea of the conservation of plants by ex-situ method has long history. In fact, ever since humans mastered agriculture, they have been forced to save plant seeds, to create their collections. The oldest and largest collection of plant seeds for scientific purposes was created in 1927 by Vavilov M.I.

There are many gene banks of plants all over the world, with the Svalbard Global Seed Vault being considered the most famous one (Kirchhoff 2019). The Seed Vault provides long-term storage of duplicates of seeds conserved in genebanks around the world (Charles 2006). As of June 2021, 87 depositors safeguard their 1,074,533 crop samples in the Seed Vault.

The European Search Catalogue for Plant Genetic Resources (EURISCO) provides information about more than 2 million accessions of crop plants and their wild relatives, preserved ex-situ by about 400 institutes. It is based on a network of National Inventories of 43 member countries and represents an important effort for the preservation of world's agrobiological diversity by providing information about the large genetic diversity kept by the collaborating institutions (Anonymous 2022).

Ex-situ conservation of animal biodiversity has some distinctive features. Animals' gene banks are a type of biorepository that preserves genetic material. For animals, this is done by the freezing of sperm and eggs in zoological freezers until further need. To establish data banks was one of the recommendations of the FAO/UNEP Technical Consultation on Animal Genetic Resources Conservation and Management, Rome, 1980 (FAO 1981). Animal Genetic Resources include all species, breeds and strains that are of economic, scientific, and cultural interest to agriculture, now and in the future. European Regional Focal Point for Animal Genetic Resources (ERFP) is the regional platform to support the in-situ and ex-situ conservation and sustainable use of animal genetic resources (AnGR) and to facilitate the implementation of FAO's Global Plan of Action for AnGR. Since 2001, ERFP facilitates the collaboration, coordination of work and exchange of information and experience between different European countries and governmental and non-governmental organizations (Anonymous 2018).

The creation and operation of living banks of cultures is the key to preserving the gene pool of bacteria, fungi, and their aboriginal strains. The worldwide importance of culture collections was originally recognized at the First Conference on Culture Collections held in Ottawa, Canada, in 1962, attended by representatives from 28 countries. In 1970, the World Federation for Culture Collections (WFCC) was established; subsequently, the World Data Center for Microorganisms (WDCM) was founded as the WFCC data center. WFCC covers 3,341,403 strains of bacteria and fungi, 832 collections of the world (Figs 1-2, World Data Center for Microorganism 2022).

The first "living" collection of cultures of microorganisms was created by Frantyšek Kralja in 1890 at the Medical Faculty of the Institute of Hygiene, Prague. It counted several hundred cultures and preparations. In 1894 the Mushroom Collection was created in Louvain-la-Neuve, Belgium (Mycotheque de l'Universite'e Catholique de Louvain (MUCL)).

According to WDCM most recent data, the largest collections of fungi by number of cultures are registered in the Netherlands (Centraalbureau voor Schimmelcultures Fungal and Yeast Collection, KNAW-CBS) and the USA (Agricultural Research Service Culture Collection, American Type Culture Collection (ATCC). Each of these collections stores from 40 to 60 thousand strains of fungi of various taxonomic and ecological groups. CBS was created in 1904 in Utrecht (Crous et al. 2005). ATCC is a nonprofit organization which collects, stores, and distributes standard reference microorganisms, cell lines and other materials for research and development. Established in 1925 to serve as a national center for depositing and distributing microbiological specimens, ATCC has since grown to distribute in over 150 countries (World Data Center for Microorganism 2022).

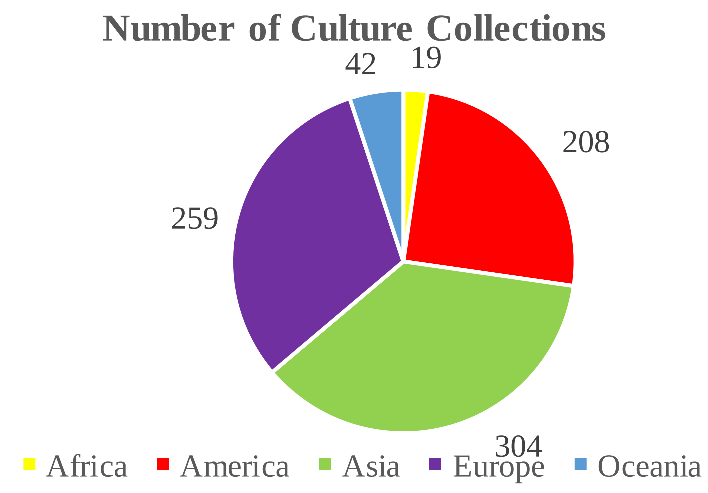

Fig. 1 – Number of Culture Collections in the world.

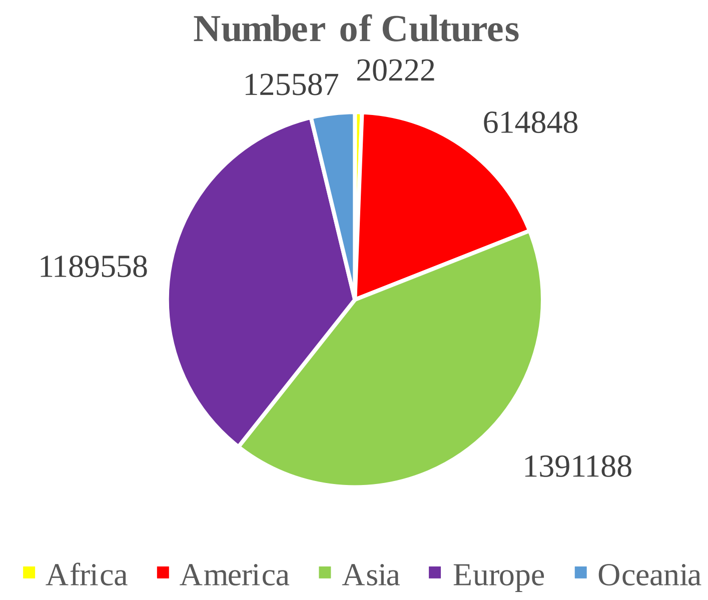

Fig. 2 – Number of Cultures in the world.

Among culture collections of macrofungi in Europe, Culture Collection of Basidiomycetes (CCBAS) of the Institute of Microbiology, Academy of Sciences of the Czech Republic, holds 292 species of the orders *Agaricales* and *Aphyllophorales*.

In Ukraine, there are several pure culture collections of macrofungi varied by number of strains and species composition. The IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine is the largest fungal culture collection in the country. Founded in 1966, it holds currently over 1110 strains of 186 species belonging to 88 genera of fungi, mostly Basidiomycota and Ascomycota (Bisko et al. 2016). Hutsulshchyna National Nature Park (NNP) has pure culture collections of aboriginal strains (Bisko et al. 2018).

So, the data about collections of plant seed, of living banks of cultures and genetic material of animals started to be recorded during last century. Therefore, ex-situ conservation of fungi of the world started even earlier than ex-situ conservation of plants and animals. But we believe that real preservation of fungal biodiversity started with appearance of rare species in the collections, even they are not edible or without medicinal value.

Fungi are the second most species-rich organism group after the insects (Dai et al. 2015); hence, it is more challenging to complete the global fungal inventory, as compared to other organisms such as plants. Based on recently generated data from culture-dependent and independent survey on same samples, the number of fungal species on the earth might be even higher. The development of molecular techniques, such as high-throughput sequencing, has contributed tremendously to identification of previously unknown fungal diversity and would reveal a higher diversity (Wu et al. 2019).

The use of DNA sequence data to infer phylogenetic relationships among fungal lineages can help to detect cryptic species (two or more distinct species classified as a single species) and the fact that many morphologically similar taxa might represent distinct lineages, or numerous well-known species are in fact species complexes (Dai et al. 2015, Wu et al. 2019).

Another novel and important aspect of fungal conservation and protection is development New Generation Sequencing (NGS). They include several massive parallel sequencing technologies that are used to determine the order of nucleotides in entire genomes or targeted regions of DNA or RNA. The basic next-generation sequencing process involves fragmenting DNA/RNA into multiple pieces, adding adapters, sequencing the libraries, and reassembling them to form a genomic sequence. NGS sequences millions of fragments in a massively parallel fashion, improving speed and accuracy while reducing the sequencing costs.

Rapid evolution of sequencing and bioinformatics methods is widely used for fungi barcoding, establishing of their relatedness with related taxa and their evolutionary trajectories, conserving of their genome data and might be used in the future for the restoring of extinct species, similarly to the plant and animal research. Unfortunately, there were not any specific genome sequencing initiative aiming sequencing of rare fungi. However, some rare species were sequenced among edible and medicinal mushrooms (Li et al. 2018). Also, many genome studies were conducted for the fungi with any relevancy for humans, including plant, animal and human pathogens, fungi important for medicine, food and energy production, extremophiles, phylogenetically important and model species. Since 1996 when the first fungal genome was sequenced, several hundred fungal genomes sequencing projects were launched (Cuomo & Birren 2010, Grigoriev et al. 2014, Cuomo 2017, Horn et al. 2020, Gryganskyi et al. 2022).

Repositories of NGS for experimental data are the Gene Expression Omnibus https://www.ncbi.nlm.nih.gov/geo/ and the Sequence Read Archive repositories https://www.ncbi.nlm.nih.gov/sra. Reference data are assembled at the Ensembl database http://www.ensembl.org/index.html, iGenomes https://support.illumina.com/sequencing/sequencing_software/igenome.html and Wormbase https://wormbase.org/#012-34-5. Other databases are also useful for exploring and downloading experimental and reference data, including UCSC Table Browser https://genome.ucsc.edu/cgi-bin/hgTables and the NCBI Genome https://www.ncbi.nlm.nih.gov/genome/ (Basenko et al. 2018).

The aim of our study is to describe the situation with protection of fungal species (in-situ and ex-situ) compared to the methods and practices for plants and animals and discuss re-situ method protection of fungi.

Materials & Methods

To compare the experience of fungal conservation, we compiled the information about the establishment of conservation organizations, ratified International Conventions, and International and National Red Lists. We identified the documents which listed fungi as objects of saving and protection. Also, we focused on the quantity of fungi in the analysed documents. We analysed since time fungi appear as objects of protection, compared to flora and fauna. We analysed the experience obtained while protecting them in Hutsulshchyna NNP in-situ (protection of the area of the fructification): *Anthurus archeri* (Berk.) E. Fisch., *Hericium coralloides* (Scop.) Pers., *Polyporus umbellatus* (Pers.) Fr., ex-situ (isolation of pure cultures and depositing of these cultures in culture collections: *A. archeri*, *H. coralloides*, *P. umbellatus*, *Sparassis nemecii* Pilát & Veselý, *S. laminosa* Fr., and re-situ (introduction of the selected substrates with rare fungi at the places they were recorded in the past: *A. archeri*, *H. coralloides*, *S. laminosa* (Pasailiuk et al. 2018, 2019a-b). Current literature data were retrieved from peer-reviewed journals published at electronic databases such as ResearchGate – https://www.researchgate.net/, NCBI – https://www.ncbi.nlm.nih.gov, Springer – http://jast-journal.springeropen.com/, ScienceDirect – www.ScienceDirect.com, Google Scholar – scholar.google.com, and subject-specific professional websites.

Results

The biodiversity preservation is one of the main missions on present days, where in-situ and ex-situ are the major trends. However, these methods are not sufficient to protect the rare species of macromycetes. Therefore, we tested re-situ preservation, a new method to protect the rare fungal species in natural habitats. The re-situ provides introduction and support of vital functions of mushroom in nature via the forming of their basidioma. For our research, we used *Anthurus archeri*, *Sparassis laminosa*, and *Hericium coralloides*, which are included in the Red Data Book of Ukraine.

We found that the substrate on which the mycelium was grown and subsequently used for natural conditions had a significant influence on the success of "reinoculation" of *A. archeri*. We used four various substrates to facilitate and evaluate the growth of this fungus: soil (for the experiment, the upper layer of a forest soil of a sod-podzolic type that was free from the plant remnants was chosen), 2. beech sawdust (received as a result of picking healthy, newly-hewed wood, the size of the pieces was 1–2 mm), 3. beech sawdust + soil (1:1), 4. beech shavings (received as a result of chipping healthy, newly-hewed wood, the size of the pieces ranged from 10 × 10 to 40 × 1 mm). Among all the investigated substrates, only beech shavings proved to be suitable for growing of sowing mycelium of *A. archeri*. The research showed that the reproduction of this fungus in nature should be held on non-forest experimental sites, mainly on meadows during the spring months (Pasailiuk et al. 2018).

We examined the growth and morphological patterns of two strains of *Hericium coralloides* cultures during competitive colonization of different nutrient media in the pure and mixed (two and three fungal species in the same vial) cultures. The nutrient chemical composition of the medium played an important role in the manifestation of antagonistic potencies of cultures. On the nutrient-poor Czapek medium with cellulose, radial growth of the monocultures was very slow. However, in triple confrontation cultures (*H. coralloides* & *Fomes fomentarius* & *Schizophyllum commune*), the rate of substrate colonization increased, and a positive effect on *H. coralloides* growth was observed. *Fomes fomentarius* (L.) Fr. was consistently antagonistic to *H. coralloides* on all examined media. The less suitable the medium for *H. coralloides* growth, the greater inhibitory effect was observed, but only in the combination of *H. coralloides* and *F. fomentarius*. This effect was observed for the *Hericium* strains. *Schizophyllum commune* Fr. displayed both an antagonistic and a stimulating influence on *H. coralloides*, depending on the medium and the strain of *Hericium* used. The morphology of cultures *H. coralloides* 2332 and 2333 on media of different compositions in dual confrontation cultures was typical of the strains, but the colony growth was mostly uneven (Pasailiuk et al. 2019b).

Sparassis laminosa is a delicious edible fungus and it is protected in many countries, including Ukraine. The study of this fungus in the laboratory and its reproduction under natural conditions are research priorities to preserve this species. We studied the growth of *S. laminosa* strain 2211 on nutrient agar media and various plant substrates. Grain-containing substrates were optimal for mycelial growth; their colonization occurred from in 10-20 days after inoculation. *S. laminosa* strain 2211 can be grown in the natural environment using the re-situ technique. However, fruit bodies were observed in only one location (near *Quercus robur* L. in a sunny area of a temperate deciduous forest next to the neighbor village) at four months after inoculation of the composite substrate containing wheat grain: coniferous sawdust: sunflower seed shells: wheat straw at a ratio of 67%:17%:8%:8%. The obtained fruit bodies were typical of the species morphology, but they had a considerably smaller size. In addition, only substrates that were completely colonized with mycelium were suitable for the application of *S. laminosa* in nature. The use of partially colonized substrates was accompanied by contamination and growth of *Schizophyllum commune* fruit bodies (Pasailiuk et al. 2019a).

We used the aforementioned results to reintroduce native strains of *A. archeri*, *H. coralloides* and *S. laminosa* into the ecosystem of Hutsulshchyna NNP. For the successful colonization of natural substrates, we considered the interference between different fungi, namely the absence of competitive fungal fruitbodies in the vicinity of our experimental plots.

Discussion

It should be noted that the protection of certain species of fungi should be carried out at the regional, national and global levels. After all, fungi, widespread in some areas, may be almost non-existent in others. In order for the inclusion / exclusion of certain species of fungi in the International Red List to be reasonable mycologists need to perform several types of tasks: a) to accumulate the information about fungi that need to be preserved and that are found in different parts of the world; b) to develop the requirements for the protection of specific species of fungi; c) to assess the possibility of preserving a particular species, even in the absence of the necessary information.

According to requirements to potentially qualify for the Global Red List at least one of the criteria of IUCN below needs to be fulfilled: the species has a very small global population (< than 2000 reproducing fungal individuals) or is confined to very few and restricted areas (< 10 locations); the species has a globally small population and is declining (the C-criteria); the species has a geographically restricted distribution globally (single – to few countries) and is declining (the B-criteria); The population of the species has globally declined by at least 15% during the last 10 to 50 years (the A-criteria) (IUCN 2022a).

And once a species is recognized as in need of global protection, certain measures should be taken to preserve fungal biodiversity. In the different countries it is important to adopt legislation on the protection of fungi and appropriate methods of their reproduction; to use the environmental knowledge about the patterns of development of fungi; to promote the natural restoration of historical foci of species, as well as the restoration of areas completely or partially destroyed by grazing and fires; to minimize anthropogenic impact in places where fungi grow; to use re-situ (namely the re-introduction of the fungus to the place where it was growing and fruiting in the past) technology to restore aboriginal strains of rare fungal species; to plan joint environmental measures, mycologists should be involved in their development.

Thus, the practical aspect of fungal conservation is a long process that is at different stages of development in different countries. The process requires not only coordinated work of mycologists and coordination of purely scientific issues related to conservation, but also requires effective support by governments of countries.

We believe that the detection of localities of rare species of fungi and their formal inclusion in the Red List without further action does not guarantee the preservation of fungi in the future and does not affect the increase in their populations in nature. Current conditions are such that in-situ measures aimed at preserving the species and its habitat should be supplemented by reintroduction measures. To this end, the second step in conserving fungal biodiversity is ex-situ measures as defined in the Convention on Biological Diversity (Table 1). The creation and operation of such living banks of cultures is the key to preserving the gene pool of fungi of aboriginal strains. Information about such collections is located at The World Data Centre for Microorganism Culture Collection Information Worldwide site, which covers 887,829 strains of fungi (World Data Center for Microorganism 2022). At the same time, the collections themselves ensure the preservation of the gene pool of fungi, but without active action do not guarantee the preservation of species in nature.

Therefore, we propose to conduct reintroduction measures in nature using experience gained in-situ and ex-situ methods. We named this technique re-situ which means the reproduction of aboriginal strains of rare species of fungi in the wild. Re-situ is a method that provides introducing and support of vital functions of mushroom in nature by forming their basidioma (Pasailiuk et al. 2018). We suggest that such kinds of investigations can become new tools of nature-protecting actions.

Some authors reported the attempts to reproduce rare fungal species in the wild. For example, Martha Crockatt (2008) described her experience with *H. coralloides* reproduction. She reported: oak dowels colonized with *H. coralloides* were inoculated into 15 standing living beech trees and 3 ash trees at Coed Gorllwyn, Bangor. *H. coralloides* was successfully established artificially in living beech trees, as revealed using molecular techniques. However, fruit bodies were not produced. Choi et al. (2003) reported the successful cultivation of *Polyporus umbellatus.* Sclerotia of *P. umbellatus* were cultivated by two methods such as burying and root inoculation methods. Again, no fruit bodies were observed. Piętka & Grzywacz (2005) reported the reproduction of *Fomitopsis officinalis.* Mycelium-overgrown chips of larch heartwood prepared in the laboratory were further used in-situ as inocula. Artificial infection of larches in the Rudka Sanatoryjna Reserve was successful: living *F. officinalis* mycelium was recorded in both cut test trees after the three years of the experiment. Authors hoped that the carpophores of this rare fungus would be produced later. In Ukraine, thanks to the use of re-situ technology the positive results have been obtained with reproduction of *Anthurus archeri* in nature. We observed systematic annual abundant fruiting of fungus withing mycological reproductive sites. These results allowed to remove *A. archeri* from the Red book of Ukraine (Domashlinets 2021). Some positive results were obtained for *Hericium coralloides*. In this case unsystematic fungus fruiting were revealed within the mycological reproductive sites. For *Sparassis laminosa* fruiting in nature using re-situ method happened for three consecutive years. At the same time, in the process of implementing re-situ technology we have a number of issues that need a clearer legal regulation, at least at the level of one state.

The fact is that in Ukraine the legislative acts regulating the implementation of any activity related to the use, reproduction and protection of rare species are the Law of Ukraine “On Flora”, the Law of Ukraine “About the Red Book of Ukraine” and the Forest Code of Ukraine. These documents are aimed at fulfilling Ukraine's obligations under the 1992 Convention on Biological Diversity. In legislative documents where fungi are a subjects of protection reproduction of rare species are mentioned but details of process are not regulated. However, in the process of practical application of reintroduction activities, there are nuances that require focusing on them in the legal field more carefully.

First of all, it is the importance of being able to control the reintroduction process to avoid the situation of "environmental explosion". The legal regulation of this aspect can be the use of only aboriginal strains of fungi for reproduction. The second aspect is a careful selection of sites for reintroduction actions, the conditions of which would not only satisfy the restoration effect, but also take into account the fungal diversity of the site and don’t disbalances the ecosystem after intervention. The third aspect is a careful substrates selection for growing mycelium in laboratory and substrates for re-situ technology in natural habitats. The fourth aspect is a question of “rarity of the substrate”. For example, if the species to be reproduced is rare, but it is an obligate parasite, and its substrate is or may be on the Red List as well, the question of reproduction of the species under these circumstances becomes rhetorical.

Thus, conservation, protection, and reproduction of rare species of fungi by re-situ is an effective tool to combat the extinction of species biodiversity. At the same time, the introduction of re-situ technology is large-scale, requires reasoned legal support to consider the effectiveness of the technology and to confirm its status.

Conclusions

The protection of certain fungal species should be carried out at the regional, national, and global levels and by in-situ, ex-situ, and re-situ methods. Fungi are not presented in any international convention. Fungal conservation became global in 2019. Therefore, experience of in-situ protection of fungi is younger than experience of protection of animals and plants. Fungi are objects of world protection for IUCN SSC Fungal Conservation Committee, The Global Fungal Red List, The IUCN Red List of Threatened Species™. To become the object of the Global Fungal Red List fungi must meet certain criteria.

The National Red Lists for fungi are not founded in many countries and even continents. South America, Australia, Africa (except Benin) haven't the National Fungal Red Lists. Thus, the practical aspect of fungal conservation by in-situ method is a long process that is at different stages of development in different countries.

The creation and operation of living banks of cultures is the key to preserving the gene pool of fungi of aboriginal strains by ex-situ method. The World Data Centre for Microorganism Culture Collection Information Worldwide site covers 887,829 strains of fungi from different countries. Culture collection and field studies widely use various molecular and genomic methods, including NGS, which helps fungal conservation in different areas including identification and ecological adaptations of rare fungal species. The records about ex-situ conservation of fungi of the world belong to the same time or are older than data about ex-situ conservation of plants and animals.

New perspective method to reintroduction of fungi is re-situ method. The method requires a legal justification for the regulation of its application at the regional, state, and global levels. The process requires not only coordinated work of mycologists and coordination of purely scientific issues related to conservation, but also requires effective support by governments of countries.

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