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Review Article

Necessity of a National Fungarium and a Culture Collection for Fungi in Sri Lanka

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ABSTRACT

Sri Lanka is rich in biological diversity, but its fungal diversity is not adequately studied and documented. Recent fungal diversity estimations have predicted that the tropical regions would harbour a large number of novel fungal species. Fungi are ubiquitous, hence it is important to carry out proper investigations to discover novel taxa in different habitats and ecosystems. These taxa represent different life modes i.e. pathogens (of plants, animals and humans), saprobes, endophytes, symbionts (lichens, mycorrhizae), and lichenicolous. Current mycology is mainly based on polyphasic

approaches (morphological, DNA based and chemical analyses) to define the species (consolidated species concept). DNA based phylogenetic analyses are widely used in higher level classification. These DNA are mainly extracted from cultures. Depositing a specimen that the fungus is present at a reputed Fungarium and depositing a culture resulted from the specimen at a reputed culture collection is important. The "International Code of Taxonomy of Nomenclature for algae, fungi and plants" stated that it is important to deposit the holotype at a reputed fungarium, while depositing the ex-type culture which is derived from the holotype at a reputed culture collection is also essential. Besides species identification and classification, these specimens and cultures are important in future studies and in genetic resource conservation. In Sri Lanka, currently a national fungarium and a culture collection for fungi do not exist. However, several institutional collections and personal collections are available. In this conceptual paper, we propose to establish a central, national fungarium to deposit holotypes and a culture collection to deposit ex-type cultures while maintaining several regional or mirror collections to replicate the specimens as isotypes and paratypes, and cultures as ex-isotypes and ex-paratypes.

Keywords: culture collections, fungal diversity, fungaria, type specimens, systematics

1. INTRODUCTION

Sri Lanka is an island in the Indian Ocean with an area of approximately 66,000 km^2 and has a shallow continental shelf which connects with India [1]. Relative to the size of the island, Sri Lanka harbours a highly diverse flora and fauna, making it a biodiversity hotspot together with the Western Ghats of India [2]. This tropical island is home for many endemic species and has the highest species density for flowering plants, amphibians, reptiles, and mammals in the Asian region [3]. There is an exceptional faunal diversity in Sri Lanka, having many point endemic species confined to smaller areas. This rich faunal diversity of the island encompasses at least 124 mammals, 237 resident birds, 120 amphibians, 95 freshwater fish, and 51 freshwater crabs [3,4]. Among these, amphibian diversity is quite significant with 85% endemism and 3.9 species per 1000 km^2 [3,5]. Considering the floral diversity in Sri Lanka, the highest diversity is recorded for flowering plants, followed by fungi, bryophytes, freshwater algae, and ferns [6]. Among the 3,154 species of flowering plants, 894 species are endemic to the island [3]. Furthermore, records also show that nearly 70% of evergreen trees, 40% of lianas, and 11% of mosses are endemic to the country [7]. Although fungi are listed next to the flowering plants in terms of diversity, the diversity of fungi is relatively under-explored in the island. Recent estimates indicate that the Sri Lankan mycota may contain up to 25,000 species, of which only slightly more than 2,000 are currently known to science, with no counts of a large number of exotic forms introduced along with food, plantations, and ornamental plants [8].

Fungi are ubiquitous and represent a vital group of organisms that play important roles in different ecosystems. Several studies have shown that fungi occur in extreme environments such as saline water and the Antarctic region [9,10]. Fungi occur as different life modes. The saprobic fungi are significant in decomposing dead organic matter, which is essential for mineral and nutrient cycling in both terrestrial and aquatic environments [11]. Phytopathogenic fungi, which cause diseases in agriculturally important crops and forests, are important since most of these species adversely impact the economy. Endophytic taxa, which inhabit healthy plant tissues without causing symptoms, are also another main life mode of fungi. In addition to these common life modes, lichenicolous taxa (exclusively parasitic on lichens

[e.g., *Lichenoconium* sp.], fungicolous taxa (parasitic on other fungi [e.g. *Ampelomyces*]), ectomycorrhizal species (e.g. Phylum Glomeromycota), insect pathogens (e.g., *Cordyceps*) are other widespread life modes in various ecosystems (Figure 1). Besides these environmental fungi, some species are important as animal pathogens, including humans [12].

Nevertheless, compared to other disciplines (e.g., lichens, and bioactive compounds in endophytic fungi), less research has been conducted on fungal taxonomy in Sri Lanka. Recently, a checklist by Adikaram and Yakandawala [13] listed 404 plant pathogenic fungi and Oomycota. However, the Oomycota are not recognized as a lineage in the fungal tree of life [14]. Consequently, Wijayawardene et al. [15] excluded this group from the 'Outline of Fungi' and listed them under 'Fungus-like taxa'. According to Adikaram and Yakandawala [13], most of the taxonomic studies of fungi (including mushrooms) had been carried out prior to 1950s by 'Berkeley and Broome [16], Petch [17,18,19,20] and Petch and Bisby [21]. Adikaram [22] and Adikaram and Yakandawala [13] estimated that Sri Lankan fungal diversity to be ca. 25,000 based on the plant: fungi ratio (1:6) proposed by Hawksworth [23]. However, Hawksworth and Lücking [24] considered that this ratio is conservative. Hence, based on recent studies, Hawksworth and Lücking [24] predicted that this ratio would be 1:9.8 (plant: fungi).

Currently, 3087 species of Angiosperms, 350 species of Pteridophytes, 02 species of Gymnosperms, 245 species of liverworts, 07 species of hornworts, and 574 species of mosses have been identified [25] In addition to those, 876 species of lichenized fungi, 48 species of planktonic green algae, 43 species of cyanobacteria, 17 species of diatoms, 09 species of euglenophytes, 05 species of diano flagellates and 139 species of marine algae have been recorded up to now [25]. As such, fungal diversity would be ca. 34,000. However, this prediction is based only on host plants and culture-based studies. Besides, insect associated fungi have not been included in to this estimation. Studies based on environmental sequences would suggest a higher diversity in soil, aquatic environments, and marine sediments.

Taxonomy and classification of fungi have been changing rapidly since the introduction of PCR technology in fungal taxonomy [26]. Morphological characters had been widely used in traditional taxonomy and classification (e.g., Sutton [27] proposed to use conidiomatal characters and conidiogenesis in taxonomy and classification of coelomycetous asexual taxa). Nevertheless, morphology-based classification and taxonomy have been challenged by DNA sequence-based phylogenetic studies [28,29,30]. Currently, mycologists widely use DNA sequence data in species identification and phylogenetic analyses. A consolidated species concept, supported by a polyphasic approach (i.e., combining ecological, morphological phylogenetic species concepts and metabolomics), is currently widely accepted by the mycological community [31]. In addition to taxonomy, the DNA sequences are used to link different morphs (i.e., asexual and sexual morphs) of pleomorphic fungi (i.e., species which show different morphs during their life cycle). Linking different morphs is important in nomenclature since dual nomenclature of fungi [32] was abandoned in 2011 [33].

It must be accepted that some of the current changes in mycology are radical (e.g., proposal for implementing sequence-based nomenclature to name dark fungi by Lücking and Hawksworth [34] and Lücking et al. [35] instead of type-based nomenclature). Currently, it is recommended to use DNA sequences when introducing novel taxa along with morphological characters. This is due to (1) A large number of asexual taxa (e.g. *Aspergillus*, *Colletotrichum*, *Penicillium* and *Phoma*) occur as species complexes (i.e., species are cryptic and difficult to distinguish by morphology [e.g., *Colletotrichum* species]); (2) Some taxa are morphologically similar but genetically distinct (i.e., divergent evolution such as the camarosporium-like taxa

Figure 1. Fungal diversity of Sri Lanka. A. Unidentified hyphomycetous taxon. B., C. Entomopathogenic fungi on dead insects. D. Lichens on rocks. E. *Ophiocordyceps myrmecophila.* F., G. Pathogenic fungal symptoms on leaves. H. *Boletellus emodensis* I. *Cyptotrama asprata* J. *Pertusaria* sp. K. *Poromycena manipularis* L. *Leucocoprinus cretaceous* M. *Coprinellus dissaminatus* N. *Favolaschia calocera* O. *Hypogymnia* sp. P. *Panus* sp. Q. *Lentinus sajor-caju*. Photo Credits: Michael Pilkington, Gunadasa Pathirana, E.Y. Fernando, R.G.U Jayalal.

in the Pleosporales) [36, 37].

Fungal cultures are the main source for extracting DNA. Specimens which harbour novel species and epitypes and generated cultures are mandatory to deposit at the fungaria and culture collections respectively. In this study, we discuss the necessity of establishing a national culture collection to maintain fungal cultures and a national fungaria for fungal specimens in Sri Lanka. It will be essential in future discoveries of novel species and revisiting old species.

2. Missing fungal species

Currently, only ca. 150,000 species are listed in Species Fungorum [38] as accepted species. Nevertheless, Hawksworth and Lücking [24] predicted that the global fungal diversity would be 2.2 to 3.8 million. Hence, a large number of species still need to be discovered. Species in biodiversity rich areas, little explored habitats and life modes, and genera with cryptic species have been recognized as the most plausible answers to the question 'where are missing species?' [24, 39,40,41]. All of these conditions and facts are important when consider the Sri Lankan fungal diversity. Adikaram and Yakandawala [13] regarded that only ca. 3000 species are currently known from Sri Lanka; thus, ca. 31,000 species which are associated with plants are still to be described. Fungi associated with insects are also an important group where species count matters. Rossman [42] predicted that 50,000 species of insect-specific fungi species exist, but currently only ca. 4000 species are known as insect parasites, insect symbiotic species [43,44]. Besides Rossman [42], Weir and Hammond [45] provided an insect: fungi ratio mainly based on beetles and species in the Laboulbeniales. Nevertheless, most research has been carried out only in the USA and Europe. Recent studies by Mongkolsamrit et al. [46] and Thanakitpipattana et al. [47] demonstrated that there is a large number of novel species to be discovered in tropical Asia (e.g., Thailand). Nevertheless, currently the knowledge of Sri Lankan insect diversity is also poor. Hence, apparently, insect-associated fungal species would represent an important group to study with relevance to Sri Lankan mycota.

3. Revisiting old species

Hawksworth and Lücking [24] mentioned that among the 120,000 known species (as of 2017), only 35,000 species have been sequenced. Since most of Sri Lankan fungi were described prior 1990 (i.e., before White et al. [26] proposed the use of PCR technology in fungal taxonomy), yet they are to be sequenced. However, some species have been recollected from other countries and subjected to morpho-molecular analyses (e.g., *Nectria gyrosa* Berk. & Broome was described from Sri Lanka by Berkeley and Broome (1875) (holotype K(M) 109807). Gryzenhout et al. [48] recollected the same taxon from New Zealand and confirmed that it was not related to *Nectria sensu stricto* and thus introduced *Amphilogia Gryzenhout*, Glen & M.J. Wingf.). Hence, besides discovering novel species, it is essential to revisit old species and epitypification (i.e., recollect, carry out morpho-molecular analyses, deposit cultures and/or specimens at international culture collections and fungaria) and confirm prevailing taxonomic understanding based on DNA sequence analyses. For example, Dai et al. [49] emphasized that it is essential to recollect *Hypocrella bambusae* (Berk. & Broome) Sacc. 1878 (basionym: *Hypocrea bambusae* Berk. & Broome, 1873) which was originally collected from Sri Lanka. In their research, Dai et al. [49] used the isotype to illustrate the species (Figure 2).

Thus, we can summarize the importance of re-visiting old species and revise the taxonomic and nomenclatural status in four aspects;

(1) Some species are cryptic and occur as species complexes. DNA sequence-based molecular taxonomy is essential to distinguish them based on phylogenetic analyses. For example, *Colletotrichum gloeosporioides* is a common pathogen which occurs on different crops in Sri Lanka (Adikaram and

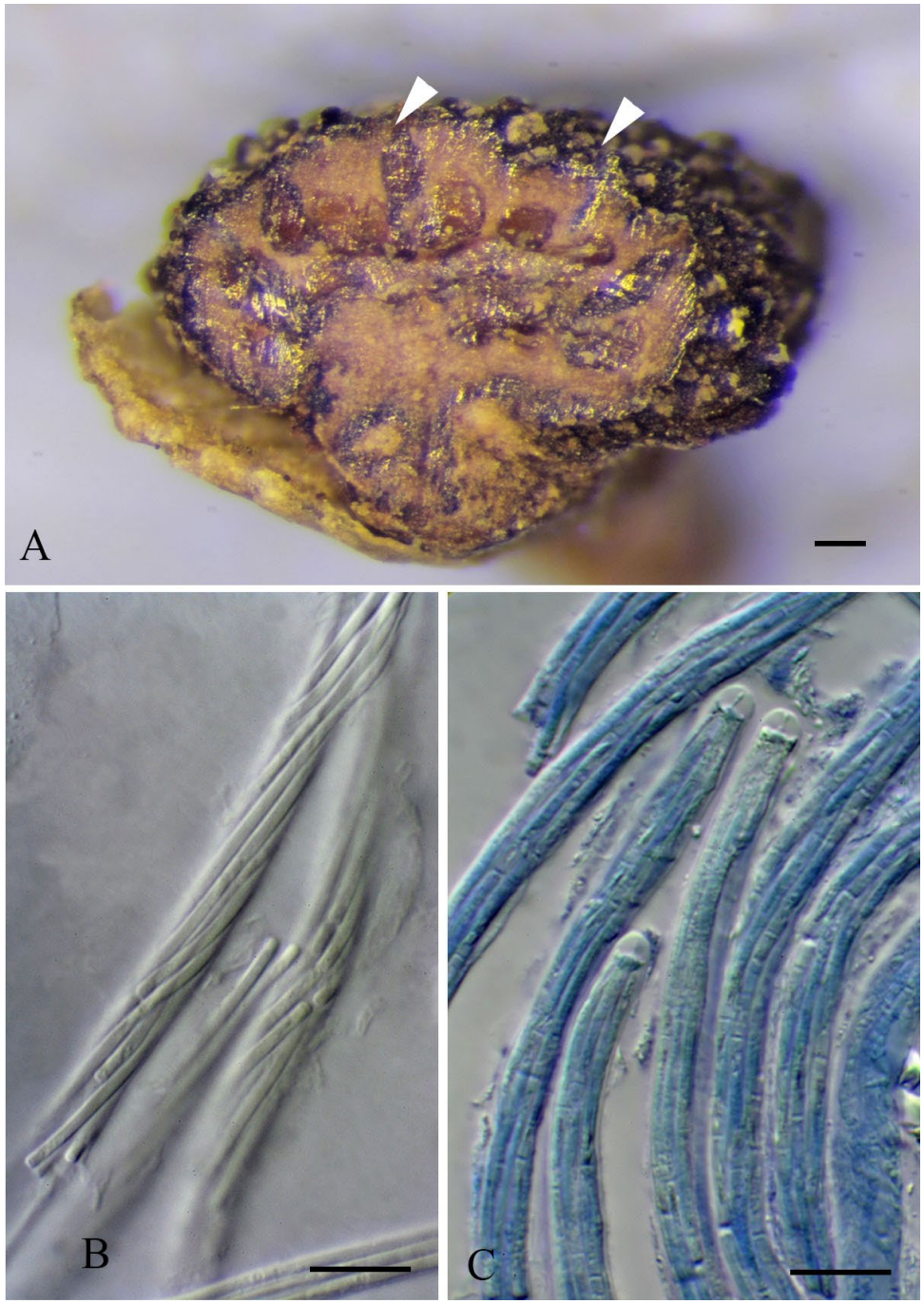

Figure 2. *Hypocrea bambusae* Berk. & Broome (Material examined. SRI LANKA, on inflorescence of bamboo, January 1855, G.H.K. Twaites s.n. (ex herb. M.J. Berkeley), K(M)52469, isotype). A. vertical section of stromata showing the perithecia locating. B. filiform ascospores. C. Asci with caps (Staining by cotton blue). A= 200 μm, B,C= 20 μm.

Yakandawala, [13] 2020). However, Weir et al. [50] discovered 22 species and one subspecies within the *Colletotrichum gloeosporioides* species complex, using multi-gene phylogenetic analyses. Similar large scale taxonomic revision based on multi-gene has not been carried out for *Colletotrichum gloeosporioides* (complex) in Sri Lanka. As such, it is uncertain how many *Colletotrichum* species occur according to Weir et al. [50] in Sri Lanka.

(2) Taxa with a similar morphology have distinct phylogenetic lineages in the *Fungi* due to divergent, polyphyletic or even paraphyletic evolution. DNA sequence-based phylogenetic analyses represent only solution to delimitate correct higher level taxonomic ranks. For example, according to Index Fungorum [44], *Phoma arundinacea*, *P. aterrima*, *P. cocoicola*, *P. coryphea*, *P. durionis*, *P. heveae*, *P. hysterioidea*, *P. lobelia*, *P. orchidearum* and *P. pelliculosa* have been described from Sri Lanka. However, phoma-like taxa are highly polyphyletic in *Dothideomycetes* (mainly in *Pleosporales*; *Didymellaceae-Phoma sensu stricto*; *Leptosphaeriaceae–Plenodomus* (*fide* de Gruyter et al. [28]. It is improper to accommodate these taxa in the correct genus without DNA-based molecular taxonomy, thus using 'Phoma' species names are incorrect.

(3) Species can have different morphological characters (e.g., conidial pigmentation and conidiogenesis) but be phylogenetically close. For example, species of *Phoma* produce hyaline, irregular, simple conidia are placed in the *Didymellaceae*. de Gruyter et al. [28] showed that some species of *Phoma* are phylogenetically related to *Coniothyrium*, which produces brown, 0-1-septate conidia consequently have been transferred to *Coniothyrium sensu stricto* (*Coniothyriaceae*). Hence, the names proposed for some taxa (such as *Phoma*) based on morphology are questionable.

(4) A large number of species described from Sri Lanka are listed as invalid or illegitimate [44] or which have been transferred to other genera or not listed in Species Fungorum [38]. Missing type specimens or specimens in which typification details were not noted are some common reasons for being invalid. Hence, it is essential to revisit older names and specimens to confirm the current status.

(5) Epitypification old species (e.g., Pratibha and Prabhugaonkar [51] designated the epitype of *Pithomyces flavus* Berk. & Broome. However, the locality was indicated as the Western Ghats in mainland India).

4. FUNGARIA AND CULTURE COLLECTIONS AS REPOSITORIES

Mycologists have deposited millions of fruiting structures of fungi (which are inhabitants of plant materials) or voucher specimens in worldwide collections known as fungaria [52]. These depositions are essential in morphology-based taxonomy and in nomenclature [53]. Taxonomists who strictly depend on morphological characters (i.e., prior to molecular taxonomy) deposited these specimens in public collections (see Index Herbariorum) since it is important for future scientific purposes [54]. Furthermore, Seifert and Rossman [53] mentioned that the collections with online databases of their holdings are an extra advantage.

The International Code of Nomenclature for Algae, Fungi and Plant (ICNafp) (Shenzhen Code) [55] mentioned that "For the name of a new species or infraspecific taxon published on or after 1 January 1990 of which the type is a specimen or unpublished illustration, the single herbarium, collection, or institution in which the type is conserved must be specified (Art. 40.7)". Hence, it is essential to indicate the type and deposit it at a mycological herbarium (or fungarium). Most of the ancient collections of Sri Lanka (i.e., in 19th century), by M.J. Berkeley (1803-1889) and T. Petch (1870-1948) were deposited at the Peradeniya Royal Botanical Garden, Sri Lanka. Currently, majority of the microfungi species and mushroom species are available at the herbarium of the Peradeniya Garden. Interestingly, most of these species were originally described from Sri Lanka. Holotypes of Petch's collections were deposited in the national herbarium at the Royal

Botanic Gardens, Peradeniya while Berkeley deposited holotypes of his species in the Royal Botanic Gardens, Kew, England, U.K. Nevertheless, still most of these taxa are lacking cultures and sequence data.

Currently, DNA sequences have been used extensively in species identification and in phylogenetic analyses. Thus, fungal cultures are being used to extract DNA [56], but direct extraction of DNA is also popular for unculturable taxa [49]. Even so, pure cultures have been used in some studies prior to the era of molecular taxonomy. For example, mycologists have been using pure cultures to confirm sexual, asexual morphs in pleomorphic species (e.g., Rogers and Samuels [57], to distinguish cryptic species in speciose genera [58] on the basis of culture characteristics; and to distinguish species of *Colletotrichum* using culture techniques (including appressoria production in slide cultures) [59,60].

Cultures can be used as types when they have been stored in a metabolically inactive state (Shenzhen Code) [55].

Art. 8.4. Type specimens of names of taxa must be preserved permanently and may not be living organisms or cultures. Nevertheless, cultures of algae and fungi, if preserved in a metabolically inactive state (e.g., by lyophilisation or deep-freezing to remain alive in that inactive state), are acceptable as types.

Art. 40.8. For the name of a new species or infraspecific taxon published on or after 1 January 2019 of which the type is a culture, the protologue must include a statement that the culture is preserved in a metabolically inactive state.

Moreover, the cultures obtained from a type specimen are referred to as ex-type (ex typo), ex-holotype (ex holotypo), ex-isotype (ex isotypo) and could be maintained as living cultures in accessible culture collections. These cultures are deposited at culture collections or 'genetic resource collection' [40].

Fungi could occur as a mixture of species in nature. Hence, for DNA-based studies (and to deposit in culture collections), it is essential to obtain DNA from a culture which is derived from a single species. Senanayake et al. [61] comprehensively discussed the different isolation techniques use to obtain pure cultures. Maintaining pure cultures (a culture generated from a single species) prior to the extraction of DNA is also important since it could be contaminated by other airborne species (due to poor storage conditions or sterilization techniques) or destroyed by mites [61]. Using contaminated strains in DNA extraction will result in a mixture of DNA and provide inaccurate results in phylogenetic analyses. At the same time, these strains are stored in accessible culture collections since maintenance of these cultures are important for future studies [40,62] and for conservation and utilization in the future [63].

5. Species introduced from Sri Lanka and typification details

According to Index Fungorum [44] (one of the repositories for registration of fungal species names *fide* F.5.1. Shenzhen Code) [55], 1,719 species (including fungi and lichens) have been described originally from Sri Lanka (i.e., as novel species). We recognize three different eras of Sri Lankan mycology and lichenology based on the indication of type and species identification protocol. These are (1) species introduced prior to 1958 (no need to indicate the type materials), (2) species introduced during 1958-1989 (type indication is essential but used only for the morphological species concept (i.e., before White et al. [26] and no DNA sequence-based analyses), and (3) species introduced during 1990-upto date (type indication is essential, species were introduced based on morphology and/or DNA sequence-based analyses).

The national herbarium at Royal Botanic Gardens, Peradeniya (PDA) was used to deposit 224 specimens, including holotypes of 213 species described before 1958 (Figure 3). These collections were mainly formed by T. Petch. These specimens are presently located at the Plant

Pathology Division of Horticultural Research and Development Institute, Gannoruwa, Sri Lanka However, 1511 species have been described from Sri Lanka prior to 1958 and other type material was deposited mainly in the Royal Botanic Gardens, Kew, England, U.K. (K(M)) (i.e., belonging to 888 species) while several were deposited at the University of North Carolina, Chapel Hill, North Carolina, U.S.A. (NCU) and The Natural History Museum, London, England, U.K. (BM).

Interestingly, type specimens of the species described during 1958-1989 (85 species) were not deposited at PDA or any other repositories in Sri Lanka. Most were deposited at K (M), Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands (CBS) and the Smithsonian Institution, Washington, U.S.A. (US).

One hundred and twenty one (121) new species have been described from 1990 to date in Sri Lanka (Figure 3). The majority of these were lichens (75), followed by non-lichenized *Ascomycota* (29) and *Basidiomycota* (16), respectively. Type specimens (holotypes) of 55 species of lichens have been deposited at PDA. However, the holotypes of non-lichenized *Ascomycota* and *Basidiomycota* taxa were not deposited at PDA or any other fungaria in Sri Lanka [44] e.g., Sivanesan [64] introduced *Coryesporasca caryotae* Sivan. (Current name = *Corynespora calicioidea* [Berk. & Broome] M.B. Ellis *fide* [38] and deposited the holotype at IMI as IMI 362840a). Nalim et al. [65] introduced *Fusarium haematococcum* Nalim et al. and deposited the holotype at BPI (BPI 881227), a dry culture ex ascospores as BPI 871363, while depositing living ex holotype culture of *F. haematococcum*: FRC S-1832 = CBS 119600). Nevertheless, a recent study by Ferdinandez et al. [66] introduced three species of *Curvularia* and deposited holotypes and ex-types at USJ-H (University of Sri Jayawardenepura Herbarium) and in USJCC (University of Sri Jayawardenepura Culture Collection). Figure 4 summarizes the deposition of holotypes of newly described species since 1990-todate.

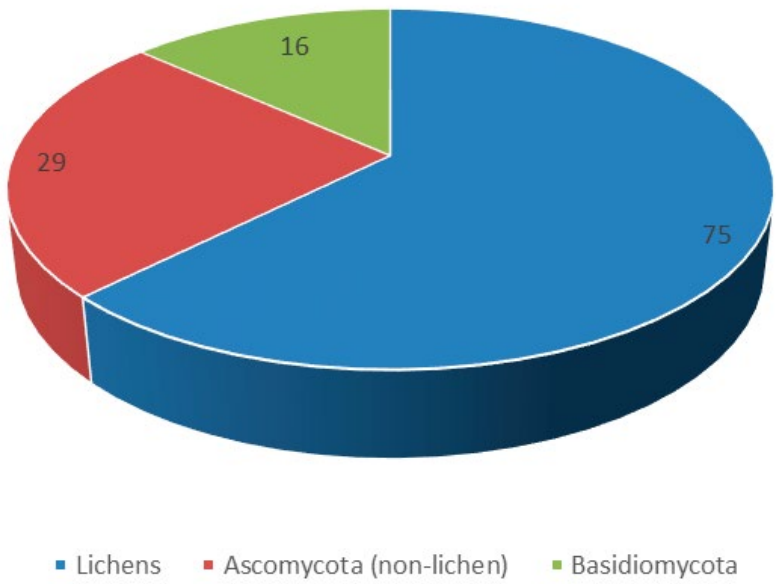

Figure 3. Species introduced during 1990-2021.

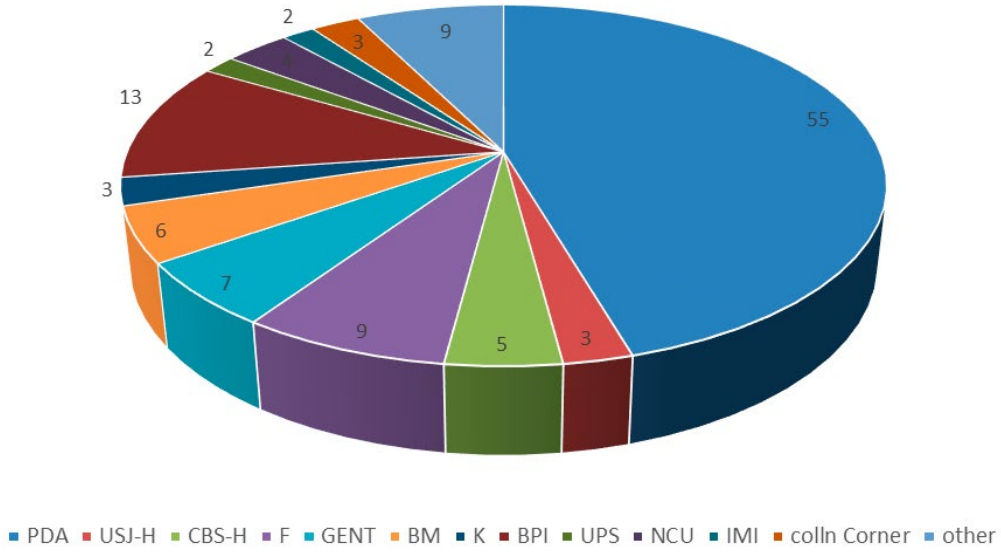

Figure 4. Deposition of holotypes in different herbaria (1990-upto date). PDA: Royal Botanic Gardens, Peradeniya, Sri Lanka; USJ-H: University of Sri Jayewardenepura Herbarium, Sri Lanka; CBS-H: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; F: Field Museum of Natural History, Chicago, Illinois, U.S.A; GENT: Ghent University, Ghent Belgium; BM: The Natural History Museum, London, England, U.K.; K: Royal Botanic Gardens, Kew, England, U.K.; BPI: U.S. National Fungus Collections, USDA-ARS, . Beltsville, Maryland, U.S.A.; UPS: Museum of Evolution Uppsala, Sweden; NCU: University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, U.S.A.; IMI: CABI Bioscience UK Centre, Egham, England, U.K.; Colln Corner: A personal collection.

6. HISTORY OF CLINICAL FUNGI IN SRI LANKA

Pathogenic fungi cause diseases in human or other organisms. Fungi which cause diseases to human bear clinical importance. They are among the first microorganisms to be recognized as etiologic agents of a disease. Approximately 300 fungi are known to be pathogenic to human. Jayasekera et. al. [67] have compared estimated annual incidence of global and Sri Lankan fungal burden (Table 1). This study estimates suggested that 639 estimated oral candidiasis cases, 100 oesophageal candidiasis cases and only 13 cases of cryptococcal meningitis among 1,317 HIV (AIDS) patients. The estimated prevalence of chronic pulmonary aspergillosis (CPA) post TB is estimated to be 1,443 and all forms of chronic pulmonary aspergillosis (including aspergilloma) 2,886 patients. They also have estimated 229 cases of invasive aspergillosis and 41 cases of mucormycosis. The projected annual incidence of candidaemia is 507 and an estimated 76 candida peritonitis cases post-surgery. According to our estimates 10,344 patients were suffering from ABPA and 13,654 were suffering from SAFS. The projected annual incidence of fungal keratitis would be 1,277 and tinea infections would affect 50 children and Pneumocystis incidence was not estimated. Candidemia is the most common form

Table 1. Annual incidence of fungal diseases (summarized from data reported by Jayasekera et al. [67])

Disease	Causative agent	Global data (million)	Local data
Vulvo vaginal candidiasis	*Candida albicans*	137	25,750
Severe asthma with fungal sensitization (SAFS)	*Aspergillus* species	3.5-15	13,654
Allergic bronchopulmonary aspergillosis (ABPA)	*Aspergillus fumigatus*	4.8	10,344
Chronic pulmonary aspergillosis (CPA)	*Aspergillus fumigatus*	3.5-15	2,886
Chronic pulmonary aspergillosis (post TB)	*Aspergillus* species	1.2	1,443
Fungal keratitis	*Fusarium* species *Aspergillus* species	1-1.2	1,277
Oral candidiasis	*Candida albicans*	10	639
Candidaemia	*Candida* species	0.4	507
Invasive aspergillosis	*Aspergillus fumigatus*	>0.2	229
Oesophageal candidiasis	*Candida* species	2	100
Candida peritonitis	*Candida albicans*	0.1	76
Tinea infections	*Trichophyton rubrum*	985	50
Mucormycosis	*Rhizopus* species *Mucor* species	Not estimated	41
Cryptococcal meningitis	*Cryptococcus neoformans*	1	13

of invasive candidiasis. *Candida* and *Aspergillus* species are the leading causative agents for fungal diseases in Sri Lanka.

Anecdotal reports on candidemia in Sri Lanka explain an upward trend [68]. This study has used molecular techniques such as 18S rRNA gene sequencing for the species identification. In addition to main fungal diseases, some rare diseases are emerging in the country. Many of the papers cited in previous literatures are short cases reported that advance our knowledge on geographical distribution, prevalence and the clinical variations of a disease. However, no systematic record keeping found in place for fungal diseases in Sri Lanka.

Though Sri Lanka doesn't have a system to calculate actual fungal disease burden in numbers, the burden has increased immensely over last nine years. There are several reasons for the increased disease burden where as increased number of immunocompromised patient population (patients who are on chemotherapy for cancers, both solid organ and stem cell transplant recipients, patients with uncontrolled diabetes, increase in HIV and tuberculosis patients, patients who are on steroids and other groups of immunocompromised populations), increased awareness about fungal infections among health-care workers and general public, increased facilities to diagnose fungal infections (specially with the introduction of various serology tests to diagnose fungal infections) and increased modalities of treatment options for patients suffering from fungal infections.

In addition to above, antifungal resistance has increased among yeast species compared to statistics 10 years back. Sri Lanka was able to identify many uncommon fungal pathogens among immunocompromised patients and also there were many uncommon clinical entities recorded with commonly isolating fungal pathogens.

8. IMPORTANCE OF FUNGARIA, BIOLOGICAL RESOURCE CENTERS OR CULTURE COLLECTIONS TO STORE INDUSTRIALLY BENEFICIAL CULTURES

Most of the Sri Lankan fungal studies have not directly related to diversity studies. However, researchers have isolated fungal species from different sources and screened them for different properties like the production of bioactive compounds, antiseptic activities, biocontrol, growth promotion activity. All types of such research have contributed to add the data to fungal diversity of Sri Lanka.

Sri Lanka is an agricultural country. Hence to improve the quality of many crop varieties; scientists have developed various types of biofertilisers together with incorporated fungal strains. Phosphate solubilizing fungi, *Trichoderma virens* and *Penicillium oxalicum* have been tested successfully in field studies as P biofertilizers for rice cultivation [69]. Some scientists have used plant growth-promoting local fungal isolates to improve the growth performances of crop varieties. Under this line, Wijesooriya and Deshappriya [70] have isolated *Acremonium* and *Arthrobotrys* from different parts of Kuruluthuda rice plants collected from a paddy field cultivated using organic fertilizers in the Gampaha district, Sri Lanka. Priyadarshani et al. [71] have isolated endophytic fungal communities associated with the rice variety Ld 368 and identified as *Trichoderma* sp. and *Chaetomium* sp. Singhalage et al. [72] have shown the presence of *Aspergillus* sp. in the rhizosphere of strawberry roots and such *Aspergillus* sp. in combination with *Enterobactor* sp. improved the yield and profitability of strawberries. *Colletotrichum* was another fungal species isolated from the soil plant system of tea in Sri Lanka and it has shown the growth promotion in tea in the fungal surface attached biofilm mode [73]. *Xanthoparmelia mexicana* inhabiting lichen thallus also showed the solubilization ability of Eppawala rock phosphate [74].

The isolation of bioactive compounds from the fungal strains isolated from different sources is another type of industrially valuable research going on in Sri Lanka. Maduranga et al. [75] has conducted a research to discover the bioactive compounds present in endolichenic fungi (ELF) living in Mangrove plants of Puttalam lagoon and 70 such fungal isolates were identified as *Aspergillus*, *Byssochlamys*, *Talaromyces*, *Diaporthe* (= *Phomopsis*), *Endomelanconiopsis*, *Schizophyllum*, *Cerrena*, *Trichoderma*, *Xylaria*, *Hypoxylon*, *Daldinia*, *Preussia*, *Sordaria*, *Neurospora*, and *Lasiodiplodia*. *Aspergillus terreus* and *Trichoderma virens* were identified as bioactive compound producers against pathogenic bacteria and were isolated from endophytic tissues of plants of Family Cyperaceae [76]. *Chaetomium globosum* which is another endophytic fungus isolated from *Nymphaea nouchali* and which showed the bioactive properties [77]. *Aspergillus flavipes* was a bioactive compound producing endophytic fungal species isolated from a demosponge collected on the west coast of Sri Lanka [78]. Endophytic *Fusarium* sp. has also been isolated from *Opuntia dillenii* obtained from South-Eastern arid zone of Sri Lanka and such *Fusarium* sp. too showed the bioactivity [79]. The *Penicillium* sp. isolated from the garden soil showed the secretion of sugar types such as *Fucose*, *Ribose*, *Arabinose* and *Xylose* when such *Penicillium* sp. combined with the *Bradyrhizobium elkanii* by forming biofilm [80]. *Acremonium* sp. and *Fusarium* sp. isolated from the litter showed high levels of cellulase secretion [81]. Ratnayake et al. [82] showed the presence of bioactive compounds pitholide E, pitholide B, pitholide D in *Pestalotiopsis microspora* isolated from the fruits of *Manilkara zapota*. The presence of antimicrobial compounds, epidithiodioxopiperazine, gliotoxin in *Hypocrea virens* the isolate from leaves and twigs of *Premna serratifolia* [83] was another example for

bioactivity of fungal metabolites. *Aspergillus terreus* and *Trichoderma virens* isolated from the plants of the family Cyperaceae also showed antibacterial activities [76]. Woodland living *Fulviformes fastuosus* also studied for cytotoxic effect [84]. Macro fungi, *Phellinus repandus* and *Inonotus porrectus* living in dry zone forest reserves of Sri Lanka also reported as the species with bioactivity [85].

Some fungal records are also available in the line of bioremediation activities. Kannangara et al. [86] have isolated aromatic hydrocarbon-degrading fungi, *Penicillium oxalicum* from ornamental leaf samples collected from highly urbanized and industrialized areas of Sri Lanka. Kannangara et al. [87] have also isolated and documented poly hydrocarbon degrading *Penicillium oxalicum*, *Nigrospora oryzae*, *Aspergillus oryzae*, *A. aculeatus*, two species of *Penicillium*, two species of *Eupenicillium* and *Mortierella* endophytic fungi from the moss plant *Macromitrium* sp. (frequently available) in Sapugaskanda (highly polluted) and Hettimulla (less polluted) areas in Sri Lanka. Kumari and Saputhanthri [88] recorded the heavy metal tolerant two fungi species isolated from Ussangoda Serpentine soil and those were identified as *Aspergillus terreus* and *Gongronella butleri*. Recently, Williams et al. [89] reported *Serpulanines* A to C, N Oxidized tyrosine derivatives isolated from the Sri Lankan fungus *Serpula* sp.

In many instances the details about the deposition and accession of such cultures are not available. The studies that are related to the bioactivity, bioremediation, bio fertilizer were conducted in the universities such as University of Colombo, University of Kelaniya and Uva Wellassa University of Sri Lanka and at the National Institute of Fundamental Studies (NIFS). However, data of the storage of such cultures were not provided in many publications. Seneviratne et al. [73] and Seneviratne and Indrasena [74] mentioned about the deposition of their fungal cultures in NIFS laboratories. Similarly, other researchers could store their fungal strains in their respective universities and institutes. Lack of facilities for deposition and accession of fungal voucher specimens and strains are a huge constraint that needs an urgent attention to protect Sri Lankan biodiversity and ex situ conservation efforts.

Further, the industries like breweries and bakeries follow the fermentations during the production of beverages and bakery products. They also use cultures such as *Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis* like species as fermentative organisms. Such yeast cultures are commercially available as starter cultures but the maintenance of yeast culture collection is beneficial for inoculum producers and for users.

9. Existing specimens and culture collections in Sri Lanka

9.1 Specimen Collections of Environmental Fungi

As mentioned above, PDA was the main repository for specimens of novel taxa described prior to 1958. However, Sutton [27] mentioned that the Tea Research Institute, Talawakelle, Sri Lanka was also maintaining specimens of tea-associated species, such as *Seimatosporium grevilleae*. Moreover, macrofungi collection at University of Colombo is also an important collection [90, 91]. University of Sri Jayewardenepura (USJH) is also currently maintaining a microfungi collection at their fungarium.

9.2 Lichen Collections

Sri Lankan main lichens collection is being maintained at the Royal Botanic Garden's Herbarium (PDA) at Peradeniya. This collection includes collections made by G.H.K. Thwaites (1849-1880), M.E. Hale (1976–1978), the first Sri Lankan lichen workshop (1999), Chandrani Wijerathne (1999–2003), R.G.U. Jayalal (2004-2006) and Gothamie Weerakoon (2009-2016). Apart from PDA collection, another unregistered collection is being maintained at the Department of Natural Resources, Sabaragamuwa University of Sri Lanka, Belihuloya, University of Colombo and University of Sri Jayewardenepura.

9.3 Culture Collections of Environmental Fungi (including Phytopathogens)

Currently, five culture collections have been registered in the World Data Centre for Microorganisms (http://www.wfcc.info/ccinfo/index.php/home/content). Three of them are being maintained at the Rubber Research Institute, Agalawatta (90 strains), while two other collections have been established at the National Institute of Fundamental Institute (NIFS) (130 strains) and the University of Sri Jayawardenepura (100 strains) (http://ccinfo.wdcm.org/index.php/search/basic/) (accession date: 29.10.2021).

9.4 Clinical Fungi

The Medical Research Institute (MRI) is the main service laboratory for processing and identification of patient samples from many parts of the country with regard to fungal infections in Sri Lanka. They process blood cerebrospinal fluid (blood CSF), sputum, body fluids, urine, stools, pus and aspirates, skin and soft tissues with regard to direct microscopy and culturing, serology for *Aspergillus* and *Candida*, histopathology, culture identification, and anti-fungal susceptibility testing.

The Department of Mycology at Medical Research Institute has a Pathogenic Fungal culture collection with 8000 isolates.

9.5 Industrially Important Fungi

Currently, Industrial Technology Institute of Sri Lanka maintaining a culture collection of fungal species which are important in industries.

10. Importance of Fungaria and Culture Collections in Current and Future Inventorying of Sri Lankan Fungal Diversity

Sri Lanka is reported to be among the 34 biodiversity hot spots in the world along with the Western Ghats. The diversity of vascular plants and different faunal groups has been well documented. However, when it comes to lower plants and microorganisms, great deals of studies are yet to be done. Our knowledge on fungal diversity in Sri Lanka is extremely poor. One of the vital prerequisites for any taxonomic study is a repository of the group under study. In Sri Lanka, the focus of the mycological studies conducted hitherto was the pathogenic fungi. Even the available mycological collections at present were mostly having those types of fungi. Therefore, it is vital to establish a central collection of free living fungi to include both phyllosphere and rhizosphere fungi. The Ministry of Environment is currently preparing a National Environment Action Plan (NEAP) and the necessity of establishing bio-repositories for lower plants has been recognized a priority and National Institute of Fundamental Studies, in Kandy has been cited as a site for a Fungal Bio repository.

11. Why Sri Lanka Needs National, Well-known Repositories

Sri Lanka ratified the UN Convention on Biological Diversity (CBD) in 1994. Article 6 of the CBD requires contracting parties to develop a National Biodiversity Strategy and Action Plan (NBSAP), or an equivalent instrument. This strategy acts as the principle instrument for the implementation of biodiversity conservation at both the national and global level. Convention on Biological Diversity has highlighted the importance of given guidelines for ex-situ and in-situ conservation of species by giving guidelines for conservation. According to the Article 8 of the CBD bring the contracting parties are bound to establish a system to protect species in their natural habitats within protected areas and outside protected areas for the conservation and sustainable use of the species. In the Article 9 of the CBD contracting parties agree to promote ex- situ conservation of species by establishing and maintaining facilities for ex-situ conservation and research on plants, animals and micro- organisms, preferably in the country of origin of genetic resources.

Biological repositories such as natural history museums, herbaria, and culture collections maintain

collections of type specimens of plants and animals, genetic resources, and microbial cultures. These repositories are critically important to ensure the continued scientific study of biological diversity on the earth and its preservation. In Sri Lanka, this requirement is addressed by institutions such as the National Museum of Sri Lanka, the National Herbarium of Sri Lanka, and the Plant genetic resource centre. Current repositories of other organisms are listed in Table 2. However, Sri Lanka lacks any globally recognized fungaria and culture collections. This is a major impediment to the exploration, and documentation of fungal diversity in this global biodiversity hotspot as any formal description of fungal taxa should be accompanied by a type specimen deposited in a globally recognized institution (ICNafp) [55].

Novel species discovered in Sri Lanka can be deposited in foreign reference collections. However, according to the fauna and flora protection ordinance of Sri Lanka, exportation of plants or any plant material is strictly prohibited even for specimens legally collected for research purposes. Thus, special permission has to be obtained from the government conservation authorities (i.e., Department of Wildlife Conservation or the Department of Forest Conservation, , Department of Agriculture and National Plant Quarantine Services) to export plant specimens that are legally collected. However, deposition of local specimens in foreign institutes hinders the accessibility of these specimens to local researchers. Thus, to serve the best interests of both global as well as local researchers, it is crucial to establish a globally recognized easily accessible national fungarium in Sri Lanka.

Sri Lanka also lacks dedicated microbial culture repositories containing cryopreserved bacteria, fungi, yeasts, cyanobacteria, algae, protozoa, and viruses. In addition to maintaining cryopreserved monoculture lineages of microorganisms, internationally recognized culture repositories such as American Type Culture Collection (ATCC), German Resource Centre for Biological Material (DMSZ) and National Collection of Industrial, Food and Marine Bacteria, UK (NCIMB) also serve as reference repositories for important industrial, marine, food, and environmental organisms. They function as important viable cell and genetic material resources for industries and researchers internationally. Cryopreserved cultures in such repositories are maintained under internationally agreed standard conditions, morphologically characterized frequently, and proliferated/passaged to maintain phenotypic and genotypic consistency.

Absence of a central microbial resources repository in Sri Lanka is a severe impediment to related research, healthcare sector, industries, and environmental/bio remediation activities. Individual institutions such as universities in the state-owned university system of Sri Lanka and

Table 2. Existing repositories for important organisms in Sri Lanka.

Group of organisms	Repository	Responsible institute/s	Important publications
Reptiles	Colombo National Museum	Herpetological Foundation of Sri Lanka, Rajarata University of Sri Lanka, University of Sri Jayewardenepura	Karunarathna et al. [92-95]
Vascular plants	National Herbarium	Department of National Botanic Gardens	Weerakoon et al. [3,25]
Environmental bacteria of industrial importance	Rajarata University of Sri Lanka	Rajarata University of Sri Lanka	Dissanayake et al. [96]

many other public-funded research institutions such as the Industrial Technology Institute (ITI), National Institute for Fundamental Studies (NIFS), and National Aquatic Resources Research and Development Agency (NARA) maintain smaller-scale microbial culture repositories. Often, their collections are confined to individual departments of these institutions and no standard protocols are followed for record-keeping, maintenance, proliferation, and storage of cultures. Furthermore, most of the isolates maintained in such repositories are characterized only for their biochemical and morphological traits. A systematic molecular characterization and taxonomic assignment based-on one or several genetic markers are lacking.

The extent of microbial biodiversity in Sri Lanka is barely understood. Given the vast habitat range found in the country, it is plausible that our current understanding of the microbial diversity in terrestrial and marine habitats is very limited. This applies to the fungi of Sri Lanka, where only a limited number of studies already conducted shed light on the local fungal diversity [66,97]. However, mycological studies conducted hitherto were mostly on the pathogenic fungi. Therefore, it is vital to establish a central collection of free living fungi to include both phyllosphere and rhizosphere fungi. In this context, the establishment of a central fungarium in Sri Lanka bears immense importance. It is also appropriate that the envisaged centrally served fungarium has tributary institutions (i.e., Sri Lankan universities, research institutions in different disciplines and in different provinces) making specimen contributions possible as and when they become available. A comprehensive centralized fungal collection will allow both the local and global community easy access of scientific information on Sri Lankan fungi. It will also allow repeatable, uniform, and standardized proliferation of reference fungal cultures that can be used in industry, agriculture, medicine, and environmental remediation.

Nevertheless, it is essential to maintain correct protocols and standards when establishing of culture collections as mentioned in World Federation for Culture Collections (http://www.wfcc.info/guidelines/) and OECD Best Practice Guidelines for Biological Resource Centres (https://www.oecd.org). 'ISO 20387:2018 Biotechnology-Biobanking-General Requirements for Biobanking' specifies the requirements of biological repositories that ensure biological material and associated data are trustworthy. ISO/TR 22758, Biotechnology – Biobanking – Implementation guide for ISO 20387 provides detailed guidance to biobanks on how to implement the quality management, management, and technical requirements of ISO 20387 and was published to help organizations get the most out of the standard. Sri Lanka Accreditation Board is currently educating the staff of culture collections including microbial culture collections in an effort of streamlining the management of culture collections as per these international standards. Maintaining living cultures is a challenge as misidentification, contamination, loss of activity etc. could jeopardize the aims of the culture users. Accreditation of biological repositories and maintenance of accreditation obtained is strenuous with respect to finances as the biological repositories are mainly non-profit entities. Hence, we strongly suggest a national level institute with direct government funding is ideal to get such responsibility. Moreover, such a key institute can easily collaborate with other institutes such as universities and industries. Maintaining a central database under the supervision of such an institute is also important to gather all the data of cultures/ strains in other institutes. Hence it fills the gap of scattered data which are not properly maintained. Furthermore, such repository can be promoted to serve as an International Depository Authority (IDA) in Sri Lanka for storing microorganisms for patent purposes by adopting the rules and regulations made in Budapest Treaty of 1980.

Here, we suggest to promote a state-owned, national institute with a mandate to conduct fundamental research as a fungarium to maintain

fungal specimens (e.g. mushrooms, lichens, plant materials with microfungi, dried cultures, and permanent slides) (herbarium code: NIFSMC) (culture collection code: NIFS-MC) while promoting regional or mirror collections based on below justifications (Figure 5).

• With vast range of diversity, Sri Lanka holds a huge potential to serve both the national and international academia with great source of ecological, conservation and biogeographical research. The significant data associate with original collections can be cooperative to describe, understand and distinguish interactions of plant and fungi, fruiting seasons, rarity, threats and other prime concerns among many branches of biology. Also, exploring fungal diversity in aquatic ecosystems should be prioritised.

• Exclusive collection of data allows scientists and researchers to compare novel specimens with current collections, making identification sturdier and describing of known species effortlessly. This will help identification of threaten and extinct species from Sri Lanka and help with getting precautions to protect rest.

• Since our collections may contain novel unidentified species, our collections may carry challengeable and strong authentication for presence of endemic species with higher values. This may create significant value in context of international trade, where those data matters over import and export process, supply chain and value addition to natural resources.

• Establishment of historical context for presence of fungi over decades will help with

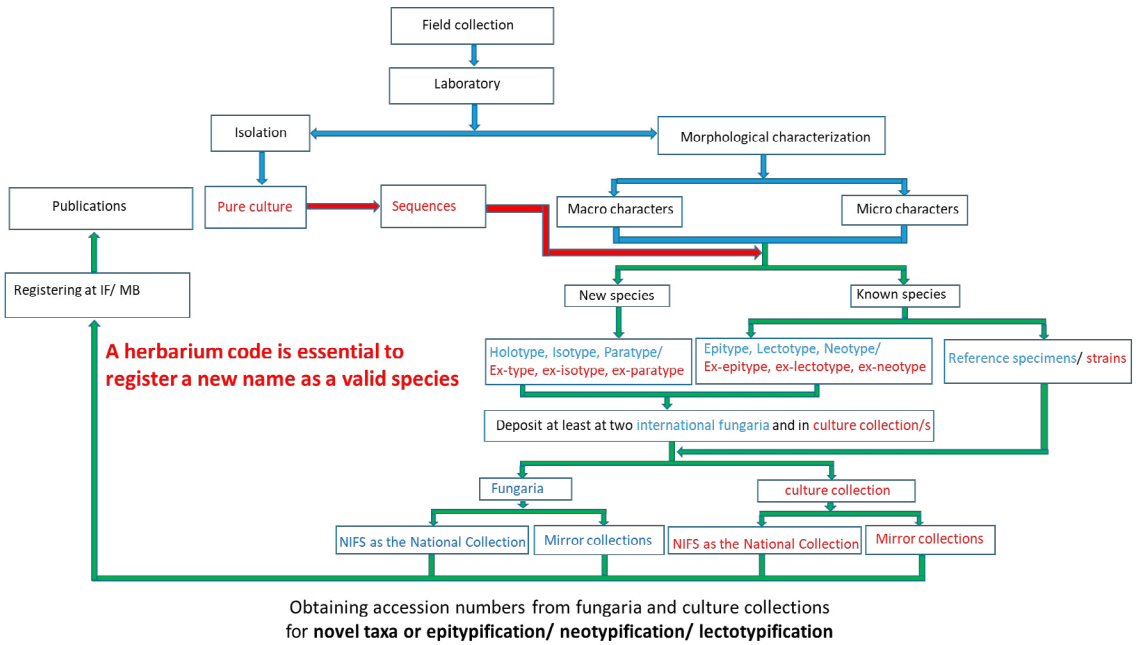

Figure 5. National Institute of Fundamental Studies (NIFS) is promoted as a national centre for depositing fungal specimens (MIFSMC) and culture collection (NIFS-MC) while promoting other mirror collections; viz., University of Rajarata (RUSLH), University of Colombo (SLCMB), University of Ruhuna (FUOR) and University of Sabaragamuwa (SUSL) (IF= Index Fungorum, MB= MycoBank).

examining variations of ecological factors relevant to different fungi. The data collection will provide better understanding about evolution of native and endemic species of Sri Lanka. Also, this will pave the path to resample specimens and to assign them to newly recognized species following their original accessions.

• As a prime concern, our collections can be introduced as historical collections of DNA which will be accessible to the international. This creates immense importance for research results with higher accuracy which opens up new findings on fungi and their interactions. Also, with accurate identification the specimens collected over decades or centuries can be studied for useful characterization.

Taxonomists, mycologists and other interested individuals are encouraged to deposit the specimens at the national fungarium while duplicating the specimens at other mirror collection/s. The Ministry of Environment of Sri Lanka is currently preparing a National Environment Action Plan (NEAP) and the necessity of establishing bio-repositories for lower plants has been recognized a priority and National Institute of Fundamental Studies, in Kandy has been cited as a site for a Fungal Bio repository.

12. Mirror collections

ICNafp ruled that specimens of newly described species (Art. 8.1, 40.1) should be maintained. Maintaining separate collections, except the holotype (i.e., isotype or paratype) and the ex-type culture (i.e., ex-isotype) is important in case of loss or poor storage conditions (damaging the specimen and then lacking the morphological characters as in the original protologue) of the holotype and cultures [53,61]. In the case of epytipification, Ariyawansa et al. [98] recommended deposition of the material (i.e., epitype) in a public collection with a duplicate (i.e., isoepitype) in another international collection.

While having different fungaria to maintain isotype or paratypes of novel taxa in Sri Lanka is also important while establishing a national collection. (e.g., Sutton [27], an outstanding monograph of coelomycetous taxa cited several specimens of *Seimatosporium grevilleae* which were deposited at the Tea Research Institute, Talawakelle, Sri Lanka. However, isotypes of recently introduced taxa (mainly lichens) are being maintained in overseas herbaria such as the Natural History Museum (BM) and the Field Museum (F), Chicago. Nevertheless, isotypes of novel mushrooms and microfungi taxa have not been designated e.g., Ferdinandez et al. [66].

Nevertheless, the institute which maintains the mirror collection would be maintained according to its expertise research area or the region where the strain was isolated (or based on the institute where laboratory work been mainly carried out). As an example, we suggest the National Aquatic Resources Research and Development Agency (NARA) to maintain specimens of marine fungal species. At the same time, in here, we promote three state universities (which are newly registered at http://sweetgum.nybg.org) as mirror collections *viz.*, University of Colombo (SLCMB), University of Ruhuna (FUOR) and University of Sabaragamuwa (SUSL) (Figure 5).

13. Conclusions

Identifying, monitoring, and conserving biological diversity are important aspects in Biodiversity Convention. Currently, several species cataloguing programs of different living organism have been carried out in Sri Lanka. However, evaluating the diversity of microorganisms, including fungi is far behind. Over 34,000 fungal species are estimated to be present in Sri Lanka based on the plants: fungi ratio but currently only ca. 3000 species have been documented. Most of the known species are based on morphological characteristics, which are not reliable due to morphological plasticity and divergent evolution. Moreover, a large number of asexually typified, monotypic genera are lacking DNA sequences thus treated as orphaned genera [99]. Hence, it is

essential to focus on discovering missing species and at the same time, revisit older type species based on the consolidated species concept [31]. It is essential to deposit holotypes in a recognized fungarium and to maintain ex-type cultures at well-known culture collections when describing a novel taxon. Moreover, fungaria and culture collections are essential to maintain epitype and ex-epitype in epitypification of older species. At the same time, duplicating specimens (i.e., isotype/paratype/epitype) and cultures (ex-isotype/ex-paratype/ex-epitype) are also important. Hence, we propose the establishment of a national fungarium and two national microbial/fungal culture collections (the first clinical fungi and the second for environmental fungi) to maintain specimens and genetic resources, respectively. At the same time, different institutes (based on their expertise fields) should be encouraged to maintain their own fungarium and culture collection. Hence, in future studies, taxonomists can deposit specimens (and duplicates) in national institutes in addition to exporting them. This will reduce illegal exporting of specimens. It is also very important to have a focal point for mycological activities within the national herbarium, which can coordinate the identification and documenting of all fungi locally.

In addition, we propose to evaluate and revisit existing specimens at PDA since no recent taxonomic revisions have been carried out. Paintings need to be well preserved. Moreover, we suggest the establishment of a virtual fungarium, which is more accessible to the international scientific community. Meanwhile, establishing an integrated fungal database for the Sri Lankan mycoflora is suggested. Finally, mycology work carried out thus far by international and local mycologists is appreciated since that has helped the development of Sri Lankan mycology.

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Conflict of interest statement

The author(s) declare(s) that there are no conflicts of interest to disclose.

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