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How to name dark fungal taxa outside the *Code*: The International Standard to Catalogue Dark Fungal Taxa (ISCDFT)

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Abstract

There is an exponential increase in the number of fungal OTUs being documented by environmental DNA (eDNA) metabarcoding. Many of these, the so-called dark fungal taxa (DFT), are known only from eDNA data, with no associated physical specimen or derived culture. Of the millions of fungal species predicted to exist, only ~205,000 are currently described, making it likely that many of these unknown or undescribed fungi are represented only by eDNA data. For more than a decade mycologists have struggled with whether the *International Code of Nomenclature for algae, fungi, and plants* (the *Code*) should allow for formal description of fungal taxa from eDNA data alone, i.e. with a DNA sequence serving as a type. It is, however, unlikely that an amendment to the *Code* with this provision will pass in the near future. It is therefore imperative to establish an informal yet structured naming system to allow unified communication about DFT. The system proposed herein applies most of the principles of the *Code* to generate designations that, while nomenclatorially invalid, can nonetheless be integrated into fungal classifications and diversity assessments. We used the 2025 Westerdijk Spring Symposium "DNA Sequences as Type" to bring mycologists together to work out community consensus-driven conventions and standards for what we call the International Standard to Catalogue Dark Fungal Taxa (ISCDFT). The guidelines established herein principally refer to naming conventions, quality and standards for the underlying data, and standards regarding the underlying analysis leading to the detection of new DFT. The ISCDFT does not compete with the *Code* but enables the community to catalogue DFT and integrate these into fungal classifications while adhering to broadly established principles. For ease of immediate implementation, we provide examples to illustrate these newly established guidelines, a checklist for publishing new DFTs under the ISCDFT, and convert some previously published fungal names based on eDNA sequences to ISCDFT-compliant names.

Keywords: classification, dark fungi, -ENAS, environmental DNA, nomenclature, taxonomy

Introduction

In terms of described species, *Fungi*, currently at ca. 205,000, represent the third largest kingdom after *Plantae* and *Animalia* (Bánki et al. 2025; Species Fungorum 2026). However, the real diversity of *Fungi* and fungus-like organisms belonging to other kingdoms–such as slime molds and *Oomycota*–has been estimated at between 500,000 to as many as 165 million species (Hawksworth 1991, 2001, 2012; Rossman 1994; Shivas and Hyde 1997; Arnold et al.

2000; May 2000; O'Brien et al. 2005; Schmit and Mueller 2007; Blackwell 2011; Mora et al. 2011; Taylor et al. 2014; Hawksworth and Lücking 2017; Larsen et al. 2017; Wu et al. 2019; Baldrian et al. 2022; Li and Wiens 2023; Hyde et al. 2024), outnumbering estimates for *Plantae* and some estimates for *Animalia* (e.g., Monastersky 2014; Larsen et al. 2017; Li and Wiens 2023; Wiens 2023).

Increasingly, environmental DNA sequencing (hereinafter eDNA) studies are being used to assess global fungal diversity, and many of the published estimates including higher-end estimates of >19 million species are based on this approach (O'Brien et al. 2005; Taylor et al. 2014; Hawksworth and Lücking 2017; Wu et al. 2019; Baldrian et al. 2022; Niskanen et al. 2023; Mikryukov et al. 2025). The large variation in estimates based on eDNA is largely due to differences in analytical pipelines, which mostly rely on pairwise alignment-based OTU clustering to infer taxonomic diversity, a "quick-and-dirty" approach that has been shown to potentially overestimate species richness by up to two orders of magnitude (e.g., Lücking et al. 2014; Niskanen et al. 2023). Indeed, a reassessment of fungal estimates derived from eDNA analyses using multiple alignment-based phylogenetic approaches narrowed previously published global estimates to between 1.76 and 3.83 million (Niskanen et al. 2023), similar to the early estimate of Hawksworth (1991) of 3 million fungal species when considering both plant and animal-associated fungi.

A staggering quantity of data has been generated by eDNA studies of fungi utilizing the fungal DNA barcode, the internal transcribed spacer (ITS) region of the ribosomal RNA gene cluster (Schoch et al. 2012). Taken together, these studies have amassed ITS data (mostly from ITS1 or ITS2 regions) from a near-global sampling of various substrata, primarily from soils but also from water, air, plants and animals (Tedersoo et al. 2014; Crowther et al. 2019; Větrovský et al. 2020; Mikryukov et al. 2023; Ovaskainen et al. 2024). The Sequence Read Archive (SRA) (Katz et al. 2022) as of late 2025 contained over 15 billion fungal ITS reads, as compared to roughly 2.2 million ITS accessions in GenBank, a ratio of 5,300:1. Just 10 years ago this ratio was about 20:1, demonstrating an exponential increase in the generation of eDNA data over the last decade. Data contained in the SRA represent the largest source of documented fungal diversity. The UNITE database, a curated collection of fungal ITS sequences (Abarenkov et al. 2010, 2024; Kõljalg et al. 2013), has increasingly incorporated fungal ITS eDNA reads into its dataset, which now comprises > 3 million high-quality fungal ITS sequences that have passed the UNITE Species Hypothesis (SH) Engine quality filters.

The exponential increase in ITS data has led to a concomitant rise in ITS sequences that cannot be confidently placed to species or, in many cases, to even higher ranks (Tedersoo et al. 2025). These sequences represent that portion of fungal diversity known as dark fungal taxa (DFT), i.e., fungi known solely from DNA sequence data (Nilsson et al. 2019), although precise estimates of the captured richness in SRAs is difficult to estimate and highlights the problems inherent in working with DFT. In addition to the difficulty in equating sequences and/or OTUs with species, there is the obvious problem of a lack of published names for application to DFT. In *Dikarya*, there are 221,950 SHs in the current UNITE ver. 10.0 (Abarenkov et al. 2024) at a 1.0% distance, of which 71,077 include one species name, 7,197 include two names, and 4,176 include more than two names (i.e., potential synonyms). Thus, nearly two-thirds of these SHs in UNITE have no available species name and it is likely that ITS reads contain hundreds of thousands of undiscovered fungal species. Moreover, intraspecific ITS variation varies substantially among fungal lineages, meaning that no fixed similarity threshold, whether used to define OTUs or species hypotheses, can reliably delimit species across all fungal groups (Vu et al. 2022). Indeed, the ITS region has been shown to lack resolution at the species rank in several groups of fungi, for example in fast-evolving

pathogens (e.g., Cai et al. 2022; O'Donnell et al. 2022; Schoch et al. 2012; Kauserud 2023; Xu 2016), leading to underestimates of species richness in some fungal groups when applying ITS data alone. Conversely, however, it should be noted that the ITS is a multicopy region, and many fungal taxa may exhibit intragenomic ITS variation, which can inflate or confound the number of ITS-based "species" in eDNA studies (Kiss 2012; Lofgren et al. 2019; Stadler et al. 2020; Bradshaw et al. 2023; Kijpornyongpan et al. 2024).

Formal fungal nomenclature is governed by the *International Code of Nomenclature for algae, fungi, and plants* (Turland et al. 2025), abbreviated herein as the *Code* and in reference to its Articles as ICNafp. For nearly two decades, mycologists have recognized the need for a system for naming fungal taxa known only from sequence data (Hibbett et al. 2009), either by modifications to the *Code* (Hibbett et al. 2011) or through creation of a new code that resides outside the formal *Code* (Taylor 2011). In recent years, several proposals were formulated to amend the *Code* to allow the use of DNA sequences as types and thus enabling formal naming of DFT, as summarized in May and Redhead (2018) and May and Bensch (2024), and the topic has received intense discussion among the mycological community (e.g., Hongsanan et al. 2018; Lücking and Hawksworth 2018; Lücking et al. 2018; Thines et al. 2018; Zamora et al. 2018). Ultimately, the proposals put forward thus far were rejected at both the International Mycological Congress (IMC) 11 in Puerto Rico in 2018 and at IMC12 in Maastricht in 2024. A community paper under the auspices of the International Commission on the Taxonomy of Fungi (ICTF) summarized the various options for how to address this issue, one of which is the implementation of an informal naming system outside the *Code* but with community-agreed upon rules and standards (Lücking et al. 2021). At the KNAW Westerdijk Fungal Biodiversity Institute's Spring Symposium in Utrecht in April 2025, dedicated to the topic of "DNA Sequences as Type", various strategies for naming DFT were debated, and in a breakout session, potential rules and standards were discussed, which we present herein.

Terminology and guidelines for use

With the advent of DNA sequencing and molecular phylogenetics, the term "dark taxa" was coined in the zoological literature for sequence-based individuals or clades that had no taxonomic names (Page 2011, 2016; Maddison et al. 2012). In those cases, specimens might have existed but not hitherto sequenced, and the formal description of taxa could not keep pace with their discovery. The emergence of eDNA sequencing in the second half of the 1980s led to the discovery of vast uncultivated diversity known only from sequence data, referred to at the time as "microbial dark matter" (Rinke et al. 2013; Hugenholtz et al. 2021). Later, the term "dark fungal taxa" was adopted and applied to those fungi only evident from eDNA sequence data, without a physical specimen or culture. The term is now sometimes shortened to "dark fungi" but "dark taxa", "dark fungal matter", and "dark fungal taxa" are also used to denote the same thing. Because the term "dark fungi" has also been applied from the 19th century onwards to melanized fungi manifesting as dark to black spots, to avoid confusion here we use dark fungal taxa (DFT). DFT are specifically those fungi–i.e., organisms traditionally treated as fungi under the *Code*, including but not limited to *Fungi*, *Oomycota*, and "slime molds"--that are solely known from DNA sequence data which, in most cases, are eDNA sequences.

Currently, the *Code* permits a valid name to be established only when the original description (the protologue) cites a physical specimen or an illustration, designated as the holotype. Therefore, any physical evidence of a given fungus, such as a specimen, culture, or *in situ* visualization removes it from being considered as belonging to DFT and instead allows for its valid naming under the *Code*. DFT, which lack a physical specimen to serve as type, cannot

be named under the *Code*. A similar situation has arisen in the cataloguing of biological diversity detected solely through eDNA under the *International Code of Nomenclature of Prokaryotes* (ICNP), where culturing of the species in question is not possible (Murray et al. 2020; Hugenholtz et al. 2021). The introduction of the informal *Candidatus* category under the ICNP in the 1990s aimed to address this gap, but the provisional names under that category have faced several limitations, including the absence of clear quality standards for etymology or for defining an appropriate nomenclatural type for such formally invalid names (Hugenholtz et al. 2021). In response to the need for permanent names for uncultured prokaryotes for which other high-quality data are available, such as genomes, an alternative system operating outside the ICNP was developed: the SeqCode, a nomenclatural code in which genome sequences serve as the nomenclatural types for prokaryotes (Hedlund et al. 2022; Whitman et al. 2024).

One possible solution for fungi is the Species Hypothesis (SH) system developed by the UNITE Community (Kõljalg et al. 2013; Abarenkov et al. 2024). This provides a stable, DNA-based framework for communicating fungal species concepts in a consistent and reproducible manner. In UNITE, publicly available rDNA ITS sequences are clustered into SHs, representing operational taxon hypotheses at approximate species-level resolution. Each SH is assigned a persistent Digital Object Identifier (DOI), allowing both described and undescribed fungal taxa to be referenced unambiguously across studies, databases, and time. This DOI-based system ensures long-term stability of species communication even as taxonomic interpretations evolve, while remaining fully interoperable with Linnaean nomenclature through links to named taxa where available. However, while the communication of SHs without attached names is readily possible in machine-operated systems, it poses challenges for the communication of fungal diversity between humans, including the needs for the implementation of conservation policies (Mueller et al. 2022; Zhou and May 2023). In addition, SHs are proxies for species, and there is not necessarily a one to one match between SHs and phylogenetic species, even at a given similarity threshold (e.g. Noffsinger et al. 2026).

In an attempt to meet all the needs of the scientific community and other end users, we have developed the International Standard to Catalogue Dark Fungal Taxa (ISCDFT). The ISCDFT resides outside the *Code*, and designations established under the ISCDFT have no formal standing under the *Code.* Nonetheless, the ISCDFT has been designed to be complementary to, and based on the same principles as, the *Code.* To minimize potential confusion, we avoid terminology that has explicitly defined meanings under the *Code*, such as the terms "name" and "type" (ICNafp Art. 6.3, Art. 7). Instead, the terms "designation" and "reference sequence", respectively, are used in the ISCDFT. The term "designation" already applies to invalidly established "names" in the *Code* (ICNafp Glossary) and the term "reference sequence" (rather than “type sequence”) is in line with the use of this term in the UNITE database for SHs (Kõljalg et al. 2013).

The framework presented here relies on the formation of operationally defined species (or higher rank) hypotheses based on rigorous analyses, as detailed in Aime et al. (2021). It is not meant to provide a means for the “naming” of single sequences. Use of the guidelines laid out herein is intended to provide a means for accurate, stable, and interchangeable communication of DFT. Finally, although the framework presented here resides outside the *Code*, it has been made compatible with the principles of the *Code* wherever possible. Therefore, when applicable, the standards presented here adopt the principles of the *Code* and allow for ease of integration of any DFT designated using these standards to the *Code*, once material that qualifies as a type, is discovered.

Table 1. Terminology used under this framework and analogous terms under the *Code*.

ASV:	Amplicon sequence variant, i.e., a unique nucleotide sequence pattern inferred to be biologically present in a set of eDNA data, and may be present in multiple copies or samples
Code:	*International Code of Nomenclature for algae, fungi, and plants* (referred to as ICNafp when individual Articles are cited)
Designation:	A unique name of a DFT, suffixed with "-ENAS"; similar to a name under the *Code*
DFT:	Dark fungal taxon/taxa, i.e., known only from eDNA
eDNA:	DNA extracted from environmental samples, including but not limited to clone libraries, DNA metabarcoding, metagenome sequencing, and environmental single-cell genome sequencing
-ENAS:	Environmental nucleic acid sequence (see Taylor 2011); proposed suffix for designations under the ISCDFT
Fungi:	Kingdom *Fungi*
fungi:	All organisms traditionally treated as fungi, including kingdom *Fungi* and fungus-like organisms, for example, *Mycetozoa* (slime molds) and *Oomycota*
ICNafp:	*International Code of Nomenclature for algae, fungi, and plants*
ISCDFT:	The International Standard to Catalogue Dark Fungal Taxa
Reference sequence:	The single sequence read from a single environmental sample attached to a DFT designation; similar to a type under the *Code*
Sequence read:	A single read from a sample of eDNA metabarcoding data, identified by a unique sequence header code generated by the underlying sequencing platform
SH:	Species Hypothesis, as developed by UNITE: a DNA-based operational species concept based on ITS sequences at various similarity thresholds

The International Standard to Catalogue Dark Fungal Taxa (ISCDFT)

Preamble

The International Standard to Catalogue Dark Fungal Taxa (ISCDFT) is herein established to provide guiding principles for the designation of dark fungal taxa (DFT) outside of the *International Code of Nomenclature for algae, fungi, and plants* (the *Code*). The *Code* states that "biology requires a precise and simple system of nomenclature that is used in all countries, dealing on the one hand with the terms that denote the ranks of taxonomic groups or units, and on the other hand with the scientific names that are applied to the individual taxonomic groups" (Turland et al. 2025). Environmental DNA sequencing and similar

technologies enable the detection and quantification of biological diversity based on environmental DNA (eDNA) for which no physical specimens are known. However, attempts to amend the *Code* to allow for formal naming of organisms solely known from DNA data have thus far failed in two consecutive International Mycological Congresses.

Considering the exponential growth and near-global coverage of eDNA data, a solution that allows for cataloguing this fungal diversity in a meaningful and interchangeable way that also allows for integration into formal fungal taxonomy is indispensable. The standards for designating taxa presented here attempt to overcome this problem by establishing community-agreed-upon standards and guidelines that provide a single methodology to designate DFT outside the *Code* while adhering to well-established principles.

This ISCDFT is to be used exclusively for DFT known only from eDNA sequence data, for which no specimen or culture or other evidence of their presence (e.g., fluorescent imaging of hyphae) is available, apart from the sequence itself. The ISCDFT should not be used for organisms for which material that would qualify as a type is available, as those can be validly named under the *Code*. Nor should the ISCDFT be interpreted as a mechanism for introducing names in situations where only a single eDNA sequence is known for a putatively undescribed species. Designations created adhering to the ISCDFT should follow best practice as applied for any taxon hypothesis, whether named under the *Code* or not.

In contrast to the *Code*, whose provisions are mandatory, the provisions of the ISCDFT presented herein cannot be enforced but instead rely on acceptance by the mycological community as a whole through peer review and subsequent take up of the names.

The ISCDFT is laid out by a set of Provisions as follows. The abbreviation ICNafp refers to Articles of the *Code* at the time of publication (currently Turland et al. 2025).

SECTION 1: PROVISIONS

Provision 1. Unambiguous indication of a designation.

1.1. A designation applied to a dark fungal taxon based on nucleic acid data is to bear the suffix "-ENAS" to that part of the designation that is based on sequence data alone; it is to be connected by a hyphen but is to be displayed in standard font instead of italics. The designation in this form is to be used when originally introduced and in subsequent usage.

Recommendation 1.1.1. When publishing a new “-ENAS” designation for the first time, authors should indicate this by including the statement “new designation”.

Example 1.1.1: *Archaeorhizomyces secundus*-ENAS Kluting, M. Ryberg & Rosling, new designation

Provision 2. Form of a designation.

2.1. A designation is formed as a binomial (genus name or designation and species designation) or in the form corresponding to its rank analogous to the corresponding stipulations in the *Code* (ICNafp Art. 16–27).

Example 2.1.1: *Archaeorhizomyces victor*-ENAS Kluting, M. Ryberg & Rosling

Example 2.1.2: *Pantelleriomycetes*-ENAS Tedersoo

Example 2.1.3: *Pantelleria*-ENAS Tedersoo

Example 2.1.4: *Pantelleria*-ENAS *saittana*-ENAS Tedersoo

2.2. Species epithets in designations are to follow the principles of the *Code* (ICNafp Art. 23).

2.3. Designations established under this framework are to be accompanied by an explanation of their etymology.

2.4. Designations of taxa ranked at genus based entirely on DFT are to follow the stipulations for such taxa laid out in the *Code* (ICNafp Art. 20). Publications that introduce genus-rank designations are to cite the name of the species-rank DFT that serves as a shorhand for the reference designation for the genus (ICNafp Art. 10).

Example 2.4.1. *Sumavosporidium*-ENAS Tedersoo & Esm.-Salest., is based on *Sumavosporidium*-ENAS *sylvestre*-ENAS Tedersoo & Esm.-Salest.

2.5. Designations of taxa at the rank above genus based entirely on DFT are to follow the stipulations for such taxa laid out in the *Code* (ICNafp Art. 16–19). They are to be formed as designations automatically derived from a genus designation. Publications that introduce designations above genus rank are to cite the DFT name at the next lowest main rank, that serves as a shorthand for the reference genus for the taxon (ICNafp Art. 10).

Example 2.5.1: *Sumavosporidiaceae*-ENAS Tedersoo & Esm.-Salest., *Sumavosporidiales*-ENAS Tedersoo & Esm.-Salest., and *Sumavosporidiomycetes*-ENAS Tedersoo & Esm.-Salest., are all based on *Sumavosporidium*-ENAS Tedersoo & Esm.-Salest.

2.6. Although not validly published under the *Code* , designations are to be written in italics, except for the suffix "-ENAS".

Provision 3. Publication.

3.1. Designations of DFT based on DNA sequence data are to be published in journals that are approved by the International Commission on the Taxonomy of Fungi (ICTF).

Note: In order to test technology platforms, workflows, and effectiveness of the ISCDFT, publication of DFT designations will for a trial period initially be mandated in the journal of the International Mycological Association, *IMA Fungus*. The ICTF will lead an annual review of the ISCDFT and its embedded processes and may change or expand the effective journal(s) for publication. The website of the ICTF will provide the current list of approved journals in which new designations for DFT can be introduced.

3.2. Keywords in manuscripts publishing designations of DFT should include the term "-ENAS" for ease of discovery of such papers, unless names bearing this suffix are already included in the abstract.

Provision 4. Evidence for new designation.

4.1. A new DFT designation at species rank is ideally to be shown by its presence in at least three (3) independent samples, corresponding to different SRA experiments. The total number of sequences representing the newly designated DFT at species rank should be at least five (5).

Note: This provision is based on the assumption of 15 billion existing SRA reads of the ITS barcoding marker and 3 million predicted fungal species, i.e., 5,000 reads per species on average. In a typical histogram, assuming few abundant and many rare species, this translates into a median of ca. 12 sequences per species. This conservative approach excludes "taxa" represented by single sequences at this time, although the ICTF will consider future revisions to these guidelines.

Provision 5. Reference sequence.

5.1. A designation of a species-rank DFT is fixed to a reference DNA sequence, in analogy to the type concept laid out in the *Code* (ICNafp Art. 7). The reference sequence should be selected from among those sequences that conform to the lineage representing the newly designated DFT for the selected marker and should be the sequence best representing the lineage, typically the longest sequence with the least amount of variation from the consensus. The reference sequence is a single sequence occurrence, i.e., a specific sequence read from a single biological sample; it is not a consensus sequence.

5.2. The reference sequence of a species-rank DFT must be lodged in a participating database of the International Nucleotide Sequence Database Collaboration (INSDC) or another repository that issues persistent identifiers for sequences, such as UNITE, and is to be publicly available at the time of publication.

5.3. Details of collection geography, date, and substrate as well as details of the sequencing platform are to be included in the reference sequence statement.

Recommendation 5.3.1: The underlying alignment providing evidence for the new DFT designation may be based on one or several markers. However, the reference sequence is to represent a single contiguous region of the genome (e.g., a larger portion of the SSU-ITS-LSU rDNA cistron), i.e., a sequence portion that can be recovered as contiguous (either directly or by unambiguous assembly) from eDNA data. In the case of ITS, when long read nuclear ribosomal rDNA sequences are available for a new designation, in particular those spanning the ITS and the first 500 to 1,000 nucleotides of the large subunit (LSU), these are preferable over the ITS alone, especially in less well documented lineages that require reliable anchoring in a broader phylogeny.

Recommendation 5.3.2: In most cases, and for broad comparison among fungi, the reference sequence will correspond to the fungal ITS barcoding marker. In particular fungal groups

where evidence points to alternative barcoding markers (e.g., TEF1), the community of experts in that group may agree on the best marker used in that case. In cases where an alternative barcoding marker is used to delimit a DFT, a detailed rationale is to be provided. The reference sequence should be of the typical length for the amplified marker region.

Provision 6. Registration of reference sequence and designation.

6.1. Publication of a designation of DFT based on DNA sequence data is to follow the stipulations of the *Code* for including citation of a registration identifier (ICNafp Art. F.5.) from one of the approved repositories for names of fungi under the *Code*, currently Fungal Names, Index Fungorum, and MycoBank. Registration of a DFT designation in an approved repository at the rank of species must include the following information: the designation name, the UNITE DFT-SH DOI, the author/s, publication details, a diagnosis, the identifier for the reference sequence (see Provision 5.2), and the number of sequences and the number of biosamples on which the DFT-SH is based (see Provision 4.1). Optional fields are the persistent identifier for the biological sample (lodged in a repository) from which the reference sequence was obtained, and the persistent identifier of the DNA sample (lodged in a repository) from which the reference sequence was obtained.

Note: Upon registration in UNITE, a DFT will be assigned to an DFT-SH and linked to a persistent DOI issued for this DFT-SH by UNITE. This DOI will then be used to register the DFT designation in one of the approved registries. This process should be initiated during manuscript revision, after initial peer-review. In the future, registration of the UNITE DFT-SH and the corresponding DFT designation in an approved repository may be completed through an integrated submission workflow, to be designed, that includes automated exchange of the required registration data and identifiers between participating systems.

6.2. At ranks higher than species, registration will include the following information: the designation name, the rank, the author/s, publication details, and the DFT name at the next lowest main rank (e.g. for the designation of a new order, the family designation is provided).

Provision 7. Priority between designations.

7.1. At all ranks, the principle of priority (ICNafp Art. 11) applies between competing designations of the same DFT based on DNA sequence data established under the ISCDFT.

Note 7.1. Although in the *Code* (ICNAfp Art. 11) the principle of priority applies only to the ranks of family and below, to maximize nomenclatural stability the ISCDFT will apply the principle of priority to all ranks.

Provision 8. Authorship.

8.1. Authorship of a designation of a DFT based on DNA sequence data is to follow the corresponding stipulations of the *Code* (ICNafp Art. 46–50).

Recommendation 8.1: Authorship of DFT designations should be reserved for individuals directly involved in the delimitation of the taxon that bears a new designation, and otherwise should follow the guidelines presented in Aime et al. (2021).

Recommendation 8.2: The full list of designation authors is required only in the original publication. If a DFT is designated by 3 or more scientists, future citations should use the first author followed by "et al.", rather than the full list of authors.

Provision 9. Diagnosis.

9.1. A designation of a DFT based on DNA sequence data is to contain a diagnosis defining the new designation based on differences in DNA sequence data from the most similar taxa. This will usually take the form of detailing positional nucleotide differences relative to the most closely related sequenced lineages in a multiple sequence alignment. The diagnostic positions given in the diagnosis are to be present in the reference sequence. The diagnosis should also include the percent identity values obtained for the best hits in BLAST searches, to demonstrate that potentially conspecific taxa are not available among already sequenced fungi.

Provision 10. Homonymy.

10.1. The creation of homonyms with *Code*-compliant names or with other DFT designations are to be avoided by checking proposed designations against those registered in, currently, MycoBank, Index Fungorum, and Fungal Names, and by avoiding, as much as is practicable, the use of designations that already exist as names for any other living organisms (e.g., as covered by the *International Code of Zoological Nomenclature*, *International Code of Nomenclature of Prokaryotes*) and otherwise following the corresponding stipulations of the *Code* (ICNafp Arts. 53, 54 and Art. F.6).

10.2. For homonyms of DFT designations, the principle of priority (ICNafp Art. 11) applies.

10.3. Homonyms with previously published *Code*-compliant names have no standing. In such cases, the DFT designation is to be replaced with a new designation (for which a new identifier is to be obtained) that lists the homonym under a section for synonyms.

Provision 11. Validation of a designation under the *Code*.

11.1. If a physical specimen, culture, or image that qualifies as a type is discovered for a fungus that corresponds to a previously designated DFT, either a completely new name can be established or the DFT designation can be validated under the *Code* by stripping the "-ENAS" from the original designation and otherwise following the stipulations for establishing a valid name (ICNafp Art. F.5.7 and Art. 40.1).

Recommendation 11.1.1: Even though a name can be validated under the *Code* by reference to a previously published diagnosis or description, and a sequence-based diagnosis is valid under the *Code*, validation of a DFT designation with a type should include a description of the phenotype of the fungus as well as an emended diagnosis that includes phenotypic characters of some kind, whether morphological, biochemical, or physiological.

Note. If a valid name is created under the *Code* to replace a DFT designation, a physical specimen, a culture (preserved in a metabolically inactive state), or an illustration (if ICNafp

Art. 40.6 concerning “technical difficulties of specimen preservation” applies) becomes the type of the validated name. The original reference sequence has no status under the *Code* but remains the reference sequence of the original DFT designation and serves to connect the DFT designation to the validated name.

Recommendation 10.1.2: The DFT designation on which a validated name is based should be cited in the protologue of the validation.

Provision 12. Governance

12.1. This set of Provisions has been approved by the ICTF.

12.2. Modifications to these Provisions can be approved by the ICTF after consultation with the broader mycological community and should be published in *IMA Fungus.* Mycologists wishing to propose changes to these Provisions should contact the ICTF.

SECTION 2: SCIENTIFIC STANDARDS

Reference samples: Where possible, researchers introducing novel -ENAS designations are encouraged to preserve a portion of the environmental sample from which they came, for example a sample of soil or water, in a metabolically inactive state in the prospect of eventual visualization of the DFT present should new technology become available. Where possible researchers are also encouraged to preserve the pooled DNA extracts from environmental samples. Such environmental and/or DNA samples should be lodged in an appropriate publicly accessible reference collection, such as a fungarium or a soil bank, and an accession number cited. While such samples have no status under the ISCDFT, they could then become available in the future to possibly serve as types of names under the *Code* or for extraction of additional data.

Analytical approach: Evidence for designation of a new designation based solely on eDNA sequence data is to be provided through phylogenetic analyses based on a multiple sequence alignment that includes the nearest relatives at the appropriate rank, provided that sequence data are available. The use of OTU clustering methods alone is insufficient for new DFT designations, although they may be part of a pipeline to initially digest larger datasets. Where available, analyses should include sequences representing relevant SH from the UNITE database to ensure appropriate taxonomic context and comparability across studies.

Datasets: General guidelines for the assembly and analysis of datasets, including taxon sampling for appropriate context, selection of outgroups, consultation with experts on taxonomic groups where appropriate, and analytical methods are to follow community standards (e.g., Aime et al. 2021). Also see the section on Data availability below.

Recommendation: In addition to phylogenetic analyses, a new designation at species rank based on eDNA sequence data may be supported by quantitative species delimitation methods, including alignment-based (e.g., ASAP, ABGD) and tree-based methods (e.g., GMYC, bPTP). ASAP and GMYC have generally been shown to give more consistent results

than ABGD and PTP. The OTU representing the new designation is to be clearly delimited in accompanying figures.

Rationale for creating new designations: When publishing a DFT designation, details are to be provided on how potentially available names for the group in question were explored, as well as why alternative options, such as culturing or direct visualization, were not employed. In particular, in cases where potential names are available (e.g., speciose genera in which few species have been sequenced), it must be convincingly shown why a new designation is unlikely to be conspecific with an existing name, or previously established -ENAS designation.

Data availability: A publication introducing one or more new DFT designations is to provide the underlying multiple sequence alignments as either supplementary data or in a publicly accessible repository (e.g., Zenodo, Figshare, etc.), available during peer review. All sequences used in the analysis (those representing new designations, and those used for taxonomic context) are to be deposited in a public sequence repository with a corresponding accession or DOI number, even if already registered in the SRA as reads forming part of an experiment and study. In case sequences reads were pulled from the SRA, the voucher table listing their subsequent INSDC accession number must also list the corresponding accession numbers for Bioproject (PRJXX000000), Biosample (SAMXX000000), Experiment (XRX000000), and Run (XRR000000) for each eDNA read, as well as its unique original sequence header. Metadata including the origin (geographical origin, substrate) of all sequences used in the analysis, voucher lists, as well as details regarding sequencing platforms used to obtain data, are to be provided for the sequence accessions. Repositories for different types of data are summarized in Fig. 1 and Table 2.

Data attribution: Papers that utilize previously published data should cite the corresponding sources that first published these data and not just the repositories from which they were retrieved.

Ecology and distribution: In addition to the data outlined within the above Provisions (Section 1), publication of a DFT designation should contain a section on Ecology and Distribution that is based on all sequences that belong to the DFT and their metadata; this includes those generated by the current authors as well as all sequences of the DFT retrieved from public sequence databases representing the same taxon; the section should include details on substrate where the sequences were recovered, as well as the corresponding ecosystems and habitats, ideally with a distribution map.

Synonyms: In addition to the data outlined within the above Provisions (Section 1), publication of a DFT designation may include a list of previously applied designations, such as the corresponding UNITE SH, that refer wholly or in part to the same DFT.

Table 2. Summary of types of data, where to register, and timing of registration/release.

Type of information	**Content [File format]**	**Registry**	**When to register**	**When to release**
Individual sequences/ genomes	Single short or long sequences generated with diverse methods [FASTA]	INSDC (NCBI GenBank, ENA, DDBJ)	upon acceptance	On deposit or upon publication
Individual reads pulled from eDNA bulk data	Short sequences pulled from metabarcoding bulk runs [FASTA]	Original data as part of runs in the SRA, ERA, DRA, KRA), individually in the INSDC	upon acceptance	On deposit or upon publication
eDNA bulk data	Complete metabarcoding runs or sets of runs from a study [FASTA, FASTQ]	SRA, ERA, DRA, KRA	prior to initial submission (as raw data independent of publication)	On deposit or upon publication
Voucher table	Assembly of medatadata and accession numbers for all sequences used in a study [TEXT]	Part of publication, either in main document or as supplementary file	provisional version with initial submission; final version upon acceptance after registering all items	Upon publication
Alignments	Integrated alignments of sequences used in a study, individual markers and/or concatenated [FASTA, PHYLIP, NEXUS]	Part of publication (as supplementary file) and/or in dedicated third-party repositories (e.g. TreeBase, Dryad, Zenodo); alignments must be available for peer-review	provisional version with initial submission; final version upon acceptance	Upon publication
Trees	Trees computed from the alignments [NEWICK, NEXUS]	Part of publication (as supplementary file) and/or in dedicated	upon acceptance	Upon publication

		third-party repositories (e.g. TreeBase, Dryad, Zenodo)		
Taxa	Group of individuals that define a taxonomic unit at a given level, in the case of species a set of sequences	UNITE DFT-SH	during revision	Upon publication
Names	Designations given to taxa under the above definition and following the guidelines and standards provided here	Allowed registries listed in the Code (MycoBank, Index Fungorum, Fungal Names) and which comply with the standards to register DFT names	during revision	Upon publication

SECTION 3: EXAMPLE

The following have been derived from Kalsoom Khan et al. (2020) to provide an example for how to create ISCDFT-compliant designations. They also serve to convert these previously published names to -ENAS designations; new designations will not have a "previously published" statement. Please note that a new ISCDFT-compliant designation would explicitly provide data tables within the publication (and not refer to their prior publication elsewhere).

***Archaeorhizomyces secundus*-ENAS** Kluting, M. Ryberg & Rosling, new designation

Identifier: MBDFT XXXXX

UNITE DFT-SH DOI: https://dx.doi.org/10.15156/BIO/SH1226833.10FU

Previously published in Kalsoom Khan et al., IMA Fungus 11: 23 (page 8 of 12) as "*Archaeorhizomyces secundus* nom. seq. Kluting, M. Ryberg & Rosling, sp. nov."

Etymology: The second most frequently recovered sequence, after *Archaeorhizomyces victor*-ENAS, from Podzol soils at Ivantjärnsheden field station.

Diagnosis: Separated from other known species in the genus by ribosomal sequences possessing the following distinctive characters in ITS1: CCGAGTCGCCACAT, at position homologous to bases 103–116 in ASV_4; and in ITS2: CCATACCTTTTTGGTGTGT, at

position homologous to bases 317–335 in ASV_4 (alignments published as Fig. 3 in Kalsoom Khan et al. (2020)).

Reference sequence: SWEDEN, Uppsala, Jädraås, Ivantjärnsheden field station, 60.816776, 16.499935, 185 m; from the organic soil horizon (approximately 0–5 cm depth) of Podzol soil in *Pinus sylvestris* forest, October 2013. From material sample ERS3508483 (UNITE). ITS1-5.8S-ITS2-LSU rDNA generated using PacBio RSII system, ASV4 (Kalsoom Khan et al. 2020), UDB0779127 UNITE, OQ676543 GenBank.

Additional sequences: AB560514 (Ishida et al. unpublished), DQ309209 (Bougoure et al. 2007), FM992980 (Kjoller and Clemmensen 2009), HM069470 (Liu et al. unpublished), JN032483 (Lindahl et al. 2007), KX289979 (Wang et al. 2017).

Ecology & distribution: Significantly associated with pine forests, mostly with ericaceous understory; also found in a range of boreal forest and alpine ecosystems. Found in both organic and mineral soil horizons but is relatively less abundant in the upper organic soil layer. Also detected from ectomycorrhizal roots of *Pinus sylvestris* and from roots of *Calluna vulgaris*. Climatic range includes temperate, boreal, and alpine climates. Detected in Austria, Finland, Norway, Sweden, and the United Kingdom. Metadata previously published as supplementary datafile 7 of Kalsoom Khan et al. (2020).

Sequences used in this analysis, alignment, and data availability: Previously published in Kalsoom Khan et al. (2020); publicly available at https://osf.io/96dkz/overview.

Notes: Despite intense culturing efforts that included the recovery of 160 fungal isolates, no isolate of *Archaeorhizomycetes* were successfully obtained (Kalsoom Khan et al. 2020).

***Archaeorhizomyces victor*-ENAS** Kluting, M. Ryberg & Rosling, new designation

Identifier: MBDFT XXXXX

UNITE DFT-SH DOI: XXXXX

Previously published in Kalsoom Khan et al., IMA Fungus 11: 23 (page 9 of 12) as "*Archaeorhizomyces secundus* nom. seq. Kluting, M. Ryberg & Rosling, sp. nov."

Etymology: The most frequently recovered sequence, and thus the "victor", from organic soils at Ivantjärnsheden field station.

Diagnosis: Separated from other known species in the genus by ribosomal sequences possessing the following distinctive characters in ITS1: ATGTGCTTTGGCGC CAAG at position homologous to bases 93–110 in ASV_3; and in ITS2: TCATACCTTCTT at position homologous to 323–333 (alignments published as Fig. 3 in Kalsoom Khan et al. (2020)).

Reference sequence: SWEDEN, Uppsala, Jädraås, Ivantjärnsheden field station, 60.816776, 16.499935, 185 m; from the organic soil horizon (approximately 0–5 cm depth) of Podzol soil in *Pinus sylvestris* forest, October 2013. From material sample ERS3508483 (UNITE). ITS1-5.8S-ITS2-LSU rDNA generated using PacBio RSII system, ASV3 (Kalsoom Khan et al. 2020), UDB0779126 UNITE, OQ676542 GenBank.

Additional sequences: AB560521 (Ishida et al. unpublished), DQ309123 (Bougoure et al. 2007), HM069408 (Liu et al. unpublished), HQ873359 (Prenafeta Boldu et al. 2014), JF300381 (Hogberg et al. 2014), JN006467 (Rosling et al. 2011), JN006468 (Rosling et al. 2011), JN032485 (Lindhal et al. 2011).

Ecology & distribution: Most frequently detected in coniferous forests, but also present in deciduous forest. Has a broad range that includes temperate, boreal, and alpine climates. Detected in Austria, Finland, Germany, the Netherlands, Sweden, and the United Kingdom. Metadata previously published as supplementary datafile 8 of Kalsoom Khan et al. (2020).

Sequences used in this analysis, alignment, and data availability: Previously published in Kalsoom Khan et al. (2020); publicly available at https://osf.io/96dkz/overview.

Notes: Despite intense culturing efforts that included the recovery of 160 fungal isolates, no isolate of *Archaeorhizomycetes* were successfully obtained (Kalsoom Khan et al. 2020).

Discussion

Here we outline a standard–The International Standard to Catalogue Dark Fungal Taxa (ISCDFT)–for naming of DFT for which no *Code*-compliant type material exists. Adoption of a single standard for naming of DFT is meant to facilitate future communication concerning DFT, avoid multiplication of variant and potentially incompatible approaches to naming DFT, and increase stability in naming of these taxa. The standards published herein were created as a community-driven effort to arrive at a balanced consensus between broadly practical applicability, scientific standards for fungal taxonomy, and established rules for the naming of fungi. The ISCDFT is laid out in twelve Provisions and provides minimal scientific standards for data and metadata to consider in publication of new DFT. A checklist summarizing steps to take to create an ISCDFT designation is provided in Fig. 2.

Designation of type specimens under the *Code* is the obvious roadblock for naming DFT known only from sequence data. Type specimens serve the foundational role in stabilization of formal nomenclature and taxonomy. This basic incompatibility between the purpose of a type specimen, and the lack of samples to serve as comparable type specimens for eDNA datasets, has at least two possible resolutions. (1) Widening the type concept to permit characters to act as type, or, (2) naming of taxa outside of the *Code* where there are no restrictions on typification, but also no hitherto agreed naming conventions to facilitate communication. A type is a physical specimen or evidence of a previously existing physical specimen (such as an illustration), from which diagnostic characters are derived. In practice, while a type does not change, the characters defining a taxon may change, based on new evidence, such as derived from additional collections and/or new methodological approaches leading to generation of new characters. A type is therefore persistent, and may allow derivation of additional and new characters, whereas the set of characters applied in a protologue are a snapshot in time. In taxa known from eDNA data, the only possible "type" is a reference sequence, which is a limited set of characters that greatly restrict comparison between other taxa that lack data from the exact same character set and from which generation of new characters is for the most part not possible.

Stability in the *Code* is achieved, at least in part, by the requirement that a new taxon name be typified with an individual specimen (or in rare cases, an illustration of that specimen) that represents that name. In the ISCDFT, that stringency is placed on the characters derived from the data and analytical workflow used to infer the existence of a taxon/taxa within a dataset. While the best diagnostic marker (or set of markers) may vary across fungal taxa, and the number of sequences for a potentially new DFT designation may vary, the advantage of working with DNA sequence data is that the analytical workflow, consisting of multiple sequence alignments, tree inference, and quantitative delimitation, is broadly established. What varies are the tools and selected parameters to perform each step of the workflow. In the

standards we therefore focus on the required workflow, which is theoretically applicable for each and every case, rather than on the specific methods. It will be critical for authors of designations to adhere to best practices to ensure that the ISCDFT has the desired outcome of providing a stable means of communicating about these unnamed but ubiquitous fungi.

A central element of the ISCDFT is the replacement of the type specimen concept with a SH system based on persistent DOIs. Unlike *Code*-compliant taxonomy, where names are anchored to individual type specimens, SHs and higher rank Taxon Hypotheses (THs) are released as citable, versioned datasets representing explicitly defined sets of individuals and their associated characters, as envisioned in Berendsohn (1995) and formalised in Kõljalg et al. (2020). Each DFT-SH DOI thus refers to a fixed, reproducible circumscription of a taxon at the time of release. Importantly, whenever individuals or traits are added, removed, or re-annotated within an SH dataset, a new SH version and DOI are issued, preserving the integrity and traceability of previous concepts rather than retroactively modifying them.

Implementation of the ISCDFT is likely to result in a substantial increase in established fungal designations, providing additional workload in terms of peer review and registration of designations and data in established repositories. However, the systems are built to place much of this workflow burden on the authors; investments in automatization of parts of the workflow may improve stringency and ease of quality checks. Nonetheless, we believe the benefits for a more global assessment and classification of fungi, such as increasing the impact of fungi on the global market of biodiversity and conservation policies, which in turn should provide increased means to support the tools and people documenting this diversity, outweigh the disadvantages.

Why the ISCDFT now, and not wait for changes to the *Code*?

The amount of data available through eDNA metabarcoding has increased exponentially over the past decade. The need to recognize the importance of these data for cataloguing the global diversity of fungi has long been recognized. At IMC10 in Thailand in 2014, an entire session was dedicated to this topic. After this session, a broader discussion was initiated (De Beer et al. 2016; Hibbett et al. 2016; Lücking and Hawksworth 2018; Lücking et al. 2018; Zamora et al. 2018; Wu et al. 2019) that led to the drafting of several proposals to amend the *Code* (Hawksworth et al. 2016) and the newly established *Chapter F* of the *Code*, respectively (Hawksworth et al. in Parra et al. 2018; Lücking et al. 2018; May and Redhead 2018). However, none of these proposals have been successful, and neither was a second round of proposals that were considered at IMC12 in Maastricht in 2024 (May and Bensch 2024). Indeed, the discussion in the mycological community continues to be contentious, and new developments are questioning the approach taken in the earlier proposals (Lücking et al. 2021). While it is possible that genomes may eventually come to be formally accepted as types of fungal names, we recognize that it is unlikely that the use of individual DNA markers as types will happen anytime soon, if ever. However, the need for a stable naming system is urgent to allow cataloguing fungi based on marker sequences alone. We believe that the proposed ISCDFT is a satisfactory compromise, as it provides a structured framework but does not threaten *Code*-compliant fungal names. Such a compromise is needed now as papers are already introducing latinized designations when classifying fungal taxa based on eDNA alone (De Beer et al. 2016; Moncada and Lücking 2017; Kalsoom Khan et al. 2020; Tedersoo et al. 2024, 2025). The publication of this standard does not preclude mycologists from submitting formal proposals to modify *Chapter F* of the *Code* to the forthcoming

Nomenclature Session of the 13th International Mycological Congress (IMC13) in Incheon, Korea, in August 2027 according to the procedures laid out in May & Bensch (2026).

The initial mandate of a single journal in which novel designations of DFT are to be introduced is designed to create confidence among the mycological community that standards will be followed. This set up also allays concerns about mass publication of new designations without appropriate documentation. The initial choice of *IMA Fungus* should be viewed as a trial of the procedures. Annual reviews of the number of DFT introduced and the efficacy of the procedures will be carried out by the ICFT. As a result of these reviews, further journals may be added and amendments made to the Provisions introduced herein.

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Figure captions

Fig. 1. Accessioning and registration of sequence data utilized in ISCDFT designations.

Fig. 2. Checklist for designating dark fungal taxa.

Individual sequences/genomes

New/unpublished data (not yet accessioned)

No accession no.

Published data (accessioned)

INSDC accession no.

From eDNA bulk sequences

Published data (accessioned)

SRA Accession Nos

Bioproject: PRJNA1450375
Biosample: SAMN57153404
Experiment: SRX32848607
Run: SRR37993329
Sequence read header: SRR37993329.1
M05520:100:000000000-LRMBR:1:1101:16545:2183 length=251

New/unpublished data (not yet accessioned)

No accession no.

Submitted to SRA

SRA Accession Nos
Bioproject
Biosample
Experiment
Run
Sequence read header

Submitted to INSDC

INSDC accession no.

Voucher table (initial submission)

Integrated alignment (MSA)

Phylogenetic tree

Submitted to INSDC

INSDC accession no.

Submitted to INSDC

INSDC accession no.

Voucher table (final publication)

Checklist for designating DFT under the ISCDFT

1. Taxonomic standards

- ❑ Ensure that the taxon meets requirements for a DFT (e.g., no physical material exists).
- ❑ DFTs are ideally shown by presence in at least three independent samples.
- ❑ The total number of sequences representing the newly designated DFT are, ideally, >five.
- ❑ Phylogenetic analyses are based on a multiple sequence alignment that includes the nearest relatives at the appropriate rank (provided sequence data are available).
- ❑ New designations are supported by quantitative species delimitation methods, whether alignment- or tree-based..

2. Designation conventions

- ❑ Ensure that the designation is unique and unlikely to be confused (verified against several sources: e.g., Index Fungorum/MycoBank and Catalogue of Life; also see Provision 10 on homonymy).
- ❑ Designated name is suffixed with "-ENAS" and include "new designation" after authors.
- ❑ Ensure all designation authors are involved directly in the designation using correct conventions for author names (see Provision 8 on Authorship).
- ❑ Includes DFT-SH DOI obtained from UNITE (obtained after peer-review).
- ❑ Includes registration identifier (obtained after peer-review).
- ❑ Includes short diagnosis based on reference sequence compared to most closely related taxa at the appropriate rank.
- ❑ Includes informative etymology indicating the person, locality, substrate or character to which the epithet refers.

3. Reference sequence designation

- ❑ Is a single representative sequence and not a consensus sequence comprising more than one ASV.
- ❑ Is available at the time of publication in a public repository such as GenBank, and accession number appears in Reference Sequence information.
- ❑ Metadata regarding sequencing platform, accession numbers, as well as locality, date, and substrate are included in sequence deposition and in the reference sequence block.
- ❑ Includes information regarding availability of material sample.

4. Designation notes and manuscript preparation

- ❑ Include non-ISCDFT synonyms from other studies if applicable.
- ❑ Include summary of ecology and distribution as inferred from all available sequences of the DFT, including those generated in other studies.
- ❑ Include a table or similar referencing all sequences used in determinations, including source of previously published data.
- ❑ Includes locality of place(s) of data deposition.
- ❑ Includes note regarding attempts to isolate or otherwise visualize the DFT.
- ❑ Keywords includes "-ENAS".

Checklist for designating DFT under the ISCDFT (cont.)

5. Submission and review process

- ❑ Submit manuscript to *IMA Fungus*.
- ❑ During revision, submit reference sequences to UNITE for generation of DOIs.
- ❑ After DOI generation, register designations with DOIs in name repository.

6. Post publication

- ❑ Ensure all appropriate data repositories are updated and provided with a PDF file.