FUNGID: a Web-based Identification Program for Phytophthora

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ABSTRACT

The genus *Phytophthora* encompasses economically important plant pathogens. To date, up to 90 species have been described in this genus. There is no comprehensive key available for the identification of the all described *Phytophthora* species. This paper, describes a web-based identification tool (which has been described by the authors under the name of Fungid) that uses morphologic features. The characters used are the same as the synoptic keys of Gerrettson-Cornell (1994) and Ho (1981) [1, 2]. For the isolate in question, the software compares the unknown isolate with more than 40 known species submitted in the database. One or more than one species is suggested to the user by the software. For some of the species, additional data such as images of the species, links to other databases like that Q-bank, Index fungorum is presented.

General Terms

Characters, Index Fungorum, Pathogen, Q-bank, Synoptic Keys

Keywords

Database, Morphologic features, Fungid, Web-based software

1. INTRODUCTION

Biodiversity is the study of the variety of living beings [3]. There are several concepts presented for species definition in literatures, while some of these concepts are theoretical, with little value for species recognition; the others are practical or a combination of both with significant value for species recognition. However, a single definition which could be applied to distinguish all domains of life is not available. The morphological species concept has widely been used by biologist in order to explore the biodiversity of different groups of organism at taxonomic level of interest. It is based on the idea that a morphological type or individual can represent the variation within the populations of an entire species. Two primary criteria for species definition under morphological species are the consistency within the species and sharp splits in consistency between species. Morphologically-based species concepts and taxonomies are useful for many purposes and serve as useful tools for initial classification of biodiversity. As pointed out by many scientists, the major strengths of the morphological species concept for fungi are the general applicability of this concept to any fungal taxon and its widespread and historical use [4]. Even though, with the advent of molecular techniques and rapid progress in gene and genome sequences technologies, DNA based approaches are becoming as a gold standard technique for species recognition in fungi, the usefulness of morphological criteria for species recognition in fungi are vital and morphological discrimination at species level is important for end users such that agriculture, medical, industry and etc.

The basis of any study of biodiversity is morphological description of the existing species as they appear to us. Such descriptions must make it possible to identify other specimens of described species. Once this done, other aspects of the well characterized species such as its biology, ecology, genome and the other potentials can be studied. But, except for some unicellular organisms such as viruses, certain bacteria and etc, the basis of all these studies will always be the morphological description, of the species [5]. The large scale identification must be done by biologists who are not professional taxonomists. It is argued that printed identification aids are too rigid and unreliable, and molecular identification aid is too limited. Only computer identification tools are flexible, reliable and general enough to give a non-taxonomist the possibility to identify any species [3]. Our knowledge about morphology and the various other scientific fields represent an enormous amount of facts, which must be classified and stored in a way that supports easy retrieval. This is particularly true if the various experts that are interested in biodiversity want to be able to access data in fields with they are not familiar. Only computer science offers some hope to put this huge mass of knowledge in order and store it in such a way that the data of interest can be retrieved easily [6]. Identification of fungal plant pathogens is commonly done using one of several well-illustrated dichotomous keys [7, 8, 9, 10, 11]. Multi-access keys are very useful for biological agents, especially for non-specialists, as it is not necessary to be able to detect all of the fine descriptions usually found in dichotomous keys [12]. The disadvantages of those printed keys are that they require the user to be able to scan a series of

tables of numbers and select those are common to the specimen being examined [13, 9]. If a universal data format were established for the descriptions and illustrations or photos of new species and used in a database freely accessible through the internet together with properly designed software, such as a database could allow any scientists with minimal training in fungi morphology to identify the specimens they are working on. For example, the multi-access lucid key to common plant pathogenic fungi causing diseases of temperate crops (http://plant-protection.massey.nz/resources/software/lucid_key.thm) or Q-bank (www.q-bank.eu/Fungi) are published in the web. Some of desktop-based programs that developed in plant mycology are: TAXADAT [14]; Simple computer program prepared by Polonelli et al. (1985) [15]; PENNAME [16]; FUSKEY [17]; PENIMAT [18]; CD-ROM designed by Sarma et al. (2000) [19]; FRIDA [20]. Identification keys for the computer are not always faster than printed ones [21], particularly if non-metrical data are used. A printed key can easily be used next the microscope, but the combination of microscope and computer necessary for utilization of identification software has only recently been applied [22]. In this paper, the concepts we will present, have been developed over the last 2 years by a team consisting of the present authors, three mycologists (ACB, MA and AB) and one computer engineer (AK). The project was called Fungid (Fungi Identification Program). We believe that our software could readily be extended to the identification of other biological groups. In this study, we prepare the *Phytophthora* section but the completion of its details will be gradual and time-consuming work.

Phytophthora (Plant destroyer) is an oomycete and the cause of the potato blight. *Phytophthora* caused Irish famine in 1845 [23]. Since the description of the genus *Phytophthora* by de Bary, over 90 species have been described in the past 100 years. Different criteria have been used to classify members of this fungi-like plant pathogenic genus The first key, by Rosenbaum (1917) [24] included 11 species and was entirely based on morphological characteristics. A later key, by Leonian (1925) [25] was instead based on physiological data. Other schemes, by Tucker (1931), Leonian (1934), Sarejanni (1936), Frezzi (1950), Waterhouse and Blackwell (1954), Waterhouse (1963), Newhook et al. (1978), Ho (1981) and Gerrettson-Cornell (1994) made use of one or both types of characters, especially morphological [26, 27, 28, 29, 30, 31, 32, 2, 1] . In spite of its comparatively small number of taxa, the genus *Phytophthora* has proven very difficult to classify because of the great variability exhibited by many species, the lack of a standard methodology for characterization and the limited information provided in many descriptions of species. A significant hindrance in *Phytophthora* studies has been the difficulty of finding media of standard composition to be used for the description of all named species and its accounts for the natural organic media mostly used by researchers in this field. The aim of this work has been to combine most of the available published taxonomically useful information on each of 41 species of *Phytophthora* of major importance in forestry and agriculture and to represent a user-friendly web-based identification key in order to facilitate quick diagnosis of plant pathogenic fungal or pseudo-fungal agents. This work includes a computer aid based mostly on morphological attributes.

2. METHODS

The program was developed in ASP.NET web language. The database was supported by SQL-Server. Fungid is freely accessible through the web, www.fungid.info . In the software for the each species of the genus *Phytophthora*, morphological characters (as characters and character states) were defined. These diagnostic features include: Mycelial growth, Cardinal temperatures, Hyphal swellings, Chlamydospores, Sporangiophores, Sporangia with more than one apex, Zoospores, Sexuality, Oogonia, Oospore and Antheridia. Details of morphologic attributes, used in Fungid are presented in Table 1. In this program, there is no limitation for the definition of other fungal groups. all species of all genera of fungi. Fungid is very user-friendly and can be extended to other types of living such as insects, plants, bacteria and etc. More information about the species is in wiki-page of each species.

Table1. Morphological attributes used in Fungid

3. DESCRIPTION OF THE USER INTERFACE

In Fungid homepage (www.fungid.info), there are two main icons, **Go to Database** and **Search in Database** (Fig. 1).

Fig. 1. Homepage of the Fungid.

In the first menu, the users must fill in the form with his/her username and password to enter and see the database contents. Certain of ID for users are supported by Administrator of the website. Users must send their request to receive the ID through the **Contact** menu. After log in, users can see the contents of the database such as **Search**, **Scan**, **Genera** and **User** icons without any possibility to edit or delete them (Fig. 2).

Fig. 2. Database layout of the Fungid.

In **Search** icon, the user can do a general exploring based on the fungus name and find it (Fig. 3).

Fig. 3. The Search section of the program.

In **Scan** icon (the most important icon of the program), the user can choose the genus name via a combo-list. After selecting the genus and clicking on **Go**, the user can fill in the form of the **Scan** page which is based on morphological features. None of the characters were defined as a required matter. By mouse clicking on Scan, the software compares the entry with submitted information in the database and finally, one or more than one species are proposed to the user (Fig. 4).

Fig. 4. The Scan section with a form that the user must fill in with morphological features of the unknown species. None of the characters were defined as required icons.

Based on the species details, the user can decide to choose the most similar species that it is highly matched to his/her isolate (Fig. 5).

Fig. 5. Search result page of the program.

We try to complete the details of the species via adding the descriptions and images. One of the major limitations in the creating web documents is copyright. Many copyright holders are reluctant to permit use of their images, because anyone can save the image to a file on their computer and use it later without obtaining permissions [33]. Although, we linked each species to useful websites like that Q-bank and Index Fungorum. So our trying to complete the program will be a gradual work. In **Genera** icon, user can see the genera that have been defined to the program with their species. Some of Genera labeled with "in progress" and/or "Forthcoming", that it means the process of completeness of the genera are not finished and it is under maintenance and the process of preparation of the genera and its species will start soon (Fig. 6).

Fig. 6. The Genera section of the program.

In both genera and species with clicking on the name of the fungus, a page is opened as "wiki page". In this page, properties of the genera and species are present. In **User** icon, every user can see her/his profile and complete and/or edit it and can see the database of the genus. A hypothetical user ID of the site is: email: <u>user@gmail.com</u>; Password: user2012.

4. RESULTS

A serious problem in biodiversity studies is the limited availability and accessibility of data which are widely spread in several books, journals and off-line databases, as well as stored in biological collections, making it very time consuming to compile the necessary information. So, we consider Fungid is a useful tool for the rapid and accurate identification of fungi. Structure of the Fungid and how-to-work of the program is presented in Fig. 7. By the way, the program can be extended to some other icons such as **gene sequence search** without substantial costs. In fact, polyphasic taxonomy is better than monophasic only based on morphologic or molecular characters. But, molecular data is not always available. In contrast, morphologic features are easier to obtain in comparison with molecular data.

5. DISCUSSION AND CONCLUSIONS

Since Fries (1921) [34] wrote his Systema Mycologicum, mycologists have tried to make accurate descriptions of the fungi they collected and to provide non-mycologists with useful identification keys. Their efforts have led to a knowledge of the most important, mainly morphological characters necessary to describe fungi and also to the different types of identification keys in use today. So far the synoptic keys and the dichotomous keys are the tools which allow

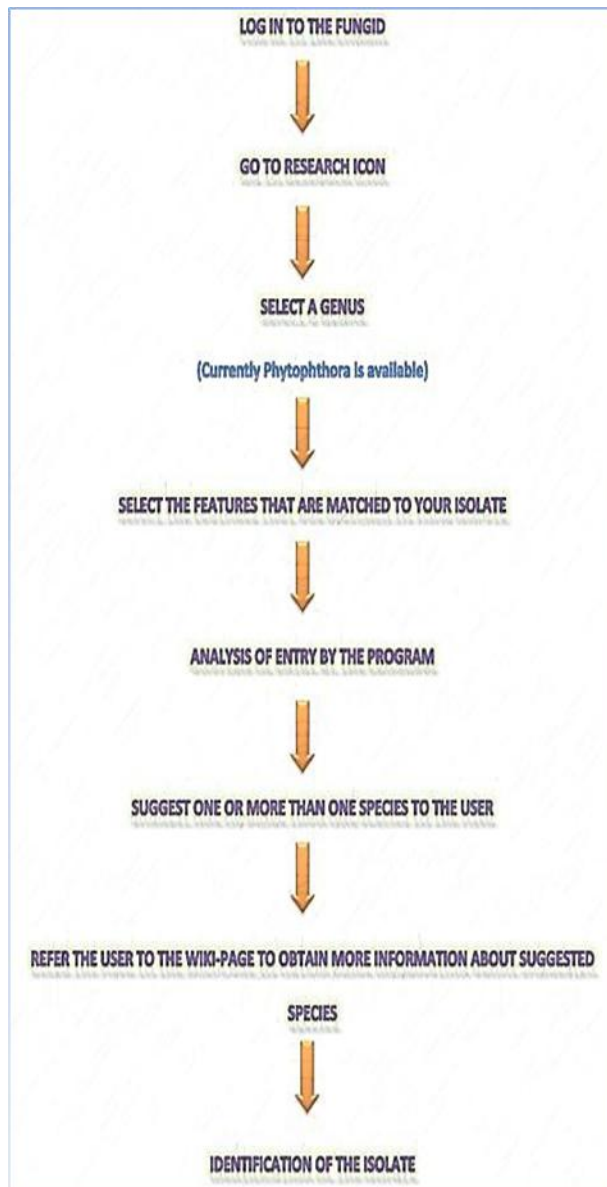mycologists to attempt the identification of living organisms to species level.

Fig. 7. Structure of the Fungid and how-to-working of the program

The synoptic key is based upon the simultaneous check of several characters and upon the elimination of the taxa not representing such features until only one taxon is left; a dichotomous key is based on a binary form of hierarchical decision-making process in which a question-making process in which a questions has to be answered by a 'yes' or 'no' in order for the next branch of the binary tree to be chosen. Both types of the keys are exhaustively discussed by Hawksworth (1974) [35] and examples of both types can be found in Sutton (1980) [9]. The use of either type of key presents specific problems. While the synoptic key is comparatively free of any kind of weighting of characters by the author, the choice of the hierarchical line of the identification steps within a dichotomous key is mainly subjective and can often lead to wrong decisions depending upon the accuracy of the choice and the skills of the user. On the other hand, the morphological and physiological characters used can be either

ill-defined or not very accurate, most of them being subjectively defined by the taxonomists: particularly with a dichotomous key a considerable amount of information is presented in the form of disjunctive (OR), conjunctive (AND) and even mixed (AND/OR) sorting or by subjectively defined frequencies of occurrence (rarely, often, etc). The identification of a fungus is thus a task which can offer many problems to the inexperienced mycologist in spite of the considerable amount of information collected over the last two centuries. The availability of a computer-assisted way such as web-based program to overcome these difficulties would be of great interest to most biologists, e.g. ecologists, plant pathologists, food microbiologists, who are confronted during their work with the identification of fungi.

Printed keys are powerful tools that can be used safely since they rely on primary identification characters [36]. The paper is a dead medium that cannot be updated, but an electronic medium offers the flexibility needed to give at least some control to the user and to make it easier to update a key [3]. Computer-aided can create a revolution, since they use in a multi-dimensional way, a wealth of morphological and physiological data plus ecological information usually hidden in the large ocean of scientific literature. Traditional keys have several drawbacks that can be avoided by computer aid tools: 1) Being printed on paper, their content is frozen and hence nomenclature-taxonomic changes and the discovery of new species render them rapidly outdated. Computerized systems, on the contrary, can be updated and corrected in real time. 2) Traditional keys are rigid. They contain a huge of information which is fixed into the format and the logical structure selected by the author. Computerized tools permit to reduce the set of organisms using different combinations of morphological, ecological, distributional characters i.e. special habitats, mycotoxin production and physiological features (temperature, water activity, pH, …). 3) A small database can be the starting point for future expansions. 4) Outputs can be edited in several different formats, from simple texts to illustrated books [20]. Some of desktop computer identification tools with their approach and scopes are : TAXADAT [14] and a simple computer designed by Polonelli et al. (1985) [15], differentiation *Candida albicans* strains based on of the production of killer toxins by each individual strain; PENNAME [16], large synoptic key for identifying 70 common *Penicillium* species; FUSKEY [17], data matrix of approx 1200 *Fusarium* isolates using DELTA system software and key generating program INTKEY, *Fusarium*; PENIMAT [18], probabilistic matching, terverticillate *Penicillium*; CD-ROM designed by Sarma et al. (2000) [19], interactive keys linked to descriptions and images of fungi, mangrove fungi; FRIDA [20], interactive tool for the identification of fungi is written in PL/SQL language, running on a Oracle database engine, airborne and food fungi.

The web-based identification programs have many advantages over the desktop-based programs of the late 1980s. First, household computer usage for example in U.S increased from 22.8% in 1993 to 61.8% in 2003 [37]. In addition, broadband internet access is becoming more common, and wireless internet success via cell technology allows access for users in remote areas. Increasingly, common smart phones also provide internet access without the expense or weight of the laptop; users can either directly access the websites or have news emailed to their phone. Another factor favoring web-based systems is the ability to access and integrate or complete data from widely separated locations. For example, definition of many administrators for the web sites as editors

is possible. So, several editors probably around the world can edit their data in the Fungid and in the other hand, simultaneous completeness of the program will happen. Perhaps, the greatest advantage of the web-based program over the desktop-based is that changes in the details of database at the server instantly propagated to all users at the next log in. So, errors can be quickly corrected without having to send out notices the users need to update their systems.

Our aim by designing this software is to facilitate the identification of fungal species and to present and further develop software which is easy to handle, useful and easy to obtain (via World Wide Web: www.fungid.info). This simple aim also implies a development for the near future; Fungid will not only support the identification key of the genus *Phytophthora*, but also of the other genera such as *Pythium, Pestalotiopsis, Colletotrichum* and etc. Existing printed references or desktop computerized identification tools cannot be trusted to non-taxonomists for identification of all the materials collected. A freely accessible identification tool via internet such as the one outlined here, probably can solve this problem.

The biggest technical problem to date has been related to different web browsers. Most of open source browsers have few problems. Among these, such as Mozilla Firefox, Internet Explorer, Maxthon and Google Chrome, Mozilla Firefox has a high compatibility with Fungid. Probably, several changes in settings of other browser must be done to ensure compatibility with Fungid.

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Table1. Morphologic attributes used in Fungid

Character	Character states
Mycelial growth	Colony radiate Colony slightly radiate Colony without any distinctive pattern "Chrysanthemun" or Coralloid growth pattern are observed "petalloid" or floral pattern "rosse" pattern "stellate" pattern Dense white colonies are observed on PDA at 27 °c Mycelium typically gnarled Less than 20mm D. growth rate in 7 days on lima bean agar, equally slow on cornmeal agar and 2% V-8 agar Moderate to fast growing 35mm D. rate at 32°c on 5% V-8 juice agar. Good growth also at 24 °c Growth on the usual media is very slow at 25 °c Cultures with variable aerial mycelium and pattern Aerial mycelium appressed Cultures fluffy Cottony like aerial mycelium over entire colony, faint lobed floral pattern Less than 20mm D. growth rate in 7 days on oat agar Less than 20mm D. growth rate in 7 days on potato agar Hyphae very unequal, often coiling in culture Granular in appearance Hyphae irregular., true hyphal swellings not formed Hyphae intercellular in host, branched Hyphae intercellular in host, provided with haustoria Non-septate hyphae in agar media, often swollen in knob like appearance at irregular intervals
Cardinal Temperature	Minimum, Optimum, Maximum degree
Chlamidospore	Absent, sparse or abundant Hyaline Rare terminal Usually or intercalary terminal Not observed Not formed by some isolates, rare to abundant in some others, thick walled Formed by a lateral swelling of the hyphae, hyaline to amber Terminal Thin walled Apical Lateral Spherical Ellipsoid Spherical ovoid Single or crowded

	Dark brown
Sporangia with more than one exit tube	Absent
	Occasionally observed
	Occasionally formed with three pores
	Rarely observed
	Usually formed with two, but even with three or four pores
	Present
	Formed with two pores

Table1. Continued

Character	Character states
Sexuality	Heterothallic
	Sterile
	Rarely homothallic
	Homothallic
	Predominantly heterothallic
Hyphal swellings	Rarely formed or in clusters of a few intercalary elements
	Terminal or intercalary
	Occasionally formed, terminal sessil or intercalary (the latter in net-like appearance, in some cultures flooded with water)
	Spherical or ovoid or in the shape of sporangia
	Not formed
	Usually abundant, single or botryose, rarely intercalary, globose and/or more less irregularly shaped, hyaline to yellowish brown
	Sub-spherical to deltoid hyphal swellings
	Intercalary in net-like clusters, abundant in clusters flooded with water or aqueous solutions, they may be elliptical, pyriform, irregular or spherical
	Clusters of intercalary swellings, angular to spherical
	Thin walled swellings, occasionally cut off by septa may occur
	Produced in mycelium transferred to distilled water and incubated for two days under light
	Variable in shape and size
	Irregular in shape and size, in chains or clusters
	Common in clusters flooded with aqueous solutions, ovoid or spherical, intercalary
	Especially in cultures flooded with water spherical, rounded or angular in chains or clusters
	Abundant, rounded, ellipsoid or angular, single or in chains (net-like type), some germinate to produce germ tubes
	Formed abundantly at 21-24°c in agar cultures flooded with water. Round or angular in chains or clusters
	Especially in water, intercalary, spherical, ellipsoidal or angular
	Terminal or sessile, globose or sub globose
	Spherical to ellipsoid, monilioid or catenulate
	Abundant on oatmeal agar, rare on cornmeal agar. Roughly spherical intercalary
Zoospores	Present
	Migrate into an evanescent vesicle like in the genus Pythium
	Presence of lateral hairs on both flagella
	Up to 40-50 zoospores may be produced in a sporangium
	Remain inside the sporangium where they germinate
	Ellipsoidal or kidney shaped
Sporangiophore	Either simple or irregularly branched
	Either simple or sympodially branched
	Proliferating through the empty sporangium
	Each branch ending in short sympodia of 1-3 sporangia
	Umbellate arrangement of sporangia is observed, especially under light conditions of incubation
	Simple
	Un-branched
	Branched
	Irregularly branched
	With globose swellings at branching points
	Swellings may occur behind the sporangia
	Branching compound, sympodial with a small swelling at the base of each branch
	Sporangia borne terminally on sporangiophores not differentiated from vegetative hyphae
	Loose sympodia
	Short, not dissimilar from hyphae

	Rather wide compared to the mycelium hyphae, especially beneath the sporangia
	Undifferentiated, often with intercalary swellings
	Undifferentiated, often with sub-sporangial swellings
	Branching in a close sympodium, not proliferating, sometimes with intercalary swellings

Table 1. Continued

Character	Character states
Sporangia	Terminal
	Intercalary
	Ellipsoid
	Obtrupinate
	Spherical
	Semi-papillate
	Papillate
	Non-papillate
	Obpyriform
	Deciduous
	Non- caduceus
	Oval to ovoid
	Regular in shape
	Non-pedicellate
	Distorted shape
	Pedicellate
	Globose
	Sub-globose
	Limoniform
	Obovate
	Pyriform
	Large vacuole often present
	Papillae usually not exceeding the width of the membrane of the sporangium or slightly pronounced
	Obclavate
	One side flattened or cured
	Bifurcate or furcate with 3-4 exit pores
	Fusiforme
	Elongated
	On one side bent or flat
	Rather irregular in shape
	Rounded apex
	Asymmetrical and lateral on stalks with rounded base
	With broadest part nearer the apex and tapering towards the base
	Obturpinate and ampullate
Antheridia	Amphigynous
	Unicellular
	Bicellular
	Often broader than long
	Nearly spherical
	Paragynous
	Close to the oogonial stalk
	Nearly spherical toirrgularly shaped
	Very variable in shape and size
	Spherical to oblong
	Oogonia stalk may be occasionally obscured in a knot of hyphae
	Spherical to cylindrical
	Ellipsoidal

Table 1. Continued

Character	Character states
Oospore	Nearly filling the oogonium
	Loose in the oogonium
	Hyaline to yellowish brown
	Plerotic
	Aplerotic
	Hyaline to yellowish at the periphery
	From aplerotic to nearly filling the oogonium
	Hyaline to yellow to tawny
	Brownish
	Spherical
	Honey yellow to brownish when old
	Pigmented
	Hyaline to faint yellow
	Colorless to light brown
	The outer wall of the oospore is distinct, thin and much darker than the thick inner wall
Oogonia	Wall smooth
	Wall wrinkled
	Wall bullate or unbullate
	Wall verrucose
	Wall reticulate
	Hyaline
	Light brown
	Brown
	Slightly rough
	Yellow
	Golden yellow in culture, colorless in host tissues
	Formed promptly often in clusters, spherical tapering to stalk
	Reddish brown
	Colorless
	The oogonia stalk is branched sympodially from immediately under the oogonia which may give the impression of intercalary ogonia
	A septum is usually present on many oogonial stalks inside the antheridium
	Orange brown
	Pyriform
	Tapering downwards to a funnel shaped base
	Oogonia are terminal but a few intercalary
	Occasionally some folds are observed in the membrane