Journal of Plant Pathology (2023) 105:429–436
https://doi.org/10.1007/s42161-023-01349-6

SHORT COMMUNICATION

FusaHelp: a web site program for the morphological identification of *Fusarium* species

Alessandro Infantino[1] · Alessandro Grottoli[1] · Valentino Bergamaschi[1] · Safa Oufensou[2] · Lester W Burgess[3] · Virgilio Balmas[2]

Received: 22 December 2022 / Accepted: 26 February 2023 / Published online: 15 March 2023

Abstract
Fusarium is one of the most important phytopathogenic fungi of agricultural and human concern. More than 300 species have been described, many of which are pathogenic to important crops, flowers, forest trees, animals, and humans. Species belonging to this genus have been detected in all environments: grassland, desert, littoral, agricultural, alpine zones, aquatic, man-made, and hospitals. Despite the importance of molecular techniques for the identification of a fungal species, morphological criteria still have an important role, including for *Fusarium* species, for which morphological identification of species requires adequate training and experience. In this paper, we present FusaHelp, a computer-based, user-friendly tool for the morphological identification of common *Fusarium* species, based on the wide experience of the authors who have devoted most of their scientific careers to the identification and characterization of these species. The web-location of FusaHelp (https://www.fusahelp.com) will greatly facilitate morphological identification and is intended to provide support for all those people who work with this important genus and need a quick clue on the identification, even incomplete, of the *Fusarium* species that they are working with.

Keywords Taxonomy · Morphology · *Fusarium* database · Synoptic keys

The accurate diagnosis of fungi is a prerequisite for many scientific fields, such as agriculture, ecology and medicine to enable rapid and efficient therapies, control strategies and to decipher the role of environmental and human effects on shaping the population structures of both beneficial and pathogenic fungal species. Despite the large number of species concept definitions in both plants and fungi (De Quieroz 2007; Giroud et al. 2008; Aldhebiani 2018; Jayawardena et al. 2021), the accurate identification through their morphological characters remains critically important. In their Handbook of Fungi (2007), Webster and Weber emphasized the importance of microscopic features in the identification of fungal species, before moving on to DNA identification, especially to introduce students to the 'art' of examining and identifying fungi. This is particularly true for fungal genera such as *Fusarium* and *Trichoderma*, or oomycetes like *Phytophthora*, consisting of many species that have been subjected to numerous revisions since they were first established. Species of the fungal genus *Fusarium* are ubiquitous, many of them are pathogenic to several important crops; in particular, two of them, *F. graminearum* and *F. oxysporum*, have been listed among the 10 most important fungal pathogens in plant pathology (Dean et al. 2012). Knowledge of the morphology of *Fusarium* is critical to an understanding of the disease cycle of *Fusarium* plant pathogens. For example, knowledge of the difference between thick walled chlamydospores and thickened hyphal and conidial walls is important to understand the persistence of some *Fusarium* pathogens such as *F. pseudograminearum* which causes crown rot of wheat and other cereals. This species does not form chlamydospores, but some isolates will form conidia and hyphae with slightly thickened cell walls in culture and

✉ Safa Oufensou
soufensou@uniss.it

[1] Council for Agricultural Research and Economics - Research Centre for Plant Protection and Certification (CREA - DC), Via C. G. Bertero 22, 00156 Roma, Italy

[2] Department of Agricultural Sciences and Desertification Research Centre (NRD), University of Sassari, Viale Italia 39a, 07100 Sassari, Italy

[3] Institute of Agriculture, Faculty of Science, University of Sydney, Sydney, Australia

soil (Wearing and Burgess 1977). This species persists primarily as hyphae in infested residues. Consequently, this is a key factor to be considered when developing disease management strategies such as the nature of crop rotations. The role of conidia in the biology and ecology of many *Fusarium* species, especially those not known to be pathogenic to plants or animals, is still poorly understood. For example, we do not know the role of the large globose conidia of *F. beomiforme* which form in culture. We do not know if they are formed in soil or in colonised plant material in nature. The morphological definition of a species is not a simple task, and it is ruled by the International Code of Nomenclature for algae, fungi, and plants (ICNafp, or simply, the Code) (https://www.iapt-taxon.org/nomen/main.php), revised on a six-year frequency at Nomenclature Section meetings of each International Botanical Congress (IBC), through the provision of detailed and precise formal requirements (Aime et al. 2021). The recent rapid and high throughput molecular identification of fungi and oomycetes has prompted proposals for the simplification of taxonomy, through the adoption of a "one name = one fungus" approach (Taylor 2011; Wingfield et al. 2012). This has also been applied to the genus *Fusarium* (Geiser 2013). Both morphological and molecular identification of fungi are useful tools: morphological and cellular structures often have direct biological significance, vital for understanding the survival, reproduction, infection and natural history of the organism. Nonetheless, DNA approaches are very good at identifying fixed allelic differences that are characteristic of the genetic isolation associated with species boundaries and for inferring evolutionary relationships among species. The importance of *Fusarium* molecular identification based on the analysis of several genes, such as the translational elongation factor 1-α (tef-1α), RNA polymerase 1 and 2 (RPB1 and RPB2), B tubulin (tub) or the nuclear ribosomal DNA intergenic spacer region (IGS rDNA), is well acknowledged (Geiser et al. 2004; O'Donnell et al. 2009, 2010, 2021). However, these analyses are costly and require specialized personnel that many laboratories cannot afford, especially those located in less developed countries. Therefore, morphological identification still has its importance, at least to provide an initial clue to the species, be subsequently confirmed by molecular approaches. The authors also consider that the requirement to include morphological details and illustrations, in addition to molecular data, in first reports of fungal pathogens or oomycetes in a country or world-wide, contribute to supporting the authenticity of the report. This can be important from an economic perspective as 'First Reports' may have a significant impact on trade in agricultural products. In recent years, different tools, using morphology as an important discriminant character, have been developed for the hierarchical classification of organisms like: PENIMAT (Kozakiewicz et al. 1993), and PENNAME (Pitt 1990) for *Penicillium* spp.; IdPhy (https://idtools.org/id/phytophthora/index.php) and FungId (Bouket et al. 2012) for oomycetes; XPER for nematodes (Palomares-Rius et al. 2022). A tool for *Fusarium* identification (FusKey) was developed using morphological features as discriminant characters. However, it covered only 17 *Fusarium* species and was not able to differentiate important *Fusarium* species such as *F. graminearum*, *F. culmorum* and *F. crookwellense* (syn: *F. cerealis*) because the author did not use macroconidia as a discriminatory character (Thrane 1991). More recently, new web-based technological tools, like FRIDA (Martellos 2010), have been implemented to generate identification keys for different organisms (Varese et al. 2010).

Conventional *Fusarium* identification procedures based on morphology are commonly undertaken by consulting several published synoptic keys (Gerlach and Nirenberg 1982; Nelson et al. 1983; Burgess et al. 1988, 1994; Leslie and Summerell 2006). The aim of FusaHelp is to facilitate the correct preliminary morphological identification of the most widespread *Fusarium* species by an open access, web-assisted interactive site, easily upgradable, and enriched by illustrations of the most relevant species. To generate FusaHelp, we have used LucidBuilder (https://www.lucidcentral.org/lucid-builder/), a platform based on JavaScript running on an independent browser application which runs on Windows, OSX and Linux and no additional software or plug-ins to run are required. The first step for the creation of FusaHelp was the thorough analysis of the literature concerning the morphological descriptions of new *Fusarium* species, along with those described in the above-mentioned synoptic keys. Where discordances among authors were encountered, or the description of some characters was missing, we examined cultures of the "true" species from International Culture Collections and confirmed by molecular analyses with tef-1α sequence. We performed a hierarchical clustering of the 82 *Fusarium* species of the dataset, using a binary distance matrix based on the Jaccard distance of the presence or absence of a certain character. The dendrogram was drawn with R version 4.2.1 (R Core Team 2022) using the "dendextend" package version 1.16.0 (Galili 2015). Except for *F. graminearum*/ *F. pseudograminearum* and *F. equiseti*/*F. compactum*, the hierarchical clustering performed on the entire dataset confirmed the unicity of the remaining species based on the combination of the morphological features observed (Fig. S1).

In developing FusaHelp, the following characters have been considered: the presence or absence of microconidia and their shape, the mode of formation of microconidia (single, in false heads or in chains); the type of conidiophores (monophialides/polyphialides); the presence or absence of the macroconidia and their shape (characteristic of the

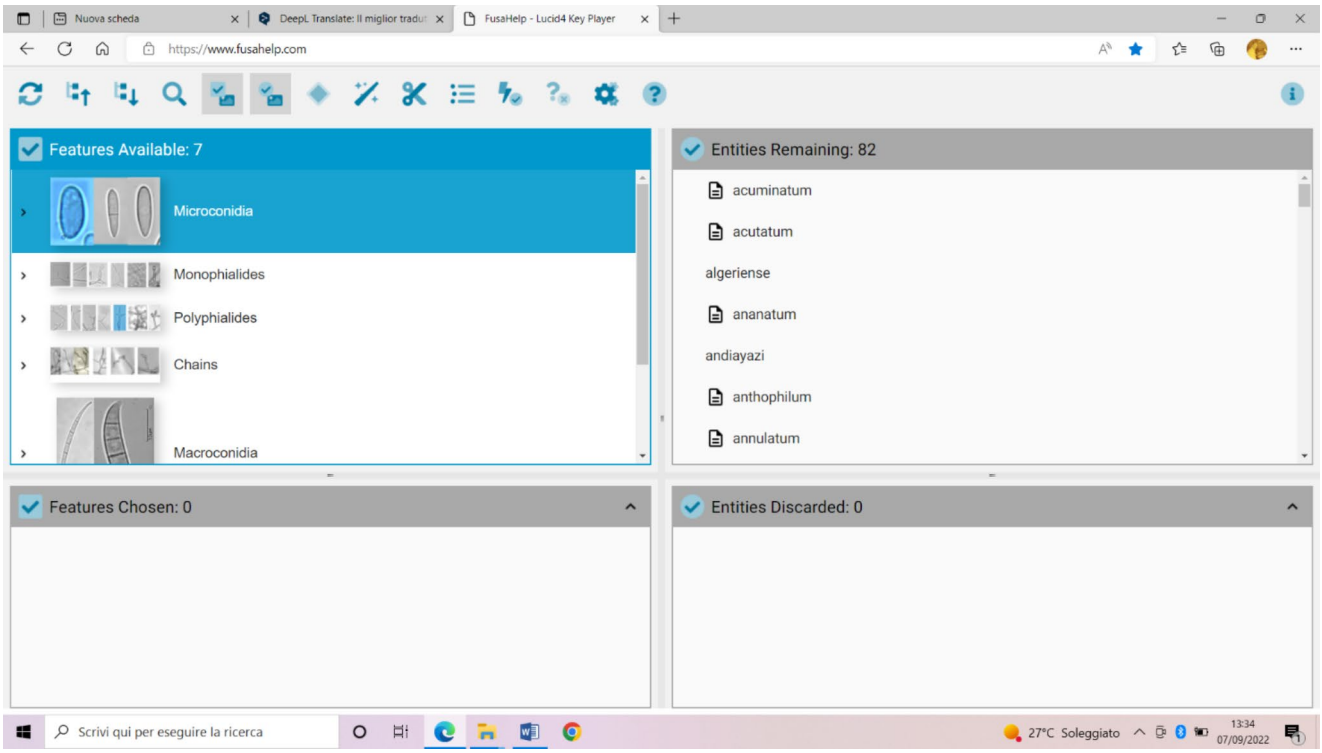

Fig. 1 Home page of FusaHelp

dorsal and ventral wall as well as the shape of the apical and basal cells); the presence and arrangement of chlamydospores. Finally, we considered the presence of pseudochlamydospores, of swollen hyphal cells and of coiled sterile hyphae as additional diagnostic features for some *Fusarium* species. We have not included the pigmentation on Potato Dextrose Agar (PDA) because this is a secondary character, that may only provide a first indication, and it has not been considered for diagnosis because it depends on the strain, type of media (commercial or homemade PDA) and cultural conditions. Before using the database, a special section has been created in which the user will be informed about which characteristics they have to consider and which to select to achieve a correct identification. We have provided a microscopic picture of each taxonomic character to help the user with the choice of the observed taxonomic character. The images used for the shape definition are reported in Fig. S2. Detailed data sheets have been created for 64 species, out of the 82 included in FusaHelp, in which additional information (important characteristics and differences with morphologically similar species) are provided. The list of *Fusarium* species included in the database can be found in Table S1.

For a correct and profitable use of FusaHelp, several rules must be followed. The morphological identification of *Fusarium* species must be based on pure single spore cultures (Fig. S3), with cultures grown on CLA (Carnation Leaf Agar) (Fisher et al. 1982) and/or SNA (Spezieller Nährstoffarmer Agar) (Nirenberg 1976). The morphological characteristics of the structures grown on PDA should not be considered as diagnostic characters. To verify the mode of formation of microconidia (false heads or chains), it is recommended to observe the culture directly under the 10× or 20× magnification, or to utilize a clear adhesive tape (Burgess et al. 1988; Harris 2000). Ideally the shape of macroconidia should be determined using macroconidia from sporodochia, when available. Therefore at least two slides are required: one from sporodochia and one from mycelium. Some authors describe the presence of "mesoconidia" as the macroconidia borne on mycelium with 3 or more septa. In this database we did not consider mesoconidia as a diagnostic criterion and we treat them as macroconidia.

The database FusaHelp is freely available at the following link: https://www.fusahelp.com. When entering the database, the first page that appears is divided into two screens. On the left frame are listed the seven morphological features available (microconidia; monophialides; polyphialides; chains; macroconidia; chlamydospores; other diagnostic features), while the right frame includes the list of 82 species in the database (Fig. 1). Each time a feature is chosen, the number of the possible species on the right will consequently be reduced. Identification starts by choosing one of the observed features. For instance, for microconidia, selecting if they are present or absent; if present, then it will appear "microconidial shape" and typing on it, the 13

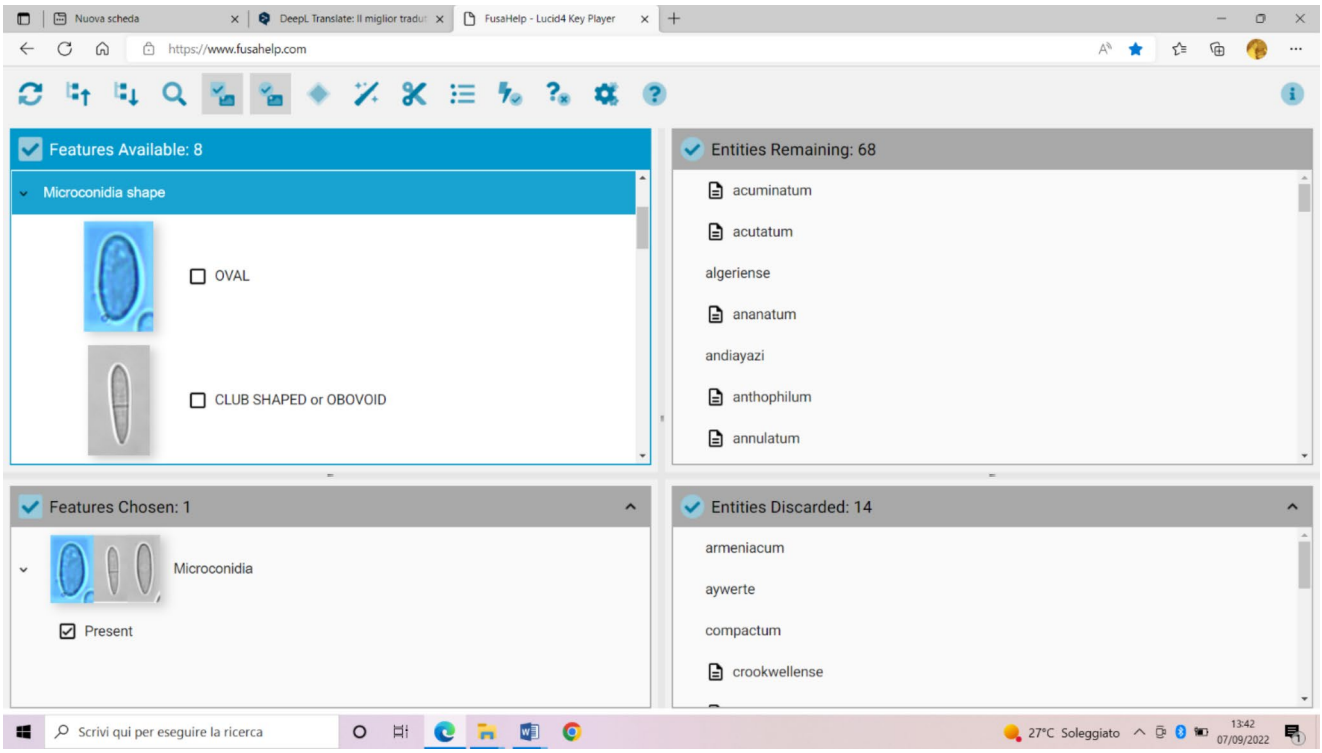

Fig. 2 Screen that appears after selecting the presence and shape of microconidia

different possible forms will appear. Choosing one or more of them, the search will be refined. As an example, if the "presence of microconidia" has been selected", in the right part of the screen, the number of species will be reduced to 68 (Fig. 2).

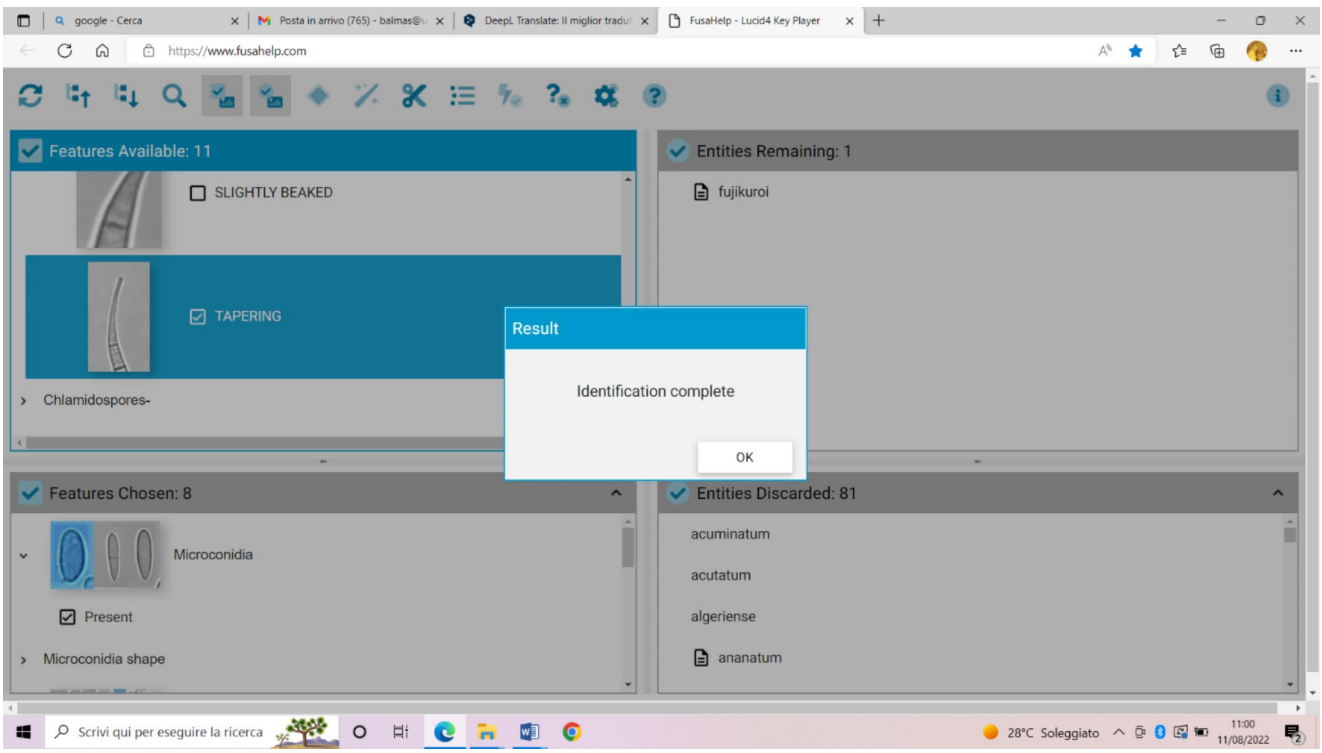

Fig. 3 Example of the completed identification of *F. fujikuroi*

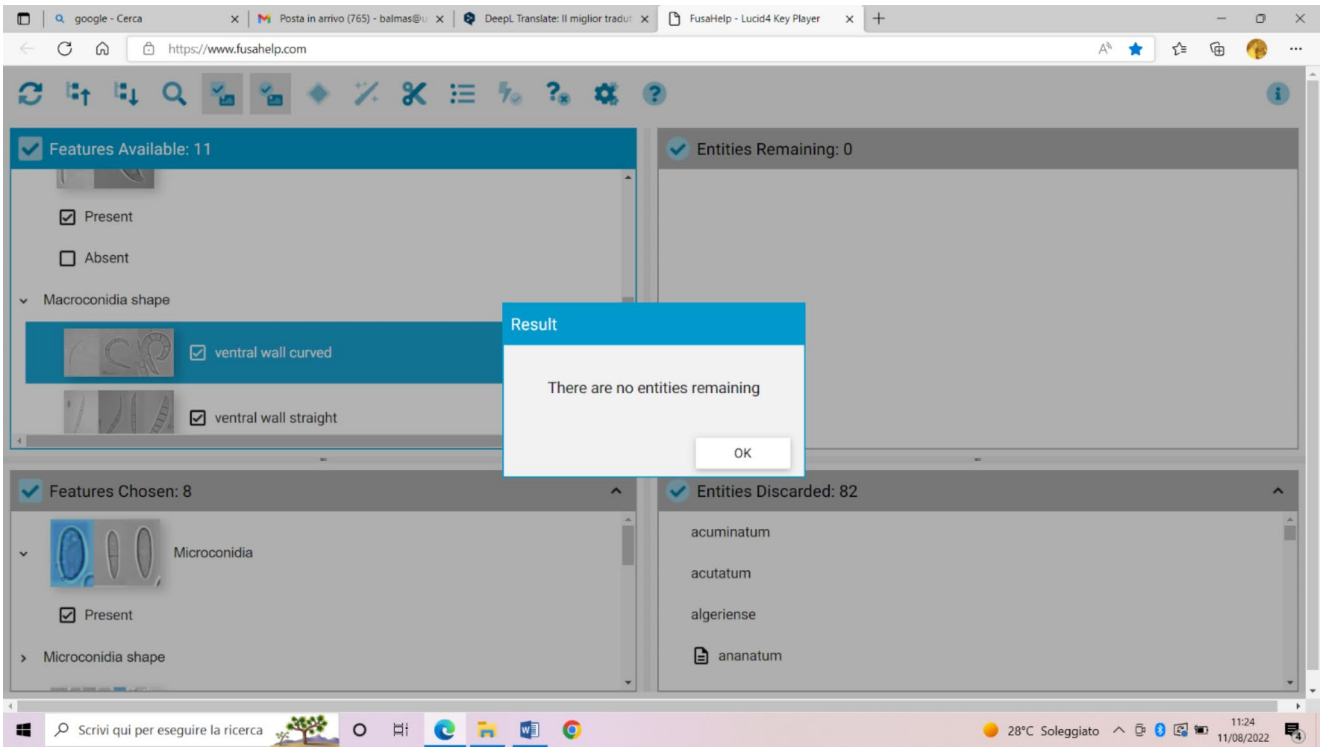

Fig. 4 Example of a failed identification

Please note that if you are not sure of the absence or presence of a character, it is better not to click on the box and leaving it blank.

Once the maximum number of the observed features has been correctly selected, the indication "identification complete" will appear and in the right part of the screen on the

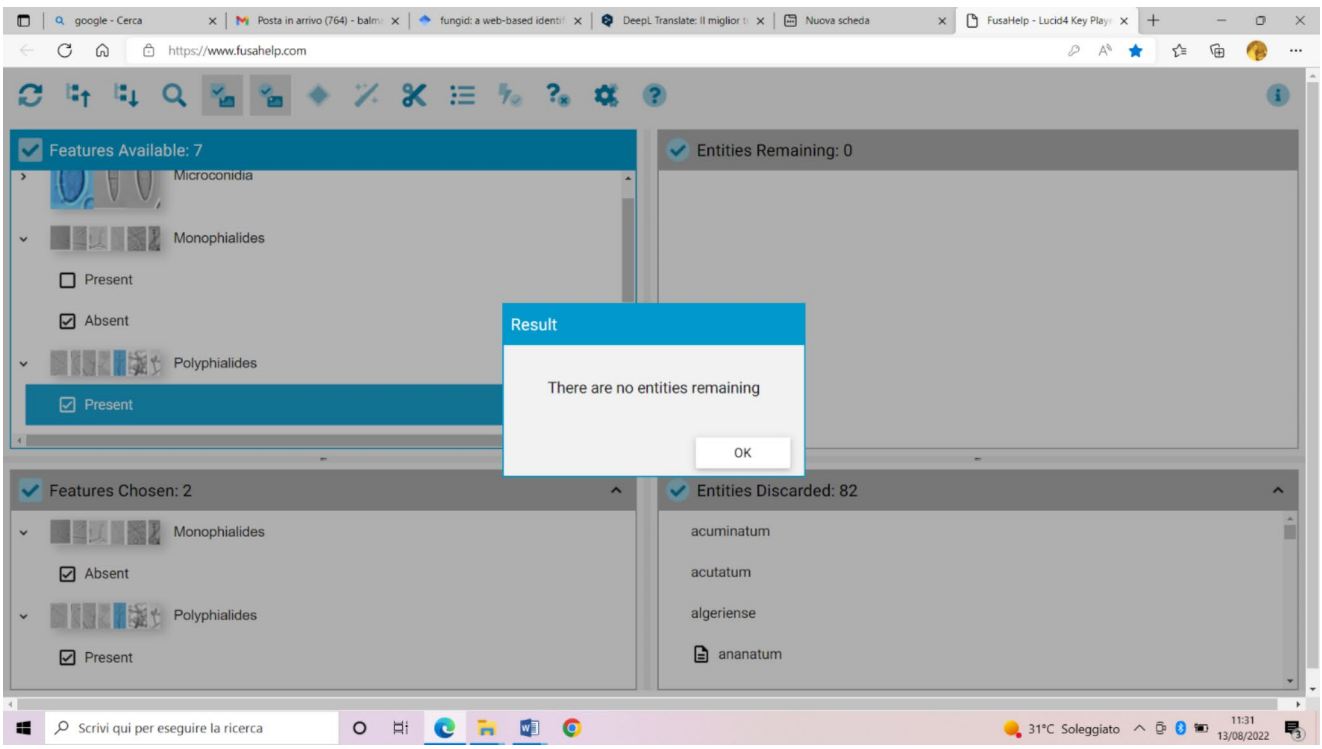

Fig. 5 Example when the absence of monophialides and presence of polyphialides is selected

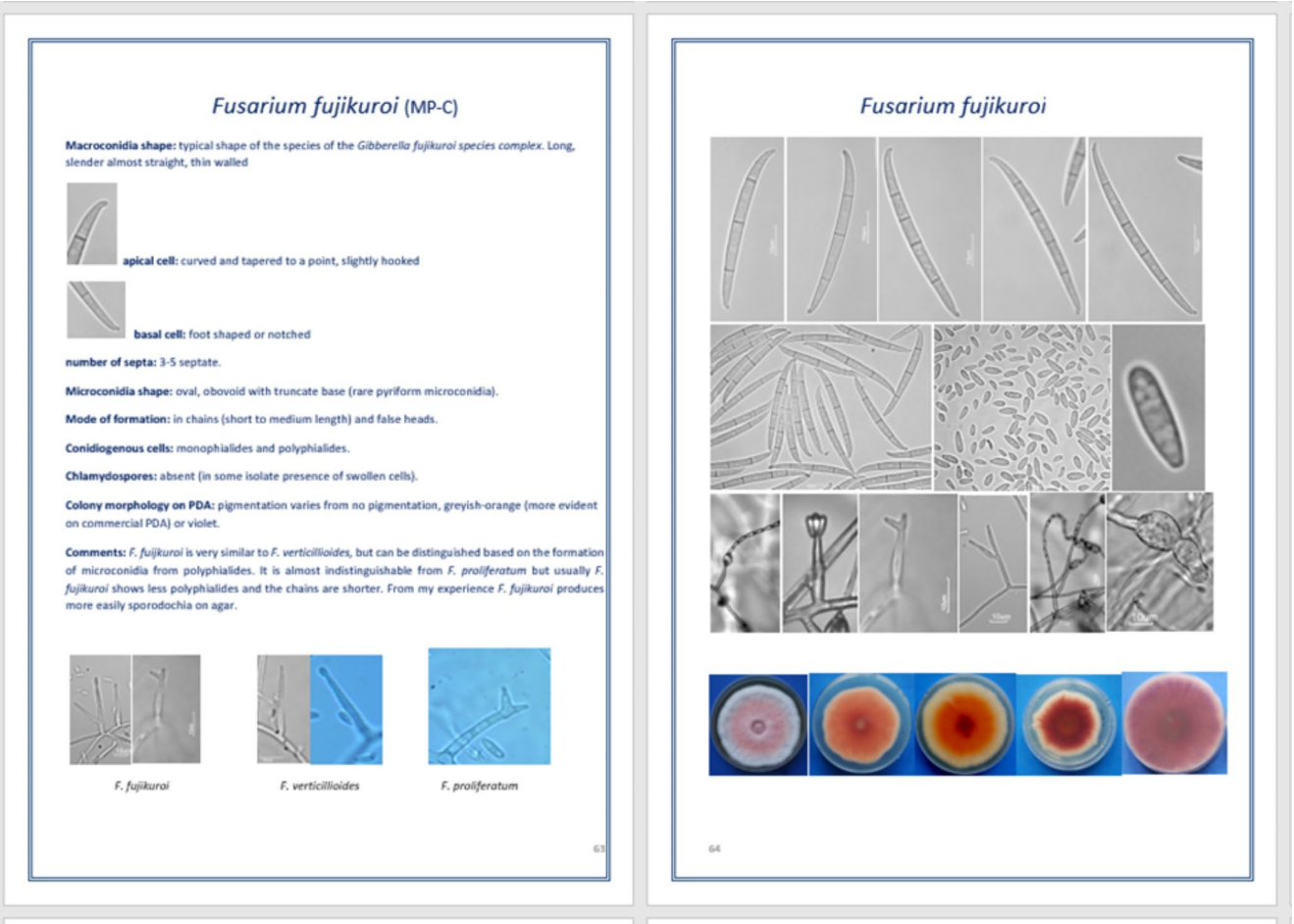

Fig. 6 Example of a detailed description of one of the species present in the database

"Entities Remaining:1", the corresponding species is identified (Fig. 3). For some species i.e., *F. graminearum* and *F. pseudograminearum*, morphologically indistinguishable, the "Entities Remaining" will be with these two species.

If the advice "There are no entities remaining" appears, it means that the characters selected for identification are not sufficient for the identification of the species present in the database and some of them must be unselected or changed (Fig. 4). Please note that when selecting polyphialides, the presence of monophialides is also implied and you may not click the box with its presence. Furthermore, if you select monophialides "absent", and "presence" in the box of polyphialides, the database does not lead to any identification (Fig. 5).

For 64 species, a detailed description with additional photos is provided by clicking (icon with page image) the linked species (Fig. 6).

Once a search is completed, or whenever needed, a new search is possible by simply clicking the icon on the top left-hand corner with two curved arrows arranged in a circle.

The classification and identification of microorganisms is constantly evolving and continuously updated according to the availability of even more advanced technologies. Currently, morphological species recognition (MSR), biological species recognition (BSR), and phylogenetic species recognition (PSR), are among the most widely adopted species concepts (Taylor et al. 2000). For many ascomycetes, including *Fusarium*, the use of one or more different approaches often leads to a long-lasting debate among research groups (Seifert et al. 2011; O'Donnell et al. 2020; Crous et al. 2021), but this is beyond the scope of the present work. Morphological identification of fungi is a key factor for the initial, immediate, and indicative diagnosis of many diseases in the agricultural, medical, veterinary sciences, as well as in food and feed industry. Furthermore, accurate morphological description of fungal structure opens the way for innovative, high throughput fungal identification techniques, like Digital Image Analysis (Papagianni 2014) and, more recently, deep learning and Artificial Intelligence approaches (Zieliński et al. 2020). FusaHelp is intended to provide a help for all those people who work with this

important genus and need a quick clue on the identification, even incomplete, of the *Fusarium* species they are working with. It is also suited for student classes and for laboratories that do not have access to technologies based on fungal DNA determination. The versatility of use of FusaHelp with any portable device (tablet, mobile phone) could represent one of the strengths of FusaHelp that can be used for training courses. The programme is easily upgradable and will be implemented by the authors whenever any additional morphologically distinguishable *Fusarium* species will be requested and/or newly recognised important *Fusarium* species is formally reported.

Acknowledgements This work was funded by EU H2020 EWA-BELT project [862848] "Linking East and West African farming systems experience into a BELT of sustainable intensification" coordinated by the Desertification Research Centre of the University of Sassari. The authors thank Antonio Moretti for providing some isolates from ITEM Collection. and Laura Orzali for helping in the documentation search. Virgilio Balmas and Safa Oufensou acknowledge the University of Sassari for the support made by the "University fund for research 2020". The authors acknowledge CREA-DC for funding the acquisition of the Software.

Author Contribution Conceptualization, V.Ba, A.I and S.O; software realisation, V.Be, A.I and V.Ba ; website creation, A.G, A.I and V.B.; data curation, A.G and V.Be; writing-original draft preparation, A.I, V.Ba and S.O; writing—review and editing, A.I, V.Ba, L.W.B.; supervision L.W.B; funding acquisition, V.Ba and S.O. All authors have approved the final version of manuscript.

References

Aime MC, Miller AN, Aoki T, Bensch K, Cai L, Crous PW, Hawksworth DL, Hyde KD, Kirk PM, Lücking R, May TW, Malosso E, Redhead SA, Rossman AY, Stadler M, Thines M, Yurkov AM, Zhang N, Schoch CL (2021) How to publish a new fungal species, or name, version 3.0. IMA Fungus 12(1):11. https://doi.org/10.1186/s43008-021-00063-1

Aldhebiani AY (2018) Species concept and speciation. Saudi J Biol Sci 25:437–440

Bouket AC, Arzanlou M, Babai-Ahari A, Khodabande A (2012) FUNGID: a web-based Identification Program for *Phytophthora*. Int J comput appl 50(2):10–18

Burgess LW, Liddell CM, Summerell BA (1988) Laboratory Manual for *Fusarium* Research 2rd Ed. *Fusarium* Research Laboratory. Department of Plant Pathology and Agricultural Entomology, University of Sydney, p 156

Burgess LW, Summerell BA, Bullock S, Gott KP, Backhouse D (1994) Laboratory Manual for Fusarium Research 3rd Ed. Fusarium Research Laboratory. Department of Crop Science, University of Sydney and Royal Botanic Garden, Australia, p 133

Crous PW, Lombard L, Sandoval-Denis M, Seifert KA, Schroers HJ et al (2021) *Fusarium*: more than a node or a foot-shaped basal cell. Stud Mycol 98:100116. https://doi.org/10.1016/j.simyco.2021.100116

Dean R, van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD (2012) The top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol 13:414–430

De Queiroz K (2007) Species concepts and species delimitation. Syst Biol56(6):879–886.https://doi.org/10.1080/10635150701701083

Fisher NL, Burgess LW, Toussoun TA, Nelson PE (1982) Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology 72:151–153. https://doi.org/10.1094/Phyto-72-151

Galili T (2015) Dendextend: an R package for visualizing, adjusting, and comparing trees of hierarchical clustering. Bioinformatics. https://doi.org/10.1093/bioinformatics/btv428

Gerlach W, Nirenberg H (1982) The Genus Fusarium- a Pictorial Atlas. Berlin-Dahlem, 406 p

Geiser DM, Mar Jiménez-Gasco M, del Kang S, Makalowska I, Veeraraghavan N, Ward TJ, Zhang N, Kuldau GA, O'donnell K (2004) FUSARIUM-ID v. 1.0: a DNA sequence database for identifying *Fusarium*. Eur J Plant Pathol 110:473–479

Geiser DM (2013) One fungus, one name: defining the genus *Fusarium* in a scientifically robust way that preserves longstanding use. Phytopathology 103:400–408. https://doi.org/10.1094/PHYTO-07-12-0150-LE

Giraud T, Refrégier G, Le Gac M, de Vienne DM, Hood ME (2008) Speciation in fungi. Fungal Genet Biol 45:791–802. https://doi.org/10.1016/j.fgb.2008.02.001

Harris JL (2000) Safe, low-distortion tape touch method for fungal slide mounts. J Clin Microbiol 38:4683–4684

Kozakiewicz Z, Bridge PD, Paterson RRM (1993) PENIMAT: a computerised identification scheme for terverticillate *Penicillium* species. Int Biodeterior Biodegradation 32(1–3):117–125. https://doi.org/10.1016/0964-8305(93)90044-3

Jayawardena RS, Hyde KD, de Farias ARG et al (2021) What is a species in fungal plant pathogens? Fungal Divers 109:239–266. https://doi.org/10.1007/s13225-021-00484-8

Leslie JF, Summerell BA (2006) The *Fusarium* laboratory manual. Blackwell Publishing, Ames, Iowa, p 388

Martellos S (2010) Multi-authored interactive identification keys: the FRIDA (FRiendly IDentificAtion) package. Taxon 59(3):922–929. http://www.jstor.org/stable/25677679

Nelson PE, Toussoun TA, Marasas WFO (1983) *Fusarium* species - an Illustrated Manual for Identification. The Pennsylvania State University Press, USA, p 193

Nirenberg HL (1976) Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium* -Sektion *Liseola*. Mitt. Biol. Bundesanst. Land-Forstw. (Berlin-Dahlem) 169:1–117

O'Donnell K, Gueidan C, Sink S, Johnston PR, Crous PW, Glenn AE, Riley R, Zitomer NC, Colyer P, Waalwijk C, van der Lee T, Moretti A, Kang S, Kim HS, Geiser DM, Juba JH, Baayen RP, Cromey MG, Bithell S, Sutton DA, Skovgaard K, Ploetz R, Kistler HC, Elliott M, Davis M, Sarver BAJ (2009) A two-locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. Fungal Genet and Biol 46:936–948

O'Donnell K, Sutton DA, Rinaldi MG, Sarver BAJ, Balajee SA, Schroers HJ, Summerbell RC, Robert VARG, Crous PW, Zhang

N, Aoki T, Jung K, Park J, Lee YH, Kang S, Park B, Geiser DM (2010) Internet-accessible DNA sequence database for identifying fusaria from human and animal infections. J Clin Microbiol 48:3708–3718

O'Donnell K, Whitaker BK, Laraba I, Proctor RH, Brown DW, Broders K, Kim HS, McCormick SP, Busman M, Aoki T, Torres-Cruz TJ, Geiser DM (2021) DNA sequence-based identification of *Fusarium*: a work in progress. Plant Dis 106(6):1597–1609. https://doi.org/10.1094/PDIS-09-21-2035-SR2022

O'Donnell K, Al-Hatmi AMS, Aoki T, Brankovics B, Cano-Lira JF, Coleman JJ et al (2020) No to *Neocosmospora*: phylogenomic and practical reasons for continued inclusion of the *Fusarium solani* species complex in the genus *Fusarium*. mSphere 5:e00810–e00820. https://doi.org/10.1128/mSphere.00810-20

Palomares-Rius JE, León-Ropero G, Clavero-Camacho I, Archidona-Yuste A, Cantalapiedra-Navarrete C, Castillo P (2022) A new interactive web-based polytomous key for species identification of pin nematodes of the genus Paratylenchus Micoletzky, 1922 (*Nematoda: Paratylenchinae*) with the use of ribosomal and mitochondrial genes. J Zoolog Syst Evol Res 6216266. https://doi.org/10.1155/2022/6216266

Papagianni M (2014) Characterization of fungal morphology using digital image analysis techniques. J Microb Biochem Technol 6:4. https://doi.org/10.4172/1948-5948.1000142

Pitt JI (1990) Penname, a computer key to common *Penicillium* species. In: Samson, R.A., Pitt, J.I. (eds) Modern Concepts in *Penicillium* and *Aspergillus* Classification. NATO ASI Series, vol 185. Springer, Boston, MA. https://doi.org/10.1007/978-1-4899-3579-3_24

R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Seifert K, Wingfield B, Wingfield M (2011) A critique of DNA sequence analysis in the taxonomy of filamentous ascomycetes and ascomycetous anamorphs. Can J Bot 73:760–767. https://doi.org/10.1139/b95-320

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher C (2000) Phylogenetic species recognition and species concepts in fungi. Fungal Genet and Biol 31:21–32

Taylor JW (2011) One fungus = one name: DNA and fungal nomenclature twenty years after PCR. IMA Fungus 2:113–120. https://doi.org/10.5598/imafungus.2011.02.02.01

Thrane U (1991) FUSKEY, an interactive computer key to common *Fusarium* species. Mycotox Res 7:50–53. https://doi.org/10.1007/BF03192184

Varese GC, Anastasi A, Voyron S, Filipello Marchisio V (2010) An interactive tool for the identification of airborne and food fungi, pp. 183–187 in: Nimis PL, Vignes Lebbe R. (eds.): "Tools for Identifying Biodiversity: Progress and Problems. Proceedings of the International Congress, Paris, September 20–22, 2010", Trieste, EUT Edizioni Università di Trieste, 2010

Wearing AH, Burgess LW (1977) Distribution of *Fusarium roseum* '*Graminearum*' Group 1 and its mode of survival in eastern australian wheat belt soils. Trans Brit Mycol Soc 69:429–442

Webster J, Weber RWS (2007) Introduction to Fungi. Cambridge University Press, UK, p 841

Wingfield MJZ, De Beer W, Slippers B, Wingfield BD, Groenewald JZ, Lombard L, Crous W (2012) One fungus, one name promotes progressive plant pathology. Mol Plant Pathol 13:604–613. https://doi.org/10.1111/j.1364-3703.2011.00768.x

Zieliński B, Sroka-Oleksiak A, Rymarczyk D, Piekarczyk A, Brzychczy-Włoch M (2020) Deep learning approach to describe and classify fungi microscopic images. PLoS ONE 15(6):e0234806. https://doi.org/10.1371/journal.pone.0234806