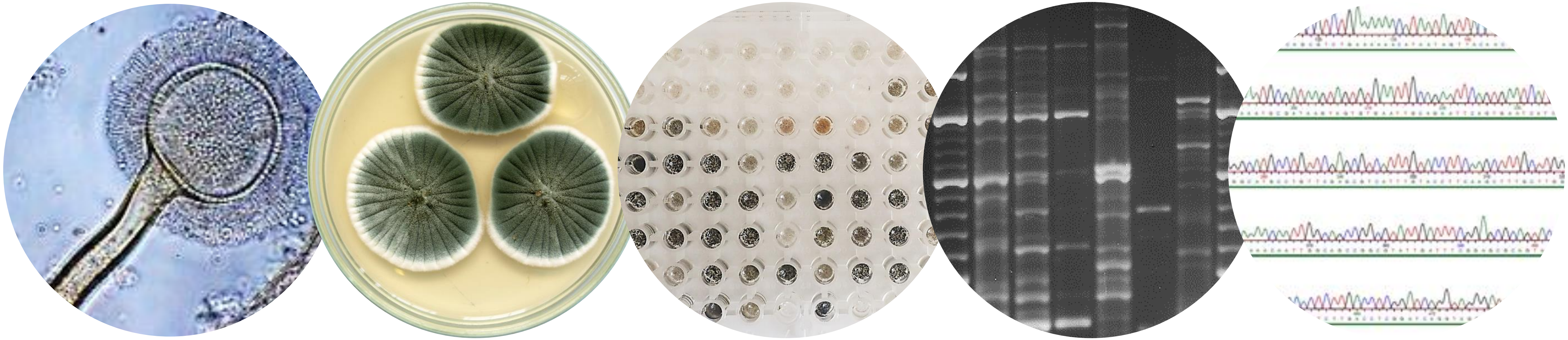
Ústav experimentální biologie
Česká sbírka mikroorganismů

Metody identifikace plísní

Monika Laichmanová, monikadr@sci.muni.cz



Identifikace mikroskopických hub

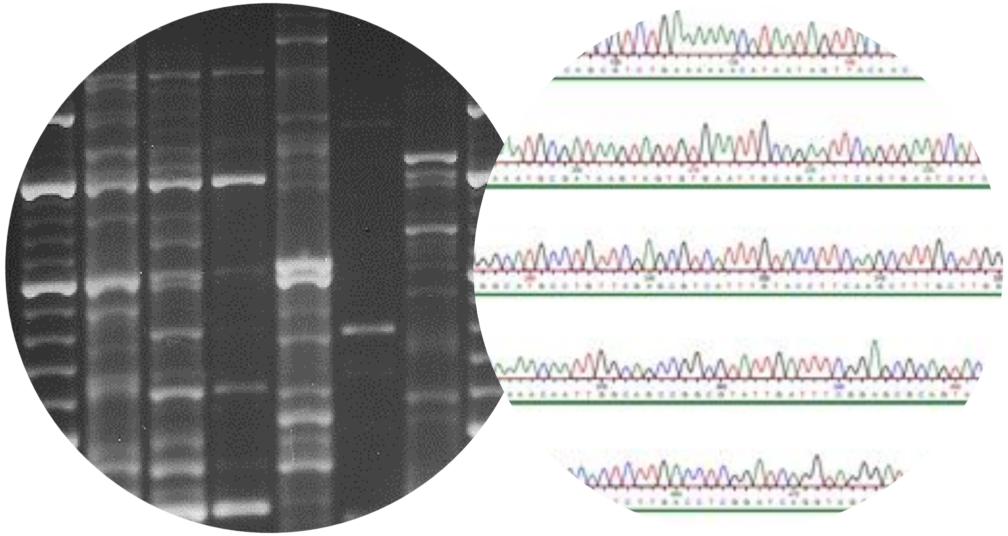
- fenotypová identifikace (morfologie – makroskopické a mikroskopické znaky)
- fyziologické vlastnosti – (růst v různých teplotních a nutričních podmínkách)
- stanovení metabolického profilu (TLC, HPLC-DAD, LC-MS)
- molekulární metody identifikace

POLYFÁZOVÁ TAXONOMIE

Molekulární metody identifikace

- sekvenování DNA
- sekvenování nové generace (NGS) - amplikonové sekvenování oblastí ITS
- molekulárně genetické typizační metody
    - multilokusová sekvenční typizace (MLST)

    - fingrprintové metody (rep-PCR, RAPD……)



Molekulární metody identifikace - nezávislé na kultivaci

Sekvenování nové generace (NGS)

- amplikonové sekvenování oblastí ITS
- necílené metoda
- detekce teoreticky všech taxonů hub přítomných ve vzorku, nezávisle na životaschopnosti a kultivovatelnosti
- umožňuje identifikaci na úroveň rodu nebo druhu
- neposkytuje kvantitativní výsledek
- výsledky NGS jsou prezentovány jako relativní četnost daného taxonu ve vzorku

Article

Indoor Bacterial and Fungal Burden in "Moldy" versus "Non-Moldy" Homes: A Case Study Employing Advanced Sequencing Techniques in a US Metropolitan Area

Bhavin V. Chauhan [1,†], Daleniece Higgins Jones [2,†], Goutam Banerjee [3], Saumya Agrawal [3], Irshad M. Sulaiman [4], Chunrong Jia [1] and Pratik Banerjee [3,*]

J Basic Microbiol. 2014 Apr;54(4):315-21. doi: 10.1002/jobm.201200507. Epub 2013 Jun 14.

Fungal high-throughput taxonomic identification tool for use with next-generation sequencing (FHiTINGS)

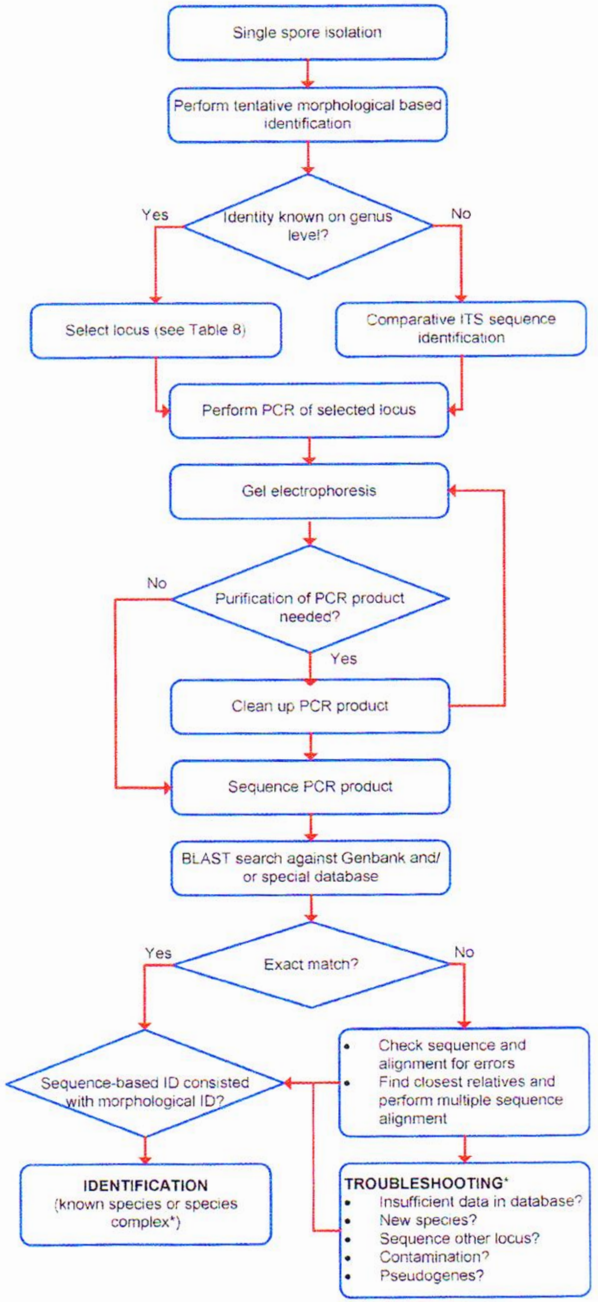
Karen C Dannemiller [1], Darryl Reeves, Kyle Bibby, Naomichi Yamamoto, Jordan Peccia

PMID: 23765392 DOI: 10.1002/jobm.201200507

Identifikace pomocí sekvenování DNA

reprodukovatelná, jednoznačná a přenosná

- extrakce DNA
- PCR amplifikace
- kontrola PCR produktu
- purifikace PCR produktu
- sekvenování PCR produktu
- srovnání sekvencí v databázích



Zdroj: SAMSON, Robert A.; HOUBRAKEN, Jos; THRANE, Ulf; FRISVAD, Jens C. a ANDERSEN, Birgitte. *Food and indoor fungi*. Second edition. Westerdijk Laboratory Manual Series. Utrecht: Westerdijk Fungal Biodiversity Institute, 2019.

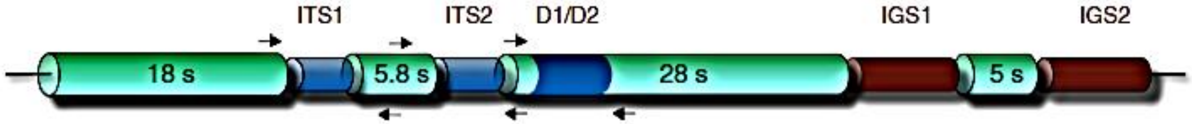
Izolace DNA

- rozrušení buněčné stěny a lýza membrán
- separace DNA centrifugací
- odstranění proteinů
- precipitace gDNA
- promytí gDNA
- rozpuštění gDNA v pufru (např. TE pufr)

http://www.wallpaperup.com/427758/DNA_3-d_structure_molecule_pattern_abstraction_genetic_psychedelic.html

Rapid Mini-Preparation of Fungal DNA for PCR
Liu, D., Coloe, S., Baird, R., & Pederson, J. (2000). Rapid mini-preparation of fungal DNA for PCR.
Journal of clinical microbiology, *38*(1), 471. https://doi.org/10.1128/JCM.38.1.471-471.2000

PCR amplifikace



Wickes, B. L., & Wiederhold, N. P. (2018). Molecular diagnostics in medical mycology. *Nature communications*, *9*(1), 5135. https://doi.org/10.1038/s41467-018-07556-5

Volba cílového genu pro PCR

<u>jaderné geny pro ribozomální RNA</u>
- vnitřní přepisovaný mezerník ribozomální DNA (ITS)
- gen pro rRNA velké podjednotky ribozomu 26S nebo 28S rDNA (LSU)

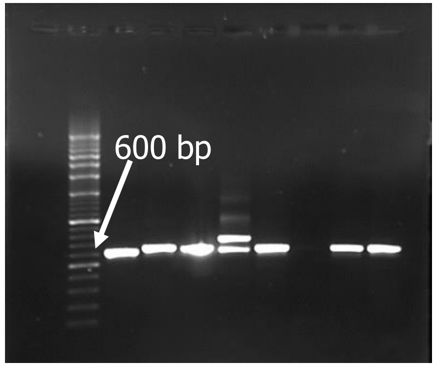
<u>jaderné geny pro proteiny</u>
- aktin (*Act*)
- β-tubulin (*BenA, tub2*)
- kalmodulin (*CaM*)
- glyceraldehyd-3-fosfát dehydrogenáza (*GAPDH*)
- druhá největší podjednotka RNA polymerázy II (*RPB2*)
- elongační faktor 1α (*TEF-1α*)

Genus	Gene/locus[1]	Primers[2]
Acrostalagmus	ITS, *TEF1α*	V9G & LS266, EF-983F & EF-2218R
Alternaria	*GAPDH*	gpd1 & gpd2
Arthrinium	*TEF1α* (and/or *BenA*)	EF1-728F & EF2 (and/or T1 & Bt2b)
Aspergillus	*CaM*	Cmd5 & Cmd6
Aureobasidium	ITS	V9G & LS266
Botryosporium	ITS	V9G & LS266
Botrytis	*GAPDH*, *RPB2*	V9G & LS266
Cadophora	ITS (and/or *TEF1α*)	V9G & LS266 (and/or EF1-728F & TEF1-LLErev)
Cephalotrichum	*TEF1α*	EF-983F & EF-2218R
Chaetomium	*BenA* (=*tub2*)	T1 & Tub4Rd
Cladosporium	*TEF1α* (and/or *ACT*)	EF1-728F & EF2 (and/or ACT-512F & ACT-783R)
Coniochaeta	ITS, LSU	V9G & LS266, LR0R & LR5
Curvularia	ITS, *TEF1α*	V9G & LS266, EF-983F & EF-2218R
Didymella	*BenA* (=*tub2*)	Btub2Fd & Btub4Rd
Epicoccum	*BenA* (=*tub2*)	Btub2Fd & Btub4Rd
Exophiala	ITS, LSU	V9G & LS266
Fusarium	*TEF1α* (and/or *RPB2*)	EF1 & EF2 (and/or RPB2-5F2 & RPB2-7CR)
Galactomyces	ITS	V9G & LS266
Gliomastix	ITS	V9G & LS266
Hyphopichia	ITS	V9G & LS266
Lichtheimia	ITS, alternative: LSU[1]	V9G & LS266, alternative: LR0R & LR5
Memnoniella	*TEF1α*, *BenA* (=*tub2*)	EF1-728F & EF2, Bt2a & Bt2b
Microascus	*TEF1α*	EF1-728F & TEF1-LLErev
Monascus	*BenA*	Bt2a & Bt2b, alternative: T10 & Bt2b
Mucor	ITS, alternative: LSU[1]	V9G & LS266, alternative: LR0R & LR5
Neocosmospora	*TEF1α* (and/or *RPB2*)	EF1 & EF2 (and/or RPB2-5F2 & RPB2-7CR)
Neurospora	ITS	V9G & LS266
Oidiodendron	ITS	V9G & LS266
Paecilomyces	*BenA*	Bt2a & Bt2b, alternative: T10 & Bt2b
Parengyodontium	ITS or *BenA*	V9G & LS266 or Bt2a & Bt2b
Penicillium	*BenA*	Bt2a & Bt2b, alternative: T10 & Bt2b
Pleurostoma	ITS	V9G & LS266
Pseudogymnoascus	ITS (and/or *TEF1α*)	V9G & LS266 (and/or EF-983F & EF-2218R)
Purpureocillium	ITS or *TEF1α*	V9G & LS266 or EF1-728F & TEF1-LLErev
Rhizomucor	ITS, alternative: LSU[1]	V9G & LS266, alternative: LR0R & LR5
Rhizopus	ITS, alternative: LSU[1]	V9G & LS266, alternative: LR0R & LR5
Saccharomycopsis	ITS	V9G & LS266
Sarocladium	ITS	V9G & LS266
Scopulariopsis	*TEF1α*	EF-983F & EF-2218R
Stachybotrys	*TEF1α* or *BenA* (=*tub2*)	EF1-728F & EF2 or Bt2a & Bt2b
Stemphylium	ITS, *GAPDH*	V9G & LS266, gpd1 & gpd2
Syncephalastrum	ITS, alternative: LSU[1]	V9G & LS266, alternative: LR0R & LR5
Talaromyces	*BenA*	Bt2a & Bt2b, alternative: T10 & Bt2b
Trichoderma	*TEF1α*	EF1-728F & TEF1-LLErev

SAMSON, Robert A.; HOUBRAKEN, Jos; THRANE, Ulf; FRISVAD, Jens C. a ANDERSEN, Birgitte. *Food and indoor fungi*. Second edition. Westerdijk Laboratory Manual Series. Utrecht: Westerdijk Fungal Biodiversity Institute, 2019.

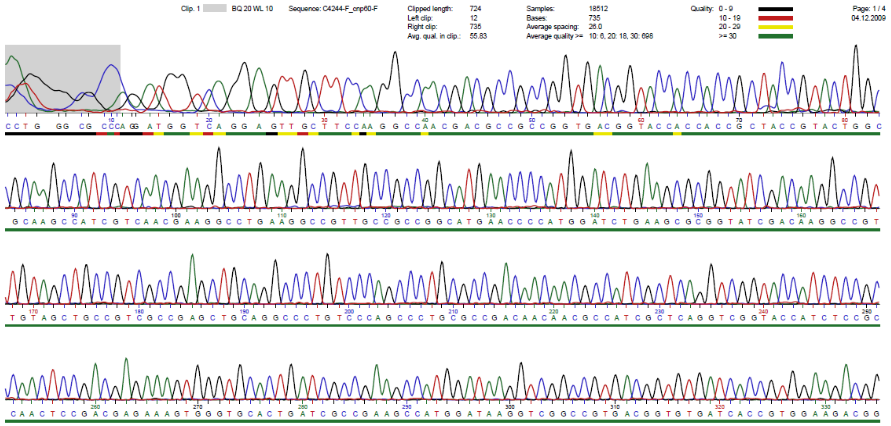
Identifikace pomocí sekvenování DNA

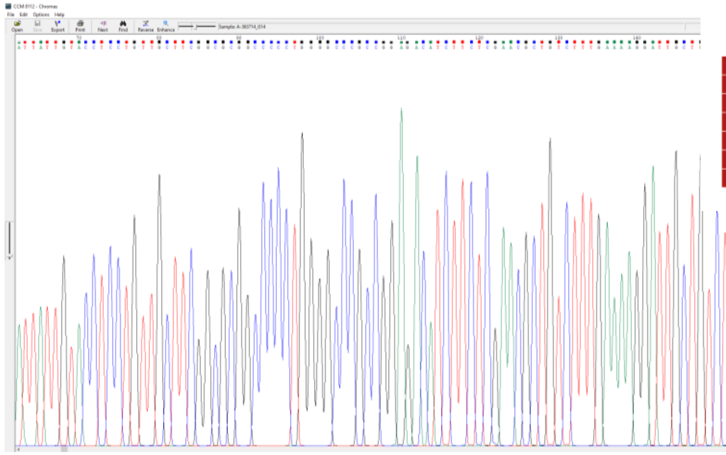
- kontrola PCR produktu
- purifikace PCR produktu
- Sangrovo sekvenování PCR produktu



FASTA formát

> CCM F-14909_ITS
CATTACAGAGTTCATGCCCTCACGGGTAGATCTCCCACCCTTGAATATCATACCTTA
GTTGCTTTGGTAGGCCGTGGAAACACTATGGGCTCCAGCTCGTACGTGCCTACCGAAGGA
AACAAACTCTGTTTCTAGTGATGTCTGAGTACTATATAATAGTTAAAACTTTCAACAACG
GATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTG
CAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCGTGGTATTCCGCGGG
GCATGCCTGTTCGAGCGTCATTACAACCCCTCAAGCTCACGCTTGGTATTGGAGCATGCG
GTCTCGCAGCTCCTAAACTCAGTGGCGGTGCCATCGAGCTCTGAGCGTAGTAAATTTTCT
CGCTATAGGGTCTCGGTGGTTGCTTGCCAACAACCCCCCATTCTATCAGGTTGACCTCGG
ATCAGGTAGGGATACCCGCTGAACTTAAGCATATC





Chromas 2.6.6 (http://technelysium.com.au/wp/chromas/)

PRABI-Doua
Pôle Rhône-Alpes de Bioinformatique Site Doua

CAP3 assembly:

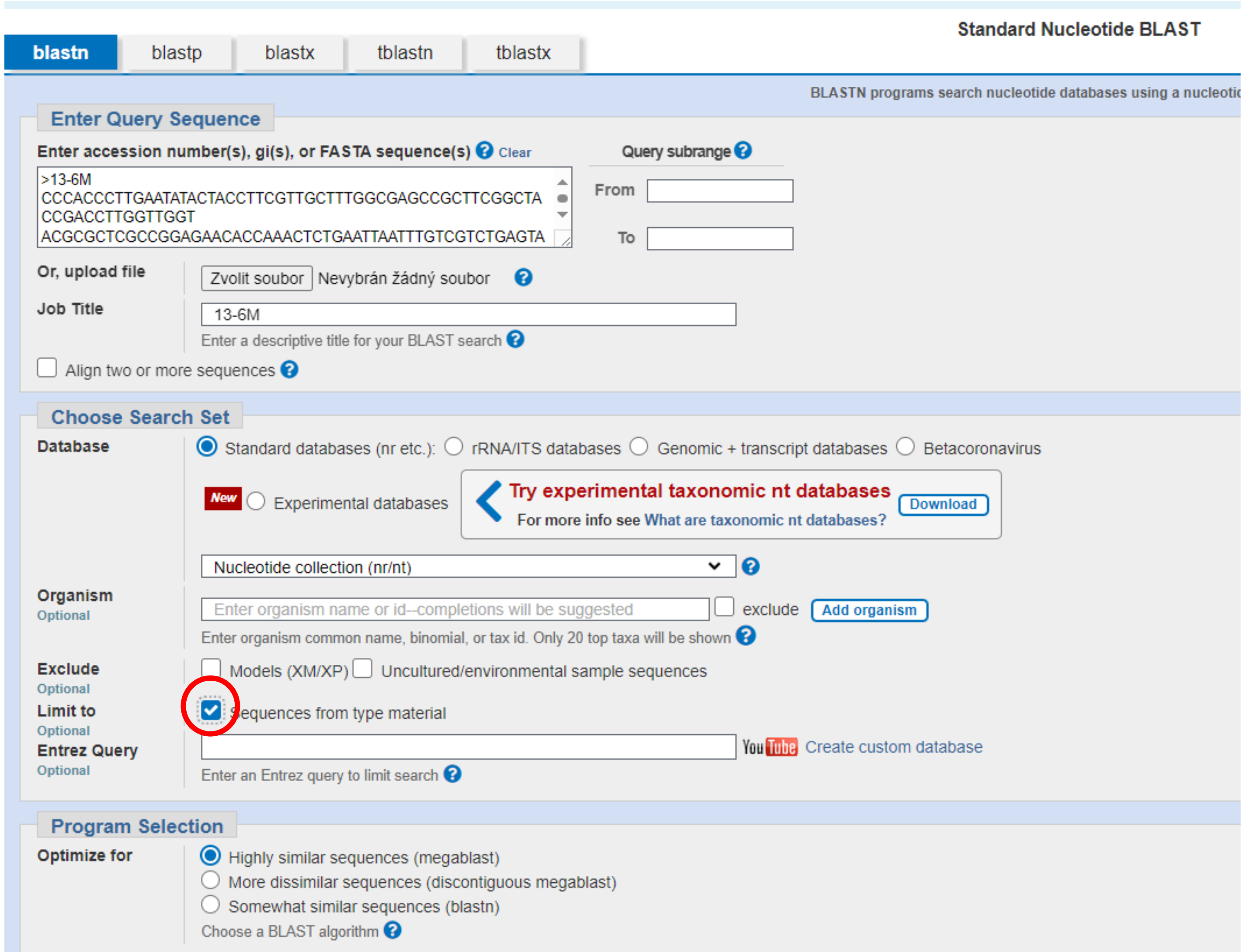
Running ... done.
Results:

Contigs
Single sequences
Assembly details
Your sequence file

https://doua.prabi.fr/cgi-bin/run_cap3
CAP3 Sequence Assembly Program (Huang & Madan, 1999)

Srovnání sekvencí v GenBank databázi

- posuzování podobnosti sekvencí
- GenBank (až 20 % chybně identifikovaných sekvencí)
- BLASTn – program pro hledání identických sekvencí DNA
- procentuální identita 100 %
  - stejný druh
  - blízce příbuzný druh



Standard Nucleotide BLAST

blastn blastp blastx tblastn tblastx

BLASTN programs search nucleotide databases using a nucleoti

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) ? Clear

```
>13-6M
CCCACCCTTGAATATACTACCTTCGTTGCTTTGGCGAGCCGCTTCGGCTA
CCGACCTTGGTTGGT
ACGCGCTCGCCGGAGAACACCAAACTCTGAATTAATTTGTCGTCTGAGTA
```

Query subrange ?

From

To

Or, upload file Zvolit soubor Nevybrán žádný soubor ?

Job Title 13-6M

Enter a descriptive title for your BLAST search ?

☐ Align two or more sequences ?

Choose Search Set

Database ● Standard databases (nr etc.): ○ rRNA/ITS databases ○ Genomic + transcript databases ○ Betacoronavirus

New ○ Experimental databases

Try experimental taxonomic nt databases Download

For more info see What are taxonomic nt databases?

Nucleotide collection (nr/nt) ?

Organism Optional: Enter organism name or id--completions will be suggested ☐ exclude Add organism

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown ?

Exclude Optional: ☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Limit to Optional: ☑ Sequences from type material

Entrez Query Optional: YouTube Create custom database

Enter an Entrez query to limit search ?

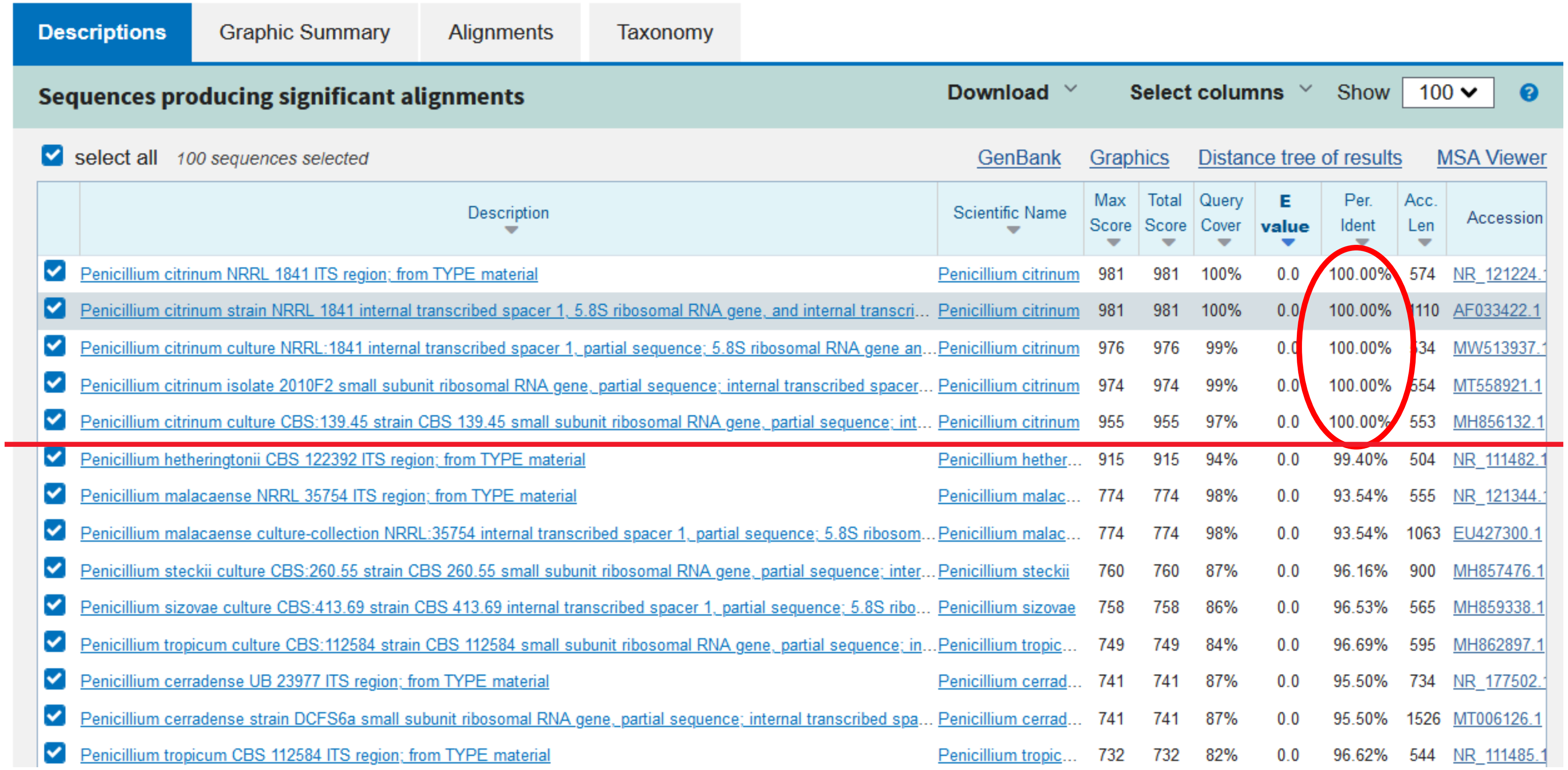
Program Selection

Optimize for

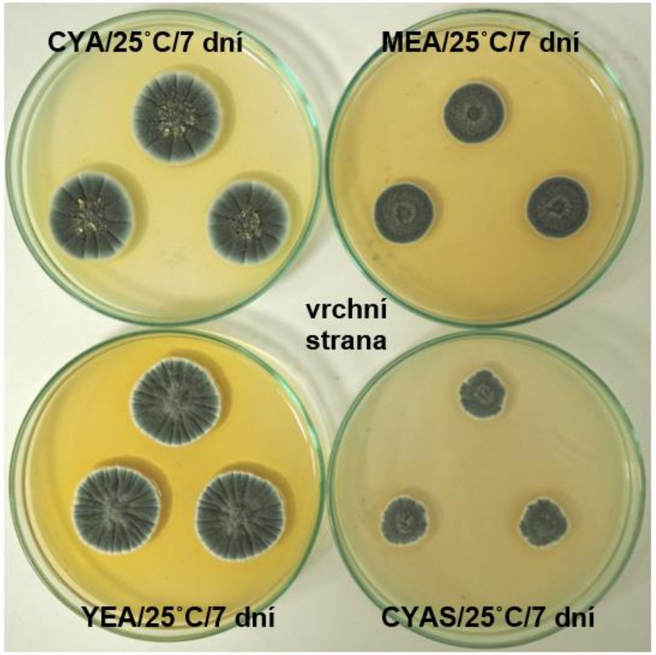
● Highly similar sequences (megablast)
○ More dissimilar sequences (discontiguous megablast)
○ Somewhat similar sequences (blastn)

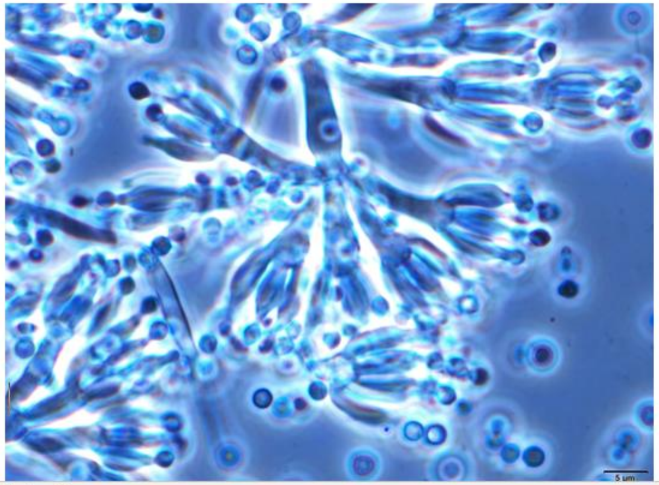
Choose a BLAST algorithm ?

Srovnání sekvencí v GenBank databázi



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Penicillium citrinum NRRL 1841 ITS region; from TYPE material	Penicillium citrinum	981	981	100%	0.0	100.00%	574	NR_121224.1
☑	Penicillium citrinum strain NRRL 1841 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcri...	Penicillium citrinum	981	981	100%	0.0	100.00%	110	AF033422.1
☑	Penicillium citrinum culture NRRL:1841 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an...	Penicillium citrinum	976	976	99%	0.0	100.00%	34	MW513937.1
☑	Penicillium citrinum isolate 2010F2 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer...	Penicillium citrinum	974	974	99%	0.0	100.00%	554	MT558921.1
☑	Penicillium citrinum culture CBS:139.45 strain CBS 139.45 small subunit ribosomal RNA gene, partial sequence; int...	Penicillium citrinum	955	955	97%	0.0	100.00%	553	MH856132.1
☑	Penicillium hetheringtonii CBS 122392 ITS region; from TYPE material	Penicillium hether...	915	915	94%	0.0	99.40%	504	NR_111482.1
☑	Penicillium malacaense NRRL 35754 ITS region; from TYPE material	Penicillium malac...	774	774	98%	0.0	93.54%	555	NR_121344.1
☑	Penicillium malacaense culture-collection NRRL:35754 internal transcribed spacer 1, partial sequence; 5.8S ribosom...	Penicillium malac...	774	774	98%	0.0	93.54%	1063	EU427300.1
☑	Penicillium steckii culture CBS:260.55 strain CBS 260.55 small subunit ribosomal RNA gene, partial sequence; inter...	Penicillium steckii	760	760	87%	0.0	96.16%	900	MH857476.1
☑	Penicillium sizovae culture CBS:413.69 strain CBS 413.69 internal transcribed spacer 1, partial sequence; 5.8S ribo...	Penicillium sizovae	758	758	86%	0.0	96.53%	565	MH859338.1
☑	Penicillium tropicum culture CBS:112584 strain CBS 112584 small subunit ribosomal RNA gene, partial sequence; in...	Penicillium tropic...	749	749	84%	0.0	96.69%	595	MH862897.1
☑	Penicillium cerradense UB 23977 ITS region; from TYPE material	Penicillium cerrad...	741	741	87%	0.0	95.50%	734	NR_177502.1
☑	Penicillium cerradense strain DCFS6a small subunit ribosomal RNA gene, partial sequence; internal transcribed spa...	Penicillium cerrad...	741	741	87%	0.0	95.50%	1526	MT006126.1
☑	Penicillium tropicum CBS 112584 ITS region; from TYPE material	Penicillium tropic...	732	732	82%	0.0	96.62%	544	NR_111485.1

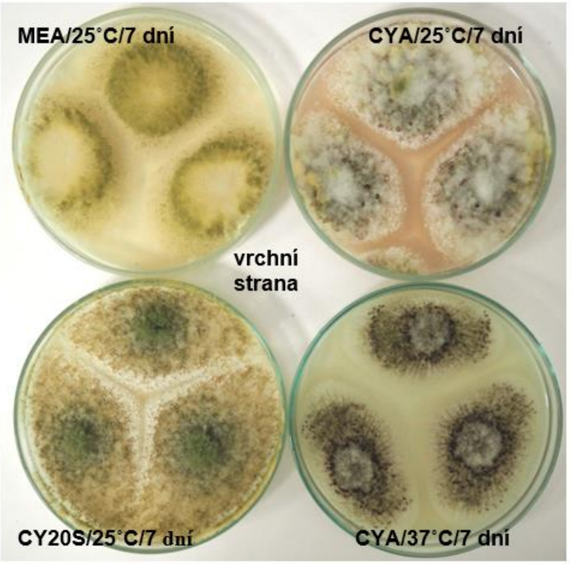


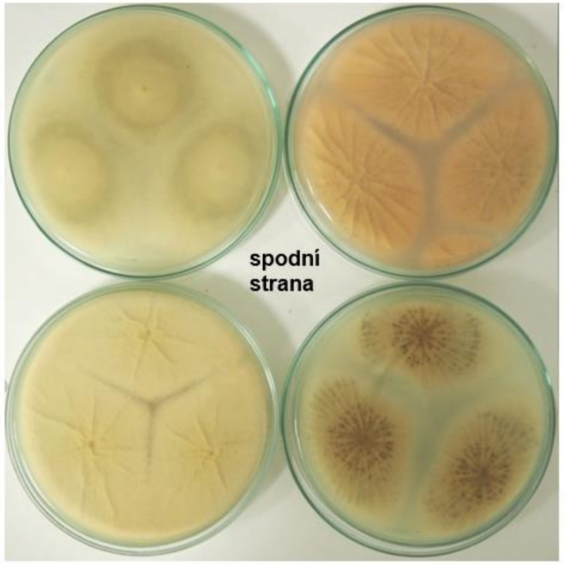


Výsledek identifikace: *Penicillium citrinum*

MUNI
SCI

Srovnání sekvencí v GenBank databázi





Procentuální identita 100 % :

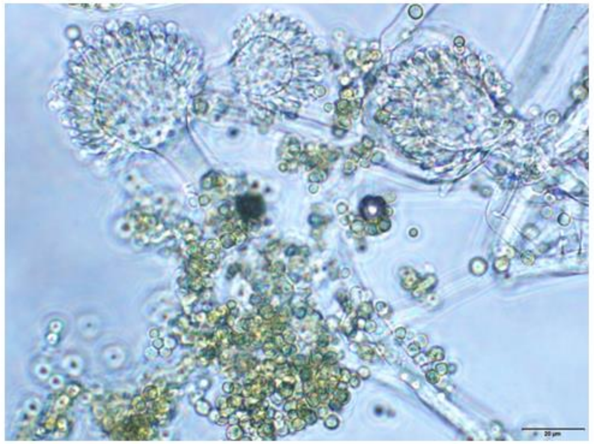
Aspergillus flavus

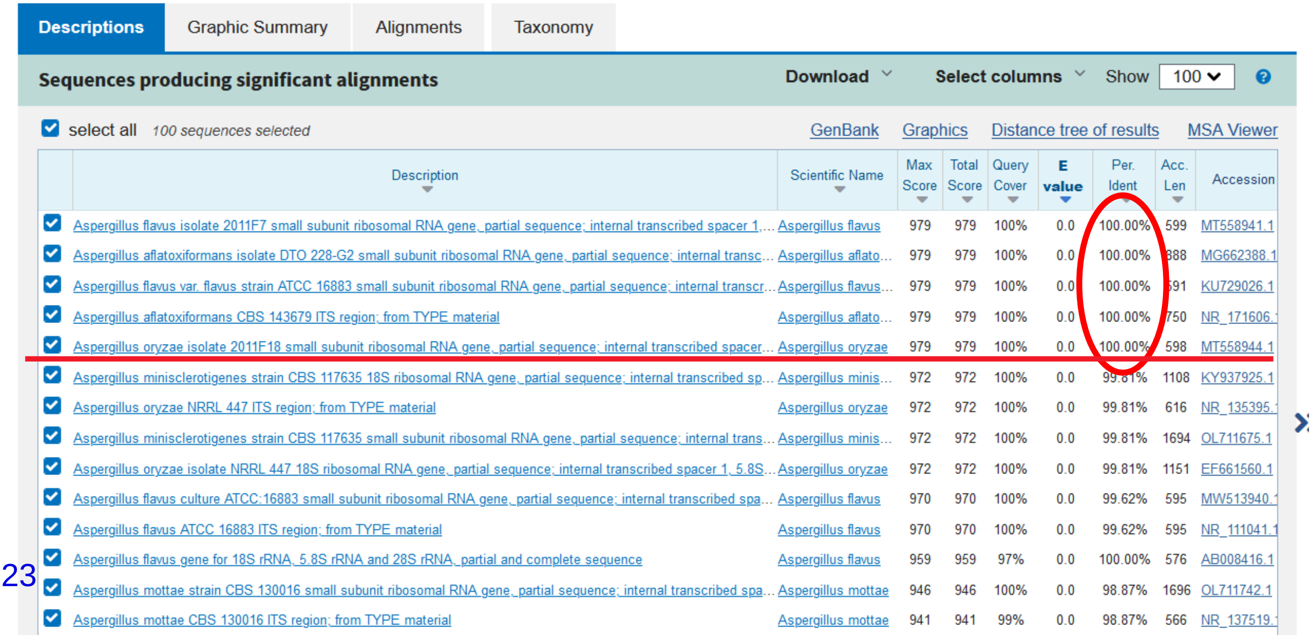
Aspergillius oryzae

Aspergillus aflatoxiformans

Frisvad, Ezekiel, Samson & Houbraken, Studies in Mycology 93: 32 (2018)

Výsledek identifikace: *Aspergillus* sekce *Flavi*



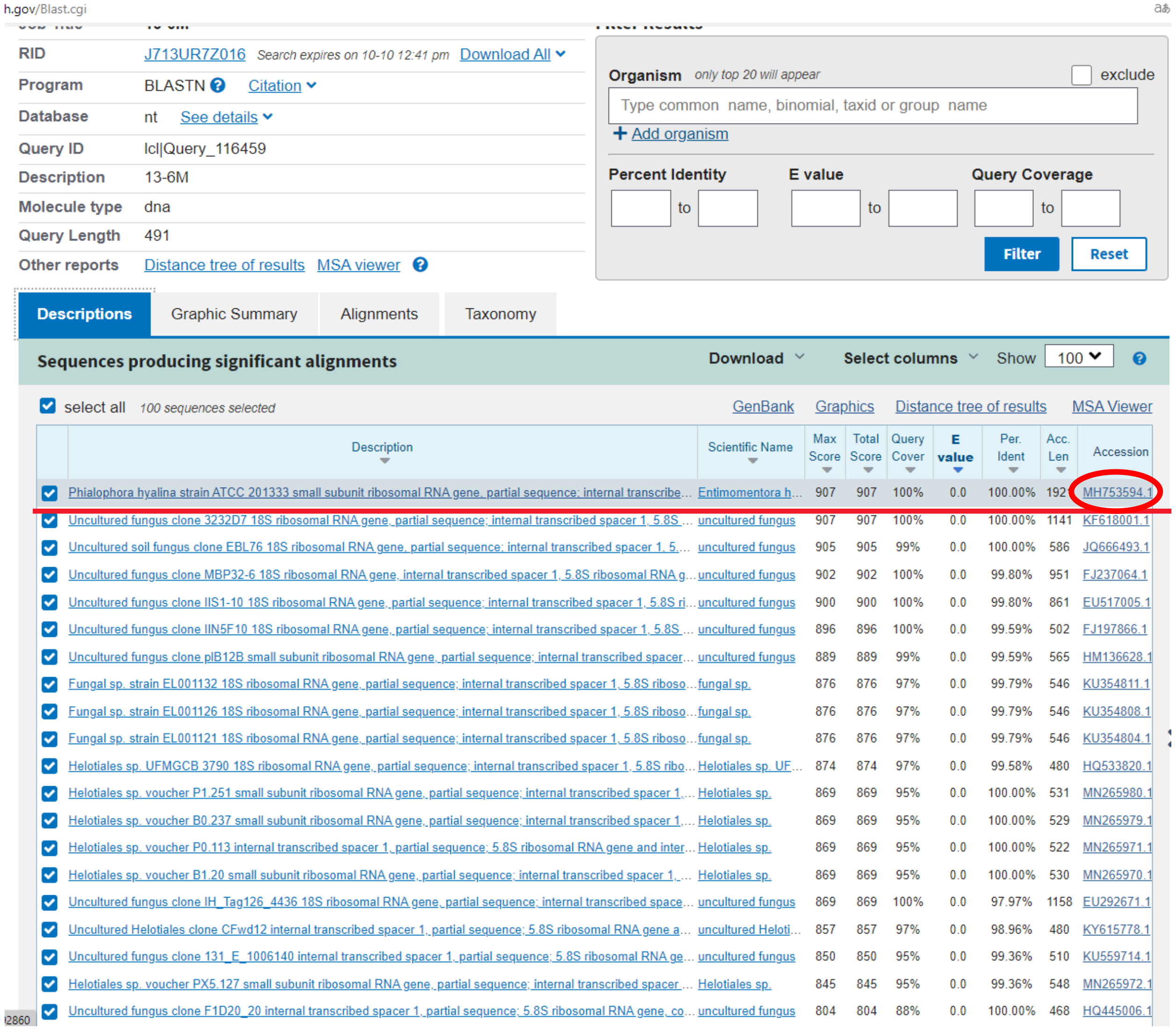


Sequences producing significant alignments

☑ select all *100 sequences selected*

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Aspergillus flavus isolate 2011F7 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1,...	Aspergillus flavus	979	979	100%	0.0	100.00%	599	MT558941.1
☑	Aspergillus aflatoxiformans isolate DTO 228-G2 small subunit ribosomal RNA gene, partial sequence; internal transc...	Aspergillus aflato...	979	979	100%	0.0	100.00%	888	MG662388.1
☑	Aspergillus flavus var. flavus strain ATCC 16883 small subunit ribosomal RNA gene, partial sequence; internal transcr...	Aspergillus flavus...	979	979	100%	0.0	100.00%	591	KU729026.1
☑	Aspergillus aflatoxiformans CBS 143679 ITS region; from TYPE material	Aspergillus aflato...	979	979	100%	0.0	100.00%	750	NR_171606.
☑	Aspergillus oryzae isolate 2011F18 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer...	Aspergillus oryzae	979	979	100%	0.0	100.00%	598	MT558944.1
☑	Aspergillus minisclerotigenes strain CBS 117635 18S ribosomal RNA gene, partial sequence; internal transcribed sp...	Aspergillus minis...	972	972	100%	0.0	99.81%	1108	KY937925.1
☑	Aspergillus oryzae NRRL 447 ITS region; from TYPE material	Aspergillus oryzae	972	972	100%	0.0	99.81%	616	NR_135395.
☑	Aspergillus minisclerotigenes strain CBS 117635 small subunit ribosomal RNA gene, partial sequence; internal trans...	Aspergillus minis...	972	972	100%	0.0	99.81%	1694	OL711675.1
☑	Aspergillus oryzae isolate NRRL 447 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S...	Aspergillus oryzae	972	972	100%	0.0	99.81%	1151	EF661560.1
☑	Aspergillus flavus culture ATCC:16883 small subunit ribosomal RNA gene, partial sequence; internal transcribed spa...	Aspergillus flavus	970	970	100%	0.0	99.62%	595	MW513940.
☑	Aspergillus flavus ATCC 16883 ITS region; from TYPE material	Aspergillus flavus	970	970	100%	0.0	99.62%	595	NR_111041.1
☑	Aspergillus flavus gene for 18S rRNA, 5.8S rRNA and 28S rRNA, partial and complete sequence	Aspergillus flavus	959	959	97%	0.0	100.00%	576	AB008416.1
☑	Aspergillus mottae strain CBS 130016 small subunit ribosomal RNA gene, partial sequence; internal transcribed spa...	Aspergillus mottae	946	946	100%	0.0	98.87%	1696	OL711742.1
☑	Aspergillus mottae CBS 130016 ITS region; from TYPE material	Aspergillus mottae	941	941	99%	0.0	98.87%	566	NR_137519.

Srovnání sekvencí v GenBank databázi



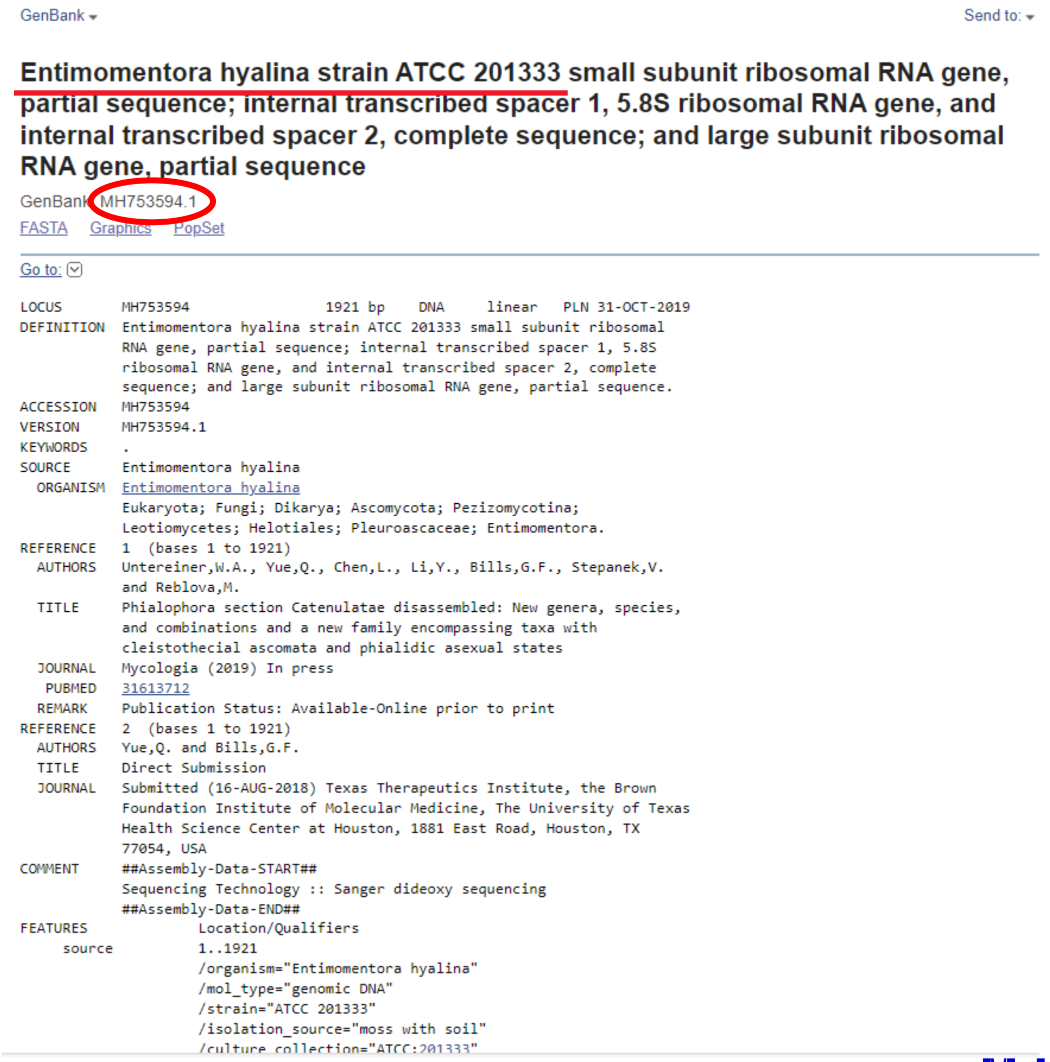
h.gov/Blast.cgi

RID	J713UR7Z016 *Search expires on 10-10 12:41 pm* Download All
Program	BLASTN Citation
Database	nt See details
Query ID	lcl|Query_116459
Description	13-6M
Molecule type	dna
Query Length	491
Other reports	Distance tree of results MSA viewer

Sequences producing significant alignments

☑ select all *100 sequences selected*

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Phialophora hyalina strain ATCC 201333 small subunit ribosomal RNA gene, partial sequence; internal transcribe…	Entimomentora h…	907	907	100%	0.0	100.00%	192	MH753594.1
☑	Uncultured fungus clone 3232D7 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S …	uncultured fungus	907	907	100%	0.0	100.00%	1141	KF618001.1
☑	Uncultured soil fungus clone EBL76 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.…	uncultured fungus	905	905	99%	0.0	100.00%	586	JQ666493.1
☑	Uncultured fungus clone MBP32-6 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RNA g…	uncultured fungus	902	902	100%	0.0	99.80%	951	FJ237064.1
☑	Uncultured fungus clone IIS1-10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ri…	uncultured fungus	900	900	100%	0.0	99.80%	861	EU517005.1
☑	Uncultured fungus clone IIN5F10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S …	uncultured fungus	896	896	100%	0.0	99.59%	502	FJ197866.1
☑	Uncultured fungus clone plB12B small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer…	uncultured fungus	889	889	99%	0.0	99.59%	565	HM136628.1
☑	Fungal sp. strain EL001132 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S riboso…	fungal sp.	876	876	97%	0.0	99.79%	546	KU354811.1
☑	Fungal sp. strain EL001126 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S riboso…	fungal sp.	876	876	97%	0.0	99.79%	546	KU354808.1
☑	Fungal sp. strain EL001121 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S riboso…	fungal sp.	876	876	97%	0.0	99.79%	546	KU354804.1
☑	Helotiales sp. UFMGCB 3790 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribo…	Helotiales sp. UF…	874	874	97%	0.0	99.58%	480	HQ533820.1
☑	Helotiales sp. voucher P1.251 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1,…	Helotiales sp.	869	869	95%	0.0	100.00%	531	MN265980.1
☑	Helotiales sp. voucher B0.237 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1,…	Helotiales sp.	869	869	95%	0.0	100.00%	529	MN265979.1
☑	Helotiales sp. voucher P0.113 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inter…	Helotiales sp.	869	869	95%	0.0	100.00%	522	MN265971.1
☑	Helotiales sp. voucher B1.20 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, …	Helotiales sp.	869	869	95%	0.0	100.00%	530	MN265970.1
☑	Uncultured fungus clone IH_Tag126_4436 18S ribosomal RNA gene, partial sequence; internal transcribed space…	uncultured fungus	869	869	100%	0.0	97.97%	1158	EU292671.1
☑	Uncultured Helotiales clone CFwd12 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a…	uncultured Heloti…	857	857	97%	0.0	98.96%	480	KY615778.1
☑	Uncultured fungus clone 131_E_1006140 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ge…	uncultured fungus	850	850	95%	0.0	99.36%	510	KU559714.1
☑	Helotiales sp. voucher PX5.127 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer …	Helotiales sp.	845	845	95%	0.0	99.36%	548	MN265972.1
☑	Uncultured fungus clone F1D20_20 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, co…	uncultured fungus	804	804	88%	0.0	100.00%	468	HQ445006.1

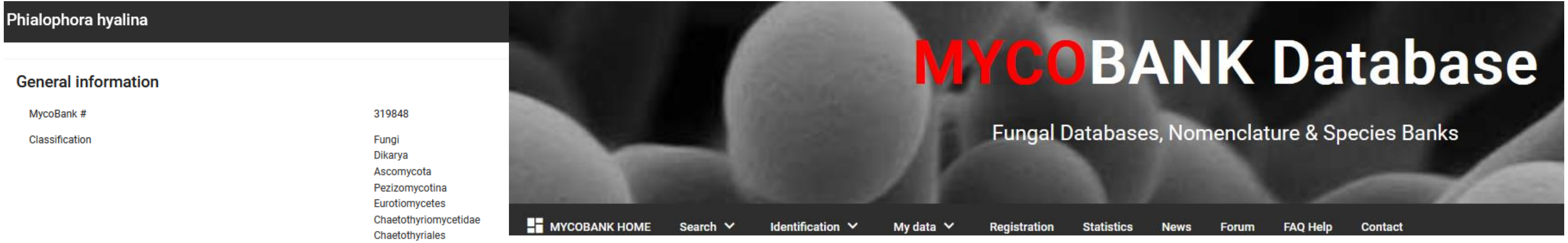


Entimomentora hyalina strain ATCC 201333 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

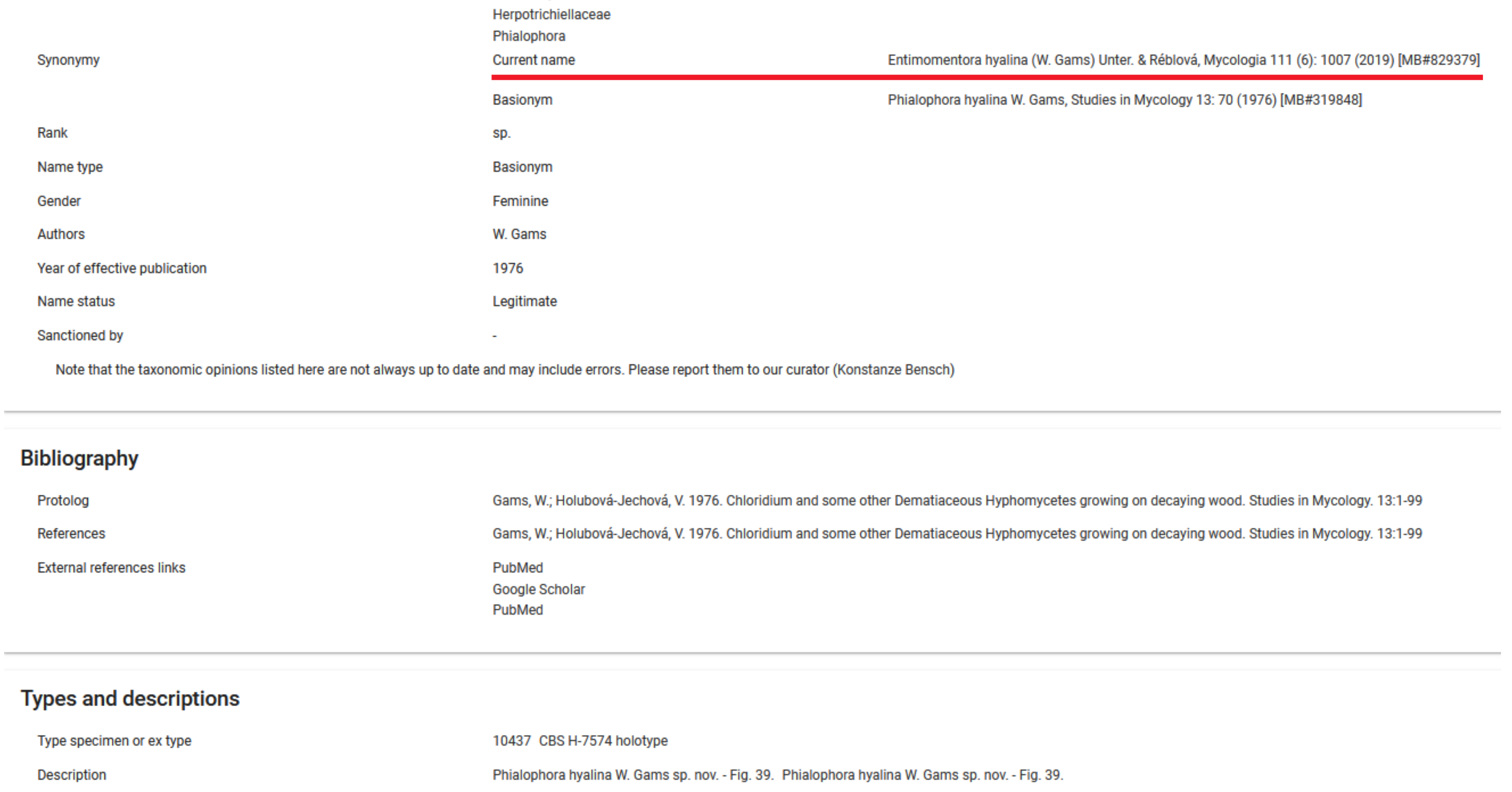
GenBank: MH753594.1

```
LOCUS       MH753594                1921 bp    DNA     linear   PLN 31-OCT-2019
DEFINITION  Entimomentora hyalina strain ATCC 201333 small subunit ribosomal
            RNA gene, partial sequence; internal transcribed spacer 1, 5.8S
            ribosomal RNA gene, and internal transcribed spacer 2, complete
            sequence; and large subunit ribosomal RNA gene, partial sequence.
ACCESSION   MH753594
VERSION     MH753594.1
KEYWORDS    .
SOURCE      Entimomentora hyalina
  ORGANISM  Entimomentora hyalina
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Leotiomycetes; Helotiales; Pleuroascaceae; Entimomentora.
REFERENCE   1  (bases 1 to 1921)
  AUTHORS   Untereiner,W.A., Yue,Q., Chen,L., Li,Y., Bills,G.F., Stepanek,V.
            and Reblova,M.
  TITLE     Phialophora section Catenulatae disassembled: New genera, species,
            and combinations and a new family encompassing taxa with
            cleistothecial ascomata and phialidic asexual states
  JOURNAL   Mycologia (2019) In press
   PUBMED   31613712
  REMARK    Publication Status: Available-Online prior to print
REFERENCE   2  (bases 1 to 1921)
  AUTHORS   Yue,Q. and Bills,G.F.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-AUG-2018) Texas Therapeutics Institute, the Brown
            Foundation Institute of Molecular Medicine, The University of Texas
            Health Science Center at Houston, 1881 East Road, Houston, TX
            77054, USA
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..1921
                     /organism="Entimomentora hyalina"
                     /mol_type="genomic DNA"
                     /strain="ATCC 201333"
                     /isolation_source="moss with soil"
                     /culture_collection="ATCC:201333"
```

Mykologické databáze - nomenklatura



Phialophora hyalina



General information

MycoBank #	319848	
Classification	Fungi Dikarya Ascomycota Pezizomycotina Eurotiomycetes Chaetothyriomycetidae Chaetothyriales Herpotrichiellaceae Phialophora	
Synonymy	Current name	Entimomentora hyalina (W. Gams) Unter. & Réblová, Mycologia 111 (6): 1007 (2019) [MB#829379]
	Basionym	Phialophora hyalina W. Gams, Studies in Mycology 13: 70 (1976) [MB#319848]
Rank	sp.	
Name type	Basionym	
Gender	Feminine	
Authors	W. Gams	
Year of effective publication	1976	
Name status	Legitimate	
Sanctioned by	-	

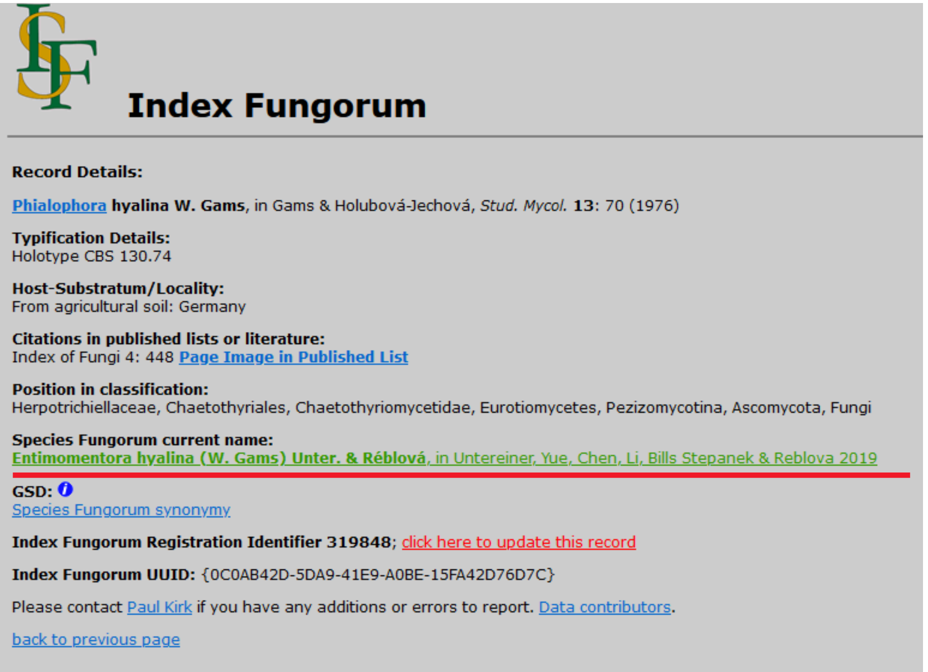
Note that the taxonomic opinions listed here are not always up to date and may include errors. Please report them to our curator (Konstanze Bensch)

Bibliography

Protolog	Gams, W.; Holubová-Jechová, V. 1976. Chloridium and some other Dematiaceous Hyphomycetes growing on decaying wood. Studies in Mycology. 13:1-99
References	Gams, W.; Holubová-Jechová, V. 1976. Chloridium and some other Dematiaceous Hyphomycetes growing on decaying wood. Studies in Mycology. 13:1-99
External references links	PubMed Google Scholar PubMed

Types and descriptions

Type specimen or ex type	10437 CBS H-7574 holotype
Description	Phialophora hyalina W. Gams sp. nov. - Fig. 39. Phialophora hyalina W. Gams sp. nov. - Fig. 39.



Index Fungorum

Record Details:

Phialophora **hyalina W. Gams**, in Gams & Holubová-Jechová, *Stud. Mycol.* **13**: 70 (1976)

Typification Details:
Holotype CBS 130.74

Host-Substratum/Locality:
From agricultural soil: Germany

Citations in published lists or literature:
Index of Fungi 4: 448 Page Image in Published List

Position in classification:
Herpotrichiellaceae, Chaetothyriales, Chaetothyriomycetidae, Eurotiomycetes, Pezizomycotina, Ascomycota, Fungi

Species Fungorum current name:
Entimomentora hyalina (W. Gams) Unter. & Réblová, in Untereiner, Yue, Chen, Li, Bills Stepanek & Reblova 2019

GSD: ℹ
Species Fungorum synonymy

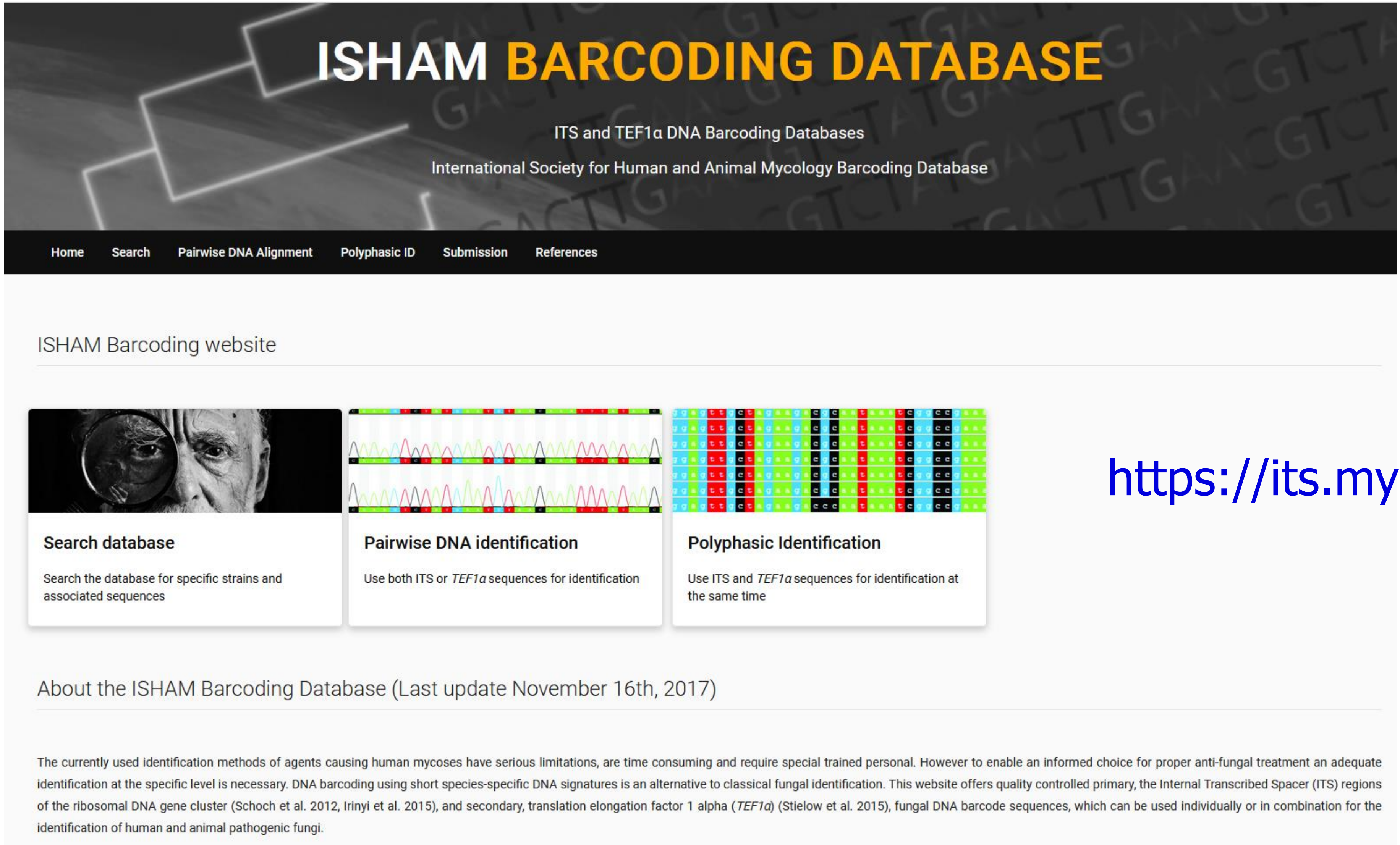
Index Fungorum Registration Identifier 319848; click here to update this record

Index Fungorum UUID: {0C0AB42D-5DA9-41E9-A0BE-15FA42D76D7C}

Please contact Paul Kirk if you have any additions or errors to report. Data contributors.

back to previous page

Srovnání sekvencí v dalších databázích



ISHAM Barcoding website

Search database

Search the database for specific strains and associated sequences

Pairwise DNA identification

Use both ITS or *TEF1α* sequences for identification

Polyphasic Identification

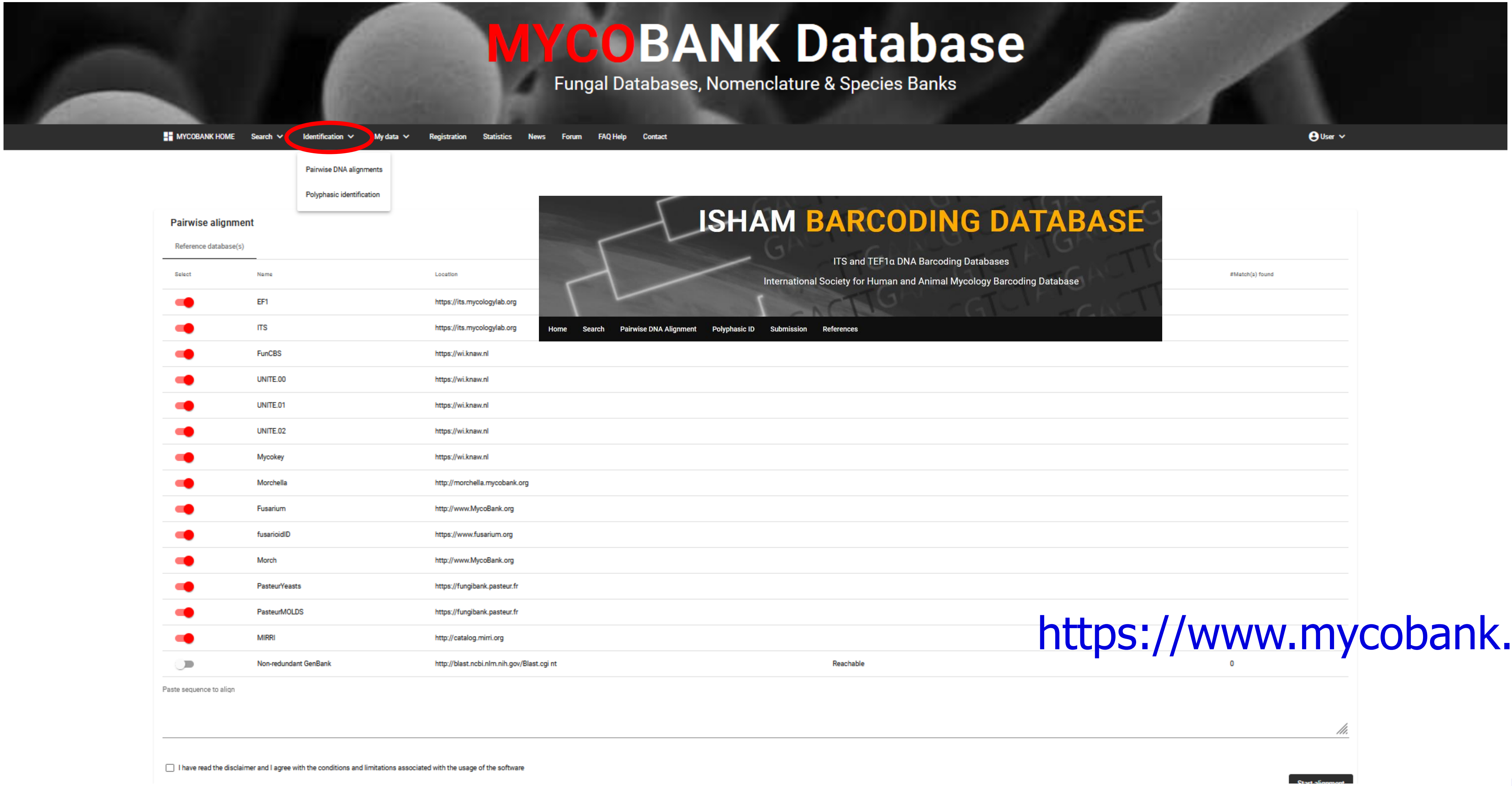
Use ITS and *TEF1α* sequences for identification at the same time

About the ISHAM Barcoding Database (Last update November 16th, 2017)

The currently used identification methods of agents causing human mycoses have serious limitations, are time consuming and require special trained personal. However to enable an informed choice for proper anti-fungal treatment an adequate identification at the specific level is necessary. DNA barcoding using short species-specific DNA signatures is an alternative to classical fungal identification. This website offers quality controlled primary, the Internal Transcribed Spacer (ITS) regions of the ribosomal DNA gene cluster (Schoch et al. 2012, Irinyi et al. 2015), and secondary, translation elongation factor 1 alpha (*TEF1α*) (Stielow et al. 2015), fungal DNA barcode sequences, which can be used individually or in combination for the identification of human and animal pathogenic fungi.

https://its.mycologylab.org/

Srovnání sekvencí v dalších databázích



Pairwise alignment

Reference database(s)

Select	Name	Location		#Match(s) found
●	EF1	https://its.mycologylab.org		
●	ITS	https://its.mycologylab.org		
●	FunCBS	https://wi.knaw.nl		
●	UNITE.00	https://wi.knaw.nl		
●	UNITE.01	https://wi.knaw.nl		
●	UNITE.02	https://wi.knaw.nl		
●	Mycokey	https://wi.knaw.nl		
●	Morchella	http://morchella.mycobank.org		
●	Fusarium	http://www.MycoBank.org		
●	fusarioidID	https://www.fusarium.org		
●	Morch	http://www.MycoBank.org		
●	PasteurYeasts	https://fungibank.pasteur.fr		
●	PasteurMOLDS	https://fungibank.pasteur.fr		
●	MIRRI	http://catalog.mirri.org		
○	Non-redundant GenBank	http://blast.ncbi.nlm.nih.gov/Blast.cgi nt	Reachable	0

Paste sequence to align

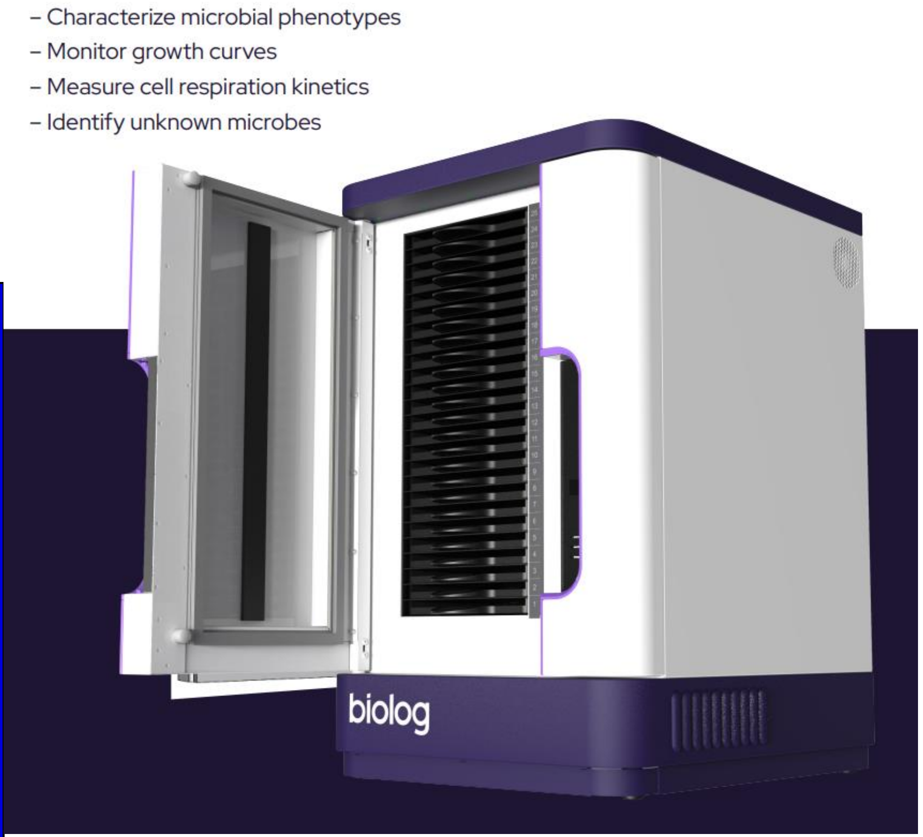
☐ I have read the disclaimer and I agree with the conditions and limitations associated with the usage of the software

https://www.mycobank.org/

Fenotypová identifikace

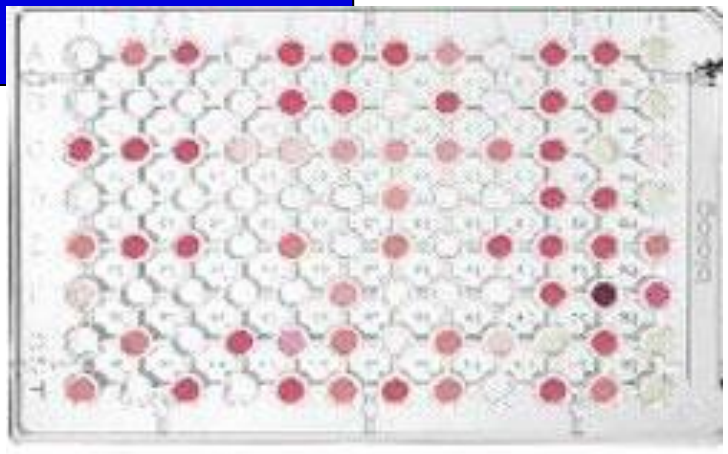
Biolog FF Microplate (Biolog)

- druhová identifikace a charakterizace vláknité houby
- rozsáhlá databáze obsahující 400 druhů vláknitých hub ze 120 rodů
- 95 fenotypových testů v mikrotitrační destičce
- testovací panel poskytuje „fenotypový fingrprint“
- vytváření vlastní databáze



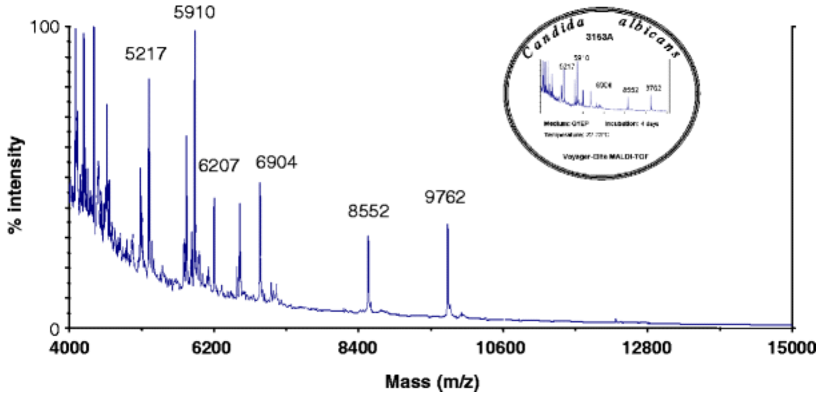
- Characterize microbial phenotypes
- Monitor growth curves
- Measure cell respiration kinetics
- Identify unknown microbes

https://www.biolog.com/products

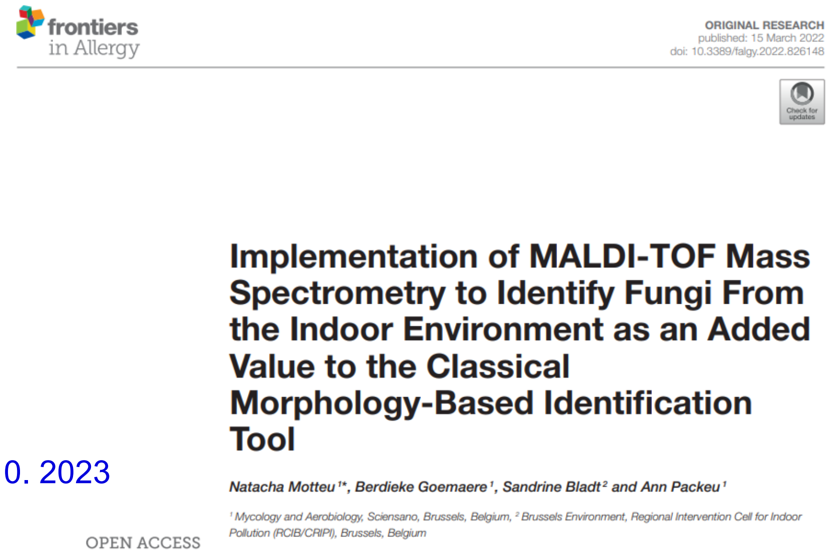


Identifikace pomocí MALDI-TOF MS

- analýza buněčných proteinů hmotnostní spektrometrií
- získaná hmotnostní spektra jsou druhově specifická
- výstupem je skóre vyjadřujícího shodu hmotnostního spektra vyšetřovaného vzorku s referenčním identifikátorem v databázi
- VITEK MS systém (bioMerieux, Francie) – kultivace na pevném médiu
- MALDI BioTyper (MBT; Bruker Scientific, Německo) – kultivace v tekutém médiu
- extrakci proteinů mimo vzorkovací desku spektrometru
- zdroj variability: kultivační podmínky, příprava vzorku, extrakční metoda a použitý spektrometr



http://link.springer.com/article/10.1007%2Fs00216-008-2288-1



frontiers in Allergy

ORIGINAL RESEARCH
published: 15 March 2022
doi: 10.3389/falgy.2022.826148

Check for updates

Implementation of MALDI-TOF Mass Spectrometry to Identify Fungi From the Indoor Environment as an Added Value to the Classical Morphology-Based Identification Tool

Natacha Motteu[1], Berdieke Goemaere[1], Sandrine Bladt[2] and Ann Packeu[1]*

[1] Mycology and Aerobiology, Sciensano, Brussels, Belgium, [2] Brussels Environment, Regional Intervention Cell for Indoor Pollution (RCIB/CRIPI), Brussels, Belgium

OPEN ACCESS

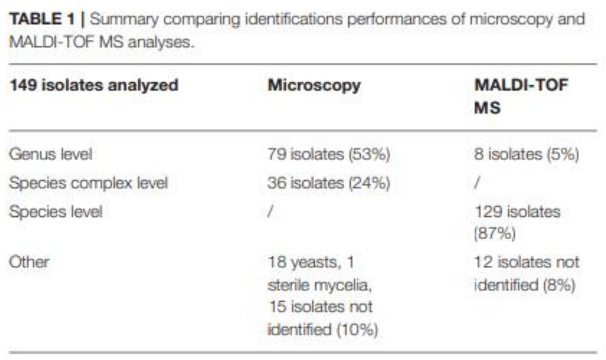


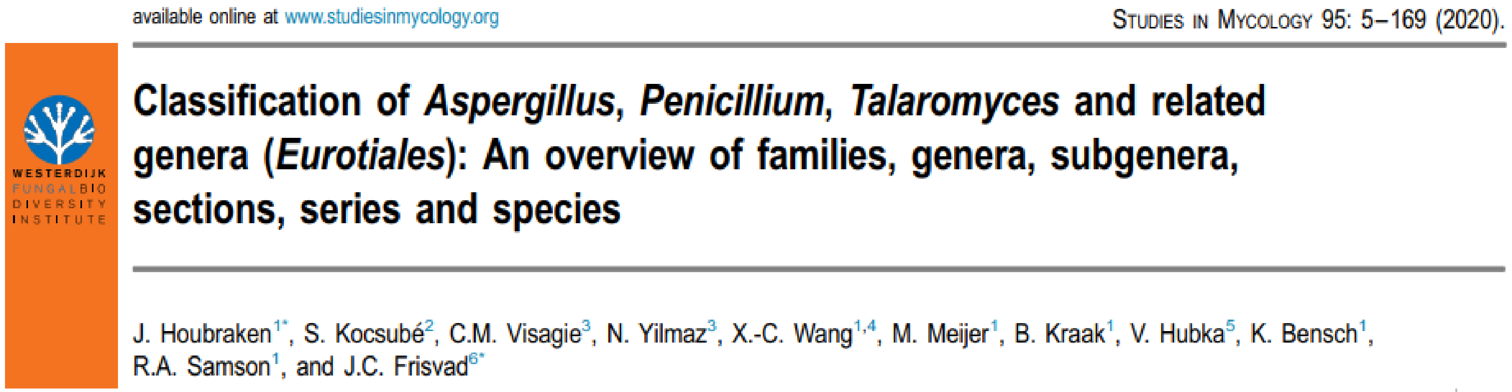
TABLE 1 | Summary comparing identifications performances of microscopy and MALDI-TOF MS analyses.

149 isolates analyzed	Microscopy	MALDI-TOF MS
Genus level	79 isolates (53%)	8 isolates (5%)
Species complex level	36 isolates (24%)	/
Species level	/	129 isolates (87%)
Other	18 yeasts, 1 sterile mycelia, 15 isolates not identified (10%)	12 isolates not identified (8%)

MUNI
SCI

Penicillium

Fungi, Dikarya, Ascomycota, Pezizomycotina, Eurotiomycetes, Eurotiomycetidae, Eurotiales, Aspergillaceae



available online at www.studiesinmycology.org

Studies in Mycology 95: 5–169 (2020).

WESTERDIJK FUNGALBIO DIVERSITY INSTITUTE

Classification of *Aspergillus*, *Penicillium*, *Talaromyces* and related genera (*Eurotiales*): An overview of families, genera, subgenera, sections, series and species

J. Houbraken[1*], S. Kocsubé[2], C.M. Visagie[3], N. Yilmaz[3], X.-C. Wang[1,4], M. Meijer[1], B. Kraak[1], V. Hubka[5], K. Bensch[1], R.A. Samson[1], and J.C. Frisvad[6*]

- 483 druhů (Houbraken et al., 2020)
- 2 podrody (Aspergilloides a Penicillium)
- 32 sekcí
- 89 sérií

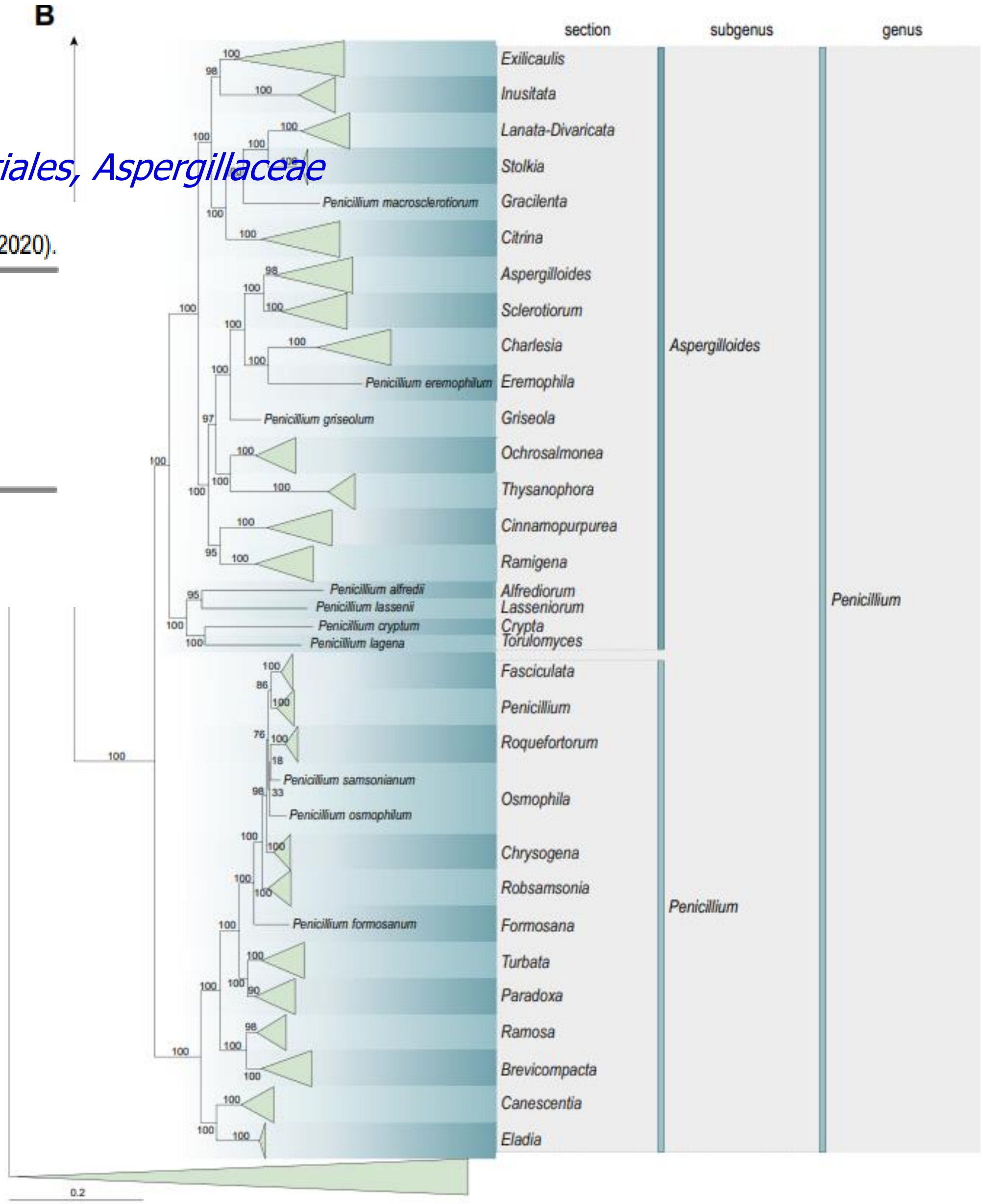
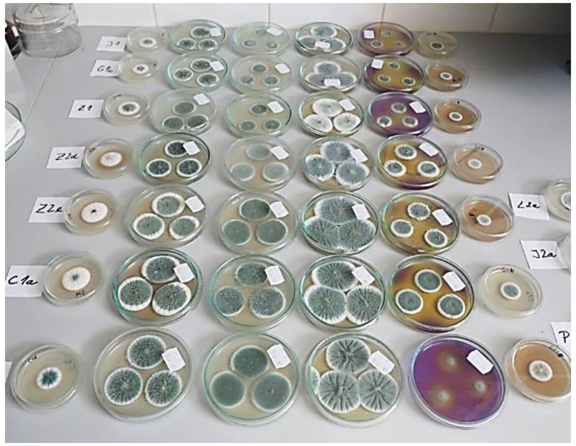


Schéma metod pro identifikaci rodu *Penicillium*



Spore suspension
- 30 % glycerol + 0.05 % agar + 0.05 % Tween80 (store at -80 °C)
- 0.2 % agar + 0.05 % Tween80 (store at 4 °C)

Micropipette (0.5–1 µl/spot)

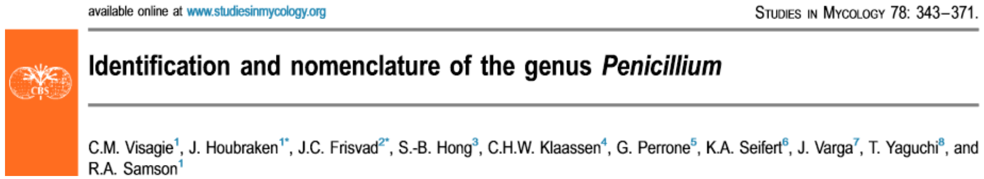
Media
- Recommended: CYA (x3), MEA,
- Optional: CZ, YES, OA, CREA, DG18, CYAS, MEAbl
- 90 mm vented Petri dish
- 20 ml media per plate

Incubate
- 7 days, 25 °C
- CYA at 30 °C & 37 °C
- Plates unwrapped
- In the dark
- Allow for sufficient aeration

Morphological characterisation
- Macromorphology
  - Characters: Colony diameter, texture, colour of conidia, mycelia, soluble pigments, exudates and reverses. Degree of growth, acid or possible base production on CREA
- Micromorphology
  - Preparations made from MEA
  - Mounting fluid: 60 % lactic acid
  - Wash excess conidia away with 70 % EtOH
  - Characters: number of branching points between stipe and phialides (i.e. solitary phialides to quaterverticilate), dimension, shape and texture of stipes, vesicles, metulae/branches (when present), phialides, conidia, cleistothecia, asci and ascospores (when present)

Extrolites
- CYA and YES
- 5 plugs per medium pooled in one vial
- Extraction: ethyl acetate/dichloromethane/methanol (3:2:1) (v/v/v) with 1 % (v/v) formic acid
- Filtered and analysed by HPLC (Frisvad & Thrane 1987, 1993, Smedsgaard 1997, Klitgaard *et al.* 2014)
- Ehrlich reaction using filter paper method (Lund 1995)

Molecular identification
- Sequencing
  - Genes for identification: ITS, *BenA*
  - Genes for phylogeny: *CaM*, *RPB2*
- Compare ITS/*BenA* to reference database
  - BLAST (↑ unreliable sequences)
  - RefSeq-BLAST (Verified ITS sequences)
  - Local BLAST (ICPA reference sequences)

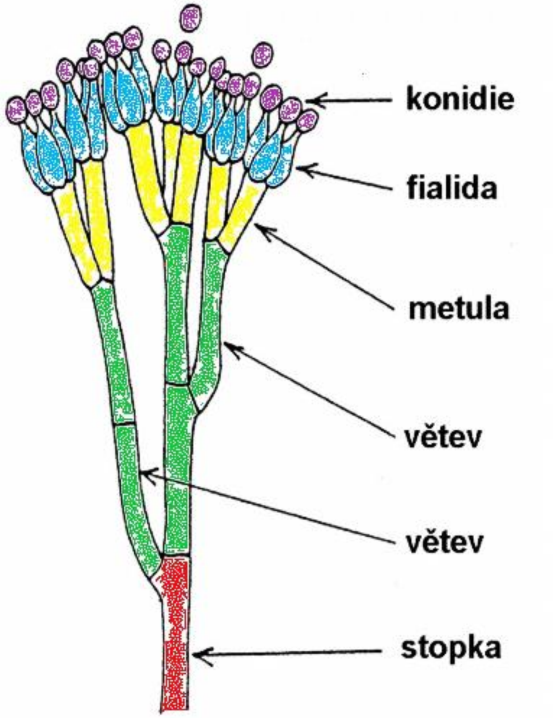


available online at www.studiesinmycology.org

Studies in Mycology 78: 343–371.

Identification and nomenclature of the genus *Penicillium*

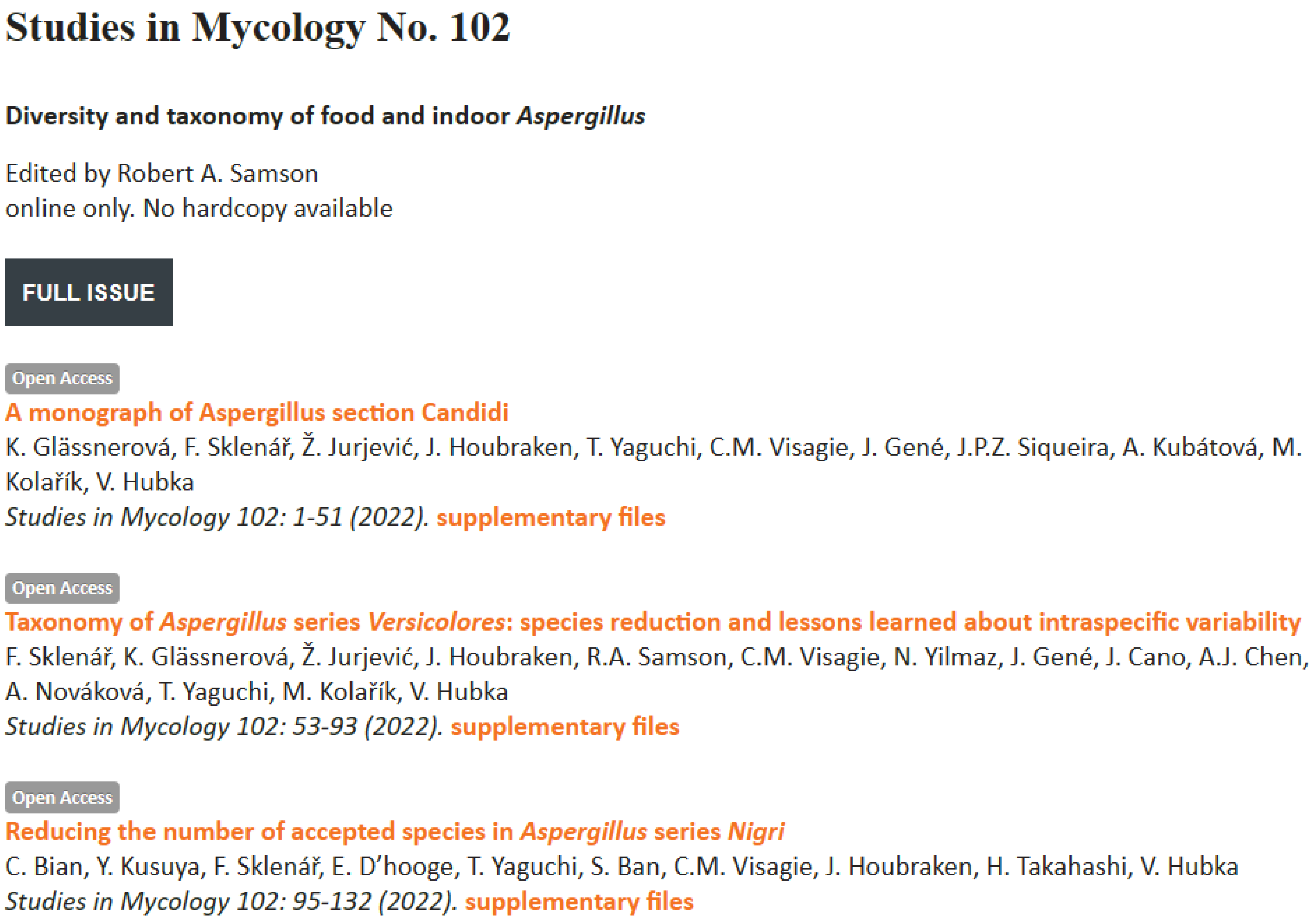
C.M. Visagie[1], J. Houbraken[1*], J.C. Frisvad[2*], S.-B. Hong[3], C.H.W. Klaassen[4], G. Perrone[5], K.A. Seifert[6], J. Varga[7], T. Yaguchi[8], and R.A. Samson[1]



Aspergillus

Fungi, Dikarya, Ascomycota, Pezizomycotina, Eurotiomycetes, Eurotiomycetidae, Eurotiales, Aspergillaceae

(Houbraken et al., 2020)
- 6 podrodů
- 27 sekcí
- 75 sérií



Studies in Mycology No. 102

Diversity and taxonomy of food and indoor *Aspergillus*

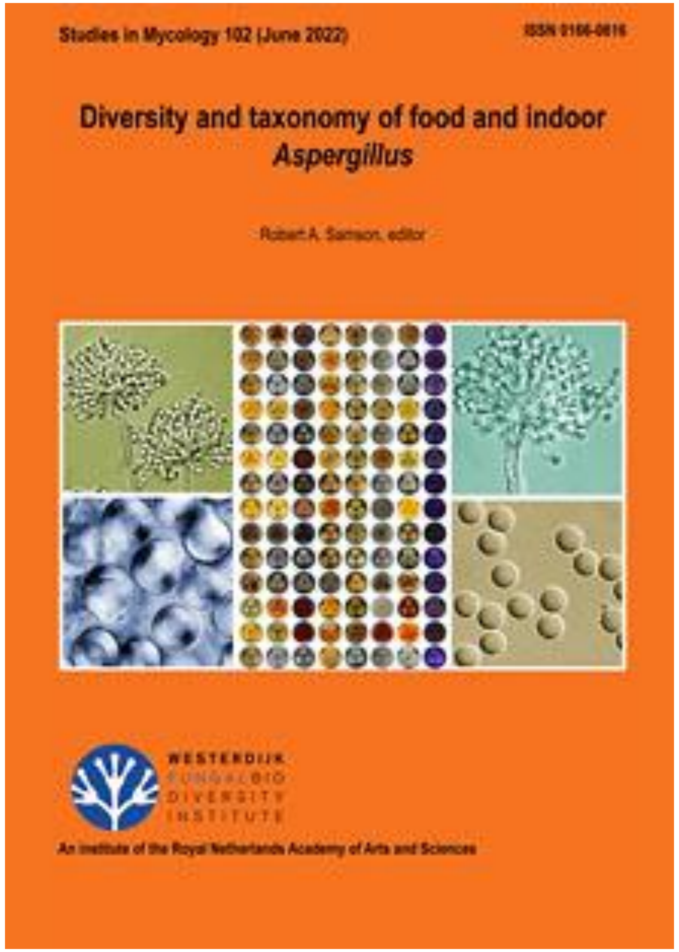
Edited by Robert A. Samson
online only. No hardcopy available

FULL ISSUE

Open Access
A monograph of Aspergillus section Candidi
K. Glässnerová, F. Sklenář, Ž. Jurjević, J. Houbraken, T. Yaguchi, C.M. Visagie, J. Gené, J.P.Z. Siqueira, A. Kubátová, M. Kolařík, V. Hubka
Studies in Mycology 102: 1-51 (2022). **supplementary files**

Open Access
Taxonomy of *Aspergillus* series *Versicolores*: species reduction and lessons learned about intraspecific variability
F. Sklenář, K. Glässnerová, Ž. Jurjević, J. Houbraken, R.A. Samson, C.M. Visagie, N. Yilmaz, J. Gené, J. Cano, A.J. Chen, A. Nováková, T. Yaguchi, M. Kolařík, V. Hubka
Studies in Mycology 102: 53-93 (2022). **supplementary files**

Open Access
Reducing the number of accepted species in *Aspergillus* series *Nigri*
C. Bian, Y. Kusuya, F. Sklenář, E. D'hooge, T. Yaguchi, S. Ban, C.M. Visagie, J. Houbraken, H. Takahashi, V. Hubka
Studies in Mycology 102: 95-132 (2022). **supplementary files**



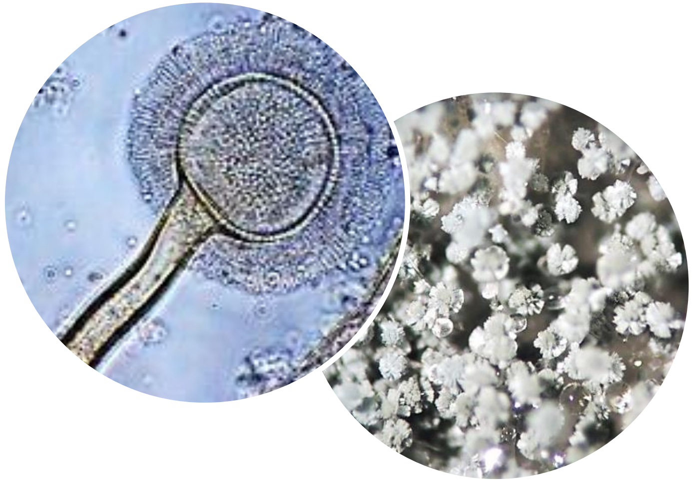
Studies in Mycology 102 (June 2022) ISSN 0166-0616

Diversity and taxonomy of food and indoor *Aspergillus*

Robert A. Samson, editor

WESTERDIJK FUNGAL BIODIVERSITY INSTITUTE
An institute of the Royal Netherlands Academy of Arts and Sciences

Schéma metod pro identifikaci rodu *Aspergillus*



Spore suspension
- 30 % glycerol + 0.05 % agar + 0.05 % Tween 80 (store at -80 °C)
- 0.2 % agar + 0.05 % Tween80 (store at 4°C)

Micropipette (0.5–1 μl/spot)

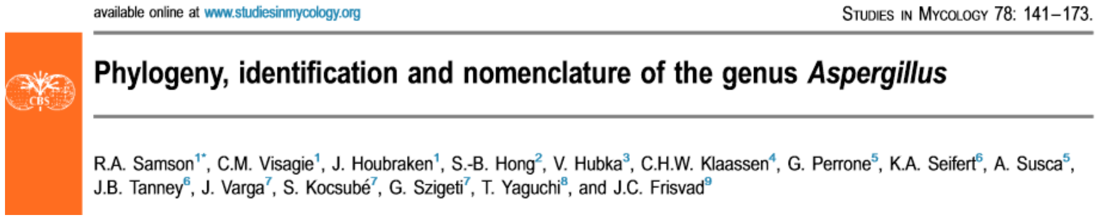
Media
- Recommended: CYA & MEA
- Optional: CZ, YES, DG18, OA, CREA, MEAbl, CY20S, ME20S
- 90 mm polystyrene Petri dish
- 20 ml media per plate

Incubate
- 7 days, 25 °C
- Additional CYA at 30, 37 & 50 °C
- Plates unwrapped
- In the dark
- Allow for sufficient aeration

Morphological characterisation
- Macromorphology
  - Characters: colony growth rates, texture, degree of sporulation, production of sclerotia or cleistothecia, colours of mycelia, sporulation, soluble pigments, exudates, colony reverses, sclerotia, Hülle-cells and cleistothecia
- Micromorphology
  - Preparations made from MEA
  - Mounting fluid: 60 % lactic acid
  - Wash excess conidia away with 70 % EtOH
  - Characters: shape of conidial heads, the number of branching points between vesicle and phialides (i.e. uniseriate or biseriate), colour of stipes, and the dimensions, shapes and textures of stipes, vesicles, metulae (when present), phialides, conidia, Hülle-cells (when present), cleistothecia, asci and ascospores

Extrolites
- CYA and YES
- 5 plugs pooled in one vial
- Extraction: ethyl acetate/dichloromethane/methanol (3:2:1) (v/v/v) with 1 % (v/v) formic acid
- Filtered and analysed by HPLC (Frisvad & Thrane 1987, 1993, Smedsgaard 1997, Klitgaard *et al.* 2014)

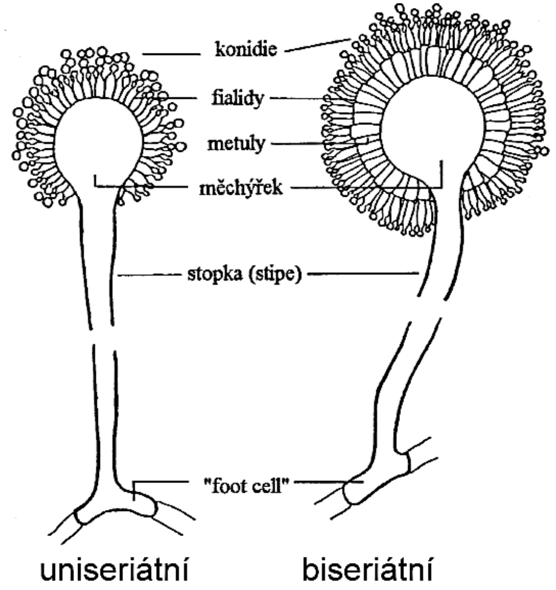
Molecular identification
- Sequencing
  - Genes for identification: ITS, *CaM*
  - Genes for phylogeny: *BenA*, *RPB2*
- Compare ITS/*CaM* to reference database
  - BLAST (↑ unreliable sequences)
  - RefSeq-BLAST (Verified ITS sequences)
  - Local BLAST (ICPA reference sequences)



available online at www.studiesinmycology.org   Studies in Mycology 78: 141–173.

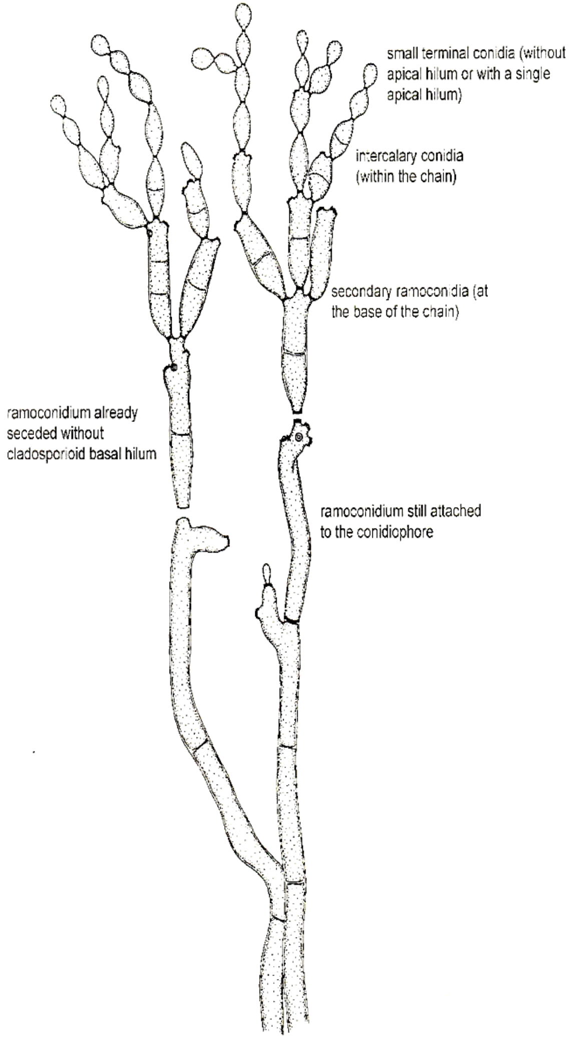
Phylogeny, identification and nomenclature of the genus *Aspergillus*

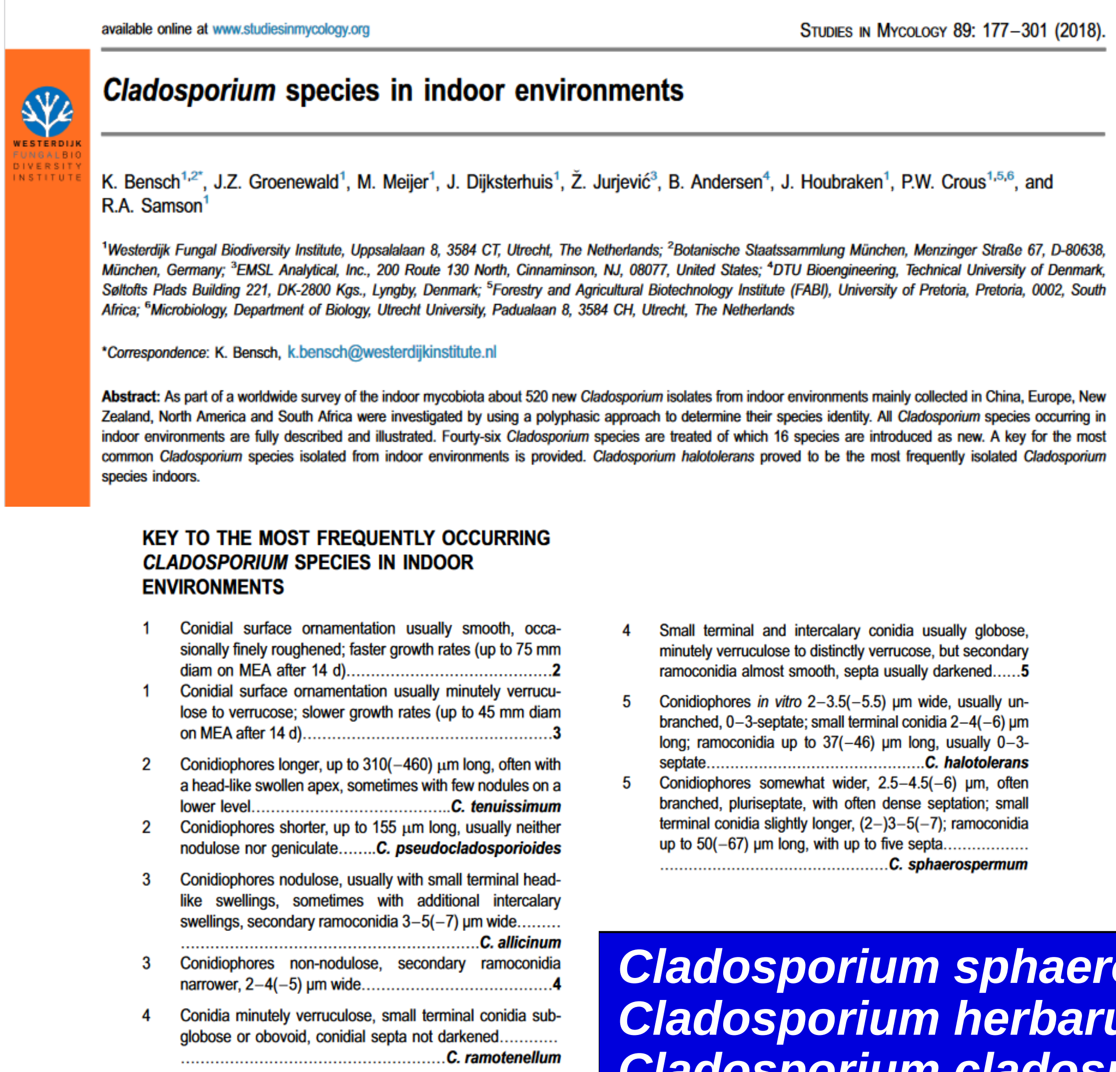
R.A. Samson[1*], C.M. Visagie[1], J. Houbraken[1], S.-B. Hong[2], V. Hubka[3], C.H.W. Klaassen[4], G. Perrone[5], K.A. Seifert[6], A. Susca[5], J.B. Tanney[6], J. Varga[7], S. Kocsubé[7], G. Szigeti[7], T. Yaguchi[8], and J.C. Frisvad[9]



Cladosporium

Fungi, Ascomycota, Pezizomycotina, Dothideomycetes, Dothideomycetidae, Cladosporiales, Cladosporiaceae





available online at www.studiesinmycology.org

STUDIES IN MYCOLOGY 89: 177–301 (2018).

Cladosporium species in indoor environments

K. Bensch[1,2*], J.Z. Groenewald[1], M. Meijer[1], J. Dijksterhuis[1], Ž. Jurjević[3], B. Andersen[4], J. Houbraken[1], P.W. Crous[1,5,6], and R.A. Samson[1]

[1] *Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT, Utrecht, The Netherlands;* [2] *Botanische Staatssammlung München, Menzinger Straße 67, D-80638, München, Germany;* [3] *EMSL Analytical, Inc., 200 Route 130 North, Cinnaminson, NJ, 08077, United States;* [4] *DTU Bioengineering, Technical University of Denmark, Søltofts Plads Building 221, DK-2800 Kgs., Lyngby, Denmark;* [5] *Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa;* [6] *Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands*

**Correspondence:* K. Bensch, k.bensch@westerdijkinstitute.nl

Abstract: As part of a worldwide survey of the indoor mycobiota about 520 new *Cladosporium* isolates from indoor environments mainly collected in China, Europe, New Zealand, North America and South Africa were investigated by using a polyphasic approach to determine their species identity. All *Cladosporium* species occurring in indoor environments are fully described and illustrated. Fourty-six *Cladosporium* species are treated of which 16 species are introduced as new. A key for the most common *Cladosporium* species isolated from indoor environments is provided. *Cladosporium halotolerans* proved to be the most frequently isolated *Cladosporium* species indoors.

KEY TO THE MOST FREQUENTLY OCCURRING *CLADOSPORIUM* SPECIES IN INDOOR ENVIRONMENTS

1 Conidial surface ornamentation usually smooth, occasionally finely roughened; faster growth rates (up to 75 mm diam on MEA after 14 d)..**2**

1 Conidial surface ornamentation usually minutely verruculose to verrucose; slower growth rates (up to 45 mm diam on MEA after 14 d)..**3**

2 Conidiophores longer, up to 310(–460) µm long, often with a head-like swollen apex, sometimes with few nodules on a lower level..***C. tenuissimum***

2 Conidiophores shorter, up to 155 µm long, usually neither nodulose nor geniculate........***C. pseudocladosporioides***

3 Conidiophores nodulose, usually with small terminal head-like swellings, sometimes with additional intercalary swellings, secondary ramoconidia 3–5(–7) µm wide......... ....***C. allicinum***

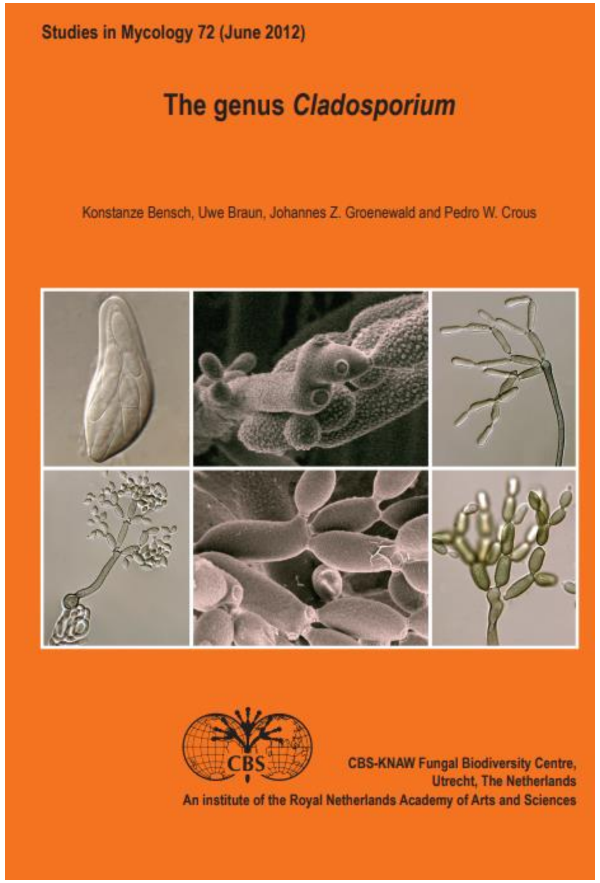
3 Conidiophores non-nodulose, secondary ramoconidia narrower, 2–4(–5) µm wide.......................................**4**

4 Conidia minutely verruculose, small terminal conidia subglobose or obovoid, conidial septa not darkened............ ....***C. ramotenellum***

4 Small terminal and intercalary conidia usually globose, minutely verruculose to distinctly verrucose, but secondary ramoconidia almost smooth, septa usually darkened......**5**

5 Conidiophores *in vitro* 2–3.5(–5.5) µm wide, usually unbranched, 0–3-septate; small terminal conidia 2–4(–6) µm long; ramoconidia up to 37(–46) µm long, usually 0–3-septate...***C. halotolerans***

5 Conidiophores somewhat wider, 2.5–4.5(–6) µm, often branched, pluriseptate, with often dense septation; small terminal conidia slightly longer, (2–)3–5(–7); ramoconidia up to 50(–67) µm long, with up to five septa................. ....***C. sphaerospermum***



Studies in Mycology 72 (June 2012)

The genus *Cladosporium*

Konstanze Bensch, Uwe Braun, Johannes Z. Groenewald and Pedro W. Crous

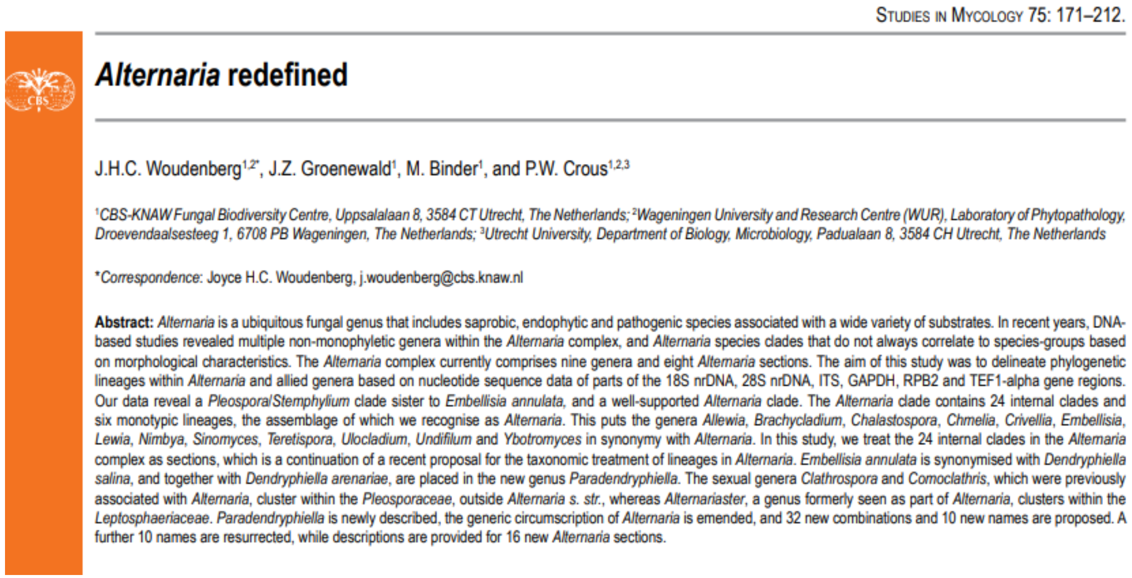
CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands
An institute of the Royal Netherlands Academy of Arts and Sciences

***Cladosporium sphaerospermum* komplex**
***Cladosporium herbarum* komplex**
***Cladosporium cladosporioides* komplex**

- aktin (*Act*), elongační faktor 1α (*TEF-1α*)

Alternaria

Fungi, *Ascomycota*, *Pezizomycotina*, *Dothideomycetes*, *Pleosporomycetidae*, *Pleosporales*, *Pleosporaceae*



Studies in Mycology 75: 171–212.

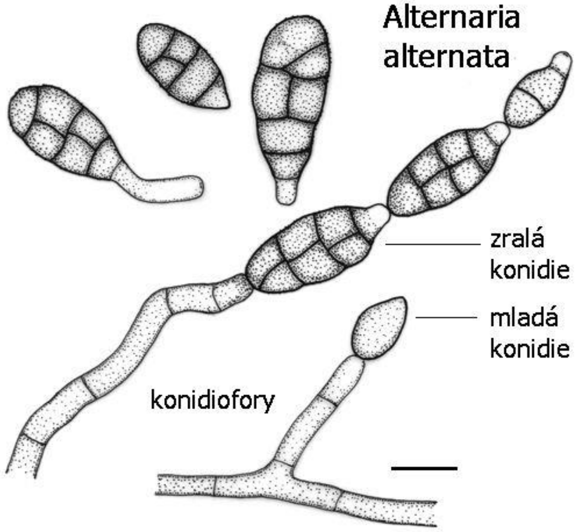
Alternaria redefined

J.H.C. Woudenberg[1,2*], J.Z. Groenewald[1], M. Binder[1], and P.W. Crous[1,2,3]

[1]CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands; [2]Wageningen University and Research Centre (WUR), Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; [3]Utrecht University, Department of Biology, Microbiology, Padualaan 8, 3584 CH Utrecht, The Netherlands

*Correspondence: Joyce H.C. Woudenberg, j.woudenberg@cbs.knaw.nl

Abstract: *Alternaria* is a ubiquitous fungal genus that includes saprobic, endophytic and pathogenic species associated with a wide variety of substrates. In recent years, DNA-based studies revealed multiple non-monophyletic genera within the *Alternaria* complex, and *Alternaria* species clades that do not always correlate to species-groups based on morphological characteristics. The *Alternaria* complex currently comprises nine genera and eight *Alternaria* sections. The aim of this study was to delineate phylogenetic lineages within *Alternaria* and allied genera based on nucleotide sequence data of parts of the 18S nrDNA, 28S nrDNA, ITS, GAPDH, RPB2 and TEF1-alpha gene regions. Our data reveal a *Pleospora*/*Stemphylium* clade sister to *Embellisia annulata*, and a well-supported *Alternaria* clade. The *Alternaria* clade contains 24 internal clades and six monotypic lineages, the assemblage of which we recognise as *Alternaria*. This puts the genera *Allewia*, *Brachycladium*, *Chalastospora*, *Chmelia*, *Crivellia*, *Embellisia*, *Lewia*, *Nimbya*, *Sinomyces*, *Teretispora*, *Ulocladium*, *Undifilum* and *Ybotromyces* in synonymy with *Alternaria*. In this study, we treat the 24 internal clades in the *Alternaria* complex as sections, which is a continuation of a recent proposal for the taxonomic treatment of lineages in *Alternaria*. *Embellisia annulata* is synonymised with *Dendryphiella salina*, and together with *Dendryphiella arenariae*, are placed in the new genus *Paradendryphiella*. The sexual genera *Clathrospora* and *Comoclathris*, which were previously associated with *Alternaria*, cluster within the *Pleosporaceae*, outside *Alternaria s. str.*, whereas *Alternariaster*, a genus formerly seen as part of *Alternaria*, clusters within the *Leptosphaeriaceae*. *Paradendryphiella* is newly described, the generic circumscription of *Alternaria* is emended, and 32 new combinations and 10 new names are proposed. A further 10 names are resurrected, while descriptions are provided for 16 new *Alternaria* sections.



http://www.sci.muni.cz/ueb/mik/Miniatlas/alt.htm

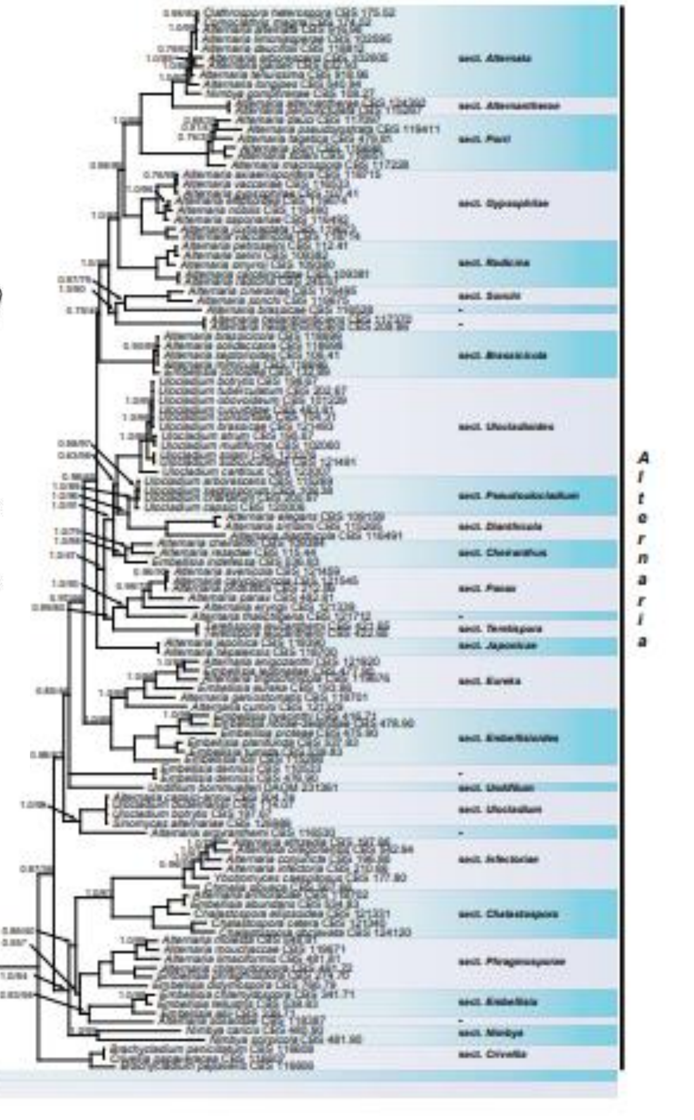


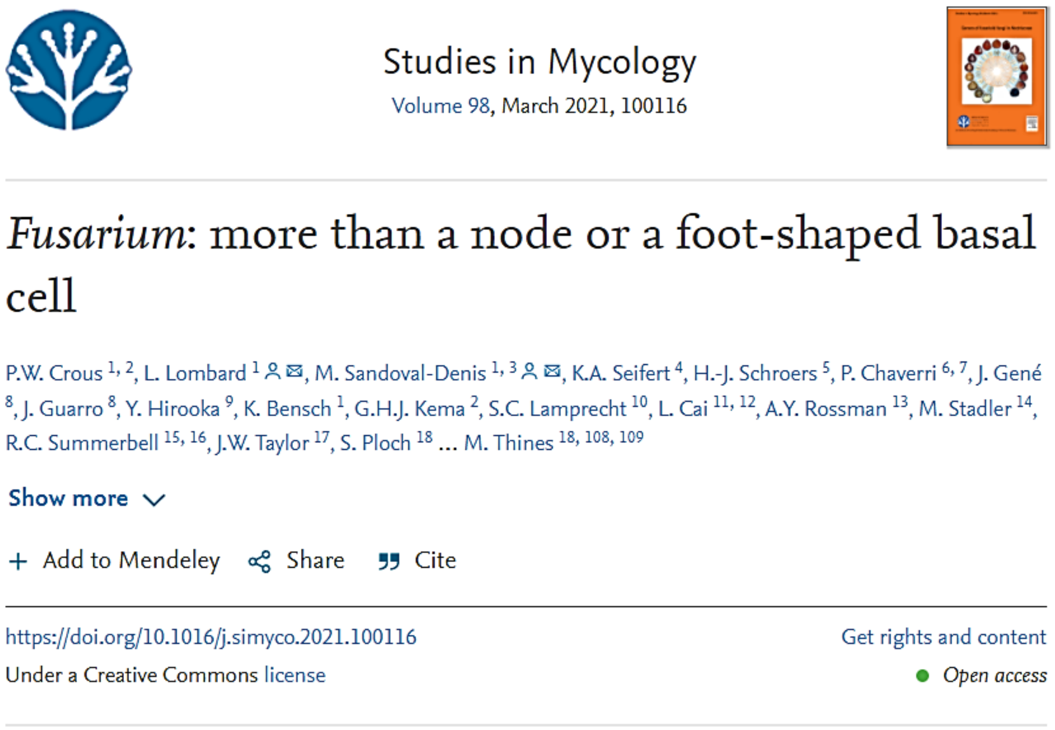
Fig. 1. Bayesian 50 % majority rule consensus tree based on the GAPDH, RPB2 and TEF1 sequences of 121 strains representing the *Alternaria* complex. The Bayesian posterior probabilities (PP) and RAxML bootstrap support values (ML) are given at the nodes (PP/ML). Thickened lines indicate a PP of 1.0 and ML of 100. The tree was rooted to *Stemphylium herbarum* (CBS 191.86). The monotypic lineages are indicated by black dots.

Rod *Alternaria* je v současné době rozdělen do 26 sekcí (STUDIES IN MYCOLOGY 82: 1–21)

- glyceraldehyd-3-fosfát dehydrogenáza (*GAPDH*)

Fusarium

Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Nectriaceae



Studies in Mycology
Volume 98, March 2021, 100116

Fusarium: more than a node or a foot-shaped basal cell

P.W. Crous [1, 2], L. Lombard [1], M. Sandoval-Denis [1, 3], K.A. Seifert [4], H.-J. Schroers [5], P. Chaverri [6, 7], J. Gené [8], J. Guarro [8], Y. Hirooka [9], K. Bensch [1], G.H.J. Kema [2], S.C. Lamprecht [10], L. Cai [11, 12], A.Y. Rossman [13], M. Stadler [14], R.C. Summerbell [15, 16], J.W. Taylor [17], S. Ploch [18] … M. Thines [18, 108, 109]

Show more

+ Add to Mendeley Share Cite

https://doi.org/10.1016/j.simyco.2021.100116 Get rights and content

Under a Creative Commons license ● Open access

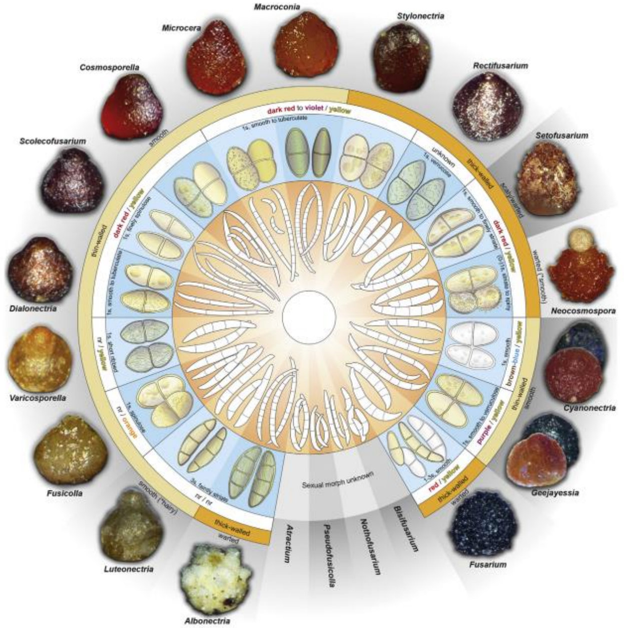
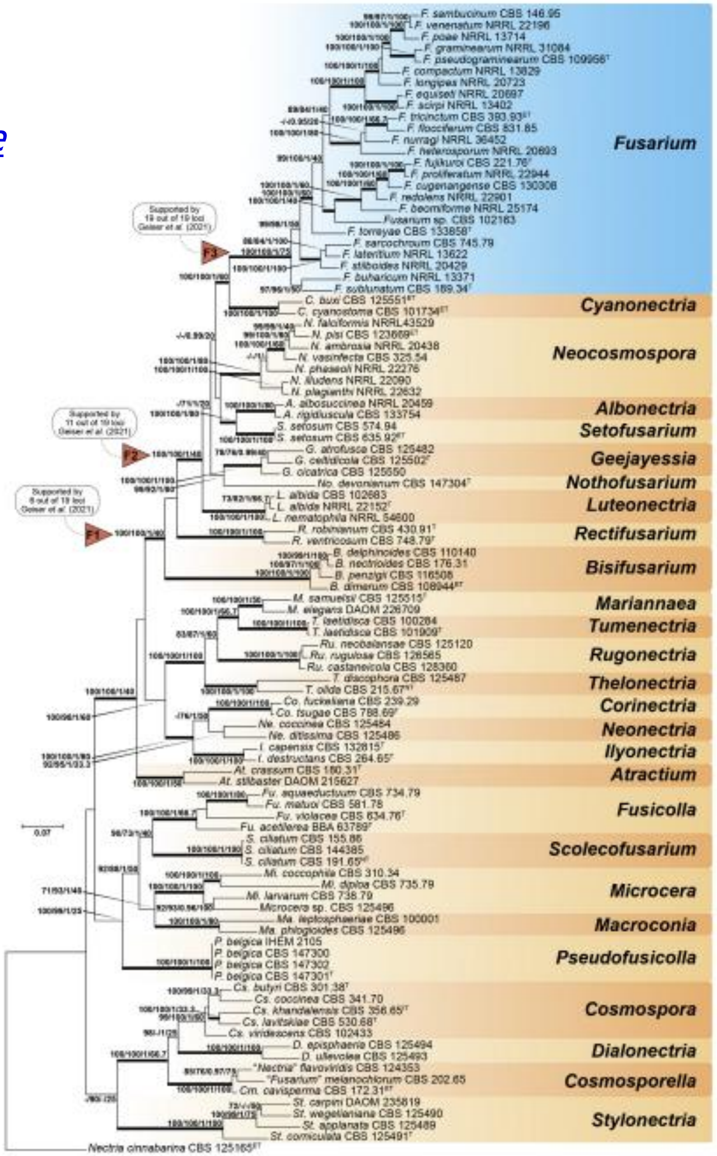


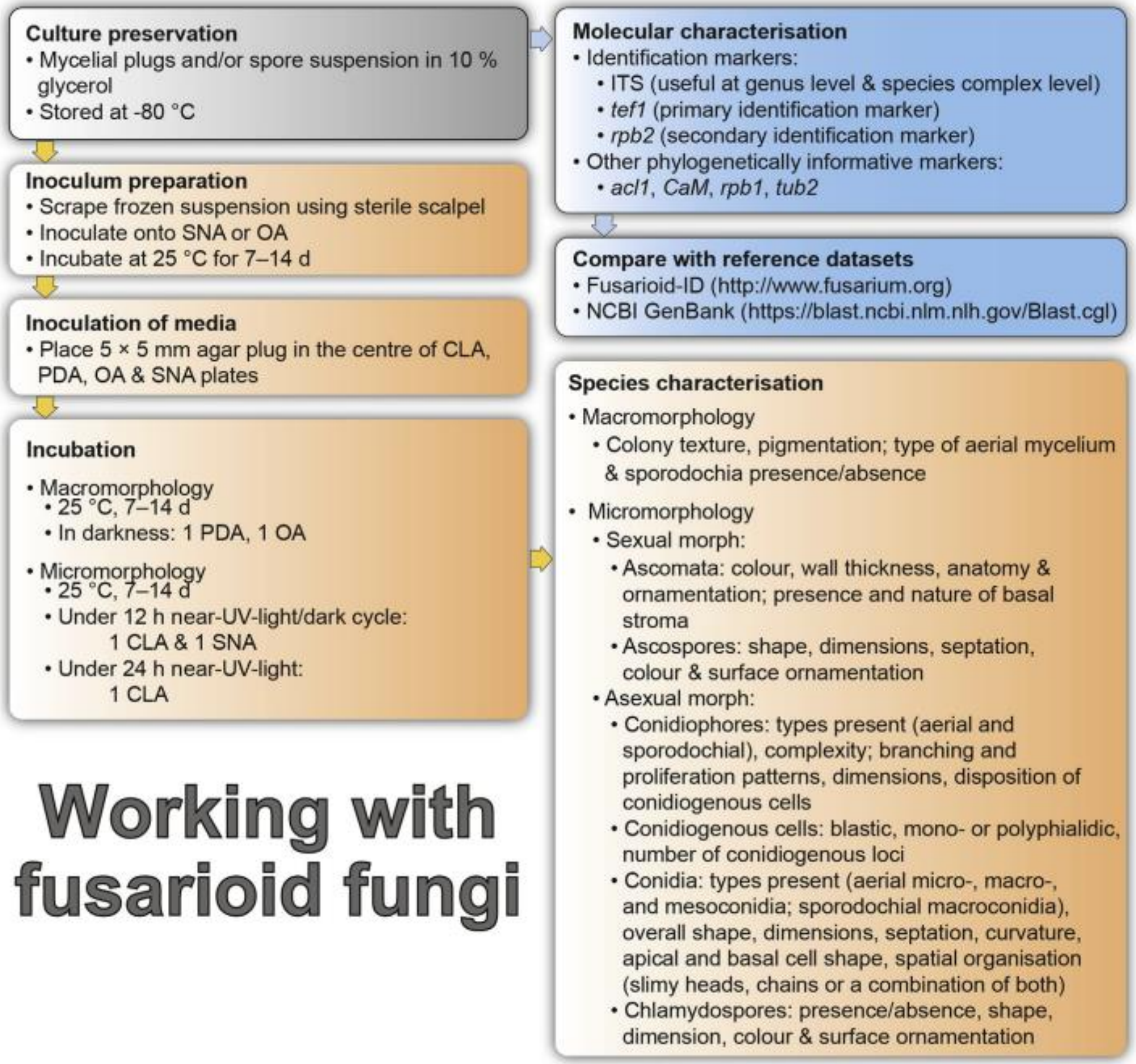
Fig. 9. Characters for morphological identification of fusarioid genera in *Nectriaceae*. The rings show, from inside to outside: conidial morphology; ascospore morphology, septation and surface; colour reaction of ascomata in 3 % KOH/lactic acid (nr = no reaction); ascomata wall thickness; and general colour, appearance and wall surface of ascomata.



Nectriaceae
- 20 rodů s jasnými synapomorfními znaky

Neocosmospora solani **syn. *Fusarium solani***

Schéma metod pro identifikaci rodu *Fusaruim* a příbuzných rodů



Culture preservation
- Mycelial plugs and/or spore suspension in 10 % glycerol
- Stored at -80 °C

Inoculum preparation
- Scrape frozen suspension using sterile scalpel
- Inoculate onto SNA or OA
- Incubate at 25 °C for 7–14 d

Inoculation of media
- Place 5 × 5 mm agar plug in the centre of CLA, PDA, OA & SNA plates

Incubation
- Macromorphology
  - 25 °C, 7–14 d
  - In darkness: 1 PDA, 1 OA
- Micromorphology
  - 25 °C, 7–14 d
  - Under 12 h near-UV-light/dark cycle: 1 CLA & 1 SNA
  - Under 24 h near-UV-light: 1 CLA

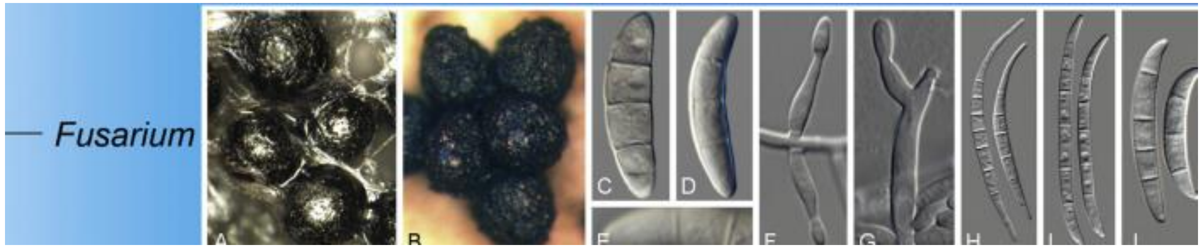
Working with fusarioid fungi

Molecular characterisation
- Identification markers:
  - ITS (useful at genus level & species complex level)
  - *tef1* (primary identification marker)
  - *rpb2* (secondary identification marker)
- Other phylogenetically informative markers:
  - *acl1*, *CaM*, *rpb1*, *tub2*

Compare with reference datasets
- Fusarioid-ID (http://www.fusarium.org)
- NCBI GenBank (https://blast.ncbi.nlm.nlh.gov/Blast.cgl)

Species characterisation
- Macromorphology
  - Colony texture, pigmentation; type of aerial mycelium & sporodochia presence/absence
- Micromorphology
  - Sexual morph:
    - Ascomata: colour, wall thickness, anatomy & ornamentation; presence and nature of basal stroma
    - Ascospores: shape, dimensions, septation, colour & surface ornamentation
  - Asexual morph:
    - Conidiophores: types present (aerial and sporodochial), complexity; branching and proliferation patterns, dimensions, disposition of conidiogenous cells
    - Conidiogenous cells: blastic, mono- or polyphialidic, number of conidiogenous loci
    - Conidia: types present (aerial micro-, macro-, and mesoconidia; sporodochial macroconidia), overall shape, dimensions, septation, curvature, apical and basal cell shape, spatial organisation (slimy heads, chains or a combination of both)
    - Chlamydospores: presence/absence, shape, dimension, colour & surface ornamentation

Identifikace musí být vždy provedena na základě **monosporické kultury** (kultura produkovaná z jedné konidie, askospory nebo z vrcholu hyfy).

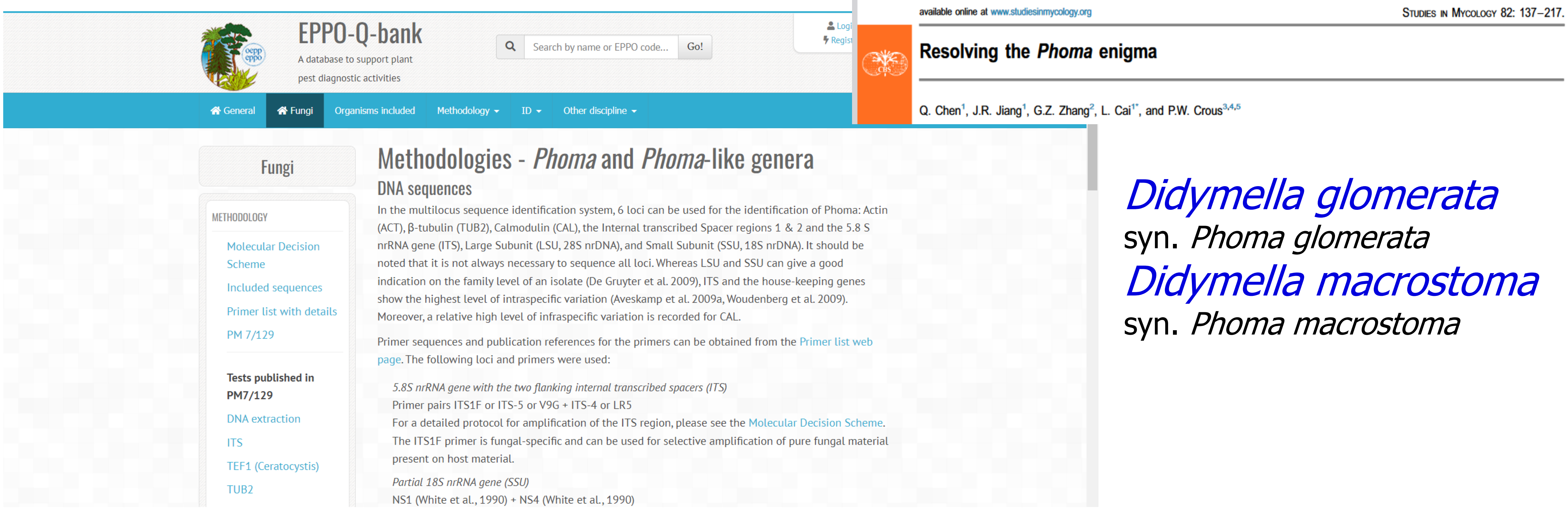


Druhy rodu *Fusarium* se vyznačují:
- tvorbou vícebuněčných zakřivených makrokonidií
- tmavě fialové až černé perithecium

P.W. Crous et al. (2021). *Fusarium*: more than a node or a foot-shaped basal cell, Studies in Mycology, Volume 98, https://doi.org/10.1016/j.simyco.2021.100116.

Phoma a příbuzné rody

Fungi, *Ascomycota*, *Pezizomycotina*, *Dothideomycetes*, *Pleosporomycetidae*, *Pleosporales*



Methodologies - *Phoma* and *Phoma*-like genera

DNA sequences

In the multilocus sequence identification system, 6 loci can be used for the identification of Phoma: Actin (ACT), β-tubulin (TUB2), Calmodulin (CAL), the Internal transcribed Spacer regions 1 & 2 and the 5.8 S nrRNA gene (ITS), Large Subunit (LSU, 28S nrDNA), and Small Subunit (SSU, 18S nrDNA). It should be noted that it is not always necessary to sequence all loci. Whereas LSU and SSU can give a good indication on the family level of an isolate (De Gruyter et al. 2009), ITS and the house-keeping genes show the highest level of intraspecific variation (Aveskamp et al. 2009a, Woudenberg et al. 2009). Moreover, a relative high level of infraspecific variation is recorded for CAL.

Primer sequences and publication references for the primers can be obtained from the Primer list web page. The following loci and primers were used:

5.8S nrRNA gene with the two flanking internal transcribed spacers (ITS)
Primer pairs ITS1F or ITS-5 or V9G + ITS-4 or LR5
For a detailed protocol for amplification of the ITS region, please see the Molecular Decision Scheme. The ITS1F primer is fungal-specific and can be used for selective amplification of pure fungal material present on host material.

Partial 18S nrRNA gene (SSU)
NS1 (White et al., 1990) + NS4 (White et al., 1990)

available online at www.studiesinmycology.org

Studies in Mycology 82: 137–217.

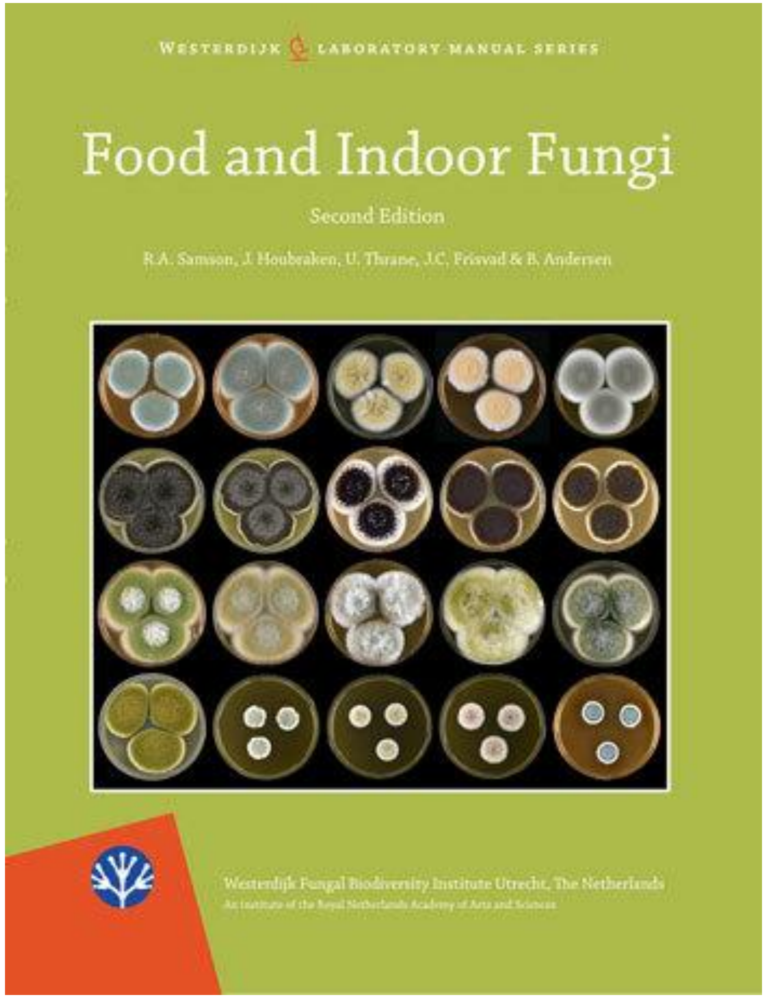
Resolving the *Phoma* enigma

Q. Chen[1], J.R. Jiang[1], G.Z. Zhang[2], L. Cai[1*], and P.W. Crous[3,4,5]

Didymella glomerata
syn. *Phoma glomerata*
Didymella macrostoma
syn. *Phoma macrostoma*

https://qbank.eppo.int/fungi/methodologies/PhomaMethodologies

Identifikace mikroskopických hub



Westerdijk Laboratory Manual Series No. 2 Food and Indoor Fungi
Second edition (2019). Robert A. Samson, J. Houbraken, U. Thrane, J.C. Frisvald and B. Andersen

Děkuji za pozornost

https://ccm.sci.muni.cz/