



2 ***Blastobotrys nigripullensis*, a new yeast species isolated**
3 **from a fungal outbreak on an ancient Roman shipwreck**
4 **in the Netherlands**

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10 **Abstract** A new species of the yeast genus *Blasto-*
11 *botrys* was discovered on ancient ship timbers in
12 the Netherlands. The species had developed on the
13 wood of a river barge dating to the Roman period.
14 The growth occurred after the preservative polyethyl-
15 ene glycol (PEG 4000) was washed out of some of
16 the timbers due to an undetected leak in the storage
17 unit. Mycological analysis of various timber sam-
18 ples revealed the presence of *Microascus melano-*
19 *sporus* (predominant), *Microascus paisii*, a member
20 of the *Acremonium chrysogenum*-clade, and a new

Blastobotrys species. The new species produced 21
sporothrix-like conidiophores with clavate blastoco- 22
nidia (3–7 × 1–3.5 μm) and were found to be osmotol- 23
erant, capable of growth on low water activity media 24
like malt yeast 50% glucose agar (MY50G). In this 25
article we formally describe and introduce *Blasto-* 26
botrys nigripullensis (CBS 17879^T) based on its mor- 27
phology, physiology and phylogenetic placement. 28

Keywords Yeasts phylogeny · Buried river barge · 29
Oak wood preservation · Polyethylene glycol · 30
Trichomonasceae · *Trichomonascus* 31

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32 **Introduction**

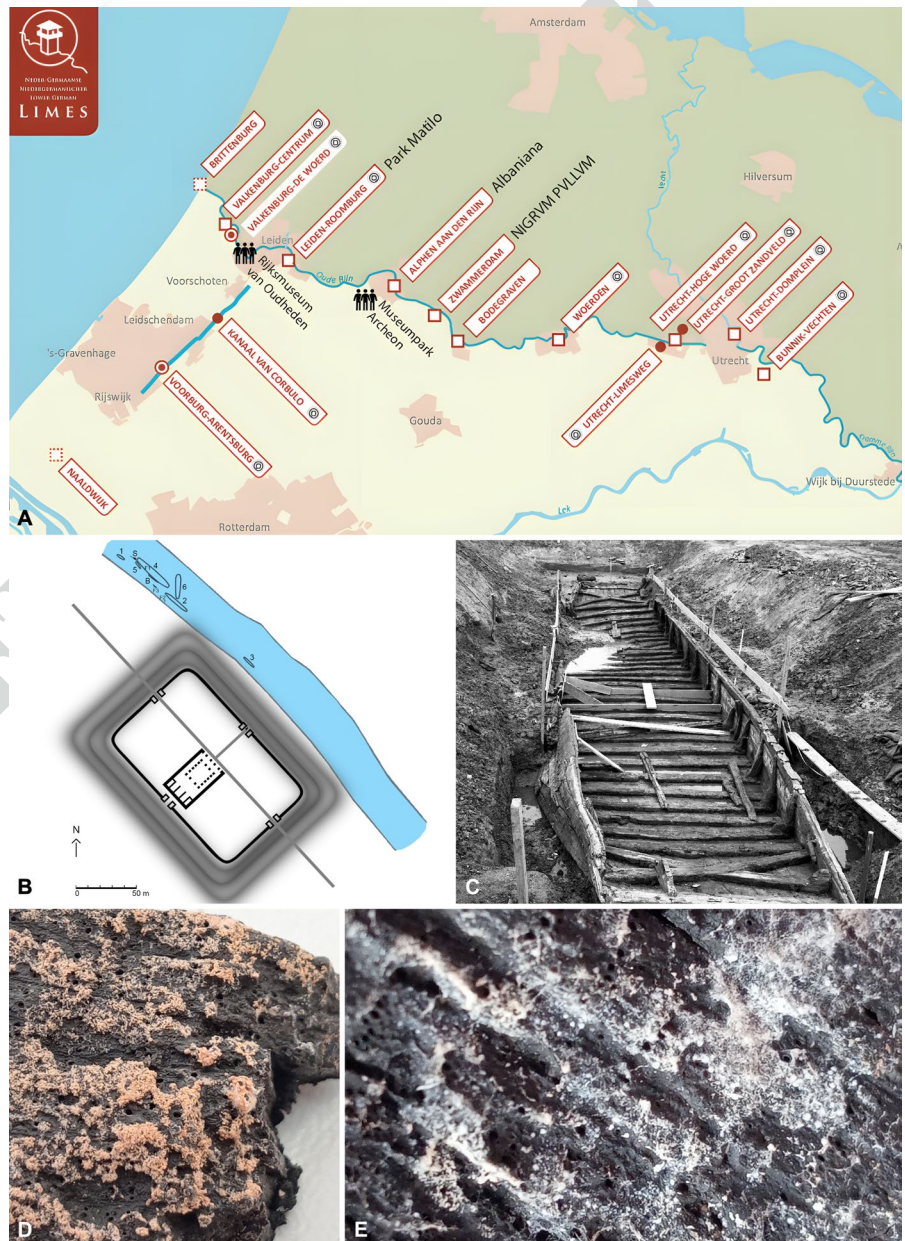
33 In the early 1970s, three large wooden river barges
 34 and three smaller Roman-era vessels were discovered
 35 during construction work in the village of Zwammer-
 36 dam in the Netherlands. At the time, the care facility
 37 ‘Ipse de Bruggen’ was being built in Zwammerdam
 38 on the Hooze Burch estate and by chance the work-
 39 ers came across the Zwammerdam vessels. The ves-
 40 sels were all made of oak and the river barges were

exceptionally large, ranging from 20 to 34 m in length
 (De Weerd 1988).

In the years leading up to the discovery, the Uni-
 versity of Amsterdam conducted archaeological
 research in the area and investigated a Roman fort
 that was marked on a Roman road map as the military
 point *Nigrum Pullum* (De Weerd 1988). This fort was
 part of the Roman *Limes*, a border where a series of
 forts along the river Rhine formed a defensive line in
 the Lower Germanic region (Fig. 1A). This defence

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Fig. 1 **A** The Roman *Limes* with the fort of *Nigrum Pullum* situated in today’s Zwammerdam, the Netherlands (drawing by T. Hazenberg). **B** The position of the vessels of Zwammerdam, the Netherlands, in front of the fort of *Nigrum Pullum*. **C** The Zwammerdam 6 ship during the excavation (photo by G. Verkuyt, collection ACASA, University of Amsterdam, the Netherlands). **D** Fungal growth of *Microascus melanosporus* (dominant) and *Acremonium* species on wood from an L-shaped chine girder. **E** White patches with *Blasbototrys nigripullensis* and *Microascus paisii* on wood from a portside wale



51 line also functioned as a transport corridor, a strictly
52 controlled long-distance waterway from the interior
53 regions inland towards the coast.

54 The vessels found near the site of *Nigrum Pullum*
55 were scattered along a section of timber shoring in
56 front of the fort, where the Rhine once flowed, and
57 were located at various depths (Fig. 1B). They were
58 essentially found in the riparian zone of a silted
59 up river bed. To document and salvage the ships,
60 construction work had to be halted and, under great
61 time pressure, only the ships were properly examined.
62 At that time, the relationship to the fort could not be
63 further explored (De Weerd 1988). Why the ships
64 sank or were scuttled in the vicinity of the fort thus
65 remains unknown.

66 An early form of crowdfunding, supported by former
67 Queen Juliana of the Netherlands, allowed the
68 vessels to be salvaged and conserved to enable future
69 display in a museum. The conservation of the wood
70 with polyethylene glycol (PEG, 4000 g/mol), a water-
71 soluble waxy solid, was carried out in batches starting
72 in 1976 and took an average of two years per load of
73 timbers. When heated, high-molecular weight PEG
74 can penetrate wood tissues and replace water mole-
75 cules. The PEG also bulks the deteriorated wood cells
76 and thus reinforces the structure of the wood, improv-
77 ing dimensional stability. After PEG treatment, the
78 wood was stored stacked in shipping containers.
79 Decades later in 2017, a project started, organized by
80 Hazenberg Archeologie and Museumpark Archeon
81 in the Netherlands, that enabled the restoration of the
82 vessels.

83 Unfortunately, an undetected leak in one of the
84 shipping containers with remains of the sixth ship of
85 Zwammerdam (Fig. 1C) had resulted in the preserva-
86 tive being washed out of some of the timbers. These
87 timbers had started to dry out, crack and warp in
88 some areas and had developed visible fungal growth
89 (Fig. 1D, E). To seek advice for the removal of this
90 growth, the project staff contacted the Westerdijk
91 Fungal Biodiversity Institute (WI) in Utrecht and
92 send in some samples to investigate further.

93 During the study of the fungal diversity present,
94 a putative new *Blastobotrys* species was isolated. In
95 the dual nomenclature era, *Blastobotrys* was con-
96 sidered the asexual genus (or anamorph) associated
97 with the sexual genus (or teleomorph) *Trichomonas-*
98 *cus*. This relationship was confirmed by Smith et al.
99 (2011a, b). Following the abandonment of dual

nomenclature for fungi (Hawksworth et al. 2011; 100
McNeill et al. 2012), a decision on what generic 101
name to apply to the *Blastobotrys/Trichomonascus* 102
lineage remains to be made. This was discussed in 103
a recent revision published for the lineage by Vis- 104
agie et al. (2023) in which *B. davinci* was described, 105
a species that has been detected from house dust 106
and ~~other~~ historically important artifacts including 107
the self-portrait of Leonardo da Vinci which dates 108
from ca 1512. The goal of the current study is to 109
report on the fungi isolated from Zwammerdam ves- 110
sel wood samples, to compare the putative new spe- 111
cies with known *Blastobotrys* and *Trichomonascus* 112
species and to formally introduce *B. nigripullensis* 113
sp. nov. For this, we provide a phylogenetic analy- 114
sis based on the internal transcribed spacer (ITS1- 115
5.8S-ITS2) rDNA region (ITS) and D1/D2 region 116
of the large subunit (LSU), and describe the new 117
species based on morphological and physiological 118
characters. 119

120 Materials and methods

121 Sampling and isolations

122 A large oak knee detached from a floor timber with
123 fungal growth as well as small pieces of a few cen-
124 timetres of oak from hull planks with small white
125 patches of suspected fungal growth on the surface
126 were sent from Archeon (Alphen aan den Rijn, the
127 Netherlands) to the Westerdijk Fungal Biodiversity
128 Institute (WI, Utrecht, the Netherlands). Here, the
129 suspected fungal material was plated directly on
130 dichloran 18% glycerol agar (DG18), malt yeast
131 50% glucose agar (MY50G) and malt extract agar
132 (MEA, Oxoid) supplemented with the antibiot-
133 ics penicillin (6600 U per plate) and streptomycin
134 (20,000 U per plate) to inhibit bacterial growth. The
135 plates were incubated for 10–14 days at 25 °C in the
136 dark. After incubation, colonies were transferred to
137 fresh agar media. The strains were preserved in 30%
138 glycerol, stored at –80 °C and accessioned in the
139 working culture collection of the Food and Indoor
140 Mycology research group (DTO) housed at WI.
141 Some of these strains were also preserved in the
142 CBS culture collection and fungarium of WI.

143 DNA extraction, sequencing and phylogenetic
144 analysis

145 DNA was extracted from 7 d old colonies grown on
146 MEA using the Ultraclean™ Microbial DNA iso-
147 lation Kit (MoBio Laboratories Inc., Solana Beach,
148 USA). The ITS was amplified for all strains using the
149 primer pair V9G and LS266 and used to make initial
150 identifications to genus level. For species level identi-
151 fications, additional regions were amplified as needed.
152 These included LSU, translation elongation factor 1- α
153 (*TEF*) and beta-tubulin (*BenA*) using primer pairs
154 LR5 and LROR, EF1 and EF2 and Bt2a and Bt2b,
155 respectively. All PCR amplifications were made fol-
156 lowing Samson et al. (2019). Amplification profiles
157 were as follow: initial denaturing 94 °C for 5 min, 36
158 cycles of 94 °C for 45 s, 55 °C for 45 s, 72 °C for
159 1 min, followed by a final elongation step of 72 °C
160 for 10 min. Sequencing reactions were prepared using
161 the BigDye Terminator Cycle Premix Kit (Applied
162 Biosystems, Waltham, USA) and were run on an ABI
163 PRISM 3730xl genetic analyser with the same primer
164 pairs used for PCR amplification. Sequence contigs
165 were assembled in SeqMan Pro v.15.0.0 (DNASTAR)
166 and newly generated sequences deposited in GenBank
167 (<https://www.ncbi.nlm.nih.gov/genbank/>) (accessions
168 OQ876811–OQ876820, OQ876860, OQ876861
169 and OQ918213). Initial identifications were made
170 by performing similarity searches using BLAST
171 against the nucleotide sequence database. To compare
172 the putative new *Blastobotrys* strains with known
173 *Blastobotrys/Trichomonascus* species, the same DNA
174 sequence reference dataset (Table 1) and phyloge-
175 netic approaches published by Visagie et al. (2023)
176 were used. Alignments and treefiles were uploaded to
177 the University of Pretoria’s research data repository
178 hosted on Figshare (<https://doi.org/https://doi.org/10.25403/UPresearchdata.23815032>).

180 Morphological and physiological characterization of
181 the yeast isolates

182 Two strains (CBS 17879 and CBS 17880) were mor-
183 phologically and physiologically characterised as
184 described by Visagie et al. (2023) using MEA, yeast
185 morphology agar (YMA), potato dextrose agar (PDA)
186 and glucose yeast peptone agar (GYPA), 5% glucose
187 in yeast nitrogen broth and Dalmau plates on yeast
188 morphology agar. The physiological growth profile

of the strains was assessed using methods commonly
used in yeast taxonomy (Kurtzman et al. 2011).

Results

Isolations and identifications

Three wood samples with visible fungal growth were
studied and isolations made from these resulted in
five strains representing four species. Light brown to
beige fungal spots were present on the wood from an
L-shaped chine girder sample (a combined bottom/
side timber in the hull of a Roman river barge) on
port side (timber code ZW6-K2 bb), and isolations
revealed the presence of *Microascus melanosporus*
(DTO 457-E5: ITS=OQ876812, LSU=OQ876817,
BenA=OQ876861) and a member of the *Acremonium*
chrysogenum-clade (DTO 457-E6: ITS=OQ876813,
LSU=OQ876818). The other two samples (knee of
a floor timber [timber code ZW6-S18] and part of a
wale on port side [timber code ZW6-BE2 bb]) showed
white spots (Fig. 1E) and direct microscopic analysis
of these spots showed (pseudo)hyphae and yeast cells.
Initial isolations only showed growth of yeast-like
colonies on the MY50G plates; however, subculturing
on MEA/PS and DG18 showed that these colonies
could also grow on these media. A *Blastobotrys* spe-
cies (CBS17879=DTO 457-E3: ITS=OQ876814,
LSU=OQ876819; CBS17880=DTO 455-G9:
ITS=OQ876815, LSU=OQ876820) was grow-
ing on the two investigated wood samples, and the
new species *B. nigripullensis* is introduced below.
Besides *B. nigripullensis*, also *Microascus paisii*
(DTO 457-E4: ITS=OQ876811, LSU=OQ876816,
BenA=OQ876860, *TEF*=OQ918213) was isolated
from wood sample ZW6-BE2 bb, but no structures of
this species were observed during the direct examina-
tion of the material.

Microascus species are both widespread and pre-
fer substrates with cellulose, the *Acremonium* species
occurs in soils and on decaying plant matter (Barron
et al. 1961, Domsch et al. 1993; Hou et al. 2023).
Considering the context of archaeological wood taken
from the ground and then decomposed in parts, the
occurrences of these species fit the picture. The sur-
faces of samples ZW6-K2 bb and ZW6-BE2 bb were
steam cleaned and seven months later no regrowth
was evident. However, the knee of the floor timber

Table 1 Strains used for phylogenetic analyses

Taxon name	Strains ^a	Location	Source	GenBank: ITS	GenBank: LSU
<i>Blastobotrys adenini-vorans</i>	CBS 8244 = NRRL Y-17692 IFO 10858 = IGC 4638 = PYCC 4638 (ex-type)	The Netherlands: Wageningen	Soil	KY101746	DQ442697
<i>Blastobotrys allociferii</i>	CBS 5166 = NBRC 10194 = IFO 10194 (ex-type)	Germany	Human	LC158134	LC158143
<i>Blastobotrys americanus</i>	CBS 10337 = NRRL Y-6844 = LRB 70B3 (ex-type)	USA: Kansas	Unknown	KY101748	DQ442699
<i>Blastobotrys arbuscula</i>	CBS 227.83 = NRRL Y-17585 (ex-type)	Finland	Indoor air	OL772655	DQ442689
<i>Blastobotrys aristatus</i>	CBS 521.75 = NRRL Y-17579 = ATCC 34215 = CCM F-410 = HEM 2672 = UAMH 4665 (ex-type)	Czech Republic	Moldy plaster	OL772656	DQ442686
<i>Blastobotrys atinorum</i>	CBS 9734 = NRRL Y-27639 = UNESP-S156 (ex-type)	Brazil: Sao Paulo	Fungal garden of nests of the leaf-cutting ant (<i>Atta sexdens</i>)	KY101749	AY442294
<i>Blastobotrys baotianmanensis</i>	CBS 16024 = CICC 33083 (ex-type)	China: Henan Province, Baotianman Nature Reserve	Gut of ground beetle (<i>Pterostichus gebleri</i>)	KU128710	KU128725
<i>Blastobotrys bombycis</i>	CBS 15274 (ex-type)	India: Dharwad	Silkworm (<i>Bombyx mori</i>)	OL772657	OL772653
<i>Blastobotrys buckinghamii</i>	CBS 13900 = NRRL Y-63727 = yHAB 196 (ex-type)	USA: Michigan, Tahquamenon Falls State Park	Mushroom associated with American beech (<i>Fagus grandifolia</i>)	OL772658	OL772654
<i>Blastobotrys capitulata</i>	CBS 287.82 = NRRL Y-17573 (ex-type)	South Africa	Flower, decaying tissue of candleabra tree (<i>Euphorbia ingens</i>)	OL772659	DQ442688
<i>Blastobotrys chiropterorum</i>	CBS 6064 = NRRL Y-17071 (ex-type)	Columbia	Liver of bat (<i>Mormoops megalophylla</i>)	KY101750	DQ442682
<i>Blastobotrys davincii</i>	CBS 16861 = DAQMC 251467 = CMW 56638 = CN 002G3 (ex-type)	Canada: Ontario, Stittsville	House dust	MW367648	MW367634
<i>Blastobotrys elegans</i>	CBS 530.83A = NRRL Y-17572 (ex-type)	Finland	Indoor air	OL772660	DQ442687
<i>Blastobotrys farinosus</i>	CBS 140.71 = NRRL Y-17593 = IGC 4592 = JCM 2935 (ex-type)	The Netherlands	<i>Hirneola auricula-judae</i>	OL772661	DQ442685
<i>Blastobotrys fungorum</i>	CBS 259.70 = CMW 17165 = UAMH 3678 (ex-type)	Germany	Old <i>Fomes fomentarius</i> basidiome	KXS90837	KXS90883
<i>Blastobotrys illinoisensis</i>	CBS 10339 = NRRL YB-1343 (ex-type)	USA: Illinois, Marion, Wohlwend farm	Tree	KY101751	DQ442696
<i>Blastobotrys indianensis</i>	CBS 9600 = NRRL YB-1950 (ex-type)	USA: Indiana, Spencer, McCormick's Creek State Park	White fungus associated with pine	KY101752	DQ442692
<i>Blastobotrys malaysiensis</i>	CBS 10336 = NRRL Y-6417 = EMMONS S3,539A (ex-type)	Malaysia	Cave soil	KY101753	DQ442695
<i>Blastobotrys meliponae</i>	CBS 14100 = URM7224 (ex-type)	Brazil: Pernambuco, Recife	Honey	KT448719	KR779217
<i>Blastobotrys mokoensis</i>	CBS 8435 = NRRL Y-27120 (ex-type)	South Africa	Soil	KY101754	DQ442694

Table 1 (continued)

Taxon name	Strains ^a	Location	Source	GenBank: ITS	GenBank: LSU
<i>Blastobotrys mucifer</i>	CBS 7409 = CCY 29-170-1 = IFO 10918 (ex-type)	Brazil: Manaus	Liver of toad (<i>Rhinella granulosa</i>)	KY102217	KY106587
<i>Blastobotrys muscicola</i>	CBS 10338 = NRRRL Y-7993 (ex-type)	USA: Louisiana, near New Orleans	Moss on fallen log	KY101755	DQ442680
<i>Blastobotrys navarrensis</i>	CBS 139.77 = ATCC 36955 = IJFM 2642 = UAMH 4664 (ex-type)	Spain: Pampelona	Black pepper (<i>Piper nigrum</i>)	OK623478	OK623486
<i>Blastobotrys nigripullensis</i>	clone CMH114	USA: Missouri, Kansas City	House dust and indoor air	KF800205	–
<i>Blastobotrys nigripullensis</i>	clone S23T_59	Unknown	Sea water	KU164259	–
<i>Blastobotrys nigripullensis</i>	CBS 17880 = DTO 455-G9	The Netherlands: Zwammerdam	Oak wood from ancient Roman ship	OQ876815	OQ876820
<i>Blastobotrys nigripullensis</i>	CBS 17879 = DTO 457-E3 (ex-type)	The Netherlands: Zwammerdam	Oak wood from ancient Roman ship	OQ876814	OQ876819
<i>Blastobotrys nivea</i>	CBS 163.67 = NRRRL Y-17581 = ATCC 18420 = HFM 26 = UAMH 4663 = MUCL 6078 (ex-type)	Germany	Municipal compost	OL772662	DQ442690
<i>Blastobotrys parvus</i>	CBS 6147 = NRRRL Y-10004 (ex-type)	Antarctic Ocean	Seawater	KY101757	DQ442693
<i>Blastobotrys peoriensis</i>	CBS 10340 = NRRRL YB-2290 (ex-type)	USA: Illinois, Peoria	Unknown	KY101758	DQ442700
<i>Blastobotrys persicus</i>	CBS 14259 = IBRC-M30238 (ex-type)	Iran: Ilam	Soil	OL772663	KU659141
<i>Blastobotrys proliferans</i>	CBS 522.75 = NRRRL Y-17577 = ATCC 34216 = CCM F-493 = HFM 2673 = UAMH 4666 (ex-type)	Brazil	Mite-infested nut (<i>Bertholletia excelsa</i>)	EU343812	DQ442684
<i>Blastobotrys raffinosisfermentans</i>	CBS 6800 = NRRRL Y-27150 (ex-type)	Unknown	Unknown	KY101759	DQ442698
<i>Blastobotrys robertii</i>	CBS 10106 = NRRRL Y-27775 (ex-type)	The Netherlands: Wageningen forest	Rotten pine wood (<i>Pinus sylvestris</i>)	KY101760	DQ839395
<i>Blastobotrys serpentis</i>	CBS 10541 = NRRRL Y-48249 = MTCC 8332 = W113A = YS W113A (ex-type)	India: Hyderabad City	Trinket snake gut	KY101761	AM410667
<i>Blastobotrys terrestris</i>	CBS 7376 = NRRRL Y-17704 = CSIR Y914 = IFO 10859 = IGC 5133 = PYCC 5133 (ex-type)	South Africa: Barberton	Soil	KY101762	DQ442683
<i>Blastobotrys vanleenenianus</i>	CBS 14902 (ex-type)	The Netherlands	Soil	MG986487	MG986492
<i>Blastobotrys xishuangbannaensis</i>	CBS 16044 = CICC 33360 (ex-type)	China: Yunnan Province, Jinghong	Rotting wood	MK682811	MK682809
<i>Trichomonascus apis</i>	CBS 10922 = NRRRL Y-48475 = NCAIM Y.01848 (ex-type)	Hungary	Mouldy honeycomb	KY105699	EU790643

Table 1 (continued)

Taxon name	Strains ^a	Location	Source	GenBank: ITS	GenBank: LSU
<i>Trichomonascus cijferrii</i>	CBS 5295 = NRRL Y-10943 = ATCC 58443 = CCRC 21427 = Goto TH-26 = IFO 1854 = IGC 4164 = IMI 344641 = JCM 7621 (ex-type)	The Netherlands	Pig	AY493435	DQ442681
<i>Trichomonascus petasosporus</i>	CBS 9602 = NRRL Y-B2092 (ex-type)	USA: Missouri, Salem	White Oak (<i>Quercus sp</i>)	KY105704	DQ42691
<i>Wickerhamiella domer-cqiae</i>	CBS 4351 = NRRL Y-6692 (ex-type)	South Africa	Wine vat	DQ911463	DQ438240

^a Acronyms of culture collections in alphabetic order: ATCC, American Type Culture Collection, Manassas, Virginia; CBS, Westerdijk Fungal Biodiversity Institute (formerly Centraalbureau voor Schimmelfcultures), Utrecht, the Netherlands; CCM, Czech Collection of Microorganisms, Brno, Czech Republic; CCRC, Bioresources Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan; CCY, Culture Collection of Yeasts, Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia; CICC, China Center of Industrial Culture Collection, Beijing, China; CMW: (culture collection housed at the Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa); CSJR, Council for Scientific and Industrial Research, Pretoria, South Africa; DAOMC, Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada; DTO, Internal Culture Collection of the Department of Applied and Industrial Mycology of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; IBRC, Iranian Biological Resources Center, Academic Center for Education Culture and Research (ACECR), Tehran, Iran; IFO, Institute for Fermentation, Osaka, Yodogawa-ku, Osaka, Japan; IGC, Center of Biology, Gulbenkian Institute of Science, Oeiras, Portugal; IJFM, Instituto Jaime Ferrán de Microbiología Consejo Superior de Investigaciones Científicas, Madrid, Spain; IMI, CABI's collection of fungi and bacteria, Wallingford, UK; JCM, Japan Collection of Microorganisms, Tsukuba, Japan; MTCC, Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India; MUCL, Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; NBRC, NITE Biological Resource Center, Department of Biotechnology, National Institute of Technology and Evaluation, Kisarazu, Chiba, Japan; NCAMM, National Collection of Agricultural and Industrial Microorganisms, Department of Microbiology and Biotechnology, University of Horticulture and Food Industry, Budapest, Hungary; NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, US Department of Agriculture, Peoria, IL, USA; PYCC, Portuguese Yeast Culture Collection, C. R. M., New University of Lisbon, Lisbon, Portugal; UAMH, UAMH Centre for Global Microfungal Biodiversity (formerly University of Alberta Microfungus collection and Herbarium), Gage Research Institute, University of Toronto, Toronto, Canada; URM, culture collection at the Federal University of Pernambuco, Recife, Brazil; Others, personal collections and isolate numbers

234 (ZW6-S18) was steam cleaned twice after *Blasto-*
 235 *botrys* reappeared after the first cleaning attempt, but
 236 seven months later the fungus again formed visible
 237 growth on the wood surface. Next, a treatment with
 238 70% alcohol is planned in the hope that this will be
 239 sufficient in the long term.

240 Species delineation and phylogenetic placement of *B.*
 241 *nigripullensis* sp. nov

242 Phylogenetic analyses based on ITS and LSU
 243 included all species currently accepted in
 244 *Blastobotrys/Trichomonascus* (Visagie et al. 2023).

The ITS and LSU alignments were 669 and 576 bp
 long, respectively, with TVM+I+G being the
 best model for the ITS partition and GTR+G the
 best for the LSU partition. *Blastobotrys nigripul-*
lensis resolved on a fully supported branch distinct
 of all other species (Fig. 2). The tree had a general
 lack of backbone support, and its closest relative(s)
 could not be determined as a result. Two DNA reads
 in Genbank, namely clones CMH114 (KF800205)
 and S23T-59 (KU164259), represent the same spe-
 cies. KF800205 originates from house dust and/or
 indoor air collected from Kansas City (USA) (Rit-
 tenour et al. 2014), and KU164259 originate from

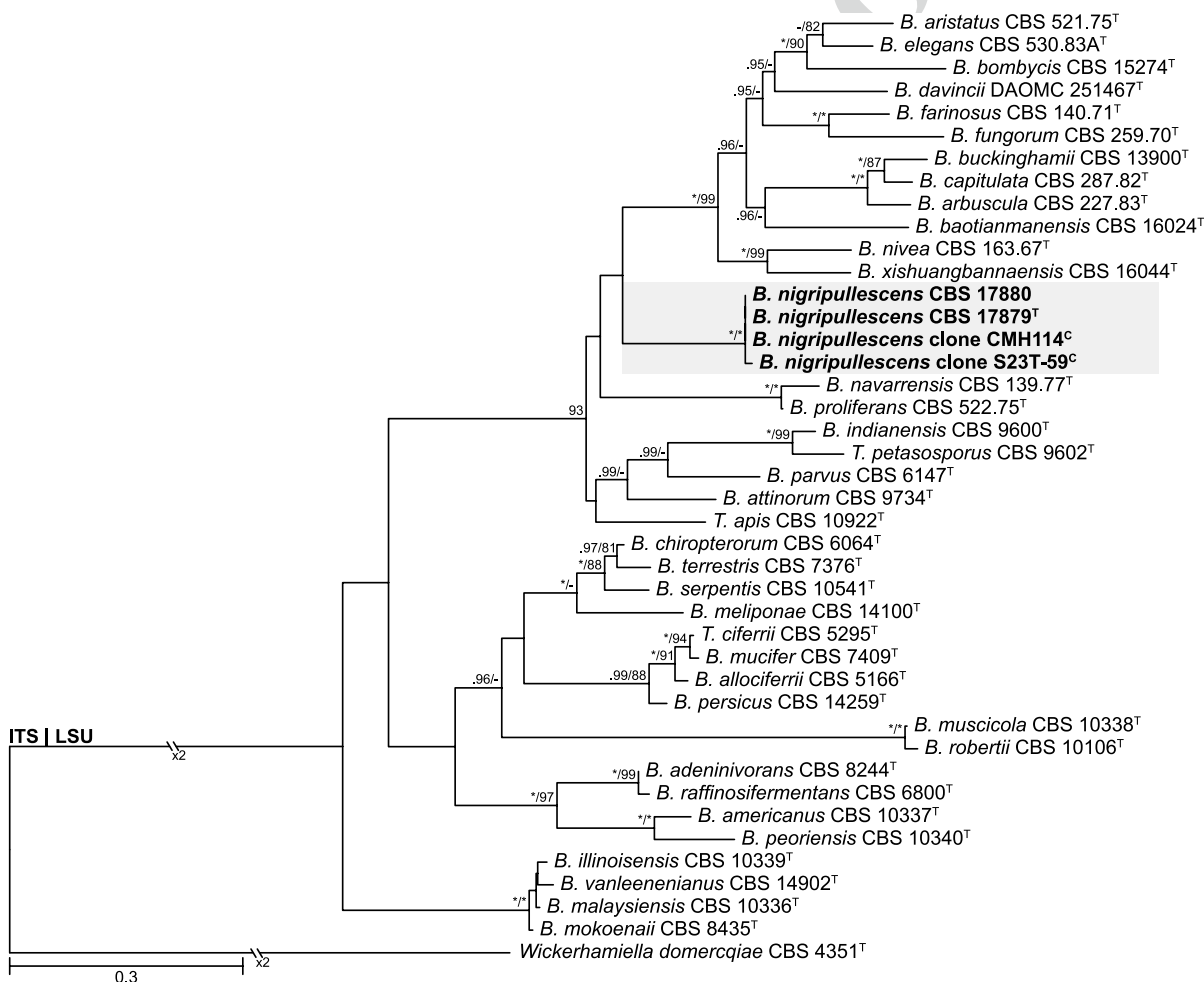


Fig. 2 Combined phylogenetic tree of described *Blastobotrys* and *Trichomonascus* species based on ITS and LSU, indicating the phylogenetic placement of *Blastobotrys nigripullensis*. The tree is rooted to *Wickerhamiella domercqiae* CBS 3451^T. Posterior probabilities (≥ 0.95) and Bootstrap support values

($\geq 80\%$) are given above branches. The new species is indicated in bold text and grey box, ^T = ex-type strain, ^c = sequence from cloned DNA fragment, and GenBank accession numbers are shown in a smaller font (ITS – green; LSU – blue)

258 sea water collected from an unknown locality. Similarities between *B. nigripullensis* (CBS 17879^T) and
 259 other species were low with LSU that differed by 42nt
 260 or more (based on alignment from 5'-ATTGCCCTA
 261 GTAACGGCGAGCG-3') and ITS by 130nt or more
 262 (based on alignment from 5'-AAGGATCATTAC
 263 CGAA-3') nucleotide substitutions, thus warranting
 264 recognition as a distinct species.

265 The new species lacks the typical morphology of
 266 most *Blastobotrys* as it did not form the branched
 267

268 conidiophores ending in conidiogenous cells produc-
 269 ing blastoconidia as seen in e.g., *B. davincii* and other
 270 *Blastobotrys* (and those formerly classified in *Tricho-*
 271 *monascus*) species (Smith et al. 2011a, b, Visagie
 272 et al. 2023). It does, however, resemble conidiophores
 273 produced by most *Sporothrix* species (Fig. 3). The
 274 nutritional growth requirements of strains CBS 17879
 275 and CBS 17880 are presented in Table 2 and can be
 276 summarized as follows: the strains ferment glucose,
 277 but no other sugars tested; they utilize many of the

Fig. 3 *Blastobotrys nigripullensis* (CBS 17879^T). **A** Colonies on, from left to right, YMA and GYPA. Colony close-ups on MYA **B**, PDA **C**, MEA **D** and YMA **E**. **F–O**. Conidiophores and conidia from YMA observed under light microscope. Scale bars 10 μm

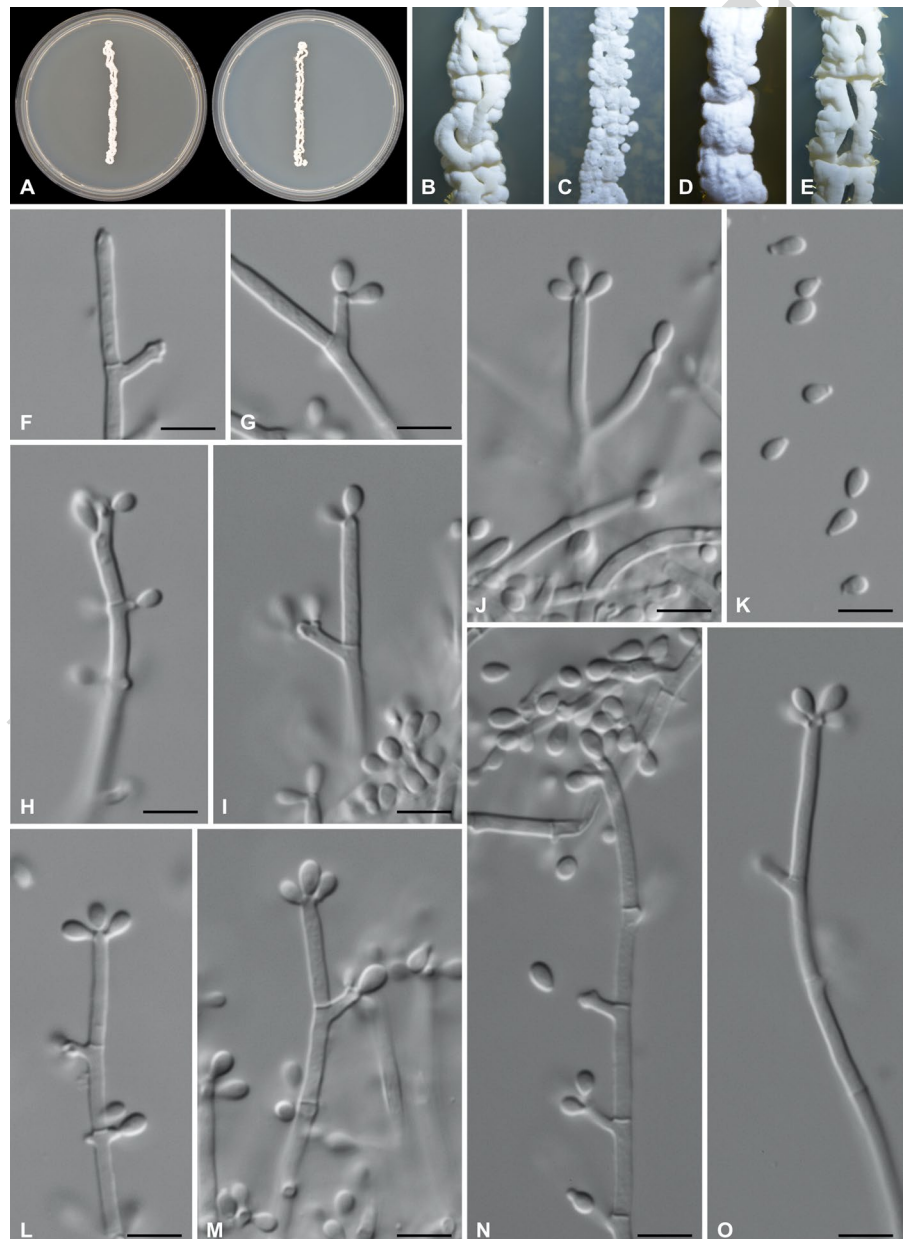


Table 2 Growth characteristics of two isolates of *Blastobotrys nigripullensis*

	CBS 17879	CBS 17880		CBS 17879	CBS 17880
Growth on carbon compounds			Fermentation		
D-Glucose	+	+	D-Glucose	d	d
D-Galactose	+	+	Galactose	-	-
L-Sorbose	+	+	Maltose	-	-
D-Glucosamine	+	+	Sucrose	-	-
D-Ribose	+	+	Trehalose	-	-
D-Xylose	+	+	Lactose	-	-
L-Arabinose	+	+	Raffinose	-	-
D-Arabinose	w,d	w	Xylose	-	-
L-Rhamnose	-,w,d	w,d	Growth on nitrogen compounds		
Sucrose	+	+	Nitrate	-	-
Maltose	-	-	Nitrite	-	v [inhibition zone]
α , α Trehalose	+	+	Ethylamine	+	+
Methyl α -glucoside	-	-	L-Lysine	+	+
Cellobiose	+	+	Cadaverine	+	+
Salicin	+	+	Creatine	-	-
Arbutin	+	+	Creatinine	-	-
Melibiose	-,w	-	Glucosamine	-	-
Lactose	+	+	Imidazole	-	-
Raffinose	-	-	Proline	-	-
Melezitose	-	-	Tryptophane	-	-
Inuline	w	w	Growth without vitamins: not done		
Soluble starch	-	w	Other tests		
Glycerol	+	+	Growth with 0,01% cycloheximide	+	+
meso Erythritol	+	+	Growth with 0,1% cycloheximide	+	+
Ribitol	+	+	Growth on 50% glucose	+	+
Xylitol	-	-	Growth on 60% glucose	-	-
L-Arabinitol	w	w	Starch production	-	-
D-Glucitol	+,d	+	Urea test	-	-
D-Mannitol	+	+	DBB reaction	-	-
Galactitol	+	+	MYA 30% glucose	+	+
myo-Inositol	+	+	MYA 50% glucose	+	+
Glucono d-lactone	-	-	MEA 20% sucrose	+	+
2-Keto-D-gluconate	-	w	MEA 40% sucrose	+	+
D-Gluconate	-	-	MEA 12,5% NaCl	+	+
D-Glucuronate	d	d	CMA 18% NaCl	+	+
D-Galacturonate	-	-	CMA 36% NaCl	+	+
DL-Lactate	-	-	Growth at various temperatures		
Succinate	-	-	4 °C	-	-
Citrate	-	-	10 °C	w	w
Methanol	-	w	18 °C	+	+
Ethanol	d	d,w	30 °C	+	+
Propane 1,2 diol	d,w	d,w			
Butane 2,3 diol	w,-	d			
Quinic acid	-	-			
Saccharate	-	-			

Table 2 (continued)

	CBS 17879	CBS 17880		CBS 17879	CBS 17880
Galactonic acid	–	–	35 °C	–	–

Abbreviations and symbols: +, growth; –, no growth; d, delayed growth; w, weak growth

278 tested carbon sources, but no maltose, and do not
279 utilize nitrate and nitrite; the species is mesophilic
280 and able to grow from 18 to 30 °C, but not at 35 °C;
281 no growth in 0.1% cycloheximide, no production of
282 starch or starch-like compounds; growth in substrates
283 with elevated sugar (50%) and NaCl (36%) concentra-
284 tions. Salient growth characteristics of the new spe-
285 cies, and species that belong to the two most closely
286 related clades are given in Supplemental Table 1. Like
287 *B. baotianmenensis* the species does not utilize malt-
288 ose, but can be separated from this species by growth
289 on L-sorbose, D-xylose, L-arabinose, D-ribose, etha-
290 nol, galactitol, mannitol, glucitol, and myo-inositol.

291 Here we adopt a phylogenetic species concept to
292 delineate our new species and formally describe it
293 below as *B. nigripullensis*.

294 Taxonomy

295 *Blastobotrys nigripullensis* Boekhout, M. Groe-
296 new., Meijer, and Visagie, sp. nov.—Mycobank MB
297 847655; Fig. 3.

298 *Etymology*: Latin, *nigripullensis*, after **Nigrum**
299 **Pullum**, the Roman fort located at Zwammerdam,
300 province Zuid-Holland, the Netherlands, along the
301 Rhine River [now Old Rhine River].

302 *Type specimen*: THE NETHERLANDS, Zwammerdam,
303 isolated from wood of Roman shipwreck, 15 Sep-
304 tember 2021, collected by Y. Vorst, (holotype CBS
305 H-25194 (preserved in a metabolically inactive state
306 in the fungarium), culture ex-type CBS 17879=DTO
307 457-E3).

308 *DNA barcodes*: ITS = OQ876815,
309 LSU = OQ876820.

310 *UNITE species hypothesis*: [https://unite.ut.ee/bl_](https://unite.ut.ee/bl_forw_sh.php?sh_name=SH2279294.08FU)
311 [forw_sh.php?sh_name=SH2279294.08FU](https://unite.ut.ee/bl_forw_sh.php?sh_name=SH2279294.08FU).

312 *Description*: Sporothrix-like. After two weeks at
313 25 °C in 5% glucose in yeast nitrogen broth, white
314 floating flocks and white sediment occur with short
315 hyphal cells, 6–16×2.5–3.5 µm, with sympodial
316 and lateral budding. After two weeks at 25 °C on
317 yeast morphology agar, colonies ca. 7 mm wide, flat,

dry, and tough, dull white to cream, under lens sur- 318
face venose, with entire margin. Hyphae branched, 319
hyaline, 1.5–3 µm wide, with lateral, clavate blasto- 320
conida, 3–7×1–3.5 µm, on short to longer denticles 321
forming a sympodial rachis of blastoconidia; thicker, 322
inflated regions, 7–130×7–20 µm, may be present 323
with finger-like outgrowths. Dalmau plate on yeast 324
morphology agar with short adhering filaments. Simi- 325
lar growth on GYP, YMA, PDA and MEA. Fermenta- 326
tion, assimilation, results from other growth and 327
biochemical tests, and growth at different tempera- 328
tures are listed in Table 2. 329

330 *Additional strains examined*: THE NETHERLANDS,
331 Zwammerdam, isolated from wood of Roman ship-
332 wreck, 15 September 2021, collected by Y. Vorst,
333 CBS 17880 = DTO 455-G9.

Discussion 334

335 The morphology of *B. nigripullensis* resembles that 336
of the genus *Sporothrix*, more than that of *Blastobot-* 337
rys/Trichomonascus (Smith et al. 2011a, b, Visagie 338
et al. 2023) as the typical inflated conidiogenous cells 339
of the latter are absent. The molecular phylogeny, 340
however, clearly shows that *B. nigripullensis* belongs 341
to the core clade of *Blastobotrys/Trichomonascus* that 342
include the generic type species, *Blastobotrys nivea*.

343 *Blastobotrys* species are common and previously 344
found on straw, rotting/dead wood, mushrooms, ~~on~~ 345
nuts and black pepper; in soil compost, the indoor 346
environment (dust and air), the Antarctic Ocean and 347
open waters. Species of the genus are usually osmo- 348
tolerant, xerotolerant and thermotolerant, and some 349
are applied in biotechnology (Visagie et al. 2023). 350
Blastobotrys nigripullensis was isolated from wood 351
of a Roman shipwreck that was preserved in alluvial 352
clay deposits near the former Roman fort of *Nigrum* 353
Pullum, located in the present-day village of Zwa- 354
mmerdam, province Zuid-Holland, the Netherlands. 355
The oak of the shipwreck was preserved with PEG 356
4000, but in this case, due to an undetected leak, the 357
preservative washed out and, consequently, the wood

358 slowly dried. This resulted in some visual growth of
 359 fungi that may be tolerant to high PEG concentra-
 360 tions. Two DNA clones that originated from house
 361 dust in the USA and seawater, had the same ITS
 362 sequences and might indicate the presence of *B.*
 363 *nigripullensis* in those samples as well. It indicates a
 364 rather broad ecological and, likely, also a broad geo-
 365 graphical preference. The new *Blastobotrys* species
 366 was isolated together with *Microascus melanosporus*
 367 (predominant), *Microascus paisii* and a member of
 368 the *Acremonium chrysogenum*-clade. Both *Microas-*
 369 *cus* species are widespread and prefer substrates with
 370 cellulose, while *Acremonium* species occurs in soils
 371 and on decaying plant matter (Samson et al. 2019).
 372 It will therefore be interesting to investigate further
 373 whether the novel species is also able to assimilate
 374 substrates such as cellulose. Preliminary experiments
 375 did not observe growth of *B. nigripullensis* strains on
 376 agar media with lignin and ferulic acid (R.P. de Vries,
 377 unpubl. observations).

378 *Blastobotrys davincii* was recently described from
 379 house dust collected in Canada and has previously
 380 been detected in environmental DNA from several
 381 historically important artifacts, including a mummy
 382 (Palermo, Italy), Leonardo da Vinci's self-portrait
 383 and cave paintings (Maijishan Grottoes, China) (Vis-
 384 agie et al. 2023). Together with *B. nigripullensis* iso-
 385 lated from a Roman shipwreck, albeit treated with
 386 PEG, it seems that *Blastobotrys* can occur on histori-
 387 cal artefacts and may be more common than previ-
 388 ously thought when dealing with such substrates. The
 389 discovery of *B. nigripullensis* raised the question of
 390 whether the yeast was already present when the wood
 391 was processed and is therefore a species from Roman
 392 times or whether it arrived via infiltration of ground-
 393 water into the archaeological site or after excavation,
 394 entering the conservation process. Since it is a spe-
 395 cies of a genus with representatives that has also been
 396 found on other historical and ancient objects, might
 397 a contemporary origin not be as likely as later con-
 398 tamination? Could *B. nigripullensis* have flourished
 399 again under the right circumstances, in this case
 400 pieces of PEG-washed out degrading wood? More
 401 research is needed to answer this question. The ini-
 402 tial isolation of *B. nigripullensis* from the wooden
 403 remains was done on MY50G, an agar medium con-
 404 taining 50% glucose, which is only used to isolate
 405 extremely xerophilic fungi. However, this medium
 406 is not routinely used by most laboratories dealing

with fungi on archaeological remains. Extreme xero-
 407 philes, such as *Aspergillus halophilicus* that grows
 408 on climate-controlled stored heritage artefacts, can
 409 only be detected and grown on media with low water
 410 activity (e.g. MY50G) (Bastholm et al. 2022), while
 411 *B. nigripullensis* can also grow on media with higher
 412 water activity. The species was able to grow on agar
 413 media with up to 36% NaCl, indicating salt tolerance.
 414 Further investigations are needed to understand why
 415 this yeast grew on PEG-contaminated remains of
 416 a Roman shipwreck. 417

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