INTRODUCTION

Phialophora verrucosa is the type species of the genus Phialophora, which belongs to the family Herpotrichiellaceae (Chaetothyriales) comprising the black yeasts and relatives. This phylogenetic affiliation excludes numerous species that have been classified in older literature in Phialophora on the basis of the combination of morphological characters of a melanised thallus and one-celled, sticky conidia that are produced through large phialidic collarettes in a poorly differentiated conidial apparatus. Gams (2000) provided an overview of phialophora-like fungi and found that according to current standards belong in nine orders of Ascomycota; for nearly all of these, separate generic names are available at present.

Numerous assexual species in the Chaetothyriales classified in Cladophialophora, Exophiala or Fonsecaeae show presence of phialophora-like synasexual morphs on nutritionally poor media, demonstrating the taxonomic coherence of species belonging to this order (De Hoog et al. 1999). Such phialidic synsexual morphs are also known in Cladophialophora carrionii, the agent of human chromoblastomycosis in arid climates and one of the nearest neighbours of P. verrucosa in molecular phylogeny. Although strictly monomorphic for phialides, P. verrucosa phylogenetically belongs to a group as the ‘carrionii-clade’ with Cladophialophora carrionii as the core species.

Several other but unrelated monomorphic phialophora-like lineages are known in the Chaetothyriales. Phialophora europaea, P. reptans, known from superficial skin infections in humans (Saunte et al. 2012), P. attae and P. capigurarae, from ant nests (Attili-Angelis et al. 2014), P. sessilis from inert surfaces (Carett et al. 2006, Zhuang et al. 2010), P. livistonae, from living plant leaves (Crous et al. 2012) and P. oxyospora are all members of the ‘europaea-clade’ (De Hoog et al. 2011, Feng et al. 2012). This clade was given family status as Cyphellophoraceae by Réblová et al. (2013) and as a consequence some of the member species were reclassified in Cyphellophora.

As a result of the above rearrangements, the genus Phialophora, for which the Index Fungorum lists 92 species names (as per 01-01-2016), from a phylogenetic viewpoint is restricted to P. verrucosa and its sister species Phialophora americana, as they both cluster in the ‘carrionii-clade’. Species of this clade, i.e. Cladophialophora carrionii, Cl. samoensis and P. verrucosa have been reported from mutilating cases of chromoblastomycosis, disseminated phaeohyphomycosis and mycetoma, which all can be chronic and refractory to therapy (McGinnis 1983, Turiansky et al. 1995, Hofmann et al. 2005, Seyedmousavi et al. 2014). Phialophora americana, a sister species of P. verrucosa is mostly regarded as being environmental. Also Cl. carrionii has an environmental sibling, viz. Cladophialophora yegresii (De Hoog et al. 2007). The bipartition clinical / environmental is however ambiguous. Phialophora verrucosa was first reported as a human pathogen a century ago (Lane 1915, Medlar 1915a, b), but fungi under this name have also been isolated from natural soils and plant debris (Gezuele et al. 1972). For most of these reports no material is known to be preserved and misidentifications with numerous phialophora-like fungi may have been concerned (Gams 2000, Lopez Martinez & Mendez Tovar 2007).

Recent studies have proven that molecular techniques have a higher precision in segregating phenotypically similar species that may differ in pathogenicity (Marimón et al. 2006, 2007). In black yeasts and allied fungi, molecular siblings may differ significantly in virulence; compare for example the neurotropic Cladophialophora bantiana and the gaselone-associated fungus Cl. psammophila (Badali et al. 2011). Internal transcribed spacer (ITS) sequencing is effective for species identification among black yeasts, as has been proven with the aid of multilocus studies (Zeng & De Hoog 2008, Heinrichs et al. 2012). Multilocus verification is available for the P. verrucosa / P. americana complex (Unterreiner et al. 2008). Molecular typing of mitochondrial DNA using restriction fragment length polymorphisms...
Genomic DNA was extracted and purified from approximately
served both on MEA and OA. For two additional weeks. In addition, gross morpho-
was fungicidal, cultures were returned to 30 °C and incubated at 30 °C for 7, 14 or 21 d in a closed plastic box with
Eclipse 80i microscope and DS Camera Head DS-Fi1/DS-5 m/
without pigments. Micrographs were taken using a Nikon
Morphology and physiology
One hundred and twenty-six isolates that were initially identified as P. verrucosa based on morphology from across the world and including 32 from clinical samples, 89 from the environment, and five from unknown sources were analysed (Table 1). Strains were obtained from the Research Center for Medical Mycology at Peking University from 1997 to 2014, and from the reference collection of the Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands. *Phialophora america*
*Capronia semimmersa*, **C. svrekiana**, **C. carri-
were included along with ecological data.
**MATERIALS AND METHODS**
**Strains studied**
One hundred and twenty-six isolates that were initially identified as P. verrucosa based on morphology from across the world and including 32 from clinical samples, 89 from the environment, and five from unknown sources were analysed (Table 1). Strains were obtained from the Research Center for Medical Mycology at Peking University from 1997 to 2014, and from the reference collection of the Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands. *Phialophora america*
*Capronia semimmersa*, **C. svrekiana**, **C. carri-
were included along with ecological data.
**Morphology and physiology**
For microscopy, small blocks were inoculated with three-point
on slants of potato dextrose agar (PDA; Difco, Detroit, USA) at 30 °C for up to 7 d until rich sporulation was obtained. Observations were done with slide cultures using corn meal agar (CMA; Difco). Agar blocks of ~ 0.5 cm² were placed on the agar plate and inoculated at the four sides. The block was subsequently covered with a sterile cover slip (~ 2 cm²). Plates were incubated at 30 °C for 7, 14 or 21 d in a closed plastic box with sterile gauze soaked with 5 mL sterile water to avoid drying of the culture. Slides were made by Shear’s mounting medium without pigments. Micrographs were taken using a Nikon Eclipse 80i microscope and DS Camera Head DS-Fi1/DS-5 m/
was fungicidal, cultures were returned to 30 °C and incubated at 30 °C for 7, 14 or 21 d in a closed plastic box with sterile gauze soaked with 5 mL sterile water to avoid drying of the culture. Slides were made by Shear’s mounting medium without pigments. Micrographs were taken using a Nikon Eclipse 80i microscope and DS Camera Head DS-Fi1/DS-5 m/
were deposited in TreeBASE (number: 19135). Phylogenetic reconstructions were done for each locus and ITS-TEF1-BT2 combined using neighbour-joining (NJ), maximum likelihood (ML) and maximum parsimony (MP) implemented in MEGA v. 6.06 (Kimura 1980, Felsenstein 1985, Saitou & Nei 1987), Sequences were aligned with Clustal W v. 1.6. Alignments were also included in the study.
**RESULTS**
**DNA extraction**
Genomic DNA was extracted and purified from approximately 1 cm² of fungal elements according to the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) with disruption of cells by glass beads (425–600 μm) (Sigma-Aldrich, Zwijndrecht, The Nether-
was fungicidal, cultures were returned to 30 °C and incubated at 30 °C for 7, 14 or 21 d in a closed plastic box with sterile gauze soaked with 5 mL sterile water to avoid drying of the culture. Slides were made by Shear’s mounting medium without pigments. Micrographs were taken using a Nikon Eclipse 80i microscope and DS Camera Head DS-Fi1/DS-5 m/DS-2Mv/DS-2MBW using NIS-Element freeware package (Nikon Europe, Badhoevedorp, The Netherlands).
Cardinal growth temperatures were determined in triplicate on 2 % malt extract agar (MEA; Difco) by measuring colony diameters for a selection of 28 strains based on phylogenetic results. Plates were incubated in the dark for 3 wk at 21, 24, 27, 30, 33, 37 and 40 °C. In order to evaluate whether 37 °C and 40 °C was fungicidal, cultures were returned to 30 °C and incubated for 2 additional weeks. In addition, gross morphology was observed both on MEA and OA.
**DNA amplification and sequencing**
The following nuclear genes were amplified by PCR: ITS and partial TEF1, BT2, SSU and LSU. PCR amplifications and sequencing primers are shown in Table 2. Amplifications were done by the 2× EasyTaq PCR Super Mix protocol (TransGen Biotech, Beijing, China). Fifty to 100 ng of DNA template and a 0.2–0.4 μM concentration of forward and reverse primers were added in a total volume of 25 μL. Amplification was performed in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) and included initial denaturation at 94 °C for 5 min, followed by 30 cycles consisting of denaturation at 94 °C for 30 s, annealing for 30 s at 54 °C (ITS, BT2, SSU and LSU) or 52 °C (TEF1), and extension for 30 s (ITS, BT2 and TEF1) or 1 min (SSU and LSU) at 72 °C. A final extension step of 72 °C for 10 min was included. Reading was done with Gel Doc XR+ system (Biorad, Hercules, CA, USA) with Trans2K Plus DNA Marker (TransGen Biotech) as size and concentration marker. Purification was performed with silica bead DNA Gel Extraction Kit (Thermo Fisher Scientific, Vilnius, Lithuania), sequencing with an ABI 3730 automatic sequencer (Applied Biosystems, Foster City, CA, USA) and sequence data were adjusted by SeqMan Pro (DNASisar, Madison, WI, USA). GenBank accession numbers are given in Table 1 except for the TEF1 region because the sequence length was less than 200 bp.
**Alignment and phylogenetic reconstruction**
Sequence data were aligned with Clustal W v. 1.6. Alignments were also included in the study.
Sequence data were aligned with Clustal W v. 1.6. Alignments were also included in the study.
| Species | Culture no. | Other reference | Source | Geography | GenBank accession numbers | References |
|---------|-------------|----------------|--------|-----------|--------------------------|------------|
| **P. americana** | CBS 40067 | | Soil | Brazil | EU514695, EU514708 | Untereiner et al. (2008) |
| | CBS 281135 | ATCC 4806; IMI 021191; MUCL 41728, NH 8719; UAMH 9609 | Chromoblastomycosis, verrucous | USA | EU514694, EU514707 | Untereiner et al. (2008) |
| | NY S 323-90 | | – | – | U31831, U31832, U31833 | Yan et al. (1995) |
| | CBS 22197 | CDC B-2723; IMI 17010; MUCL 40613 | – | Uruguay | U31833, U31834, U31835 | Yan et al. (1995) |
| | CBS 22097 | ATCC 51962; CDC 5; MUCL 40612 | Tree bark | Virginia, USA | U31836, U31837, U31838 | Yan et al. (1995) |
| **P. americana, originally identified as Capronia semilimosa** | UAMH 10875 (T) | CDC 10; Conant 333 | Wood pulp | USA | EU514696, EU514712 | Untereiner et al. (2008) |
| | UAMH 10876 | C.J.K. Wang 1050; WUC 402 | Wood | USA | EU514697, EU514713 | Untereiner et al. (2008) |
| | MUCL 40572 | AF-TOL 655 | – | France | AF05259, AF05260 | Untereiner & Naveau (1999), Untereiner et al. (2008) |
| | MUCL 39979 | | Rotten wood | USA | AF05260, EU514702 | Untereiner et al. (2008) |
| **P. americana, originally identified as Capronia svrcekiana** | UAMH 10874 | | Wood | Czech Republic | EU514693, EU514706 | Untereiner et al. (2008) |
| | UAMH 10873 | | Wood | Czech Republic | EU514692, EU514705 | Untereiner et al. (2008) |
| | UAMH 10872 | | Wood | Czech Republic | EU514691, EU514704 | Untereiner et al. (2008) |
| **P. americana, originally identified as P. verrucosa** | BMU 01245 | CBS 140292 | Chromoblastomycosis | North China | KF819141, KF819142 | This study |
| | BMU 01244 | CBS 140291; DCU-600, ATCC 38561, IFM 4928 | Subcutaneous cyst | Japan | AB190375, KF819143 | Iwasu & Miyaji (1978); this study |
| **Capronia svrcekiana** | BMU 01215 | CBS 140309 | Tree bark | Jiamusi, northeast China | KF819147, KF819148 | This study |
| | BMU 06860 | CBS 140311 | Soil of patient's garden | Hebei, north China | KF819150, KF819151 | This study |
| | BMU 04641 | CBS 140312 | Leaf of Changbai Shan | Changchun, northeast China | KF819151, KF819152 | This study |
| | BMU 00131 | | Dead wood | Beijing, north China | KF819152, KF819153 | This study |
| | BMU 00132 | | Wheat | Jiamusi, northeast China | KF819154, KF819155 | This study |
| | BMU 05997 | | Soil of patient's garden | Hebei, north China | KF819156, KF819157 | This study |
| | BMU 04693 | | Soil | Changchun, northeast China | KF819158, KF819159 | This study |
| | BMU 04607 | | Tree bark | Changchun, northeast China | KF819161, KF819162 | This study |
| | BMU 04622 | | Leaf | Changchun, northeast China | KF819162, KF819163 | This study |
| | BMU 00121 | | Leaf | Jiamusi, northeast China | KF819163, KF819164 | This study |
| | BMU 00101 | CBS 140307 | Soil | Xingjiang, northwest China | KJ700955, KM658110 | This study |
| | BMU 00107 | | Soil | Xian, northwest China | KJ700945, KM658122 | This study |
| | BMU 00109 | | Soil | Xian, northwest China | KJ700945, KM658122 | This study |
| | BMU 00110 | | Soil | Xian, northwest China | KJ700949, KM658125 | This study |
| | BMU 00111 | | Soil | Xian, northwest China | KJ700951, KM658127 | This study |
| | BMU 00114 | | Soil | Harbin, northeast China | KJ700955, KM658132 | This study |
| | BMU 00117 | | Soil | Xian, northwest China | KJ700962, KM658138 | This study |
| | BMU 00118 | | Soil | Xian, northwest China | KJ700964, KM658140 | This study |
| | BMU 00117 | | Soil | Xian, northwest China | KJ700957, KM658133 | This study |
| | BMU 00170 | | Soil | Beijing, north China | KJ700944, KM658120 | This study |
| | BMU 00206 | | Soil | Beijing, north China | KJ700958, KM658134 | This study |
| | BMU 00432 | | Soil | Jiamusi, northeast China | KJ700967, KM658143 | This study |
| | BMU 04506 | CBS 140329 | Soil | Changchun, northeast China | KJ700965, KM658131 | This study |
| | BMU 04524 | | Soil | Changchun, northeast China | KJ700963, KM658139 | This study |
| | BMU 04528 | | Soil | Changchun, northeast China | KJ700950, KM658126 | This study |
| | BMU 04532 | | Soil | Changchun, northeast China | KJ700968, KM658144 | This study |
| | BMU 04538 | | Soil | Changchun, northeast China | KJ700954, KM658130 | This study |
| | BMU 04554 | | Tree bark | Changchun, northeast China | KJ700959, KM658135 | This study |
| | BMU 07607 | | Soil | Shanghái, east China | KJ700969, KM658145 | This study |
| | BMU 07608 | | Soil | Shanghái, east China | KJ700970, KM658146 | This study |
| | BMU 07617 | | Soil | Shanghái, east China | KJ700976, KM658082 | This study |
Table 1 (cont.)

| Species | Culture no. | Other reference | Source | Geography | GenBank accession numbers<sup>1</sup> | References |
|---------|-------------|-----------------|--------|-----------|--------------------------------------|------------|
| P. americana, originally identified as | BMU 07625 | CBS 140305 | Leaf | Huangzhou, east China | KJ700981 KJ700982 | This study |
| P. verrucosa (cont.) | BMU 07626 | CBS 140327 | Leaf | Chongqing, southwest China | KJ700985 KJ700986 | This study |
|              | BMU 07645 | CBS 140313 | Leaf | Chongqing, southwest China | KJ700987 KJ700988 | This study |
|              | BMU 07650 | CBS 140310 | Leaf | Chongqing, southwest China | KJ700993 KJ700994 | This study |
|              | BMU 07653 | CBS 140312 | Leaf | Chongqing, southwest China | KJ700996 KJ700997 | This study |
|              | BMU 07643 | CBS 140315 | Wood | Lijiang, southwest China | KJ701005 KJ701006 | This study |
|              | BMU 07660 | CBS 140301 | Decaying wood | Lasa, southwest China | KJ701010 KJ701011 | This study |
|              | BMU 07696 | CBS 140302 | Decaying trunk | Shanghai, east China | AF050283 EU514711 | Untereiner & Naveau (1999), Untereiner et al. (2008) |
|              | BMU 07610 | IFM 41871 | Soil | Colombia | AB550778 – | Takizawa et al. (2011) |
|              | CBS 840.69 | g281331169 | Japanese flounder | Japan | AB536235 – | – |
|              | PBS 102234 | CBS 140300 | Decaying trunk | Brazil | KU306358 KU306351 | – |
| P. chinensis, originally identified as | BMU 02669 | CBS 140300 | Chromoblastomycosis | Guangdong, south China | KF881930 KF881964 | This study |
| P. verrucosa | BMU 01890 (T) | CBS 140326 | Chromoblastomycosis | Guangdong, south China | KF881948 KF881955 | This study |
|              | IFM 51934 | – | Human | China | AB50779 – | Takizawa et al. (2011) |
|              | BMU 00441 | CBS 140310 | Wood | Hakou, south China | KF881948 KF881949 | This study |
|              | BMU 00127 | CBS 140308 | Tree bark | Hakou, south China | KF881949 KF881950 | This study |
|              | BMU 00447 | CBS 140303 | Bank | Zhanjiang, south China | KF881950 KF881951 | This study |
|              | BMU 00104 | CBS 140304 | Soil | Xian, northwest China | KJ700960 KJ700961 | This study |
|              | BMU 00112 | CBS 140305 | Soil | Haerbin, northeast China | KJ700961 KJ700962 | This study |
|              | BMU 00150 | CBS 140306 | Soil | Haerbin, northeast China | KJ700962 KJ700963 | This study |
|              | BMU 00105 | CBS 140328 | Soil | Xian, northwest China | KJ700963 KJ700964 | This study |
|              | BMU 07601 | CBS 140311 | Soil | Shanghai, east China | KJ700964 KJ700965 | This study |
|              | BMU 07612 | CBS 140314 | Wood | Shanghai, east China | KJ700965 KJ700966 | This study |
|              | BMU 07613 | CBS 140318 | Wood | Shanghai, east China | KJ700966 KJ700967 | This study |
|              | BMU 07615 | CBS 140319 | Bamboo | Shanghai, east China | KJ700967 KJ700968 | This study |
|              | BMU 07616 | CBS 140320 | Soil | Shanghai, east China | KJ700968 KJ700969 | This study |
|              | BMU 07621 | CBS 140321 | Soil | Guangzhou, south China | KJ700969 KJ700970 | This study |
|              | BMU 07622 | CBS 140322 | Banyan leaves | Guangzhou, south China | KJ700970 KJ700971 | This study |
|              | BMU 07642 | CBS 140330 | Leaf | Chongqing, southwest China | KJ700971 KJ700972 | This study |
|              | BMU 07643 | CBS 140304 | Leaf | Chongqing, southwest China | KJ700972 KJ700973 | This study |
|              | BMU 07649 | CBS 140305 | Leaf | Chongqing, southwest China | KJ700973 KJ700974 | This study |
|              | BMU 07654 | CBS 140306 | Leaf | Chongqing, southwest China | KJ700974 KJ700975 | This study |
|              | BMU 07627 | CBS 140312 | Soil | Nanning, south China | KJ700975 KJ700976 | This study |
|              | BMU 07629 | CBS 140313 | Soil | Nanning, south China | KJ700976 KJ700977 | This study |
|              | BMU 07636 | CBS 140314 | Dead wood | Nanning, south China | KJ700977 KJ700978 | This study |
|              | BMU 07637 | CBS 140315 | Dead wood | Nanning, south China | KJ700978 KJ700979 | This study |
|              | BMU 07646 | CBS 140306 | Wheat straw | Guangzhou, south China | KJ700979 KJ700980 | This study |
|              | BMU 07650 | CBS 140307 | Leaf | Chongqing, southwest China | KJ700980 KJ700981 | This study |
|              | BMU 07653 | CBS 140308 | Leaf | Chongqing, southwest China | KJ700981 KJ700982 | This study |
|              | BMU 07657 | CBS 140309 | Leaf | Chongqing, southwest China | KJ700982 KJ700983 | This study |
|              | BMU 07658 | CBS 140310 | Leaf | Chongqing, southwest China | KJ700983 KJ700984 | This study |
|              | BMU 07659 | CBS 140311 | Leaf | Chongqing, southwest China | KJ700984 KJ700985 | This study |
|              | BMU 07664 | CBS 140302 | Molded leaf | Nanning, south China | KJ700985 KJ700986 | This study |
|              | BMU 07665 | CBS 140303 | Molded leaf | Nanning, south China | KJ700986 KJ700987 | This study |
|              | BMU 07666 | CBS 140304 | Molded leaf | Nanning, south China | KJ700987 KJ700988 | This study |

<sup>1</sup> ITS = Internal Transcribed Spacer; B2 = 18S ribosomal RNA gene.
| NH 258 | Environment | Japan | AB498320 | -- | Hamada & Abe (2010) |
| R70D1 | Leaf of living tree | Brazil, Bahia state, Sao Paulo, Brazil | KC445295 | -- | Research database |

**P. ellipsoidea**, originally identified as

| CBS 286.47 (T) | Environment | Brazil, Bahia state, Brazil | AF050282 | EU154175 | Unterreiner & Naveau (1999), Unterreiner et al. (2008) |

**P. verrucosa**

| CBS 224.97 | Mycotomba hand | Texas, USA | U31848 | KU006354 | Yan et al. (1995) |
| CBS 140242 | Chromoblastomycosis | China | KF881934 | KF971734 | This study |
| BMU 02333 (T) | Chromoblastomycosis | China | KF881937 | KF971737 | This study |
| BMU 07676 | Face phaeohyphomycosis | Wuhan, central China | KJ701006 | KM658113 | This study; Tong et al. (2013), Wang et al. (2014) |
| BMU 07163 | Phaeohyphomycosis skin; case 2 | Hebei, north China | KF360975 | KF971725 | This study; Zhang et al. (2015) |
| BMU 04480 | Chromoblastomycosis face | North China | KF881927 | KF971726 | This study |
| BMU 03336 | Chromoblastomycosis hand | East China | KF881928 | KF971727 | This study |
| BMU 03362 | Chromoblastomycosis | East China | KF881938 | KF971738 | This study |
| BMU 00849 | Chromoblastomycosis | East China | KF881945 | KF971746 | This study |
| BMU 07066 | Chromoblastomycosis upper limb | Tiantian, north China | KF881933 | KF971759 | This study |
| CBS 226.97 | Human, facial burn | Tennessee, USA | U31846 | KU030639 | Yan et al. (1995), Unterreiner & Naveau (1999), Unterreiner et al. (2008) |

**P. expanda**, originally identified as

| BMU 1245 | Wood Sweden | EU514701 | EU514716 | Unterreiner et al. (2008) |
| CBS 138.67 | LCP 971; dH 15364 | France | KU030356 | KU030635 | This study |
| WM 03.287 | Environment | -- | KU030357 | -- | Research database |
| LY2 | -- | -- | KU030359 | -- | Research database |
| CBS 273.57 | -- | -- | KU030360 | -- | Research database |

**P. tarda**, originally identified as

| CBS 111589 (T) | Invasive Chromoblastomycosis; case 13 | Libya | KU030632 | KU030637 | This study |

**P. macrospora**, originally identified as

| BMU 00106 | Soil | Xian, northwest China | KJ700946 | KM658124 | This study |
| BMU 00115 | Soil | Xian, northwest China | KJ700961 | KM658137 | This study |
| BMU 00149 | Soil | Xian, northwest China | KJ700952 | KM658128 | This study |
| CBS 839.69 | ATCC 34159; MUCL 15541 | Wood | EU514701 | EU514716 | Unterreiner et al. (2008) |
| CBS 138.67 | LCP 971; dH 15364 | -- | KU030356 | KU030635 | This study |

**Cladophialophora carrionii**

| CBS 160.54(T) | Chromoblastomycosis | Australia | EU137266 | EU137201 | This study; De Hoog et al. (2007) |

**Cladophialophora yegresii**

| CBS 114406 | UNEFM SgSR1 = dH 13275 | Stenocereus griseus cactus | EU137322 | EU137209 | De Hoog et al. (2007) |
| CBS 114405(T) | UNEFM SgSR3 = dH 13274 (ex-T of C. yegresii) | Stenocereus griseus cactus | EU137323 | EU137208 | De Hoog et al. (2007) |

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1 CBS: Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; ATCC: American Type Culture Collection, Virginia, USA; MUCL: Mycotheque de l'Université de Louvain, Louvain-la-Neuve, Belgium; UAMH: Mycofungus Herbarium and Collection, Edmonton, Canada; NIH: National Institutes of Health, Bethesda; WAD: Wadsworth Center for Laboratory and Research, New York; NYS: New York State Department of Health, New York; CDC: Centers for Disease Control and Prevention, Atlanta, USA; AFTOL: Assembling the Fungal Tree of Life; BMU: Department of Dermatology, Beijing Medical University, Beijing, China; DCC: Department of Vascular School of Medicine, China University, Chiba, Japan; IFM: Research Center for Pathogenic Fungi and Mycotoxicoses, Chiba University, Chiba, Japan; PMR: Facultad de Medicina Ciencias de la Salud, Reus, Spain; PMC: Facultad de Medicina, Caracas, Venezuela; UTSC: University of Texas Health Science Center, San Antonio, TX, USA; IMTSP: Instituto de Medicina Tropical, São Paulo, Brazil; IHEM: The BCCM/IHEM Biomedical Fungi and Yeasts Collection, Brussels, Belgium; Conant: research collection of N.F. Conant; MR: research collection of M. Reblová; WUC: research collection of W.A. Unterreiner; CJK Wang: research collection of C.J.K. Wang; dH: research collection of G.S. de Hoog.

2 ITS: internal transcribed spacer, BT2: β-tubulin, TEF1: translation elongation factor 1-α.
Growth at different temperatures indicated an optimum at 27–30 °C (Fig. 1) for most of the strains. No growth was observed at 40 °C. The following eight isolates were unable to grow at 30 °C (Fig. 1) for most of the strains. No growth was observed.

**Molecular phylogeny**

Phylogenetic reconstruction based on the ITS region and using NJ (Fig. 2), ML, MP and BI algorithms showed similar, more or less congruent topologies (data not shown), but generally with poor resolution. Three main aggregates of strains were preponderantly found and MUCL 39979) and P. americana Ca. semiimmersa comprised four environmental isolates. Strains identified as Ca. semiimmersa (UAMH 10875, UAMH 10876, MUCL 40572 and MUCL 39979) and P. americana were preponderantly found in the environmental clades. The study set also contained the type strain of P. macrospora, CBS 273.37; it was located in a cluster that mainly contained strains from clinical samples. Strains of Ca. semiimmersa were indistinguishable from those of P. americana; a small group of strains denominated Ca. svrekiana took an unresolved position paraphyletic to the P. americana / Ca. semiimmersa clade. Cladophialophora carrionii and Cl. yegresii, which are known to be phylogenetically close to P. verrucosa, were selected as out-groups and were clearly distinguishable by ITS (Fig. 2). Phylogenetic reconstruction based on SSU and LSU did not distinguish species of the P. verrucosa complex or related groups (data not shown).

To verify the ITS results and to explore a more detailed clustering, we analysed the BT2 and TEF1 regions of 118 strains phenotypically identified as P. verrucosa / P. americana, with the addition of CBS reference strains. Topologies were congruent with that of ITS, but at a higher level of resolution. Results of PHT showed that three gene lineages were congruent (P > 0.01). The tree of the combined 3-gene locus dataset (Fig. 3) revealed a topology similar to those of individual ITS, TEF1 and BT2 genes. The multilocus tree was used as a basis for a new taxonomic system for the P. verrucosa complex. The complex contained seven species, consistently separated with all partitions at high statistical support. Only a single cluster contained a type strain, i.e. CBS 273.37 of Phialophora macrospora. Strains generally identified and published in the literature with case reports as P. verrucosa comprised a small group of strains from five patients, two of which had been proven to have a CARD9 immunodeficiency, the two strains are BMU 07678 and BMU 07506, and this group kept the species name P. verrucosa (Gao et al. 2013, Tong et al. 2013, Wang et al. 2014, Zhang et al. 2015). A single, slow-growing isolate from a girl with a disseminated, severely mutilating chromoblastomycosis-like infection in Libya (Hofmann et al. 2005) took an isolated position in all analyses. One environmental cluster with 32 isolates

**Table 2** Primers used for PCR amplification and sequencing.

| Gene region | Primer name | Primer sequence (5′ -> 3′) | Reference |
|-------------|-------------|---------------------------|-----------|
| ITS         | V9G         | 5′-TTACGTCCCTGCCCCTTGTGTA-3′ | De Hoog & Gerits van den Ende (1998) |
|             | LS266       | 5′-GCATTCCCAAACAACTCGACTC-3′ | Masclaux et al. (1995) |
|             | ITS1        | 5′-TCCTAGTGTGAACCTCGGCCG-3′ | White et al. (1990) |
|             | ITS4        | 5′-TCCCTCCGCTTATGATATGC-3′ | O’Donnell et al. (2000) |
| BT2         | B2a         | 5′-GGTAACCAAATCGGTGCTGCTTTC-3′ | Carbone & Kohn (1999) |
|             | B2b         | 5′-GCATACCAAAATCGGTGCTGCTG-3′ | O’Donnell (1993) |
| TEF1        | EF1-726F    | 5′-TCCTCCGCTTATTGATATGC-3′ | White et al. (1990) |
|             | EF1-986R    | 5′-TCCTCCGCTTATTGATATGC-3′ | O’Donnell (1993) |
| LSU         | NL1         | 5′-TCCGTAGGTGAACCTCGGCCG-3′ | White et al. (1990) |
|             | LR5         | 5′-TCCTCCGCTTATTGATATGC-3′ | O’Donnell (1993) |
| SSU         | NS1         | 5′-GTAGTCATATGCTCGTGTTCTC-3′ | White et al. (1990) |
|             | NS24        | 5′-AAACCTTGTTACGACCTTAA-3′ | Gargas & Taylor (1992) |

![Fig. 1 Colony diameters at various temperatures ranging from 21–40 °C, measured after 3 wk on 2 % MEA.](image-url)
Fig. 2 Neighbour-Joining tree obtained from the 141 ITS sequences data. Bootstrap values above 80 % are shown at the nodes. The carrionii-clade is selected as outgroup. P, E, A, U after strain number mean sources: patient, environment, animal and unknown.
A Phialophora verrucosa
B Phialophora chinensis
C Phialophora americana
D Phialophora tarda
E Phialophora expanda
F Phialophora ellipsoidea
G Phialophora macrospora

Fig. 3 Maximum-Parsimony (MP) tree obtained from the combined DNA sequence data from three loci (ITS, BT2 and TEF1). Bootstrap values of Neighbour-Joining (NJ), Maximum-Likelihood (ML) and MP above 80 % / Bayesian (BS) posterior probability value above 0.80, are shown at the nodes (NJ/ML/MP/BS). Type strains and supported branches are drawn in bold. The carrioni-clade is selected as outgroup. Sources of isolation are mentioned at each strain.
Fig. 4  Phialophora verrucosa (CBS 140325). a. Colonies grown on MEA for 3 wk; b. colonies grown on OA for 3 wk; c–k. micromorphology showing phialides and conidia. — Scale bar = 10 μm.
contained strains collected in China from diverse environments such as soil, wood and plant debris, in addition to two isolates (BMU 01890 and BMU 02669) from human patients. Two small groups of strains with human-derived strains only were clearly separate from the main groups at high statistical support. A further, predominantly environmental group (4 clinical of 56 in total) contained strains that were identified in the literature (Untereiner & Naveau 1999) as *P. americana* and its sexual morph *Ca. semiimmersa*. For sequences deposited under the name *Ca. sveciakiana* no multi-locus data were available, but the position of these strains in the ITS tree, i.e. unresolved and adjacent to the *P. americana* group, suggested that the same taxonomic entity was concerned; for extended data see Untereiner et al. (2008).

**TAXONOMY**

**Clade A**

*Phialophora verrucosa* Medlar, Mycologia 7: 203. 1915 — MycoBank MBT203396, Fig. 4

Typus. Lectotype designated herewith f. 1 in Medlar (1915b: 201), an illustration of the fungus from a culture derived from a lesion in the buttock of a 22-yr-old Italian immigrant to Boston, USA. Whether original material of this strain has been preserved could not be ascertained. China, from skin lesions of human disseminated phaeohyphomycosis patient with CARD9 deficiency, epitype designated here: CBS 140325 (preserved at CBS in metabolically inactive condition in liquid nitrogen). Living strain also deposited as BMU 07506.

![Fig. 5 Phialophora chinensis (CBS 140326). a. Colonies grown on MEA for 3 wk; b. colonies grown on OA for 3 wk; c–h. micromorphology showing phialides, conidia, torulose hypha and muriform-like cells. — Scale bar = 10 µm.](image)
Description of CBS 140325 after 3 wk incubation on OA, 30 °C: Colonies growing slowly, olivaceous brown, with black olivaceous in the centre and slightly pink margin. Reverse olivaceous black. On MEA, 30 °C: Colonies growing slowly, pale grey, woolly with smooth, moist margin; reverse olivaceous brown. No diffusable pigment produced. *Hyphae* olivaceous brown, irregularly separate, flexuous, 2.5 ± 0.5 (1.5–3.5) μm wide. *Conidia* hyaline, 4.5 ± 0.5 (3.0–5.5) × 2.5 ± 0.5 (2.0–3.5) μm, smooth-walled, teardrop-shaped with protruding beak on one end and remain aggregated around the phialides. Sexual morph unknown. Cardinal temperatures: minimum below 21 °C, optimum 30 °C, maximum 37 °C.

Additional material examined. Table 1.

Notes — The type isolate has been preserved at Research Center of Medical Mycology, Peking University and at CBS. Isolates belonging to this species were derived from five patients, including four from China, and two of them concerned cases of CARD9-related immunodeficiency phaeohyphomycosis reported by Wang et al. (2014).

Clade B

*Phialophora chinensis* Yali Li, de Hoog & R.Y. Li, *sp. nov.* — MycoBank MB815345; Fig. 5

Typus. *China*, from skin lesions of human chromoblastomycosis patient, holotype CBS 140325 (preserved at CBS in metabolically inactive condition in liquid nitrogen). Living strain also deposited as BMU 01890.

Description of BMU 01890 after 3 wk incubation on OA, 30 °C: Colonies growing slowly, olivaceous black, with pale olivaceous centre. Reverse olivaceous black. On MEA, 30 °C: Colonies growing slowly, woolly, pale olivaceous grey with brown, smooth margin; reverse olivaceous brown. No diffusable pigment produced. *Hyphae* brown, regularly septate, 4.0 ± 0.5 (3.0–4.5) μm wide. *Conidiophores* absent. *Phialides* broadly flask-shaped. *Conidia* hyaline, smooth-walled, spherical to broadly ellipsoidal, 4.5 ± 0.5 (3.0–6.0) × 3.5 ± 0.5 (2.0–5.5) μm, some larger conidia developing a median septum resembling muriform cells, or show budding. Sexual morph unknown. Cardinal temperatures: minimum below 21 °C, optimum 24 °C, maximum 40 °C.

Additional material examined. Table 1.

Notes — The species grows with short cells producing thick-walled, swollen cells with median septa strongly resembling muriform cells on routine media. Nevertheless, nearly all strains known of *P. chinensis* are environmental, mostly being isolated from soil and plant debris. Two of the strains examined (Table 1) were derived from human patients, causing chromoblastomycosis.

Clade C

*Phialophora americana* (Nannf.) S. Hughes, Canad. J. Bot. 36: 795. 1958 — MycoBank MB203397; Fig. 6

Basionym. *Cadophora americana* Nannf., in Melin & Nannf., Svensk Skogs-vårdsförening Tidskr. 3–4: 412. 1934.

*Dictyochiricella semiimmerma* Cand. & Sulmont, Rev. Mycol. 36: 242. 1972.

= *Capronia semiimmerma* (Cand. & Sulmont) Unter. & F.A. Naveau, Mycologia 49: 82. 1996.

= *Capronia svrcnikiana* Réblővá, Czech Mycol. 49: 82. 1996.

Typus. USA, Wisconsin, woodpulp, A. Richards, holotype of *P. americana* slide 6320-2 (UPS). Living strain also deposited as UAMH 10875 = CDC 10.

Description of CBS 281.35 after 3 wk incubation on OA, 30 °C: Colonies growing moderately rapidly, olivaceous brown and pale at the centre. Reverse olivaceous black. On MEA, 30 °C: woolly, olivaceous grey; reverse olivaceous black. No diffusible pigment produced. *Hyphae* irregular, 2.5 ± 0.5 (1–3) μm wide. Distinct conidiophores absent. *Phialides* variable, flask-shaped to cylindrical or elongated, with darker, vase-shaped or tubular collarettes, which may also be sessile directly on undifferentiated hyphae. *Conidia* hyaline, 5.0 ± 0.5 (3.5–7.0) × 3.0 ± 0.5 (2–4) μm, subpherical to broadly ellipsoidal, occasionally subcylindrical, of variable size, mostly adhering in loose clumps at the collarette openings, rarely arranged in loose strings. Cardinal temperatures: minimum below 21 °C, optimum 30 °C, maximum 37 °C.

Additional material examined. Table 1.

Notes — The strain taken by several authors as representative for the species, CBS 281.35 was derived from a verrucous dermatosis of the legs of a human chromoblastomycosis patient, USA. The isolate was first described as *Phialophora verrucosa* by Schol-Schwarz (1970) as representative of that species, but later it was redescribed as *P. americana* by Yamagishi (in Yamagishi et al. 1997), Unterreiner (in Unterreiner et al. 2008) and Takizawa (in Takizawa et al. 2011). The species was also reported as *Capronia semiimmerma* from a herbarium specimen by Candrousseau & Sulmont (1971). Unterreiner & Naveau (1999) judged living strain MUCL 40572, parasitizing a lichen on *Populus* wood in France, identical to the type specimen and provided an illustration of its monomorphic *Phialophora* asexual morph with deep, vase-shaped phialidic collarettes. Strains UAMH 10872, 10873, 10874 are representative of *Ca. svrcnikiana* and are also identical to *P. americana* in the ITS tree (Fig. 2), confirming conclusions of Unterreiner et al. (2008).

Clade D

*Phialophora tarda* Yali Li, de Hoog & R.Y. Li, *sp. nov.* — MycoBank MB815349; Fig. 7

Typus. *Leva*, from tissue of disseminated chromoblastomycosis-like infection in human patient (Hofmann et al. 2005), holotype CBS 111589 (preserved at CBS in metabolically inactive condition in liquid nitrogen).

Description of CBS 111589 after 3 wk incubation on OA, 30 °C: Colonies growing slowly, olivaceous brown, with black olivaceous centre. Reverse olivaceous black. On MEA, 30 °C: Colonies growing slowly, pale olivaceous grey, woolly, with narrow smooth margin; reverse olivaceous brown. No diffusible pigment produced. *Hyphae* olivaceous brown, flexuous, 2.0 ± 0.5 (1.5–2.5) μm wide. *Conidiophores* absent. *Phialides* regularly flask-shaped to elongate; adelophialides uncommon. *Collarettes* slightly darker than the rest of the phialide, narrow funnel-shaped to almost cylindrical, up to 5.6 μm long. *Conidia* hyaline, variable in shape, mostly broadly ellipsoidal, 3.5 ± 1.0 (2.0–5.5) × 2.5 ± 0.5 (1.5–3.5) μm, smooth-walled. Sexual morph unknown. Cardinal temperatures: minimum below 21 °C, optimum 27 °C, maximum 40 °C.

Notes — The species is known from a single strain causing a severely mutilating, disseminated infection in a girl from Libya, initially identified as *P. verrucosa* (Hofmann et al. 2005). The patient was judged to be immunocompetent, but at that time the existence of CARD9- or STAT1-based or other rare inherited genetic immune defects was not known. Muriform cells in tissue had a variable appearance without typical cruciate septation.

(text continues on p. 17)
Fig. 6 *Phialophora americana* (CBS 281.35). a. Colonies grown on MEA for 3 wk; b. colonies grown on OA for 3 wk; c–j. micromorphology showing phialides, conidia and torulose hypha. — Scale bar = 10 μm.
Fig. 7  *Phialophora tarda* (CBS 111589). a. Colonies grown on MEA for 3 wk; b. colonies grown on OA for 3 wk; c–k. micromorphology showing phialides and conidia. — Scale bar = 10 μm.
Fig. 8  *Phialophora expanda* (CBS 140298). a. Colonies grown on MEA for 3 wk; b. colonies grown on OA for 3 wk; c–l. micromorphology showing phialides, conidia and torulose hypha. — Scale bar = 10 μm.
Fig. 9 *Phialophora ellipsoidea* (CBS 286.47). a. Colonies grown on MEA for 3 wk; b. colonies grown on OA for 3 wk; c–k. micromorphology showing phialides and conidia. — Scale bar = 10 μm.
Fig. 10  *Phialophora macrospora* (CBS 273.37). a. Colonies grown on MEA for 3 wk; b. colonies grown on OA for 3 wk; c–k. micromorphology showing phialides and conidia. — Scale bar = 10 μm.
Clade E

*Phialophora expanda* Yali Li, de Hoog & R.Y. Li, *sp. nov.* — MycoBank MB815350; Fig. 8

*Typhus*. *Chna*, from skin lesions of chromoblastomycosis patient, holotype CBS 140298 (preserved at CBS in metabolically inactive condition in liquid nitrogen). Also deposited as living strain CBS 140298 = BMU 02323.

Description of BMU 02323 after 3 wk incubation on OA, 30 °C: Colonies growing slowly, olivaceous black, with brown, woolly hyphae near the centre. Reverse olivaceous black. On MEA, 30 °C: Colonies growing moderately rapidly, woolly, pale olivaceous grey, with smooth margin; reverse olivaceous brown. No diffusible pigment produced. *Hyphae* olivaceous brown, often emerging from torulose hyphae and flexuous, 2.0 ± 0.5 (1.5‒2.5) μm wide. *Conidiophores* absent. Part of the phialides ceous brown. No diffusible pigment produced. *Hyphae* olivaceous brown, often emerging from torulose hyphae and flexuous, 2.0 ± 0.5 (1.5‒2.0) μm × 2.5 ± 0.5 (1.5–3.5) μm, smooth-walled, occasionally budding, aggregated in heads, sometimes in short chains. *Sexual morph* unknown. Cardinal temperatures: minimum below 21 °C, optimum 30 °C, maximum 40 °C.

*Additional material examined.* Table 1.

Notes — This isolate was collected by Peking University First Hospital from a chromoblastomycosis patient in 2000. It always clustered with the isolate BMU 01245 that was collected in 1999 from another patient.

Clade F

*Phialophora ellipsoidea* Yali Li, de Hoog & R.Y. Li, *sp. nov.* — MycoBank MB815351; Fig. 9

*Typhus*. *Brzul*, from human patient, holotype CBS 286.47 (preserved at CBS in metabolically inactive condition in liquid nitrogen). Living strain CBS 286.47 = ATCC 9541 = MUCL 9768 = UAMH 3635.

Description of CBS 286.47 after 3 wk incubation on OA, 30 °C: Colonies growing moderately rapidly, olivaceous brown, with black and purple granules at the centre. Reverse olivaceous black. On MEA, 30 °C: woolly, olivaceous grey; reverse olivaceous brown. No diffusible pigment produced. Hyphae pigmented with slightly brown, separate uniform with 2 ± 0.5 (1.5–2.5) μm wide. Distinct conidiophores absent. Part of the phialides flask-shaped, later enlarge to become subellipsoidal; some of the phialides give rise to a second phialide. *Collarettes* mostly small, sometimes longer, 1.5 ± 0.5 (0.5–2.0) μm. *Conidia* hyaline, ellipsoidal, 3.0 ± 0.5 (2.0–4.5) × 1.5 ± 0.5 (1.5–2.0) μm. *Sexual morph* unknown. Cardinal temperatures: minimum below 21 °C, optimum 27 °C, maximum 37 °C.

*Additional material examined.* Table 1.

Notes — This isolate had been identified as *P. verrucosa* (Unterreiner & Naveau 1999, Unterreiner et al. 2008, Heinrichs et al. 2012), or *P. americana* (Yamagishi et al. 1997).

DISCUSSION

The present study aims to investigate the biodiversity and taxonomy of *Phialophora verrucosa*, which has been reported in older literature as one of the uncommon agents of human chromoblastomycosis (Guerrero et al. 1998). However, also other types of infection have been ascribed to this species, among which are mycetoma (Turiansky et al. 1995), disseminated (Hofmann et al. 2005, Tong et al. 2013) and particularly different kinds of subcutaneous infection, often with cystic encapsulation (Iwatsu & Miyaji 1978, Schnadig et al. 1986, Kimura et al. 2003). Most infections were noted in patients with apparently good health; the share of immunocompromised patients, such as transplant recipients (Lundstrom et al. 1997), those with AIDS (Duggan et al. 1995) or with chronic use of antibiotics (Hochfelder & Fetto 2013) are relatively limited. In addition to human infection, the species has also been isolated from the environment, by enrichment in a mammal vector (Gezuele et al. 1972) but also with methods that are standard for direct black yeast isolation (Iwatsu et al. 1981). The majority of these isolates have, however, not been preserved, and their identity thus can no longer be verified.

Our data provide evidence that separate species are concerned, with different predilection and possibly causing different disorders. The combined ITS-*TEF1*-BT2 tree showed seven clades, six of which were supported by high bootstrap values and the seventh took an isolated position in all partitions. It was concluded that seven putative phylogenetic species exist in the *P. verrucosa* complex. Most of the recognised phylogenetic species exhibited a high degree of origin specificity, with species significantly differing in apparent pathogenicity. Two of the main environmental clusters contained 6.3–7.1 % strains from human patients, whereas in the combined ‘pathogenic’ clusters this ratio was 83.3 %.

Species identification for black yeasts in general (Zeng et al. 2013) including members of the genus *Phialophora* s.str. (Chowdhary et al. 2014) is possible by ITS sequencing. Our study shows the taxonomy of *P. verrucosa* in more detail. The phylogenetic trees of both ITS and BT2 distinguish the *P. verrucosa* complex unambiguously from its close the relatives *Cl. carrioni* and *Cl. yegresii*. Within the complex, rDNA ITS provides insufficient resolution in that the seven species-clusters have statistical support due to strains in paraphyletic position. Nevertheless, characteristic ITS-profiles are recognizable for each species, so that ITS can be used as barcode for routine identification (Schoch et al. 2012).
Only a single sexual morph of *P. americana*, *Ca. semiimmersa*, is known in the *P. verrucosa* complex (Untereiner et al. 2008). Sexual connections (*Ca. semiimmersa* including *Ca. svvocediana*) were made by isolation of ascospores from natural samples, and sequences of cultures invariably clustered in *P. americana* (Untereiner et al. 2008, Rébélová 1996). *Phialophora americana* is a preponderantly environmental species and is predicted to have low human pathogenicity judging from isolation sources. Pathogenicity and virulence is known to differ significantly between closely related species of black fungi (Chowdhary et al. 2014). Virulence factors listed thus far include melanin and carotene, thick cell walls, muriiform cells, yeast-like phases, thermo- and perhaps also osmoterlance, adhesion, hydrophobicity, amatic hydrocarbon assimilation, and production of siderophores, factors exerting variable influence upon location and severity of the infection (Seyedmousavi et al. 2014). These are general factors attributed to the entire family *Herpotrichiellaceae*: significant differences between species as yet have not been found. It remains difficult to explain why closely related species, as in *P. verrucosa* and its allies, differ significantly in this respect, while on the other hand agents of a highly specific disease as chromoblastomycosis are scattered over the family. Infections caused by members of the *P. verrucosa* complex can be destructive and highly refractory to therapy. Clinical isolates collected in the course of our study mostly were derived from patients with chromoblastomycosis or phaeohyphomycosis, while treatment outcomes of those patients were quite different (Tong et al. 2013, Wang et al. 2014). Remarkably, two patients were ultimately proven to have a mutation in the CARD9 signaling pathway interfering with Dectin-1 immunity. *Phialophora verrucosa* isolates caused recalcitrant infections, and a species named *P. tarsa* was collected from an invasive disseminated mycosis in Libya (Hofmann et al. 2005) in a patient that may also have had a CARD9 immune defect. It is not understood why such patients acquire just a single mycotic infection, and why black fungi are relatively frequent in these hosts. Infections by *P. americana* and *P. chinensis* are environmental fungi with opportunistic behaviour after local trauma. Isolates used in this study had been recovered from diverse environmental sources across the world such as plant debris, soil and rotten wood. These environmental isolates tended to aggregate in a limited number of clusters, different from the subgroups with preponderantly human sources of isolation according to the phylogenetic trees. The overabundance of Chinese strains probably is a sampling effect; we expect that all environmental species have a global distribution. The most enigmatic species in the complex is *P. tarsa*, originating from a severe human infection and without known environmental source. Notably, despite extensive environmental sampling, *P. verrucosa* (s.str.), *P. expanda* and *P. ellipsoidea* were not encountered either.

**CONCLUSIONS**

Distinction of six clades described here and summarised in Fig. 1 was achieved with molecular characters, phenotype and ecology. Optimum temperatures differ between strains and are therefore compared below at an average of 27 °C after 3 wk. *Phialophora chinensis* (B), nearly exclusively derived from environmental sources, in culture nevertheless has a strong tendency to production of isodiametric cells resembling muriiform cells of chromoblastomycosis, and shows some yeast-like cells; hyphae are scant and conidiophores are absent. Growth is moderately slow (19–42 mm). *Phialophora verrucosa* s.str. (A) contains clinical strains only. Phialides have a wide base and a dark, funnel-shaped collarette. Growth 22–31 mm. *Phialophora tarsa* (D) is known only from a moderately slow-growing (32 mm) clinical strain with well-differentiated, flask-shaped phialides. *Phialophora expanda* (E), with slow or fast-growing colonies (15–44 mm), has more slender phialides and very dark collarettes which have a huge expansion when young. *Phialophora macrospora* (G) has expanding, woolly colonies; phialides are slender, nearly cylindrical, with ellipsoidal conidia. *Phialophora ellipsoidea* (F), known from two clinical cases grows moderately rapidly (22–45 mm), has flask-shaped phialides but with short collarettes. *Phialophora americana* (C) is an environmental species with moderate growth (26–37 mm), differentiated conidiogenous cells with dark, funnel- to vase-shaped collarettes and broadly ellipsoidal conidia. Several species need further study when additional material becomes available.

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