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ORIGINAL ARTICLE

A new species of the oligotrophic genus *Ochroconis* (Sympoventuriaceae)

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Abstract *Ochroconis globalis*, a novel species of the melanized genus *Ochroconis* (Sympoventuriaceae, Venturiales), is described and illustrated and distinguished phenotypically and molecularly from existing ochroconis-like species. Phylogenetic analyses using nuclear ribosomal DNA genes (nuSSU, ITS, nuLSU) and coding gene fragments (*ACT1*, *BT2*, *TEF1*) revealed clustering of all strains as a monophyletic clade which was well separated from known *Ochroconis* species. Most strains of the new species were obtained from human-made environments, while the natural habitat of the species remains enigmatic. The new combination *Ochroconis musae* is introduced for one of the most commonly encountered *Ochroconis* species, and a phenotypic key to all species is provided.

Keywords *Ochroconis* · Ant fungus · Opportunist · Phylogeny · Venturiales

Taxonomic novelties: *Ochroconis globalis* Samerpitak, Duarte, Attili-Angelis & de Hoog, **sp. nov.**, *Ochroconis musae* (G.Y. Sun & Lu Hao) Samerpitak & de Hoog, **comb. nov.**

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Introduction

The genus *Ochroconis*, typified by *O. constricta*, was described by de Hoog and von Arx (1973) for melanized fungi with sympodial conidiogenesis and septate, ellipsoidal conidia. The genus was differentiated from the sister genus *Scolecobasidium* Abbott (1927) which was originally described with T- or Y-shaped conidia. Samerpitak et al. (2014) showed that the status of *Scolecobasidium* was doubtful and combined species with forked conidia in *Ochroconis* on phylogenetic grounds as members of Sympoventuriaceae, although some of the species with elaborate morphology still remain outside the family and are in need of correct classification. Colonies of members of the expanded genus *Ochroconis* are characteristically rust- to olivaceous-brown and produce small, brownish conidiophores bearing small numbers of mostly rough-walled conidia. All members of the genus share rhexolytic conidial liberation (Ellis 1971). Several species with more elaborate, branched conidia had been added (Matsushima 1971; 1975), and during the following decades more species, such as *O. tshawytschae* (Kirilenko and All-Achmed 1977) and *O. gamsii* (de Hoog 1985), were recognized in addition to the classical species *O. anellii*, *O. constricta* and *O. humicola*. Samerpitak et al. (2014) introduced a new genus, *Verruconis*, for a group of thermophilic species around *Ochroconis gallopava*. Almost synchronously with the paper of Samerpitak et al. (2014), Hao et al. (2013) proposed a new species of *Scolecobasidium*, and being unaware of the doubtful status of the generic type species *S. terreum* opted for maintenance of *Scolecobasidium* at the expense of *Ochroconis* for the entire species group. Given the ambiguity of *Scolecobasidium* this choice is less appropriate and is corrected in the present paper; some necessary name changes are proposed.

Machouart et al. (2014) investigated conserved genes (nuSSU, nuLSU, mtSSU, and *RPB2*) of the species at hand and found that both *Ochroconis* and *Verruconis* belonged to the order Venturiales, family Sympoventuriaceae. Detailed taxonomy was elaborated by Samerpitak et al. (2014) using nuclear ribosomal (nuSSU, nuLSU, ITS) and partial coding genes (actin: *ACT1*, β-tubulin: *BT2*, translation elongation factor 1-α: *TEF1*). In this revision, 13 species were recognized in *Ochroconis* and three in *Verruconis*. The latter genus comprised the human opportunistic neurotroph, *Verruconis gallopava* and two related species. Remarkably large phylogenetic distances were noted among and within the species of *Ochroconis* and *Verruconis*, which indicated the possible existence of additional, presently unrecognized taxa.

Some basic ecological trends can be observed in *Ochroconis* and *Verruconis*. *Ochroconis* species are mesophilic and often oligotrophic. *Ochroconis anellii* was isolated from a stalactite (Graniti 1962), *O. lascauxensis* from ancient drawings on a cave wall, and *O. anomala* from sediment in the same cave, i.e. the Lascaux Cave in France (Martin-Sanchez et al. 2012). *Ochroconis* species morphologically similar to *O. humicola* were reported from wet areas in the domestic environment, such as bathrooms. Several ochroconis-like strains were isolated from soil or water (Lian and de Hoog 2010). *Verruconis* species differ by being thermophilic and have repeatedly been recovered from hot water and from brains of warm-blooded animals including humans.

The present paper presents a taxonomic study of eleven ochroconis-like strains from various sources. Phenotypic and genotypic characters of the strains were evaluated in view of refined species delimitations and a novel *Ochroconis* species is introduced.

Materials and methods

Phenotypic studies

Eleven strains of an unknown *Ochroconis* species (Table 1) were cultured on oatmeal (OA) and malt extract agars (MEA), respectively, and incubated at 24 °C for 14 days. Morphological observations were carried out as described by Samerpitak et al. (2014). To investigate the optimal temperature for growth, all strains were cultured on MEA and incubated for three weeks at temperatures varying from 4 to 40 °C with 3 °C intervals. Colony diameters were measured after 3, 7, 11, 14, 18, and 21 days.

Phylogeny

Eleven unknown strains including 18 type and reference strains of *Ochroconis* and *Verruconis* species (Table 2, Samerpitak et al. 2014) were included in phylogenetic analyses. DNA extraction was performed according to Feng et al. (2014). Six markers, viz. nuSSU, nuLSU, ITS, *ACT1*, *BT2*, and *TEF1* were amplified and sequenced by PCR using primers and conditions as reported earlier (Badali et al. 2008; Najafzadeh et al. 2009; Feng et al. 2014). BIONUMERICS v. 4.61 (Applied Maths, Sint-Martens-Latem, Belgium) was employed for first iterative alignments. Sequences of nuLSU, nuSSU and ITS were aligned with the web-based program MUSCLE (www.ebi.ac.uk/Tools/msa/muscle), and *ACT1*, *BT2*, and *TEF1* with the program MAFFT (http://www.ebi.ac.uk/Tools/msa/mafft). Sequence alignments were adjusted using BIOEDIT v. 7.0.5.2. Mole% G+C of ITS was calculated using BIOEDIT v. 7.0.5.2, and distances between species by MEGA5 (Tamura et al. 2011).

Table 1 Investigated *Ochroconis globalis* strains

CBS No.	Alternative number	Original ID	Source	Geography	Year of isolation
CBS 119643	dH 12398	*O. constricta*	Foot, 40 year old man	Venlo, the Netherlands	2001
CBS 119644(T)	CBS-H 21940, dH 12983	*Ochroconis* sp.	Domestic sample	Düsseldorf, Germany	2002
CBS 124172	dH 19904	*O. constricta*	Shower filter, bathroom	Austria	2008
CBS 131956	dH 22893, AP171	*Ochroconis* sp.	Gynes cuticle of ant, *Atta capiguara*	São Paulo, Brazil	2008
CBS 135766	U111109	*Ochroconis* sp.	Fish, *Salmo salar*	Uppsala, Sweden	2011
CBS 135921	R985	*Ochroconis* sp.	Bathroom; black biofilm, sink drain	Erftstadt, Germany	2009
CBS 135922	R474	*Ochroconis* sp.	Bathroom; black biofilm, shower head	Erftstadt, Germany	2009
CBS 135923	R565	*Ochroconis* sp.	Sediment, municipal water distribution system	Cologne, Germany	2010
CBS 135924	R806	*Ochroconis* sp.	Bathroom; black biofilm, sink drain	Aachen, Germany	2009
CBS 135925	R1077	*Ochroconis* sp.	Bathroom; black biofilm, bathtub, water tap	Cologne, Germany	2011
CBS 135926	V152-75	*Ochroconis* sp.	Water	Nijmegen, the Netherlands	2010

CBS = Centraalbureau voor Schimmelcultures

Sequences were concatenated following Samerpitak et al. (2014). Multi-locus analysis was performed using the Bayesian approach with MRBAYES v. 3.1.2 from the CIPRES Science Gateway (Miller et al. 2010). Two parallel runs of 10,000,000 generations were done with a sampling frequency of 1,000 trees. A burnin tree sample of 10 % was discarded. Maximum likelihood (ML) using Tamura-Nei and GTR+I as the best model with 1,000 bootstrap replicates, and maximum parsimony (MP) with 1,000 bootstrap replicates were also carried out in MEGA5 (Tamura et al. 2011). Presented tree was obtained with Bayesian approach. Tree reconstruction, visualization, and editing were done with TREEVIEW v. 1.6.6, FIGTREE v. 1.1.2 and MEGA5.

Results

The overall mean distance of ITS sequences among 15 *Ochroconis* species and the unidentified *Ochroconis* was 13.4 %. ITS sequences of the unidentified *Ochroconis* were 687 bp in length including 317 bp for ITS1, 157 bp for 5.8S, and 213 bp for ITS2. All 11 strains of the unknown species were 99.8−100 % identical in rDNA genes (data not shown). Judging from ITS phylogeny of all *Ochroconis* and *Verruconis* species sequenced to date (Samerpitak et al. 2014), the strains were at considerable distance to the described species, *O. tshawytschae, O. anellii*, *O. anomala*, *O. verrucosa*, and *O. lascauxensis*. A minimum distance of 6.5 % was found with *O. tshawytschae*. ITS length and mole% G+C seem to be specific to each *Ochroconis-Verruconis* species including the unidentified one, ranging from 566 bp (*O. cordanae*) to 754 bp (*O. anomala*), and from 48.82 % (*O. sexualis*) to 60.96 % (*O. tshawytschae*) (Table 3).

The dataset of six concatenated sequences contained 4,913 characters of which 1,271 were parsimony-informative. A multi-locus analysis, applying all algorithms mentioned above confirmed that the investigated strains formed a separate, strongly supported cluster at 1/100 %/100 % (BI/ML/MP) and that the 1 strains represented a hitherto undescribed member of the *O. tshawytschae* species group with smallest distances to *O. anellii* and *O. lascauxensis* (Fig. 1).

Taxonomy

Ochroconis globalis Samerpitak, Duarte, Attili-Angelis & de Hoog, **sp. nov.** – Figs. 2 and 3. Mycobank MB807506

Etymology: the name refers to the fungus' wide geographical distribution.

Holotype: CBS 119644 (living)=CBS H-21940 (dried), from indoor sample, dwelling house, Germany, Düsseldorf, 2002.

Description based on CBS 119644 at 24 °C after 2 weeks in darkness.

On OA, colonies 25–30 mm in diameter after 2 weeks, moderately expanding, smooth, dry, flat, greyish brown to dark brown. On MEA, colonies attaining 18–20 mm in diameter after 2 weeks, flat, velvety to floccose with some shallow radial fissures, brownish olive green to dark olive green with a 0.5 mm submerged colony margin, reverse as dark olive, green and brown in the central portion, on MEA, hyphae subhyaline to pale brown, smooth- and thick-walled; 1.4–3.0 µm wide, coiled hyphae usually present. Conidiophores mostly arising laterally from vegetative hyphae, erect or flexuous, cylindrical with 1–2 septa,

Table 2 GenBank numbers of strains of *Ochroconis* and *Verruconis* used in phylogenetic analyses

Strains	Culture voucher	GenBank accession numbers					
		SSU	ITS	LSU	*ACT1*	*BT2*	*TEF1*
O. globalis	CBS 119643	KF961109	KF961085	KF961096		KJ867432	KF961074
O. globalis	CBS 119644(T)	KF961108	KF961086	KF961097	KF956086	KF961065	KF961075
O. globalis	CBS 124172	KF961109	KF961087	KF961098	KF956088	KF961066	KF961076
O. globalis	CBS 131956	KF961117	KF961088	KF961100	KF956094	KF961067	KF961081
O. globalis	CBS 135766	KF961116	KF961094	KF961106	KF956087	KF961072	KF961082
O. globalis	CBS 135921	KF961112	KF961089	KF961101	KF956090	KF961068	KF961077
O. globalis	CBS 135922	KF961113	KF961090	KF961102	KF956091	KF961069	KF961078
O. globalis	CBS 135923	KF961114	KF961091	KF961103		KJ867434	KF961084
O. globalis	CBS 135924	KF961107	KF961092	KF961104	KF956092	KF961070	KF961079
O. globalis	CBS 135925	KF961115	KF961093	KF961105	KF956093	KF961071	KF961080
O. globalis	CBS 135926	KF961111	KF961095	KF961099	KF956089	KF961073	KF961083
Reference strains							
O. anellii	CBS 284.64(T)	KF156070	FR832477	KF156138	KF155912	KF156184	KF155995
O. anomala	CBS 131816(T)	KF156065	HE575201	KF156137	KF155935	KF156194	KF155986
O. crassihumicola	CBS 120700	KJ867431	KJ867429	KJ867430	KJ867427	KJ867433	KJ867428
O. constricta	CBS 211.53	KF156073	HQ667519	KF156148	KF155941	KF156187	KF156005
O. cordanae	CBS 475.80(T)	KF156058	KF156022	KF156122	HQ916976	KF156197	KF155981
O. gamsii	CBS 239.78(T)	KF156088	KF156019	KF156150	KF155936	KF156190	KF155982
O. humicola	CBS 116655(T)	KF156068	HQ667521	KF156124	KF155904	KF156195	KF155984
O. lascauxensis	CBS 131815(T)	KF156069	FR832474	KF156136	KF155911	KF156183	KF155994
O. longiphora	CBS 435.76	KF156060	KF156038	KF156135	KF155908	KF156182	KF155978
O. macrozamiae	CBS 102491	KF156092	KF156021	KF156152	KF155938	KF156191	KF155983
O. minima	CBS 510.71(T)	KF156087	HQ667522	KF156134	KF155945	KF156172	KF156007
O. musae	CBS 729.95	KF156082	KF156029	KF156144	KF155948	KF156171	KF155999
O. sexualis	CBS 131765(T)	KF156089	KF156018	KF156118	KF155902	KF156189	KF155976
O. tshawytschae	CBS 100438(T)	KF156062	HQ667562	KF156126	KF155918	KF156180	KF155990
O. verrucosa	CBS 383.81(T)	KF156067	KF156015	KF156129	KF155910	KF156185	
V. calidifluminalis	CBS 125818(T)	KF156046	AB385698	KF156108	KF155901	KF156202	KF155959
V. gallopava	CBS 437.64(T)	KF156053	HQ667553	KF156112	HQ916989	KF156203	KF155968
V. verruculosa	CBS 119775	KF156055	KF156014	KF156106	KF155919	KF156193	KF155974

T = type strain

15–60 × 2–4.7 µm, brown, smooth-walled, with sympodially proliferating conidiogenous cells bearing one or more denticles in the apical region, denticles cylindrical, subhyaline, up to 2 µm long. Conidia ellipsoidal to cylindrical sometimes slightly apiculate at the base, 5.5–10.5 × 3.0–5.5 µm, smooth-walled, pale brown, mostly 2-celled, becoming verrucose and constricted at the median septum when old. Conidial secession rhexolytic, frills remaining visible on denticle and on conidial base. Cardinal temperatures on MEA: minimum at 4 °C, optimum at 24–27 °C, maximum at 33 °C. No growth at 37 °C, leading to death after 4 weeks of incubation.

Note: To clarify the new taxonomic position of the new taxon *O. globalis* in the *Ochroconis* lineage, the available type and representative strains of species in the genera *Ochroconis* and *Verruconis* (Table 2) were included in a phylogenetic comparison. *Ochroconis atlantica* ATCC 32684 (Wellman 1975) was not available for study, but with its muriform conidia and its different habitat, this species was judged to be significantly different from *O. globalis*. Morphologically, *O. globalis* resembles *O. constricta*, but conidia of *O. globalis* are larger and more variable in shape, i.e. ellipsoidal to cylindrical, 2- to 4-celled, with less rough walls at young age, and with longer, cylindrical conidiophores. Conidia of *O. globalis* are also similar to those of *O. musae* and *O. cordanae*, but have a constriction at the median septum and have more prominently verrucose walls at later stages of development.

Table 3 ITS characters of *Ochroconis* and *Verruconis* species

Species	ITS characters		
	Length (bp)	G+C%	Distances of *O. globalis* to neighboring species (substitution/site)
Ochroconis globalis sp. nov.	687	57.06	
O. anellii	649	51.16	0.077
O. anomala	754	58.49	0.083
O. crassihumicola	579	55.27	0.240
O. constricta	678	49.71	0.089
O. cordanae	566	56.54	0.145
O. gamsii	678	53.54	0.140
O. humicola	674	54.90	0.144
O. lascauxensis	584	59.93	0.077
O. longiphora	651	56.22	0.125
O. macrozamiae	668	51.80	0.126
O. minima	580	59.31	0.117
O. musae	636	54.40	0.121
O. sexualis	592	48.82	0.150
O. tshawytschae	707	60.96	0.065
O. verrucosa	699	55.22	0.107
Verruconis calidifluminalis	668	51.80	0.150
V. gallopava	672	52.23	0.157
V. verruculosa	597	60.47	0.174

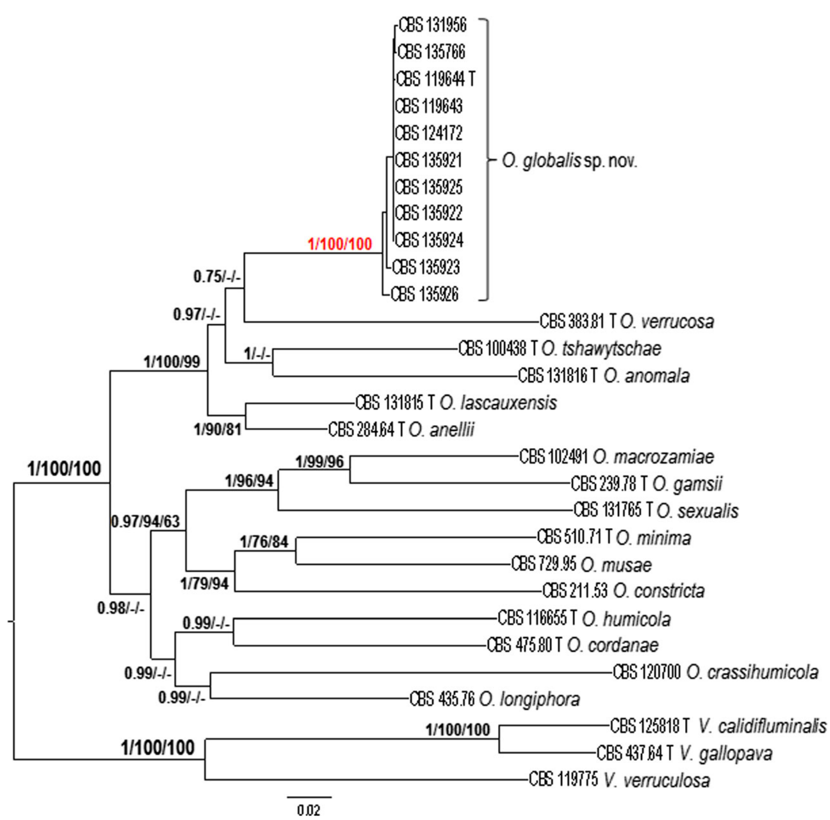

Fig. 1 Bayesian tree from a concatenated dataset including the gene regions nuSSU, ITS, nuLSU, *ACT1*, *BT2*, and *TEF1*. Numbers on the branches are bootstrap values for Bayesian posterior probabilities (PP), MEGA5-maximum likelihood (ML), and MEGA5-maximum parsimony (MP). Type strains are highlighted by a T

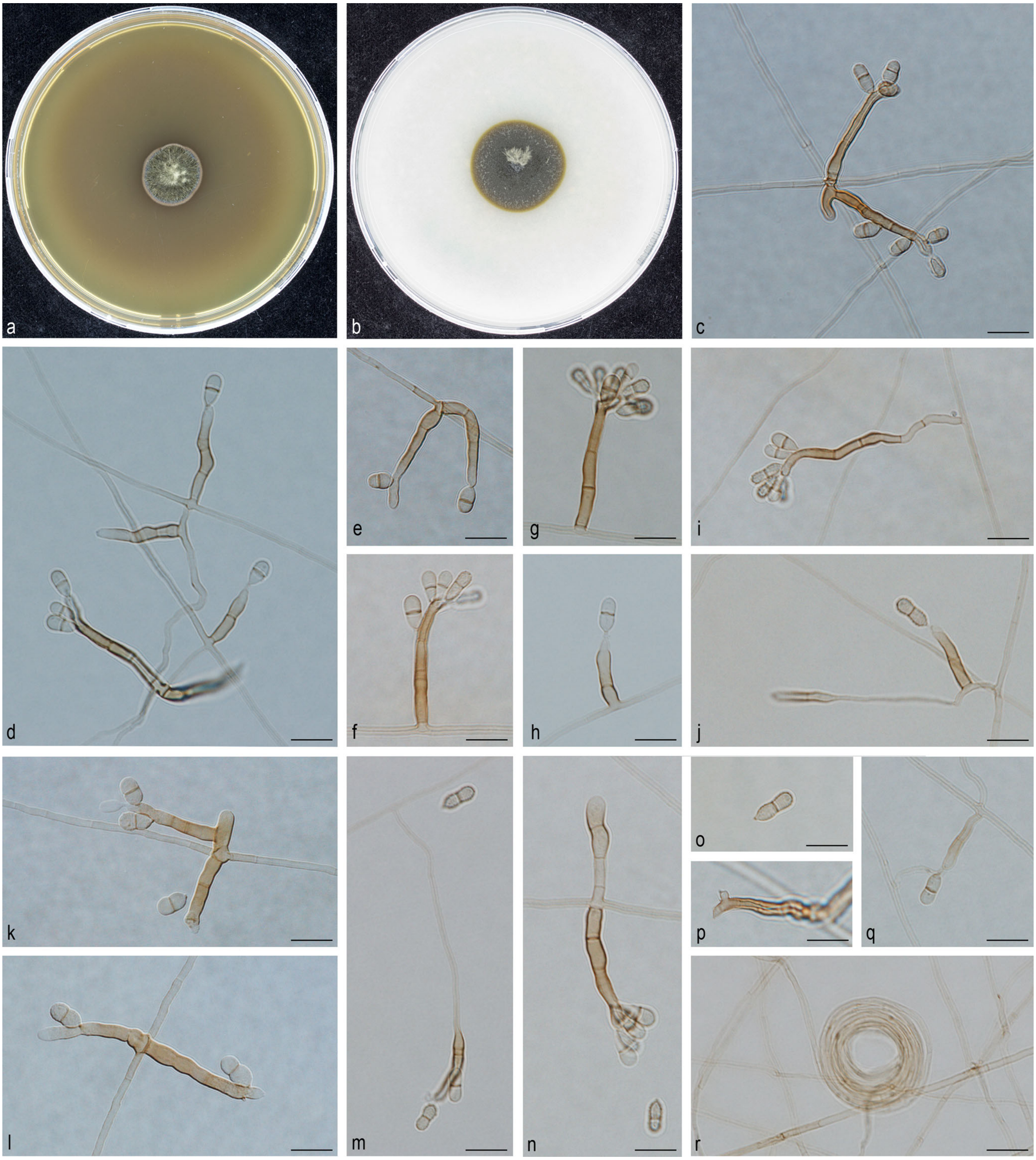

Fig. 2 *Ochroconis globalis*, CBS 119644. a. Colony on MEA 2 weeks. b. Colony on OA, 2 weeks. c−q. Conidial apparatus with rhexolytic conidia produced from simple conidiophores. r. Hyphal coil. Scale bar=10 μm

Conidiophore lengths varied from long cylindrical – similar to those of *O. gamsii* and *O. humicola* – to shorter, like those of *O. cordanae* and *O. musae*. Therefore, given the high degree of variation (Fig. 3), morphological characters are insufficient to unambiguously differentiate *O. globalis*. However, when compared within members of the *O. tshawytschae* species group (*O. anellii*, *O. lascauxensis*, *O. anomala*,

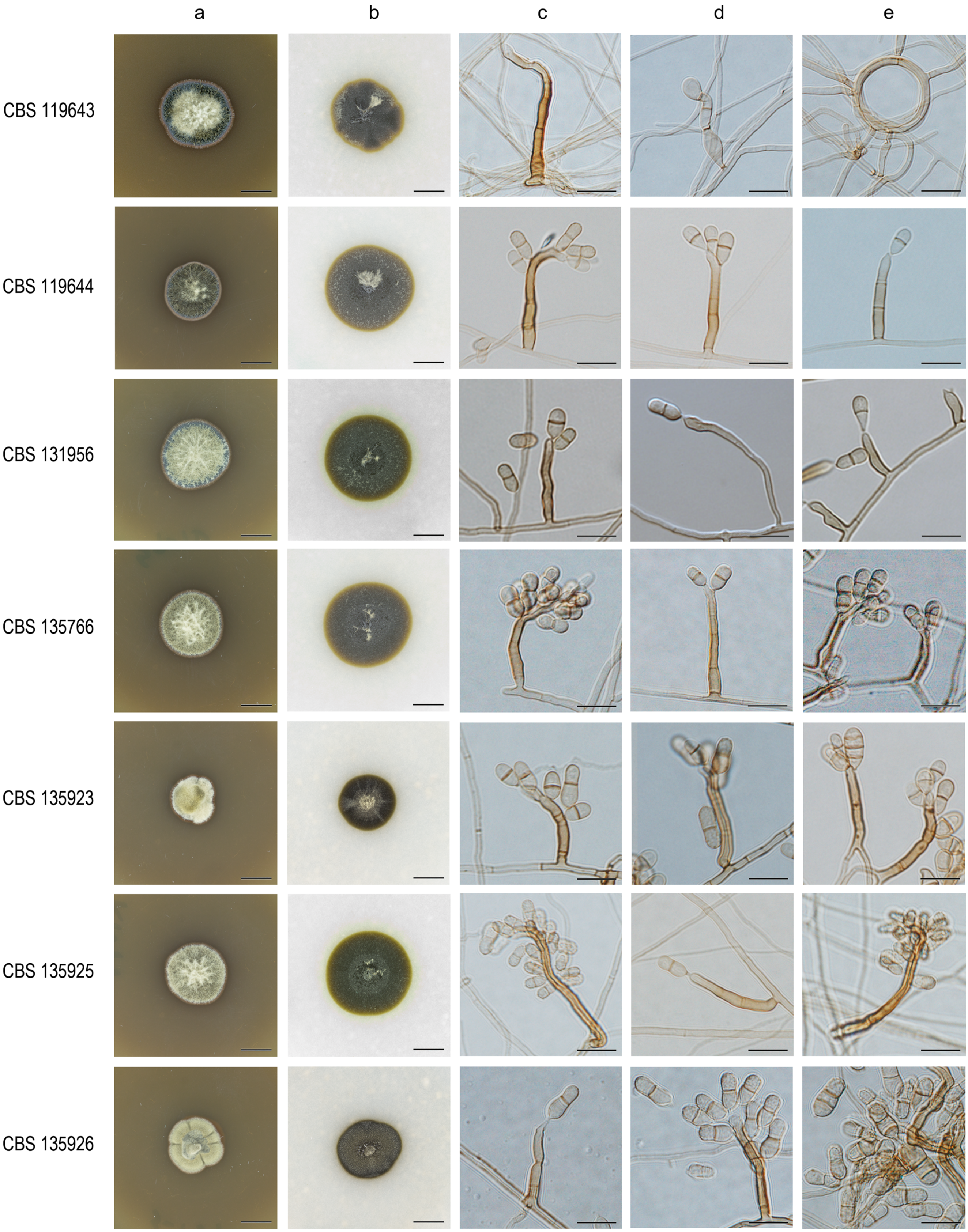

Fig. 3 Cultural and morphological variability of *Ochroconis globalis*. A. Colony on MEA 2 weeks. B. Colony on OA, 2 weeks. C−E. Conidial apparatus with rhexolytic conidia, conidiophores and somatic structures. A and B, scale bar=1 cm. C−E, scale bar=10 μm

O. verrucosa, and *O. tshawytschae*), *O. globalis* can be differentiated morphologically from other species by features listed in the key and summarized in Fig. 4.

Ochroconis musae (G.Y. Sun & Lu Hao) Samerpitak & de Hoog, **comb. nov.** Mycobank MB808843

≡ *Scolecobasidium musae* G.Y. Sun & Lu Hao, Mycol. Prog. 12: 492, 2013 (basionym)

= *Ochroconis mirabilis* Samerpitak & de Hoog, Fungal Divers. 65: 110, 2014.

Holotype (dried culture): HMAS 243664 from fruit surface of Japanese fiber banana, *Musa basjoo*, China, Hainan Province, Haikou City, Ledong county, L. Hao; culture ex-type CGMCC 3.14990=0HLHKBJ-22.

Note: Full descriptions of this species were given by Hao et al. (2013) and by Samerpitak et al. (2014) under its synonymous name *O. mirabilis*. The species was classified in *Scolecobasidium* (Hao et al. 2013) without consideration of the doubtful identity of the genus *Scolecobasidium*. The large phylogenetic distances among *Ochroconis* and *Verruconis* species were neglected. Samerpitak et al. (2014) described *O. mirabilis* for the most common *Ochroconis* species, which mostly had been reported under the name of the phenotypically similar species *O. constricta*; the authors were unaware of the almost synchronous description of the same species as *S. musae* by Hao et al. (2013). The ITS sequence of strain GS-2012 (=0HLHKBJ-22) had been deposited at GenBank as *Scolecobasidum* sp. (JQ364738) and was included by Samerpitak et al. (2014) under *O. mirabilis*. LSU sequence (JQ364739) of *O. musae* CGMCC 3.14990 (=HLHKBJ-22) is almost identical to that of *O. mirabilis* CBS 729.95 (KF156144). Consequently, to solve this taxonomic dilemma with two names having been introduced for the same fungus, the new combination *Ochroconis musae* is proposed in the present study.

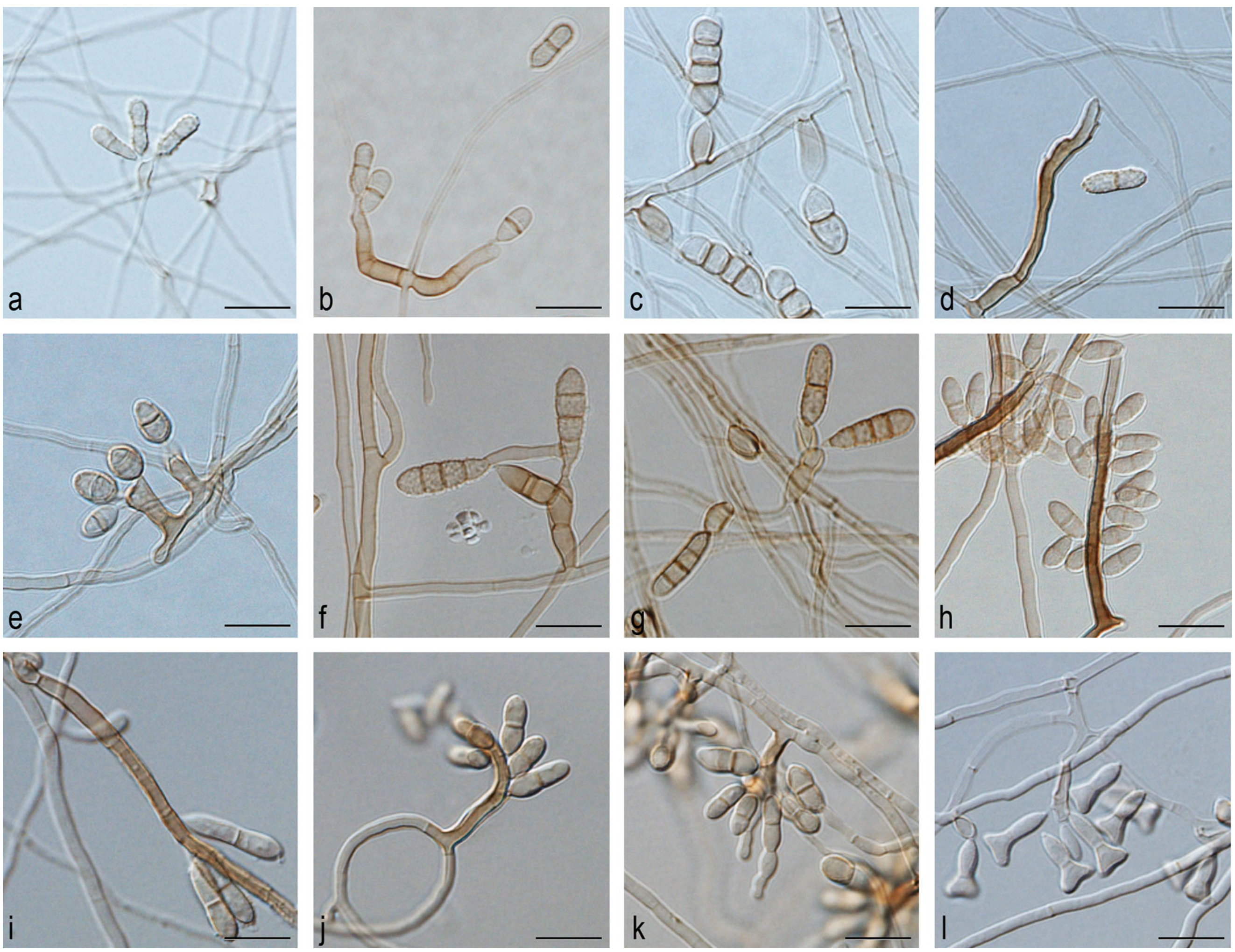

Fig. 4 Overview of *Ochroconis* species with septate conidia. a. *O. constricta* CBS 211.53. b. *O. globalis* CBS 119644. c. *O. anellii* CBS 284.64. d. *O. lascauxensis* CBS 131815. e. *O. anomala* CBS 131816. f. *O. verrucosa* CBS 383.81. g. *O. tshawytschae* CBS 100438. h. *O. gamsii* CBS 239.78. i. *O. humicola* CBS 116655. j. *O. cordanae* CBS 475.80. k. *O. musae* CBS 729.95. l. *O. minima* CBS 510.71. Scale bar=10 µm

Key to species of *Ochroconis*

1a. Conidia ovoid to cylindrical……………………………2
1b. Conidia muriform………………………...*O. atlantica*
2a. Conidia mostly 2-celled……..………………….…….3
2b. Conidia mostly 4-celled…………………….…..……12
3a. Conidia smooth-walled, clavate to T- or Y-shaped…...............………………………*O. minima*
3b. Conidia smooth-walled to verrucose, ovoid to cylindrical……..............………………….......…..…4
4a. Conidiophores flask-shaped to cylindrical………..…5
4b. Conidiophores long cylindrical mostly unbranched…..….…..10
5a. Conidia constricted at the septum…....................……6
5b. Conidia rarely constricted at the septum….............…7
6a. Conidia verrucose, cylindrical, mostly 2-celled………….......................................*O. constricta*
6b. Conidia smooth-walled to verrucose, cylindrical, sometimes 3- or 4-celled……………......…………*O. globalis*
7a. Conidia ovoid to cylindrical, 2- to 3-celled..……*O. anomala*
7b. Conidia cylindrical, mostly 2-celled…......….............…8
8a. Conidiophores subhyaline to pale brown, flask-shaped to cylindrical, branched……….............….…*O. musae*
8b. Conidiophores brown, cylindrical, erect, mostly unbranched……………………............……………..….9
9a. Conidiophores 10–50×2.5–3.5 µm, mostly arising laterally; conidia cylindrical...................…*O. cordanae*
9b. Conidiophores 10–100×2.5–3.5 µm, arising both terminally and laterally; conidia ovoid to cylindrical …......................………….....…......…*O. crassihumicola*
10a. Conidiophores brown, cylindrical, 20–100×2–3 µm, erect, sometimes short rhizoid present; conidia long cylindrical, 8–20×3–5 µm ……......……..*O. humicola*
10b. Conidiophores dark- to red brown, cylindrical, 20–80×2–3 µm, erect to flexuous; conidia mostly one septum, straight to curved and unilaterally flattened…………...……11
11a. Conidia 6–9×2–3 µm, straight to curved……..….*O. gamsii*
11b. Conidia larger, 8–12×3–4 µm, mostly straight………..................…….…*O. macrozamiae*
12a. Conidia cylindrical, 2- to 4-celled.………………..13
12b. Conidia cylindrical to slightly fusiform, mostly 4-celled...................……………………………….15
13a. Conidiophores cylindrical; conidia long cylindrical, 20–50×10–20 µm, 2- to 4-celled, round at both ends, verruculose….……………….................*O. longiphora*
13b. Conidiophores cylindrical to acicular; conidia smaller than 20–50×10–20 µm, smooth-walled, verrucose or tuberculat..…14
14a. Conidia cylindrical, mostly 4-celled, smooth-walled to verrucose…..............................…………*O. sexualis*
14b. Conidia 2- to 4-celled, verrucose to tuberculated…........…......….....…..*O. lascauxensis*
15a. Conidia smooth-walled to verrucose, often constricted at septa……………………….............…*O. anellii*
15b. Conidia verrucose, rarely constricted at septa…........16
16a. Conidiophores short to long cylindrical, erect to flexuous; conidia cylindrical, verrucose, single; sometimes chlamydospores present…...…*O. tshawytschae*
16b. Conidiophores cylindrical; conidia cylindrical to slightly fusiform, coarsely verrucose, sometimes in branched or unbranched chains; chlamydospores absent……..………*O. verrucosa*

Discussion

In phylogenetic analyses of both conserved and coding genes, *O. globalis* could easily be distinguished from described *Ochroconis* species. All 11 strains of the new species were 99.8−100 % identical in rDNA genes and three coding genes, and clustered together concordantly in all single-gene analyses (data not shown). A multi-locus analysis of 11 available strains showed strict concordance in all investigated genes. Because of the high degrees of variability found even in conserved markers, ITS and LSU were recommended as the best diagnostic and barcoding candidates for *Ochroconis* and *Verruconis* species (Samerpitak et al. 2014), which also holds true for *O. globalis*. Distances between species were large enough to recognize *O. globolis* unambiguously in all investigated genes, each having satisfactory usability as barcoding markers. The large barcoding gaps between *Ochroconis* and *Verruconis* species are reflected by differences in length and mole% G+C of ITS (Table 3). For routine diagnostics of *Ochroconis* and *Verruconis* species, ITS sequencing is the most effective tool.

The majority of investigated *O. globalis* strains were isolated from water or from domestic wet cells such as bathrooms. A similar habitat choice was observed in *O. musae* [as *O. mirabilis* in Samerpitak et al. (2014) or as *O. humicola* in Lian and de Hoog (2010) and Heinrichs et al. (2013a, b)]. These habitats suggest oligotrophism accompanied by low competitive ability, as is known for black yeasts (Sudhadham et al. 2008). Water might play an important role in their distribution (Heinrichs et al. 2013a, b). One of the investigated strains, CBS 119643, was found as a superficial opportunist on skin of a human foot, but no proven case report has as yet been published. Lian and de Hoog (2010) suggested that ability to grow at 37 °C was not necessary for cutaneous infection. Strains may have entered softened human skin during bathing, as hypothesized by Satow et al. (2008) and Lian and de Hoog (2010). CBS 135766 was isolated from a living salmon (*Salmo salar*) with visceral infection. The Brazilian strain of *O. globalis*, CBS 131956, was isolated from the cuticle of gynes of an ant species, *Atta capiguara*, and shared a similar habitat with some strains of *O. cordanae* and *O. sexualis* (Samerpitak et al. 2014). CBS 131956 is the only strain from a tropical climate, while remaining *O. globalis* strains originated from temperate zones. The tropical ant-associated strain grew optimally at 27 to 30 °C. Investigations on fungal diversity and ecology associated with social insects are currently in progress. Given the high diversity of sources of isolation of *O. globalis* and the scant information on their ecology other than yield in culture, the actual natural habitat of these oligotrophic fungi remains enigmatic.

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