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DOI
10.1007/s11557-015-1023-5

Publication date
2015

Document Version
Final published version

Published in
Mycological Progress

Citation for published version (APA):
Samerpitak, K., Duarte, A. P. M., Attilli-Angelis, D., Pagnocca, F. C., Heinrichs, G., Rijs, A. J. M. M., Alfjorden, A., Gerrits van den Ende, A. H. G., Menken, S. B. J., & de Hoog, G. S. (2015). A new species of the oligotrophic genus Ochroconis (Sympoventuriaceae). Mycological Progress, 14(2), [6]. https://doi.org/10.1007/s11557-015-1023-5
A new species of the oligotrophic genus *Ochroconis* (Sympoventuriaceae)

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Received: 30 June 2014 / Revised: 22 September 2014 / Accepted: 26 September 2014 / Published online: 8 February 2015
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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. tschawytschae* (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 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). BIOPHINERICS 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).
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 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 19004 | 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
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 conidiphores. 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 2** GenBank numbers of strains of *Ochroconis* and *Verruconis* used in phylogenetic analyses

| Strains   | Culture   | GenBank accession numbers      |
|-----------|-----------|--------------------------------|
|           | voucher   | 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 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

| Strains   | Culture   | GenBank accession numbers      |
|-----------|-----------|--------------------------------|
|           | voucher   | SSU    | ITS     | LSU     | ACT1   | BT2   | TEF1 |
| *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 | KF156144 | KF155948 | KF156171 | KF155999 |
| *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
### Table 3 ITS characters of *Ochroconis* and *Verruconis* species

| Species                     | 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. lascaussensis*          | 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.
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 Scolecobasidium 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 ........................ *O. constricta*  
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. anomalala*  
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 verruculose .......... 14  
14a. Conidia cylindrical, mostly 4-celled, smooth-walled to verrucose .......... *O. sexualis*  
14b. Conidia 2- to 4-celled, verrucose to tuberculcated ........................ *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. globalis* 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 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.

**Acknowledgments** We would like to thank K. Luijsterburg for his assistance in making fungal photographs.

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