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Taxonomy and phylogeny of the xerophilic genus *Wallemia* (Wallemiomycetes and Wallemiales, cl. et ord. nov.)

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Abstract

The genus *Wallemia* comprises xerophilic species. Based on parenthesome ultrastructure it has been linked to the Filobasidiales (basidiomycetes). Species show a unique type of conidiogenesis, including basauxic development of fertile hyphae, segregation of conidial units more or less basipetally, and disarticulation of conidial units into mostly four arthrospore-like conidia. *Wallemia* is known from air, soil, dried food (causing spoilage), and salt. It can be isolated from hypersaline water of man-made salterns on different continents. Based on analyses of the nuclear small subunit ribosomal DNA (SSU rDNA) *Wallemia* has been placed into a highly supported clade together with Ustilaginomycetes and Hymenomycetes (Basidiomycota). Within this clade, it possesses an isolated position distantly related to the Filobasidiales and was characterized by numerous nucleotide substitutions not shared by any other fungus. Tests on xerotolerance indicated that *Wallemia* presents one of the most xerophilic fungal taxa. Xerotolerance is otherwise rare in the Basidiomycota. To acknowledge its unique morphology, evolution, and xerotolerance, a new basidiomycetous class Wallemiomycetes covering an order Wallemiales, is proposed. Based on differences in conidial size, xerotolerance, and sequence data of the rDNA internal transcribed spacer regions (ITS rDNA), at least three *Wallemia* species are segregated, identified as *Wallemia ichthyophaga*, *Wallemia sebi*, and *Torula epizoa* var. *muriae*, for which the combination *Wallemia muriae* is proposed. The three species are neotypified. *W. ichthyophaga* differs from *W. sebi* and *W. muriae* in numerous nucleotides of the SSU and ITS rDNA. This high variation within *Wallemia* indicates existence of at least two cryptic genera not distinguishable by morphological characters.

Introduction

Wallemia Johan-Olsen is a genus of cosmopolitan xerophilic fungi, frequently involved in food spoilage. Strains can be isolated from sweet (fruits, jams, cakes, pure sugar), salty (fish, meat, peanuts) and dried foods (Samson et al. 2002), from sea salt (Høye 1902), soil (Domsch et al. 1980), as well as

from indoor and outdoor air (Takahashi 1997). Initially, *Wallemia* was described as being halophilic (Høye 1902; Schoop 1937; Frank and Hess 1941). Later it was recognized to be xerophilic because growth on artificial media proved to be independent of the solute used to lower the water activity (a_w) (Vaisey 1955; Pitt and Hocking 1977). Certain strains of *Wallemia* can produce the toxins walleminol and walleminon (Wood et al. 1990; Frank et al. 1999) and cause subcutaneous infections in humans (de Hoog et al. 2000) and probably also allergological problems resulting in farmer's lung disease (Lappalainen et al. 1998; Roussel et al. 2004).

The genus *Wallemia* was introduced by Johan-Olsen (1887) for the single species *W. ichthyophaga* Johan-Olsen, which was described as slow growing, xerophilic, and forming peculiar conidia. The conidiogenesis was later interpreted or illustrated as phialidic (Barron 1968; von Arx 1970; de Hoog et al. 2000) but is now understood as a variation of the basauxic mode of development of fertile hyphae, which segregate into larger conidiogenous units, in a more or less basipetal succession, each unit disarticulating into four arthrospore-like conidia that remain connected long time by means of connectives or disconnect (Hashmi and Morgan-Jones 1973; Madelin and Dorabjee 1974; Cole and Samson 1979).

von Arx (1970) synonymized *Sporendonema* Desm. with *Wallemia* and established the combination *Wallemia sebi* for the species *Sporendonema sebi* Fr. *Wallemia sebi* (Fr.) v. Arx is today the most frequently cited *Wallemia* species and encompasses a large number of synonyms (Ciferri and Redaelli 1934; Ciferri 1958; Cannon 1990). Frank and Hess (1941) distinguished a second species, *Sporendonema epizoum* Cif. & Red., based on a study of numerous strains, but this study remained unacknowledged by subsequent workers.

Terracina (1974) showed dolipore-like septal structures in *W. sebi*, similarly to those formed by many basidiomycetes and some ascomycetous yeasts. He explicitly ascribed the structures surrounding the pores to the endoplasmatic reticulum. However, they were later interpreted as a special kind of parenthesome and used as an argument to describe a new family, the Wallemiaceae, which was placed into the Filobasidiales (basidiomycetes) (Moore 1986, 1996). While the Filobasidiales contain two other xerophilic taxa, namely the black yeast genera *Moniliella* Stolk & Dakin and *Trichosporonoides* Haskins & Spencer (de Hoog 1979), xerophily is rarely observed in basidiomycetes but more frequently encountered in ascomycetes (Samson et al. 2002). Wu et al. (2003) developed SSU rDNA primers for the detection and identification of airborne fungal species, including *W. sebi*. They phenotypically compared *Wallemia* sequences with those of other airborne taxa, mainly ascomycetes and zygomycetes, among which *Wallemia* took an isolated, unresolved position.

The present study aims to clarify aspects of the phylogeny, taxonomy, and ecology of the genus *Wallemia*. Recently isolated strains were examined and compared with various reference strains, including ex-type cultures of taxa considered synonymous with *W. sebi*. A higher-rank phylogeny of the genus was inferred from analyses of the small subunit ribosomal DNA gene cluster (SSU rDNA). For the species level, sequence data of the rDNA internal transcribed spacer regions 1 and 2 (ITS), including the 5.8S rDNA, as well as morphological and physiological characteristics were studied.

Material and methods

Strains and culture conditions

Strains studied are listed in Table 1. They were isolated from the hypersaline water of salterns in Mediterranean (Slovenia, Spain), Dominican Republic and Namibia using the method described by Gunde-Cimerman et al. (2000) and from spoiled prsutto using general microbiological techniques and isolation media for xerophilic fungi (Pitt and Hocking 1997). The strains were deposited at the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands), the Culture Collection of the National Institute of Chemistry (MZKI, Ljubljana, Slovenia), and the Culture Collection of Extremophilic Fungi (EXF, Ljubljana, Slovenia). Reference strains were obtained from the CBS and from the personal collection of one of us (J.M.F). The strains were maintained on 50% glucose medium (MY50G, Pitt and Hocking 1997) and on Malt Extract Agar (MEA, Gams et al. 1998) with or without the addition of 5 or 10% NaCl, and were preserved either in lyophilized condition or under liquid nitrogen.

Table 1. Isolates studied and their strain numbers, substrate, origin, and GenBank accession numbers.

Strain number	Substrate	Country	History	*Current identification*	GenBank accession numbers (*ITS rDNA; ** SSU rDNA)
CBS[a] 200.33	Unknown	Italy	Received as *Oospora d'agatae*	*W. sebi*	*AY302519
CBS 202.33	Sea salt	Unknown	*Torula sacchari* T[b]	*W. sebi*	*AY328912
CBS 213.34	Unknown	Italy	Received as *Sporendonema epizoum*	*W. sebi*	*AY302520
CBS 196.56	Chronic ulcerative skin lesion of human	The Netherlands	Received as *Hemispora stellata*	*W. sebi*	*AY302526 **AY741379
CBS 633.66	Date honey	Unknown	Received as *Hemispora stellata*	*W. sebi*	*AY328916
CBS 453.80	Air of banana container on ship	Unknown	*Sporotrichum navale* T	*W. sebi*	*AY328914
CBS 818.96	Sunflower seed	Sweden		*W. sebi* NT[c]	*AY328915
CBS 110582 (=EXF-585)	Hypersaline water of saltern	Namibia		*W. sebi*	*AY302499
CBS 110584 (=EXF[d]-1261)				*W. sebi*	*AY302501
CBS 110585 (=EXF-1263)	Barley	UK		*W. sebi*	*AY302502
CBS 110587 (=EXF-1281)	Cake	UK		*W. sebi*	*AY302503
CBS 110588 (=EXF-1274)	Peanuts	Indonesia		*W. sebi*	
CBS 110589 (=EXF-1277)	Dried salted fish *Ophiocephalus striatus*	Indonesia		*W. sebi*	*AY302505
CBS 110590 (=EXF-1266)	Hay sample associated with livestock toxicosis	UK		*W. sebi*	*AY302506
CBS 110591 (=EXF-1268)	Rye	Sweden		*W. sebi*	*AY302507
CBS 110592 (=EXF-1264)	Wheat	UK		*W. sebi*	*AY302508
CBS 110593 (=EXF-1279)	Straw hat	Philippines		*W. sebi*	*AY302509
CBS 110594 (=EXF-1280)	Domestic interior	UK		*W. sebi*	*AY302510
CBS 110595 (=EXF-1270)	Domestic interior	UK		*W. sebi*	*AY302511
CBS 110597 (=EXF-1281)	Wheat	UK		*W. sebi*	*AY302512
CBS 110598 (=EXF-1284)	Domestic interior	UK		*W. sebi*	
CBS 110600 (=EXF-1053)	Hypersaline water of Dead Sea	Israel		*W. sebi*	*AY302513
CBS 110620 (=EXF-1282)	Domestic interior	UK		*W. sebi*	*AY302515
CBS 110622 (=EXF-1265)	Catwalk in silos	UK		*W. sebi*	
CBS 116627 (MZKI[e] B-953)	Hypersaline water of saltern	Slovenia		*W. sebi*	*AY302535
EXF-483	Hypersaline water of saltern	Spain		*W. sebi*	
EXF-622	Hypersaline water of saltern	Spain		*W. sebi*	*AY302528
EXF-757	Hypersaline water of saltern	Dominican Republic		*W. sebi*	*AY302531 **AY741380
MZKI B-384	Seed	Hungary		*W. sebi*	*AY302532
MZKI B-451	Dead grasshopper	Slovenia		*W. sebi*	*AY302533
CBS 103.10	Culture contaminant	France	*Hemispora stellata* T	*W. muriae*	*AY302524
CBS 184.28	Sugar of Arenga (sugar palm)	Unknown	Received as *Torula sacchari*	*W. muriae*	*AY302525
CBS 411.77	Decaying fruit of *Phoenix dactylifera*	Tunesia		*W. muriae*	*AY302527
CBS 110583 (=EXF-1054)	Hypersaline water of Red Sea saltern	Israel		*W. muriae*	*AY302500

Table 1. Continued.

Strain number	Substrate	Country	History	*Current identification*	GenBank accession numbers (*ITS rDNA; ** SSU rDNA)
CBS 110599 (=EXF-1272)	Domestic interior	UK		*W. muriae*	*AY863021
CBS 110619 (=EXF-1285)	Milking parlor	UK		*W. muriae*	*AY302514
CBS 110621 (=EXF-1269)	Domestic interior	UK		*W. muriae*	*AY302516
CBS 110623 (=EXF-985)	Wheat grain	Denmark		*W. muriae*	*AY302518
CBS 110624 (=EXF-1275)	Domestic interior	UK		*W. muriae*	*AY863022
CBS 116628 (MZKI B-952)	Hypersaline water of saltern	Slovenia		*W. muriae* NT	*AY302534 **AY741381
EXF-753	Salty butter	Slovenia		*W. muriae*	*AY302529
EXF-755	Hypersaline water of saltern	Dominican Republic		*W. muriae*	*AY328918
EXF-756	Hypersaline water of saltern	Dominican Republic		*W. muriae*	*AY302530
CBS 113033 (=EXF-994)	Hypersaline water of saltern	Slovenia		*W. ichthyophaga NT*	*AY302523 ** AY741382
CBS 116629 (=EXF-1059)	Salted ham (prsutto)	Slovenia		*W. ichthyophaga*	*AY302521
CBS 116630 (=EXF-759)	Hypersaline water of saltern	Namibia		*W. ichthyophaga*	

[a]Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
[b]Ex-type strain.
[c]Ex-neotype strain.
[d]Culture Collection of Extremophilic Fungi, Ljubljana, Slovenia.
[e]Culture Collection of the National Institute of Chemistry, Ljubljana, Slovenia.

Morphology and cultural characteristics

Macromorphological characters such as size of colonies, spreading tendency, colony structure, exudate production, colony colour, and production of soluble pigments were described on five different media, which are as follows: (i) *Wallemia* morphology medium I (W-4) with $a_w = 0.95$, containing glycerol and NaCl as controlling solutes [yeast extract 2.5 g; KH_2PO_4 0.64 g; $MgSO_4 \cdot 7H_2O$ 0.12 g; glycerol 120 g; NaCl 40.9 g; H_2O 839 ml; agar 15 g; Borrows micronutrients 2 ml ($ZnSO_4$ 1 g; $FeSO_4$ 1 g; $CuSO_4$ 150 mg; $MnSO_4$ 100 mg; K_2MoO_4 100 mg; H_2O 1 l)], (ii) *Wallemia* morphology medium II (W-10) with $a_w = 0.90$ [as W-4 but containing 100 g NaCl]; (iii) MEA with $a_w = 0.998$, (iv) MY50G with $a_w = 0.890$ and (v) MY10-12 with $a_w = 0.916$ (Pitt and Hocking 1997). Macroscopical characters and general growth rates were reported from point-inoculated media in plastic Petri dishes (9 cm diam) incubated at 24 °C for 14 days. Micromorphological characters were studied from slide cultures (Gams et al. 1998) using media (i)–(iv) incubated at 24 °C for 7 days and phase-contrast microscopy. The reported characters and measurements derived from MY50G. Medium (v) was used for isolation purposes. Water activities of media used for morphological examinations were verified by the DECAGON CX-1 Water Activity System (Campbell Scientific Ltd). The pH of media (i)–(iii) and (v) was set to 6.5 prior autoclaving according to Pitt and Hocking (1977).

Physiology

Colony growth of strains marked below was measured on media with 11 different water activities ranging from $a_w = 0.99$ to 0.77 (measured as described above). For all, glycerol, NaCl, or glucose was used as controlling solute. The basic medium contained 10 g malt extract, 10 g yeast extract, 1 g K_2HPO_4 and 20 g agar (Wheeler et al. 1988) and had a water activity of $a_w = 0.999$. The pH of the media was adjusted as described above. The following strains were included in physiological experiments: CBS 113033, CBS 116629, CBS 116630 (all *W. ichthyophaga*); CBS 202.33, CBS 196.56, CBS 633.66, CBS 453.80, CBS 818.96, CBS 110582, CBS 110600, CBS 116627, EXF-483, EXF-622, EXF-757, MZKIB-384, MZKI B-451 (all *W. sebi*); CBS 103.10, CBS 184.28, CBS 411.77, CBS 110583, CBS 110599, CBS 110619, CBS 110621, CBS 110623, CBS 110624, CBS 116628, EXF-753, EXF-755, EXF-756 (all *W. muriae*). For each medium and each water activity, accomplished by different NaCl concentrations, mean values of three replicates per strain were calculated. The mean of these mean values was calculated for strains belonging to one species. The standard deviation was calculated as well.

Molecular methods

DNA was extracted from ca. 1 cm^2 of 14 days old cultures by mechanical lysis (Gerrits van den Ende and de Hoog 1999). For the amplification of SSU rDNA and the internal transcribed spacer regions 1 and 2 including the 5.8S rDNA (hereafter referred to as 'ITS'), primers NS1 (White et al. 1990) and NS24 (Gargas and Taylor 1992) and V9G (de Hoog and Gerrits van den Ende 1998) and LS266 (Masclaux et al. 1995), respectively, were used. PCR conditions were applied as described by de Hoog et al. (2000). PCR fragments were purified using the GFX^{TM} purification kit (Amersham Pharmacia Biotech Inc., Roosendaal, Netherlands). Sequence reactions containing primers ITS1 or ITS4 (White et al. 1990) for the ITS, and Oli1, Oli9, Oli3 (Hendriks et al. 1989), BF83, BF951, BF163, BF1438, and BF1419 (de Hoog et al. 2004) for the SSU rDNA as well as aliquots of the BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) were analyzed on an ABI Prism 3700 (Applied Biosystems). Sequences were assembled and edited using SeqMan 3.61 (DNAStar, Inc., Madison, USA).

Sequence data

Newly generated DNA sequences of the SSU rDNA have been deposited in GenBank (http://www.ncbi.nlm.nih.gov) (Table 1). Accession numbers of published sequences are juxtaposed to their taxon names in the phylogenetic tree of Figure 1. References for these sequences are the following: AJ495820, AJ495823, AJ495830 (Bacigalova et al. unpublished), U00969, U00971, U00972 (Berbee and Taylor 1993), AJ271380, AJ271381 (Doering

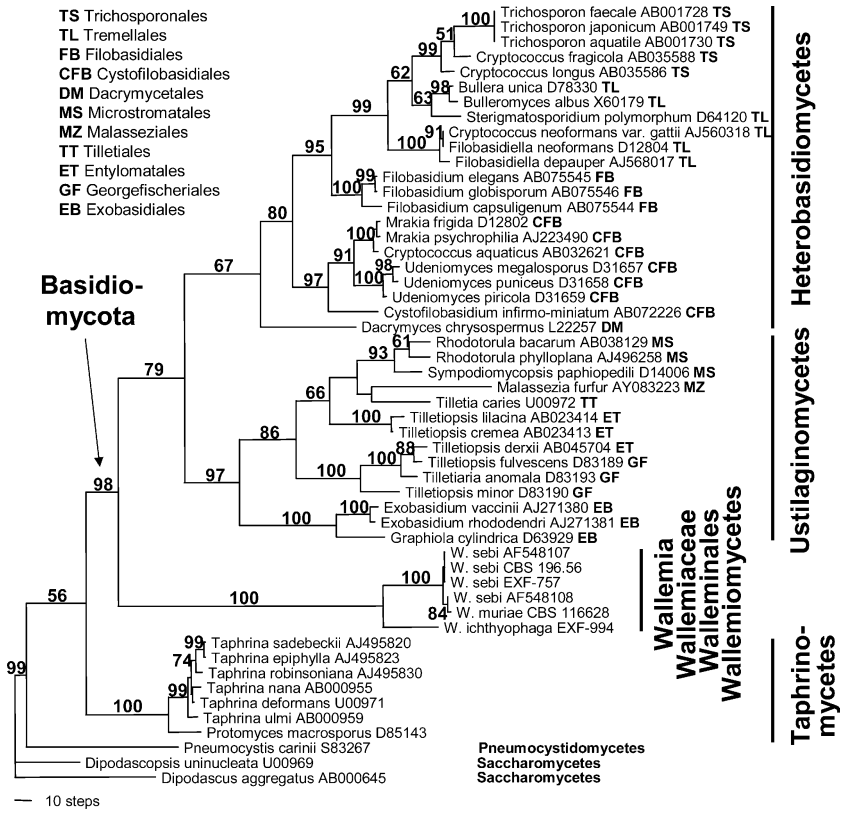

Figure 1. Phylogenetic tree based on SSU rDNA. One of 504 equally parsimonious phylograms inferred from heuristic analyses of the partial SSU rDNA sequences rooted with *Dipodascus agregatus*. Bootstrap intervals from 1000 replicates are indicated above branches. *Wallemia* forms the supported sistergroup of a clade comprising Ustelaginomycetes and Heterobasidiomycetes (bootstraps = 99%), which suggests that it is related to the Basidiomycota, within which it comprises a unique phylogenetic position. CI = 0.473; RI = 0.806.

and Blanz unpublished), AB023413, AB023414 (Hamamoto and Nakase unpublished), AJ568017, AJ560318 (Kidd unpublished), AJ496258 (Lopandic et al. unpublished), AY083223 (McIlhatton and Curran unpublished), AB038129 (Nagahama et al. unpublished), D85143 (Nishida et al. 1998), AB072226 (Niwata et al. 2002), S83267 (Shah et al. 1996), AB000955, AB000959 (Sjamsuridzal et al. 1997), AB001728, AB001730 (Sugita and Nakase 1998a), AB001749 (Sugita and Nakase 1998b), AB035586, AB035588 (Sugita et al. 2000), D63929 (Sugiyama et al. 1995), D31657, D31658, D31659 (Suh and Nakase 1995), D12802, D12804 (Suh and Sugiyama 1993), D14006 (Suh and Sugiyama 1994), D64120 (Suh et al. 1996b), D78330 (Suh et al. 1996a), L22257 (Swann and Taylor 1993), D83189, D83190, D83193 (Takashima and Nakase 1996), AB032621 (Takashima and Nakase 1999), AB045704 (Takashima and Nakase 2001), AB075544, AB075545, AB075546 (Takashima and Nakase unpublished), AB000645 (Ueda-Nishimura and Mikata 2000), X60179 (van de Peer et al. 1992), AF548107, AF548108 (Wu et al. 2003), AJ223490 (Xu et al. unpublished).

Phylogenetic analyses

Wallemia sequences were compared with published and unpublished data available at the National Center for Biotechnology Information or the CBS, respectively. SSU rDNA sequences were aligned with sequences of basidiomycetous and ascomycetous taxa, partly selected using the BLAST server at http://www.ncbi.nlm.nih.gov/Blast/ (Altschul et al. 1990). Incomplete 3′ and 5′ parts of sequences were coded as missing characters. Sequences were automatically aligned using

ClustalX 1.81 (Jeannmougin et al. 1998). The alignments were adjusted manually using BioEdit 5.0.9 (Hall 1999). Phylogenetic relationships of the taxa were estimated from the aligned sequences by the maximum parsimony criterion as implemented in PAUP 4.0b10 (Swofford 2003). Heuristic searches were performed using parsimony informative, unordered, and equally weighted characters; branch robustness was tested by 1000 search replications, each on bootstrapped data sets. Gaps were treated as missing characters. For the SSU rDNA analyses, starting tree(s) were obtained via random, 100 times (10 times in bootstrap analyses) repeated sequence addition. For the ITS rDNA sequence addition was simple. A maximum number of 1000 trees were allowed.

Results

Molecular phylogeny

Heuristic parsimony analyses of the SSU rDNA aligned sequences yielded 252 equally most-parsimonious trees 1558 steps long with a consistency index (CI) of 0.476 and a retention index (RI) of 0.813. The data set contained 2151 bp alignment positions including an intron of 339 bp only encountered in *Protomyces macrosporus* Unger and 446 parsimony informative characters (PIC). The main topology of the 252 trees, one of which is shown in Figure 1, was identical. *Wallemia* was located in a highly supported clade (bootstrap value = 98%) of Heterobasidiomycetes (Trichosporonales, Tremellales, Filobasidiales, Cystofilobasidiales, and Dacrymycetales) together with a moderately supported monophyletic group (bootstrap value = 79%) of Ustilaginomycetes (Microstromatales, Malasseziales, Tilletiales, Entylomatales, Georgefischeriales, and Exobasidiales). Within the former clade, *Wallemia* clustered at the base and formed an isolated, 165 steps long branch. No other genus was found to cluster with *Wallemia* and no fungal SSU rDNA sequence was available sharing any of the numerous nucleotide substitutions unique for *Wallemia*. A highly supported clade (bootstrap value = 100%), comprising species of the ascomycetous genus *Taphrina* Fr. and *Protomyces macrosporus*, was placed outside of the basidiomycetous clade, but sistergroup relationship between these clades was weakly supported (bootstrap value = 56%). *Pneumocystis jirovecii* Frenkel and *Dipodascopsis uninucleata* (Biggs) L.R. Batra & Millner, two other primitive yeast-like fungi with ascomycete affinity, received an unresolved position near the root of the tree. The number of nucleotide substitutions between the sequences of *W. sebi* (CBS 196.56) and *W. ichthyophaga* was 81 bp.

Based on the heuristic parsimony analysis of the 623 aligned position of the partial large ribosomal subunit (LSU rDNA) sequences, *Wallemia* was placed among Basidiomycota (bootstrap value = 99%), next to Heterobasidiomycetes (Dacrymycetales and Tremellales), the later not being supported in bootstrap analysis (results not shown). In the LSU rDNA tree as well none of the deeper branches were supported.

Using 5.8S rDNA sequences of *W. ichthyophaga* in BLAST searches, the species appeared related to ascomycetes such as the members of Dothideales and Helotiales. 5.8S rDNA sequences of other *Wallemia* strains appeared similar for example to the members of Urediniomycetes, Basidiomycota (e.g. *Sporobolomyces* spp., *Rhodotorula* spp.). None of the ITS1 and ITS2 rDNA sequences of any *Wallemia* strains could be compared with any fungal sequences published thus far, which is why no additional out- or sistergroup was added to the phylogenetic analyses of the ITS rDNA (Figure 2). ITS rDNA sequences of *W. ichthyophaga* were 63–78 bp longer than those of other *Wallemia* strains included. Therefore, gaps had to be introduced at numerous alignment positions. Heuristic parsimony analyses of the ITS rDNA aligned sequences (570 bp alignment positions containing 101 PIC) were incomplete, yielding an unspecified number of equally parsimonious trees, of which 1000 were collected and 1 is shown in Figure 2. The trees were 122 steps long and had a CI of 0.926 and RI of 0.974. The tree presented was either rooted with *W. ichthyophaga* (Figure 2a) or unrooted (Figure 2b). ITS rDNA sequences of *Wallemia ichthyophaga* appeared unrelated to those of other included *Wallemia* taxa, which clustered in a highly supported clade (bootstrap value = 100%). Within this clade, three supported subgroups were encountered. Two of the subgroups formed a supported clade (bootstrap value = 81%) containing sequences of strains morphologically identified as *W. sebi*. The other subgroup was highly supported (bootstrap value = 98%) and contained sequences of strains

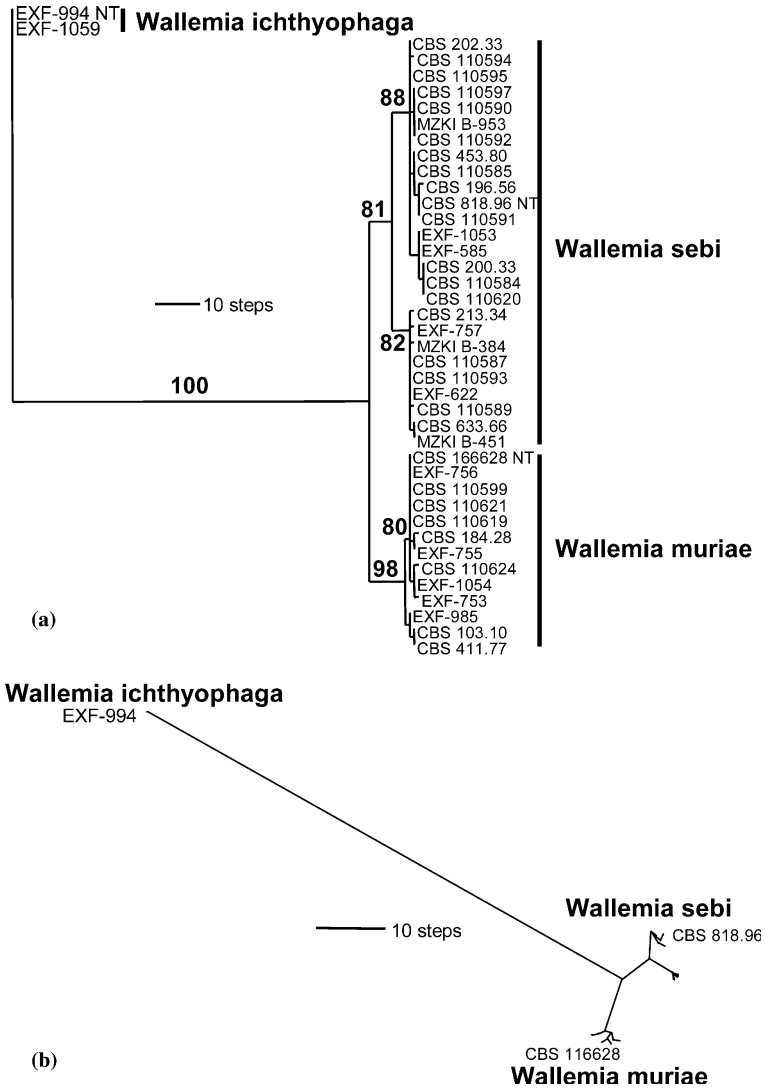

Figure 2. Phylogenetic trees based on ITS rDNA. (a) One of more than 1000 equally and most parsimonious phylograms from an incomplete heuristic phylogenetic analyses inferred from aligned sequences of the ITS1, 5.8S, and ITS2 rDNA rooted with *W. ichthyophaga*. The incomplete analyses yielded an unspecified number of equally parsimonious trees, of which 1000 trees were collected. Bootstrap intervals from 1000 replicates higher than 70% are indicated near their respective branches. (b) Unrooted radial tree based on the same analyses as in (a) not showing bootstrap values. The relatedness of *W. sebi* and *W. muriae* is highly supported and numerous molecular steps distinguish *W. ichthyophaga* from these two species. The moderately supported clade of *W. sebi* (bootstraps = 81%) is segregated into two different monophyletic, but phenotypically indistinguishable groups. CI = 0.926; RI = 0.974.

morphologically identified as *Torula epizoa* Corda var. *muriae* Kickx.

Discussion

Analyses of the SSU rDNA (Figure 1) and LSU rDNA (not shown) strongly suggest that the genus *Wallemia*, represented by *W. sebi* and *W. ichthyophaga*, is a member of or at least a close relative of the Basidiomycota. Phylogenetic analyses thus are in support to earlier interpretations based on the dolipore-like hyphal septum morphology in *W. sebi*, which was seen similar to those formed generally by basidiomycetes and few ascomycetous yeast (Terracina 1974; Moore 1986). Formation of arthrospore-like conidia in *Wallemia* possibly also indicates its relationship with the Basidiomycota,

in which the arthric development of conidia is frequently encountered (Stalpers 1978; Walther et al. 2004). Based on the parenthosome ultrastructure of *W. sebi*, relationship of *Wallemia* with the Filobasidiales has been predicted (Moore 1986), but this interpretation is rejected by the molecular phylogeny based on SSU rDNA sequences (Figure 1).

Based on the SSU rDNA data, *Wallemia* takes an isolated position as a sister group of a clade comprising members of the Ustilaginomycetes and Heterobasidiomycetes. In BLAST searches, only sequences of the Heterobasidiomycetes, Ustilaginomycetes, and Taphrinomycetes appeared comparable to sequences of *Wallemia*. Both the Heterobasidiomycetes and Ustilaginomycetes encompass isolated phylogenetic positions among other basidiomycetes (Scorzetti et al. 2002; Stoll et al. 2003) and are rich in taxa with yeast-like anamorphs such as *Rhodotorula* F.C. Harrison, *Cryptococcus* Vuill., *Malassezia* Baill., *Tilletiopsis* Derx, and *Tilletiaria* Bandoni & B.N. Johri (Boekhout et al. 1998) and biotrophic, plant parasitic life styles such as *Tilletiopsis*, *Tilletiaria*, and *Exobasidium* Woronin (Blanz and Döring 1995; Begerow et al. 2000). These features are considered to represent phylogenetically old life forms. Biotrophic life style and yeast anamorphs are also encountered in the Taphrinomycetes (Eriksson and Winka 1997), which, in respect to certain morphological features, encompass an intermediate between ascomycetes and basidiomycetes and are regarded as phylogenetically old taxa (Nishida et al. 1995). Because of its relative relatedness to taxa with yeast-like anamorphs and biotrophic life-styles, it can be assumed that *Wallemia* is a phylogenetically ancient taxon.

Other supposedly primitive or phylogenetically old ascomycetes, like *Protomyces* Unger, *Pneumocystys* P. Delanoë & Delanoë, *Schizosaccharomyces* Lindner (Archiascomycetes) did not show any relatedness to *Wallemia* but were placed at unique positions in the tree on long terminal branches. Huge molecular distances which resulted in hardly alignable sequences have also been reported for example for two morphologically similar groups of the Dipodascaceae (Ueda-Nishimura and Mikata 2000) and Zygomycota (Voigt et al. 1999), respectively. Also in *Wallemia*, several subregions of the SSU rDNA as well as the LSU rDNA were rich in nucleotide substitutions. Within the LSU rDNA, numerous synapomorphies were encountered, not being shared by any other fungal taxon. The large amount of nucleotide substitutions in these taxa probably accumulated over extended time during evolution or is due to the fact that their close relatives became extinct and may therefore indicate that they are phylogenetically old.

Large molecular distances, however, were also encountered in the LSU rDNA of *W. ichthyophaga* and *W. sebi* and in sequences of the ITS1 and ITS2 rDNA of *W. ichthyophaga* on the one and *W. muriae* and *W. sebi* on the other hand (Figure 2). While the sequences of *W. muriae* and *W. sebi* were well alignable, sequences of *W. ichthyophaga* were hardly alignable to those of *W. muriae* and *W. sebi* and multiple gaps had to be included at various places. It is therefore possible that the genus *Wallemia* in fact comprises a complex of several phylogenetically remote genera and that taxa in between and linking the extant *Wallemia* species have become extinct or have not yet been isolated and analyzed. The formation of sarcina-like structures only in *W. ichthyophaga* (Figure 3m) may indicate that *W. ichthyophaga* is also morphologically distinct from the other two species. A potentially close relative of *Wallemia* might be the genus *Arthrowallemia* Castañeda Ruiz, a genus of leaf-inhabiting fungi because of morphologically similar conidia formed by holothallic conidiogenesis (Castañeda Ruiz et al. 1998). No material from living cultures, however, was available to test this hypothesis.

In spite of considerable molecular distances between *W. ichthyophaga* to *W. sebi* and *W. muriae*, their close relatedness is particularly well corroborated by morphological characters. In all three species, similar arthrospore-like conidia in units of mostly four were formed from morphologically similar conidiophores (Figure 3c, g, h, l). The morphology of the conidiophores and the basauxic mode of conidiogenesis were considered to be unique in the fungal Kingdom and highly typical for *W. sebi* (Hashmi and Morgan-Jones 1973; Madelin and Dorabjee 1974; Cole and Samson 1979) but present in the other two species as well. All three species were also characterized by xerophily, which is relatively rare in basidiomycetes, but more often encountered in various, but unrelated ascomycetes such as *Aspergillus* Link

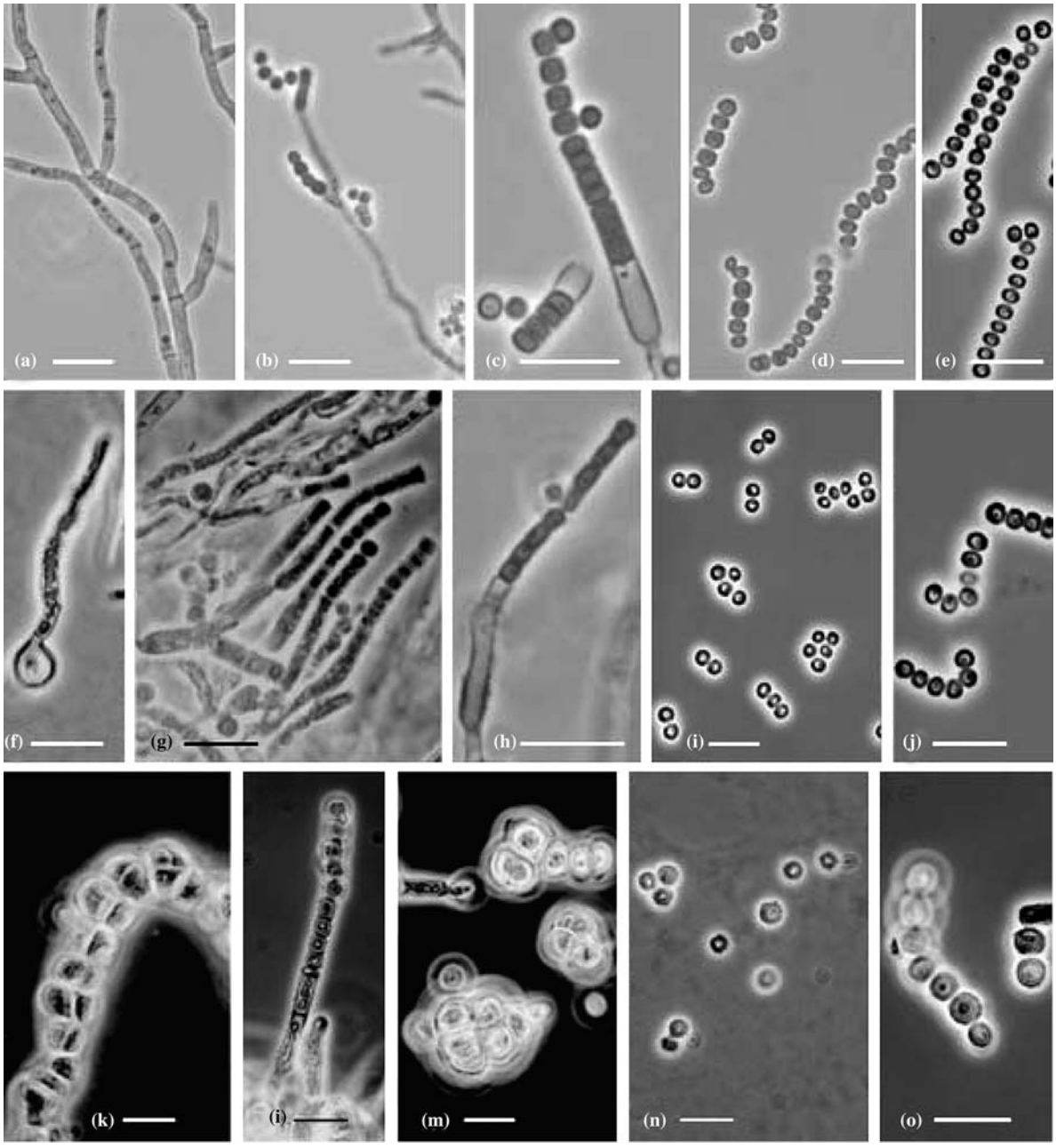

Figure 3. Micromorphology of *Wallemia* spp. (a–e) *Wallemia sebi*, MY50G. (a) Hyphae (CBS 196.56); (b) sympodially elongating conidiophor (CBS 196.56); (c) conidiogenous cell producing conidia in packages of four (MZKI B-953); (d, e) conidia in chains (MZKI B-384, CBS 196.56, respectively); (f–j) *Wallemia muriae*, MY50G. (f) Swollen conidium germinating into hypha (EXF-753); (g, h) conidiogenous cells producing conidia (CBS 110624; EXF-755, respectively); (i) single conidia (CBS 411.77); (j) conidia in chains (EXF-755). (k–o) *Wallemia ichthyophaga*, MY50G. (k) Muriform hypha (EXF-759); (l) Conidiogenous cell forming conidia (EXF-1059). (m) sarcina-like structures of cells (EXF-994); (n) single conidia (EXF-759); (o) conidia in chains (EXF-759). Scale bars on all the figures indicate 10 μm.

Eurotium Link, *Chrysosporium* Corda, *Xeromyces* L.R. Fraser, and *Hortaea* Nishim. & Miyaji (Samson et al. 2002). Of these, *Xeromyces* and *Wallemia* exclusively comprise species that are xerophilic or at least xerotolerant, while xerophily in other genera typically is observed only in certain

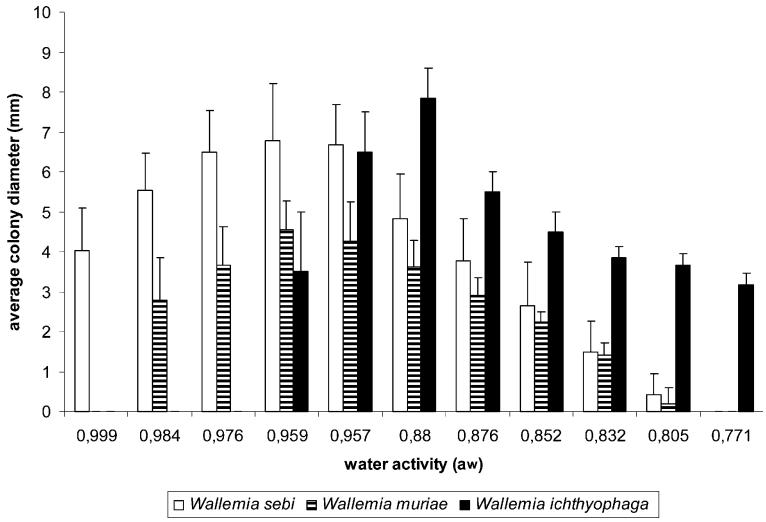

Figure 4. Physiology of *Wallemia* spp. Growth of *W. ichthyophaga*, *W. sebi* and *W. muriae* (average values) on media of different water activities accomplished by different NaCl concentrations. The measures of standard deviation are indicated by bars on the columns.

specialized taxa but not in all species of the respective genus.

Particularly because of its phylogenetic position (Figure 1), but also because of its unique morphology (Hashmi and Morgan-Jones 1973; Madelin and Dorabjee 1974; Cole and Samson 1979) and physiology, a new class and order, the Wallemiomycetes and Wallemiales, respectively, were introduced in this paper.

Species of *Wallemia* are distinguished mainly by morphological and physiological characters, particularly the size range of conidia, presence and absence of sarcina-like structures in *W. ichthyophaga*, and differences in the degree of their xerophily. Because of having their growth optimum in media with additional solutes (Figure 4), all three *Wallemia* species have been considered to be xerophilic. *W. sebi*, however, differed from *W. ichthyophaga* and *W. muriae* in that it showed growth also on media without additional solutes, while the other two species grew only in their presence (Figure 4). Because *W. ichthyophaga* showed positive growth still at a water activity of 0.77, it can be distinguished from both, *W. sebi* and *W. muriae* (Figure 4). The difference in xerophily among the three species apparently is not correlated with occupations of different habitats because representatives of all three species were isolated from the brine of salterns and from dried foods (Table 1). However, the suggested distinction of the three species that also differed from each other in their degree of xerophily (Figure 4) may prove to be highly significant in applied areas such as food industry dealing with materials and substrata exhibiting different water activities.

The relatively wide, irregularly swollen, as well as irregularly branched hyphae, from which also sarcina-like structures develop, were seen in *W. ichthyophaga* but not in *W. muriae* and *W. sebi*. These structures, which are reminiscent of meristem-like structures also observed in other xero- or halophilic fungi such as *Trimmatostroma salinum* Zalar et al. (1999a) and *Phaeotheca triangularis* de Hoog & Beguin (de Hoog et al. 1997; Zalar et al. 1999b), possibly present special adaptations for a growth under relatively low water activities. Sarcina-like structures, however, were formed frequently in *W. ichthyophaga* independently of the medium and solute used and the water activity applied.

The adapted concept of *Wallemia* species is also supported by molecular data (Figure 2). ITS rDNA sequences in *W. sebi* showed high molecular variation that mainly resulted in two moderately supported subclades within *W. sebi*. These subclades probably present two distinct phylogenetic species. To segregate these taxa based on phenotypic data needs additional studies because

relatively strongly variable size ranges of conidia were encountered in both subgroups and no difference in their xerophilic behaviour was obvious.

The name *Hemispora stellata*, of which the extype strain CBS 103.10 was identified as *W. muriae*, has been used repeatedly for clinical isolates identified as such (Ciferri and Redaelli 1934). Following older concepts, taxa of *Wallemia* might be considered as opportunistic human pathogens with low virulence, but in recent years only a single *Wallemia* strain has been isolated from a skin lesion of a human patient (Table 1). Because of its rare occurrence in skin disorders, it is possible that previously isolated *Wallemia* strains actually presented culture contaminants of microsporidial dermatophytes that demand specific media and conditions for growth. In contrast, recent reports attribute a possible role of *W. sebi* in other clinical cases such as lung diseases (Lappalainen et al. 1998; Roussel et al. 2004).

Taxonomy

Wallemiomycetes Zalar, de Hoog et Schroers, cl. nov.

Classis Basidiomycotis vulgo xerophilis, basidiosporis ignotis. Septae cum doliporis. Conidioonenesis basauxis. Conidia arthrosporidia.

Type order. Wallemiales (see below).

Wallemiales Zalar, de Hoog et Schroers, ord. nov.

Ordo Basidiomycotis vulgo xerophilis, basidiosporis ignotis. Septae cum doliporis. Conidiogenesis basauxis. Conidia arthrosporidia.

Type family. Wallemiaceae R.T. Moore.

Wallemiomycetes Zalar, de Hoog and Schroers cl. nov. et **Wallemiales** Zalar, de Hoog and Schroers ord. nov.

Conidiophores unbranched or sympodially proliferating, continuous with conidiogenous cells, smooth-walled. Conidiogenous cells verruculose, basauxially extending, distally disarticulating into arthrospore-like conidia. *Conidia* verruculose, short cylindrical, becoming spherical. *Hyphal septa* with a single pore, flaring out near the periphery of the pore, barrel-shaped, dolipore-like. *Ecophysiology*. Typically xerophilic.

Type familiy. *Wallemiaceae* R.T. Moore, *in* Sneh B, Jabaji-Hare S, Neate S. and Dijst. G. (eds), *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Kluwer Acad. Publ., Dordrecht, The Netherlands. p. 20, 1996.

Type genus and type species. *Wallemia ichthyophaga*.

Phylogenetic affinities. Basidiomycota.

Habitat. Hypersaline water, food with low water activity due to salt and other solutes and dried plant products.

Distribution. Cosmopolitan.

References. For conidiogenesis: Cole and Samson (1979), Hashmi and Morgan-Jones (1973), Madelin and Dorabjee (1974) and Moore (1986); for ultrastructure of septal pores: Moore (1986, 1996) and Terracina (1974); for life style: Frank and Hess (1941).

Commentary. Moore (1996) classified the Wallemiaceae into the Filobasidiales based on ultrastructure of the septal pore in *Wallemia sebi*, particularly on the parenthesomes, which were described as vesiculate.

Wallemia ichthyophaga Johan-Olsen 1887. Christiania Videnkabs-Selskab Forhandl. no. 12, pp. 1–20. – Figure 3k–o.

Colony characteristics. Colonies punctiform, cerebriform, dusty, soft, and slimy typically spreading deeply into the agar, on W-4, W-10 and MY50G 3.0–5.0 mm diam, about 3 mm high; on W-4 and W-10 dark reddish brown with dark greenish brown reverse; on MY50G light brown; on media with glycerol and glucose variously pigmented, beige, yellow, light green, or brown, sometimes shiny with an irregular surface, consisting of one to several, up to 3–5 mm diam large globular structures; not growing on MEA or media without additional solutes; exudates not observed.

Microscopy. Hyphae hyaline, smooth, 4.0–10.0 μm wide, with up to 2.0 μm thick walls, forming an irregularly branched, transversely and sometimes also longitudinally septate mycelium (Figure 3k). Conidiophores more or less solitary, erect, subhyaline, unbranched, smooth-walled, slightly constricted below the apex, continuing into fertile conidiogenous cells (Figure 3l); conidiogenous cells cylindrical, verruculose, basauxically extending, at ca. 10–12.5 μm height, basipetally disarticulating into packages of four arthrospore-like conidia. Conidia one-celled, pale brown, initially short cylindrical, soon becoming spherical, with thick, echinulate walls, 4.0–5.0 μm diam,

forming fragile chains (Figure 3 n, o); conidia typically swelling to 10.0 μm diam, dividing irregularly to form sarcina-like structures up to 200 μm diam, from which new conidiophores arise (Figure 3m).

Physiology. Growth requiring additional solutes, no growth on ordinary media such as MEA; growth not depending of the solute type; growth at a minimum $a_w = 0.96$, optimum at around $a_w = 0.90$, maximum at $a_w = 0.77$ (Figure 4).

Type. Norway, isolated from salted klip-fish (cited by Ciferri 1958). No authentic material preserved. NEOTYPE: Slovenia, Sečovlje salterns, isolated in Oct 1999 by L. Butinar from hypersaline water; dried MY10-12 culture of CBS 113033 (=EXF-994) preserved in herb. CBS.

Habitat. Occurring in hypersaline water of salterns in Slovenia and Namibia; on salted meat and fish; also recorded from stock fish (salted cod), where it produced sarcina-like structures on salt crystals (J.C. Frisvad, personal communication); until now not recorded from environmental substrates with high sugar content, although it can grow also on sugary culture media.

Diagnostic characters. Growth only on media with additional solutes, best growth at $a_w = 0.90$ (e.g. by using 10% of NaCl and 12% of glucose); minimal water activity required is $a_w = 0.96$; conidial size range; presence of sarcina-like structures.

References. Frank and Hess (1941) and Ciferri (1958).

Commentary. The identity of *W. ichthyophaga* is supported by the size range of the conidia, which was given in the original description as 3–4 μm diam and by the presence of sarcina-like cells that derive from the swelling and irregular dividing of conidia (Ciferri 1958). Strains identified here as *W. ichthyophaga* fit remarkably well to the fungus described by Johan-Olsen, however, they form slightly larger conidia. *Wallemia ichthyophaga* is xerophilic because it grows at water activity below 0.85. Wang (1965) reported it several times from paper, but the identity of these strains could not be confirmed.

Wallemia sebi (Fr.) von Arx 1970. The Genera of Fungi Sporulating in Pure Culture. Cramer Verlag, p. 181 – Figure 3a–e.

= *Sporendonema sebi* Fr., Syst. Mycol. 3: 435. 1832.

? = *Torula epizoa* Corda, Deutschl. Fl., ed. J. Sturm, Nürnberg 1: 9. 1829.

≡ *Sporendonema epizoum* (Corda) Ciferri et Redaelli, J. Trop. Med. Hyg. 37: 170. 1934.

= *Sporotrichum navale* Joly, Revue Mycol. 26: 98. 1961. Supported by ex-type strain CBS 453.80.

? = *Torula minuta* Høye 1902. = *Sporendonema minutum* (Høye) Frank & Hess, J. Fish. Res. Board Can. 5: 291. 1941. Characterized as halotolerant, which is why it could be a synonym of *W. sebi*.

Colony characteristics. Colonies punctiform, typically spreading deeply into the agar, on MEA, 3–6 mm diam, compact, powdery, rust brown to purplish-brown, with brown reverse; on MY50G 4.0–12.5 mm diam, with a yellowish brown reverse; on W-4 4.0–8.0 mm diam; colony shape variable, typically domed without or sometimes with short marginal spreading area; margin white or of same colour as the colony, shaggy to irregular; surface smooth but velvety in the central part or, on MY50G, powdery due to strong sporulation; exudates randomly present as yellow droplets, mainly formed on W-10.

Microscopy. Hyphae hyaline, smooth- and thin-walled, 1.5–2.5 μm wide, forming a compact mycelium (Figure 3a). Conidiophores erect, densely, parallel arranged, subhyaline, unbranched or sometimes sympodially elongating, smooth-walled, slightly or not constricted at the apex just below a darker integument, which continues into fertile conidiogenous cells (Figure 3b, c); conidiogenous cells cylindrical, verruculose, basauxically extending, at ca. 7.5–20 μm length basipetally disarticulating into packages of four arthrospore-like conidia (Figure 3c). Conidia one-celled, pale brown, initially short cylindrical, soon becoming spherical, verrucose, thick-walled, (1.5–)2.0–2.5 μm diam, forming up to 1 mm long, straight or bending chains (Figure 3d, e). Conidial chains of neighbouring conidiophores densely aggregated, remaining intact in undisturbed colonies or easily falling apart leading to randomly distributed satellite colonies.

Physiology. Growth not depending of the solute type, also on media without additional solutes having $a_w = 0.99$; growth optimum between $a_w = 0.976$ and 0.957, maximum at $a_w = 0.83$ (Figure 4).

NEOTYPE: Sweden, isolated from sunflower seed, dried MEA culture of CBS 818.96 deposited in herb. CBS, designated herewith.

Habitat. Occurring in salty environments (hypersaline water of salterns or salty fish), air in

indoor environments, on seeds (rye, wheat), dry plant material (hay); also isolated from air in indoor environment.

Diagnostic characters. Growth on media without additional solutes such as MEA; conidial size range.

References. Domsch et al. (1980), de Hoog et al. (2000) and Samson et al. (2002).

Commentary. *Wallemia sebi* is the only species of *Wallemia* capable of growth on media without additional solutes, such as MEA. Because it grows at water activity below 0.85, *W. sebi* is xerophilic. Fries (1832) synonymized *Torula epizoa* Corda, which originated from salty meat in Belgium, as *Sporendonema sebi* Fr., which was described from tallow (tasteless solid fat extracted from animal fat). Both authors did not mention measurements of morphological structures. In contrast, the concept of *W. sebi* proposed here, is based on morphological and physiological characteristics. Although originally described from animal fat, *W. sebi* has been isolated mainly from various substrata other than meat or tallow but once from a salted fish. These substrates share a certain degree of dryness, but are not exceptionally osmotic. In order to stay as close as possible to the strain reported in the protologue of *S. sebi*, we select CBS 818.96 from sunflower seed in Sweden as NEOTYPE of *Wallemia sebi*. Our concept is in consistency with previous concepts (Ciferri and Redaelli 1934; Ciferri 1958; Cannon 1990), which all consider *Sporendonema sebi* as the basionym of the taxon, that later von Arx combined as *W. sebi*.

Wallemia muriae (Kickx) Zalar & de Hoog, comb. nov. – Figure 3f–j.

≡ *Torula epizoa* Corda var. *muriae* Kickx, Fl. Crypt. Flandres 2: 299. 1847.

= *Hemispora stellata* Vuillemin, Bull. Soc. Mycol. Fr. 22: 1–6. 1906. Colonies star-shaped, 0.5–2.5 mm diam. Conidia 2.6–3.5 μm diam, verruculose. The ex-type strain of *H. stellata*, CBS 103.10, is a representative of *W. muriae*. Vuillemin (1931) redescribed this strain, which he originally described as a culture contaminant, but listed the species as originating from a human patient.

? = *Oidium morrhuae* Farlow, Bull U.S. Fish., p. 4. 1886. The species was isolated from salted fish. Its habitat and description indicates that it is likely a synonym of *Wallemia muriae*.

Colony characteristics. Colonies punctiform, typically spreading deeply into the agar, on W-4 and W-10 3.0–5.0 mm diam, walnut- to purplish-brown, with dark greenish-brown reverse; on MY50G 5.5–8.0 mm diam; not growing on MEA or media without additional solutes; colony shape somewhat domed, typically with a spreading marginal area: on MY50G flat, extremely dusty due to strong sporulation, developing in concentric rings; exudates occasionally present on W-10, as yellow droplets.

Microscopy. Hyphae hyaline, smooth- and thin-walled, 2.5–3.5 μm wide, forming a compact mycelium. Conidiophores aggregated, subhyaline, unbranched, rising laterally from hyphae, smooth-walled, slightly constricted below the apex, continuing into fertile conidiogenous cells (Figure 3g, h); conidiogenous cells cylindrical, verruculose, basauxically extending, at ca. 10–12.5 μm height basipetally disarticulating into packages of four arthrospore-like conidia (Figure 3h). Conidia one-celled, pale brown, initially short cylindrical, soon becoming spherical, verrucose, with up to 1 μm thick walls, 2.5–3.0 μm diam, forming chains (Figure 3i, j).

Physiology. No growth on media without additional solutes such as MEA; growth not depending of the solute type; growth minimum at $a_w = 0.985$, optimum at around $a_w = 0.96$, maximum at $a_w = 0.83$ (Figure 4).

Type. Belgium, described from fish brine. No authentic material preserved. NEOTYPE: Slovenia, Sečovlje salterns, isolated in July 1997 by P. Zalar from hypersaline water; dried MY10-12 culture of CBS 116628, deposited in herb. CBS, designated herewith.

Habitat. Occurring on sugary food products (date honey, cake), salty food (peanuts), hypersaline water of salterns world-wide, dry substrates (straw, seeds), and in indoor environments; one record from an insect is available.

Diagnostic characters. Growth only on media with additional solutes, best growth at $a_w = 0.985$ (e.g. by using 2% NaCl, 10% glucose, or 6% glycerol); conidial size range.

Commentary. The identity of *W. muriae* is supported by the size range of the conidia, which was given in the original description as 3–3.5 μm diam based on *in vivo* measurements. Strains identified here as *W. muriae* have 2.5–3 μm large conidia based on *in vitro* measurements and are interme-

diate of *W. ichthyophaga* and *W. sebi*. *Wallemia muriae* is xerophilic because it grows at water activity below 0.85.

Dichotomous key to accepted Wallemia species

(1a) Colonies well growing at 24 °C on MEA, reaching 3–6 mm diam in 14 days; conidia shortly cylindrical, 1.5–2.5 μm diam; sarcina-like structures absent ..... *W. sebi*

(1b) Colonies only growing on MEA with additional solutes (NaCl, glucose); conidia larger than 2.5 μm diam, sarcina-like structures absent or present 2

(2a) Colonies on MY50G dark brown, with a cerebriform surface; conidia 3.5–5.0 μm in diam, sarcina-like structures present ..... *W. ichthyophaga*

(2b) Colonies on MY50G walnut brown, with a powdery surface; conidia 2.5–3.0 μm diam; sarcina-like structures absent. ..... *W. muriae*

List of doubtful names listed as synonyms of *Wallemia sebi* (Fr.) von Arx.

Bergellinia monospora Borzi, Malpighia 2: 469–476. 1888. Described from an infected external ear canal of human from Sicily, Italy. The description could not be interpreted.

Catenularia fuliginea Saito, J. Coll. Sci. Imp. Univ. Tokyo 18: 51. 1904. Saccardo synonymised this species, isolated from cheese, with *Torula simplex* (Lindner) Sacc. The original description of the fungus could not be interpreted.

Oidium pulvinatum Cooke, Rav. Am. Fungi n. 770. 1883–1884. The species was described from a living leaf of *Carya tomentosa* in South Carolina, probably accidentally included by Ciferri (1958) in a list of synonyms of *W. sebi*.

Oospora d'agatae Saccardo, in: Pasini, Atti Riun. Soc. Ital. Dermat. Sifil. 26: 106. 1930. The species was described on the basis of a clinical isolate. Its identity is doubtful.

Oospora ochracea Corda, Ic. Fung., vol. I, p. 16, 1837. The citation is reproduced from Saccardo (1884) but not listed in this publication of Corda. The name was assigned to an isolate from condensed sap of *Sambucus nigra* berries in Bohemia (Czech Republic). The conidia were reported to be 35 μm diam. The species is of doubtful identity.

Penicillium simplex Lindner, Mikrosk. Betriebsk. Gärungsgew. p. 170. 1895. The name was introduced for an isolate from wine must in Germany. Raper and Thom (1949) listed it as a doubtful species.

Sporendonema terrestre Oudemans, Meded. Kon. Akad. Wetensch., Afd. Natuurk., 3e Reeks, 2: 115. 1885. The species was described from soil, cannot be interpreted and is doubtful.

Torula pulvinata (Bonorden) Saccardo, (= *Alysidium pulvinatum* Bonorden) – Abh. Geb. Myk. 2: 86. 1886. The species was described from wood of *Abies* sp., which is an unusual habitat for *Wallemia*. The morphological description cannot be interpreted.

Torula rubiginosa Rivolta, Paras. Veget. Introd. Studio Mal. Parass., p. 254. 1873. The species was isolated from hay in Italy. Its conidial size was given as 9 μm, which excludes *Wallemia*.

Torula rufescens Fresenius, Beitr. Mykol, p. 36. 1850–1863. The species was described for a white fungus with spherical, reddish conidia measuring 5–8 μm in diam. The description was based on an isolate from human eye. Its identity is doubtful.

Torula acchari Corda, Ic. Fung. 4: 23. 1840. The species was described for a white fungus on sugar, probably an *Acremonium* species forming conidia in chains.

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