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Novel anamorphic mite-associated fungi belonging to the Ustilaginomycetes: *Meira geulakonigii* gen. nov., sp. nov., *Meira argovae* sp. nov. and *Acaromyces ingoldii* gen. nov., sp. nov.

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Three novel mite-associated basidiomycetous species are described in two new anamorph genera as *Meira geulakonigii* gen. nov., sp. nov. (type CBS 110052^T = NRRL Y-27483^T = AS 004^T), *Meira argovae* sp. nov. (type CBS 110053^T = NRRL Y-27482^T = AS 005^T) and *Acaromyces ingoldii* gen. nov., sp. nov. (type CBS 110050^T = NRRL Y-27484^T = AS 001^T). Morphologically, these fungi are similar to the yeast-like fungi classified in the Ustilaginales, such as *Pseudozyma* species. However, analysis of the D1/D2 domain of the LSU rDNA suggests that they belong to two different lineages within the Exobasidiomycetidae of the Ustilaginomycetes (Basidiomycota). Furthermore, these fungi may be of interest for the biocontrol of mites, as they reduced mite numbers by approximately 80 % after inoculation.

INTRODUCTION

Mites (Acari) are among the major pests of commercial crops that annually require costly control measures. Prominent amongst the phytophagous Acari are spider mites (Tetranychidae) and rust mites (Eriophyidae). Many spider mites have developed extensive resistance to most available pesticides (Helle & Sabelis, 1985), whereas rust mites, although less resistant to pesticides, are difficult to control because of their short generation time and their propensity to hide in galls and buds (Lindquist *et al.*, 1996). These difficulties have engendered much interest in additional control options, especially in using natural enemies, such as other mites (Gerson & Smiley, 1990). Interest in acaropathogenic fungi as biocontrol organisms for pest mites has increased in recent years, culminating in reviews dealing with acarine mycoses (van der Geest *et al.*, 2000) and with the use of fungi in mite control (Chandler *et al.*, 2000). The two best-known acaropathogenic fungi are *Neozygites floridana* (Weiser & Muma) Remaud & S. Keller and *Hirsutella thompsonii* Fisher; the former is mostly apathogenic to spider mites, while the latter attacks mainly rust mites.

Within an ongoing project intended to develop fungi for mite control (Sztejnberg *et al.*, 1997), we obtained several fungal isolates from cadavers of the citrus rust mite, *Phyllocoptruta oleivira* (Ashmead) (Eriophyidae), occurring on citrus leaves and fruit. These fungi, along with an isolate obtained from a dead carmine spider mite, *Tetranychus cinnabarinus* (Boisduval) (Tetranychidae), infesting castor beans, and another isolated from a citrus leaf, constitute the subject matter of this article.

A preliminary examination revealed that the fungi isolated from the mites belong to the Ustilaginales, or smut fungi, and share a number of morphological features with *Pseudozyma* Bandoni emend. Boekhout, from which they differ by rDNA sequences. In this paper, we suggest names for these novel fungi, describe their morphology, taxonomy and phylogeny and comment on the effect of one species on mites and on its potential use as a biocontrol agent.

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Abbreviations: ITS, internal transcribed spacer; MEA, malt extract agar; PDA, potato dextrose agar; YMA, yeast extract/malt extract agar; YPGA, yeast extract/peptone/glucose agar.

The GenBank/EMBL/DDBJ accession numbers for isolates AS 001^T to AS 006 are respectively AY158665–AY158670 (D1/D2 domain of LSU rDNA) and AY158671–AY158676 (ITS domain).

METHODS

Organisms studied. All fungi were collected in Israel and are deposited at Centraalbureau voor Schimmelcultures (CBS; Utrecht, The Netherlands), the ARS culture collection (NRRL; Peoria, IL, USA) and in the Department of Plant Pathology and Microbiology, Rehovot, Israel (AS numbers). Strain AS 001^T (=CBS 110050^T=NRRL Y-27484^T) was isolated from the citrus rust mite on grapefruit (*Citrus paradisi*) leaves, south of the Sea of Galilee, in late 1995. Strain AS 002 (=CBS 110051) came from the citrus rust mite on pummelo (*Citrus grandis*), at Ga'aton, in the northern coastal plain. Strain AS 003 originated from the same mite and citrus hosts as AS 001^T, but at Beit She'an, south of the Sea of Galilee, in October 1996. Strain AS 004^T (=CBS 110052^T=NRRL Y-27483^T) also came from the same mite and plant hosts, collected in Dan (Upper Galilee) in October 1996. Strain AS 005^T (=CBS 110053^T=NRRL Y-27482^T) was isolated in autumn 1996 at Nes Ziona (coastal plain) from a carmine spider mite on leaves of castor bean (*Ricinus communis*). Strain AS 006 came from the same area, from lemon leaves (*Citrus limon*) that were not infested by any mites, in November 1999. Mite cadavers or leaf particles were placed on 2 % potato dextrose agar (PDA; Difco) in Petri dishes and observed for fungal growth. After 2–3 days, hyphae or conidia were isolated from the cadavers or the leaves and replated onto PDA.

Inoculation, morphology and physiology. Isolates AS 001^T, AS 004^T and AS 005^T were mass-cultured in the laboratory at 25 °C on PDA and 2 % malt extract agar (MEA; Difco). Maximal spore production occurred within 4–5 days. In order to explore biocontrol effects on mites, spores of isolate AS 004^T (=CBS 110052^T) were washed off from cultures grown on PDA with deionized water and their concentration was adjusted to 10^9 spores ml^{-1} with a haemocytometer. Citrus seedlings infested with the citrus rust mite and (separately) with the citrus red mite (*Panonychus citri* McGregor) and cucumbers infested by the carmine spider mite were sprayed with a suspension of 10^9 spores ml^{-1}, whereas control plants were sprayed only with water. Citrus seedlings were sprayed only once due to the small size of the citrus rust mite and the glabrous leaves. Cucumbers were sprayed six times, because the mites are much larger, they may move onto younger leaves and the leaves are hairy.

The morphology of the isolates was investigated using line inoculations on the following media: 1 % yeast extract/0·5 % peptone/4 % glucose agar (YPGA), yeast extract/malt extract agar (YMA, Difco), yeast morphology agar (Difco), MEA, oatmeal agar (OA; Gams *et al.*, 1987) and PDA. Plates were kept at 25 °C for between 5 days and several weeks. Slides were made in water. Comparative nutritional tests were performed according to Boekhout (1991) and Yarrow (1998). Scanning electron microscopy (SEM) of mites that died during inoculation experiments was performed as described by Staugaard *et al.* (1990) and Weidenbörner *et al.* (1989).

rDNA sequencing and sequence analysis. Isolation of DNA was performed as described by Boekhout *et al.* (1995). The internal transcribed spacer (ITS) domains were amplified using primers V9 (5′-TGCGTTGATTACGTCCCTGC) and RLR3R (5′-GGTCCGTGTTTCAAGAC) in 50 µl reaction volumes containing 30 µM $MgCl_2$, 200 µM of each dNTP, 1 µM of each primer and 1 U DNA polymerase. The following PCR conditions were used: initial denaturation of 5 min at 94 °C, followed by 35 cycles each with a denaturation step of 45 s at 94 °C, annealing for 30 s at 52 °C and an elongation step of 2 min at 72 °C and a final elongation step of 6 min at 72 °C. The amplicons were purified using the GFX PCR DNA purification kit (Amersham Pharmacia Biotech). Aliquots of the PCR products containing 10–40 ng DNA were used in cycle-sequencing reactions in a total volume of 10 µl, containing 1 µl 5× sequencing buffer and 2 µl BigDye terminator RR mix (both from PE Biosystems) and 400 nM primer. The sequencing primers used for the ITS 1, 5·8S rDNA and ITS 2 were ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG) and ITS4 (5′-TCCTCCGCTTATTGATATGC) and primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG) and RLR3R (5′GGTCCGTGTTTCAAGAC) were used for the LSU rDNA. Purification of these amplicons was performed by using the MultiScreen filtration system (Millipore) in combination with Sephadex G-50 Super fine (Amersham Pharmacia Biotech). Sequences were obtained with an ABI 3700 capillary sequencer (PE Biosystems) and further analysed using the Lasergene software package (DNASTAR Inc.). Phylogenetic trees were made with the PAUP 4.0b8a program using parsimony analysis, random step-wise addition and tree bisection-reconnection. Bootstrap values below 50 % were not reported. Unfortunately, the available databases of the D1/D2 and ITS domains are not fully congruent. Therefore, it was not possible to use the same set of species in both analyses.

RESULTS AND DISCUSSION

Morphology and physiology

Approximately two-thirds of the mites used for isolation of fungi became covered with dense mycelia, which are described below. Colonies of isolate AS 001^T remained whitish on PDA, whereas those of the other isolates showed various tinges of brown. Isolates AS 002, 003, 004^T, 005^T and 006 formed a brownish pigment on YPGA, YMA or PDA. Microscopically, all isolates shared the presence of acropetally formed, branched or unbranched, short chains of fusiform conidia (Figs 1 and 2), giving the colonies a somewhat velvety appearance (Fig. 3). Judging from the presence of the chains of blastoconidia, frequently originating from lateral sterigma-like structures occurring near the septa of narrow, hyaline hyphae, we initially identified these isolates as belonging to the genus *Pseudozyma*. SEM showed that blastoconidia were also formed on leaves and on dead mites (Fig. 2). The physiological characteristics of the isolates are presented in Table 1. The basidiomycetous nature of the fungi was further supported by positive Diazonium blue B (DBB) tests and urease activities. The isolates did not ferment glucose, nor did they form extracellular starch-like compounds. Isolates AS 002, 003, 005^T and 006 showed nearly identical assimilation patterns of carbon and nitrogen compounds, which differed from those of AS 001^T and AS 004^T. The physiological patterns of these latter two isolates also differed from each other (Table 1). For instance, in contrast to strain AS 004^T, strain AS 001^T was able to grow on melezitose, *myo*-inositol, nitrate and nitrite.

rDNA analysis and comparison with closely related species

Due to the rather uniform morphological characteristics of these fungi, we analysed the D1/D2 domain of the LSU rDNA, the internally transcribed spacers (ITS) and the 5·8S rDNA (Fell *et al.*, 1995, 2000; Begerow *et al.*, 2000, 2001, 2002). Sequence analysis of the D1/D2 domains of the LSU rDNA demonstrated that the novel isolates cluster within the Exobasidiomycetidae of the Ustilaginomycetes (Fig. 4). The closest relatives appeared to be *Dicellomyces scirpi* Raitv.

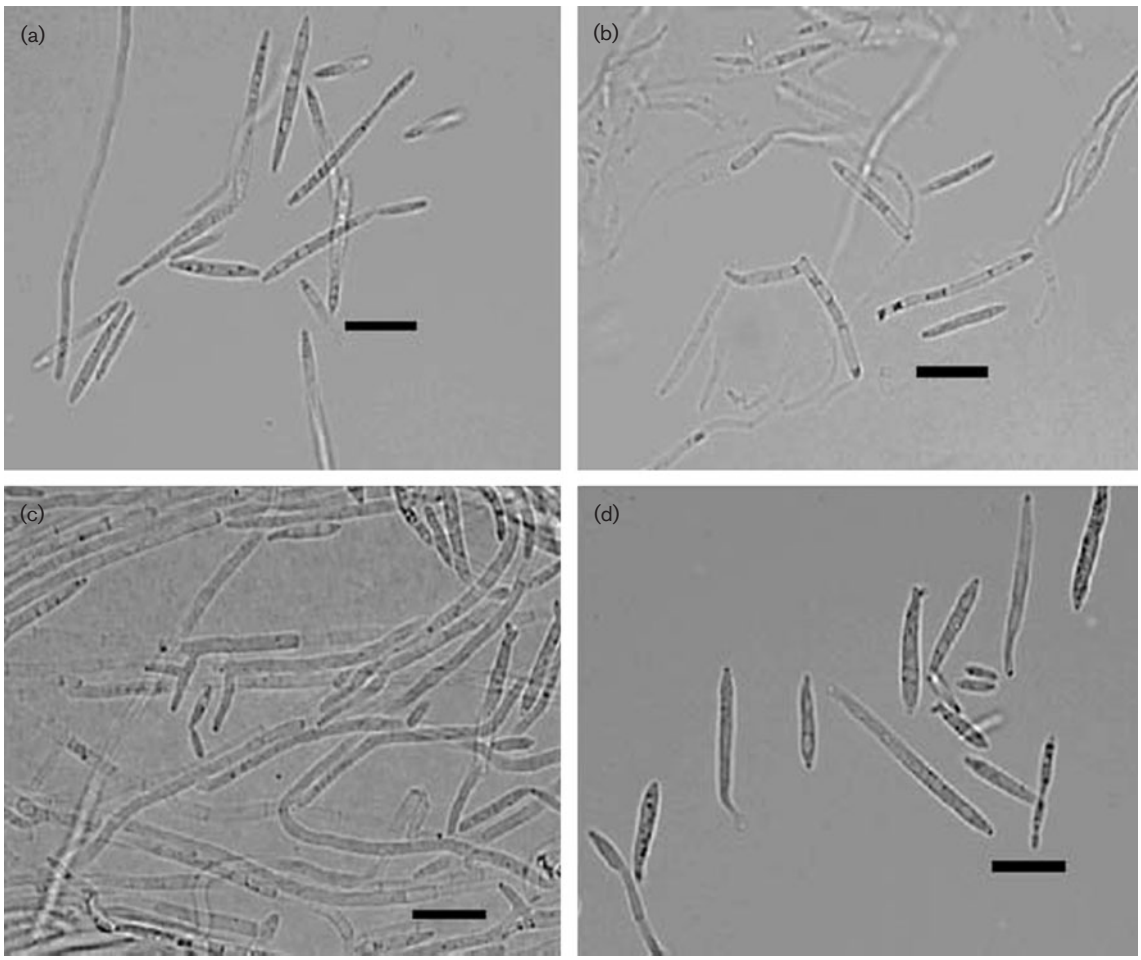

Fig. 1. Microscopic morphology of *Meira argovae* (a), *Meira geulakonigii* (b) and *Acaromyces ingoldii* (c, d), showing fusiform conidia, sterigma-like structures and hyphae. Bars, 10 μm.

and *Kordyana* spp., although the bootstrap values were lower than 50 %. These species have been classified in the Brachybasidiaceae of the Exobasidiales (Exobasidiomycetidae, Ustilaginomycetes) (Begerow *et al.*, 2002). *Graphiola phoenicis* (Mougeot) Poiteau (Graphiolaceae) appears more distantly related (Fig. 4). Fungi belonging to the Brachybasidiaceaea have sexual states and are known as plant parasites (Kirk *et al.*, 2001). Isolates AS 002, 003, 005^T and 006 shared the same D1/D2 and ITS sequences. Isolate AS 004^T belonged to the same cluster, but differed in 5 nt in the D1/D2 sequence and 45 nt plus three deletions in the ITS sequences. The isolates could not be identified with any known taxon of the Exobasidiales and we propose a new anamorphic genus, *Meira* gen. nov. Boekhout, Scorzetti, Gerson & Sztejnberg. The systematic position of the genus *Meira* within the Exobasidiales is not yet clear, because of low bootstrap values. The Brachybasidiaceae may be a candidate family to accommodate these fungi, but Graphiolaceae or Exobasidiaceae are possible alternatives. It appears that additional sampling is required within this group of fungi to resolve this intraordinal phylogeny. The plant pathogen *Muribasidiospora indica* Kamat & Rajendren is also classified in the Exobasidiales (Rajendren, 1969; Begerow *et al.*, 2001), but the culture of that species differs from that of *Meira* by the presence of pigmented hyphae and abundant chlamydospores (Rajendren, 1970).

Strain AS 001^T differed from the remaining isolates in a considerable number of nucleotides and formed a well-supported cluster (bootstrap value 100 %) with *Clinoconidium bullatum* H. Syd. The cluster of these two species with *Coniodictyum chevalieri* Har. & Pat. was supported by a bootstrap value of only 55 % (Fig. 4). We concluded that strain AS 001^T may belong to the Cryptobasidiaceae of the Exobasidiales (Exobasidiomycetidae, Ustilaginomycetes, Basidiomycetes) but, again, further sampling and expansion of the set of organisms and genes are required to settle this issue. However, this phylogenetic position of isolate AS 001^T suggests that it belongs to a genus separate from the other five mite-associated fungi. Strain AS 001^T could not be identified with any known taxon within the Exobasidiomycetidae and we therefore propose a new genus, *Acaromyces*

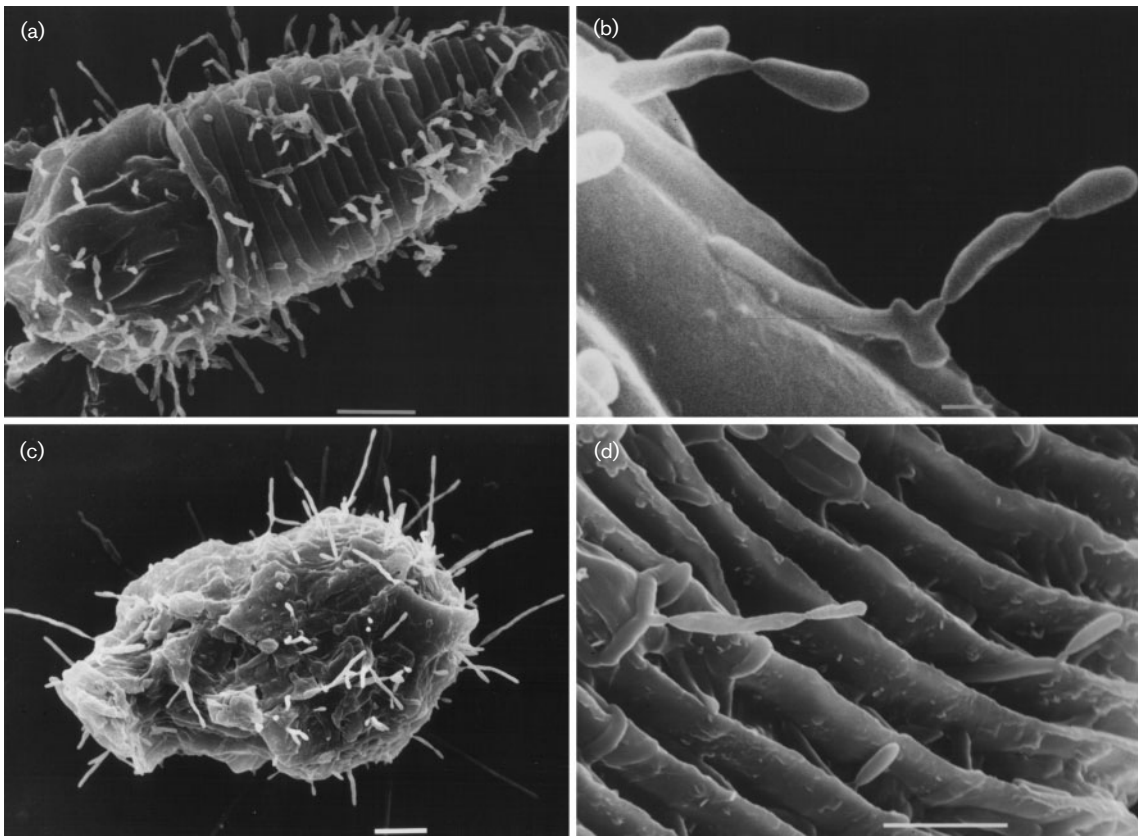

Fig. 2. Scanning electron micrographs of *Meira geulakonigii* and *Meira argovae*. (a) Sporulation of *Meira geulakonigii* on a mite. (b) Detail of acropetally formed chain of blastoconidia of *Meira geulakonigii* on a mite. (c) Sporulation of *Meira argovae* on a mite. (d) Detail of acropetally formed chain of blastoconidia of *Meira argovae* on a mite. Bars, 10 (a, c), 5 (d) or 1 (b) μm.

gen. nov. Boekhout, Scorzetti, Gerson & Sztejnberg, to accommodate this fungus. In contrast to most other genera of the Exobasidiomycetidae, which have sexual stages and mainly occur as plant parasites (Begerow *et al.*, 2002), *Acaromyces* is an asexual genus known from mites. Interestingly, the conidial state of *Coniodyctium chevalieri*, as depicted by Malençon (1953), has a gross morphology similar to that of *Acaromyces*.

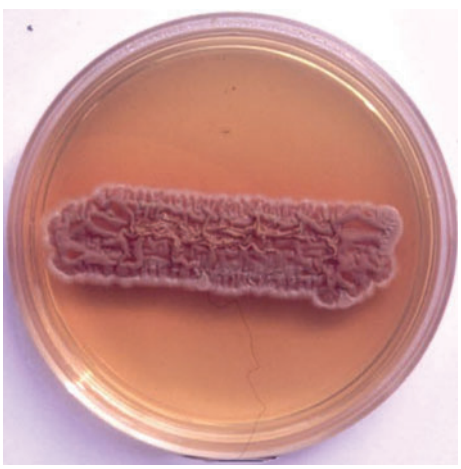

Fig. 3. Culture of *Meira geulakonigii* CBS 110052^T (isolate AS 004^T) on PDA after 11 days.

A number of other fungi form similar acropetally fusiform blastoconidia in chains. The most notable are *Hyalodendron* Diddens, *Fusidium* Link (and close relatives), *Pseudozyma* Bandoni emend. Boekhout and *Sympodiomycopsis paphiopedili* Sugiyama *et al.* Molecular studies showed that *Hyalodendron* belongs to the Hymenomycetes (Guého *et al.*, 1993), *Pseudozyma* species cluster within the Ustilaginales (Ustilaginomycetidae) (Begerow *et al.*, 2000) and *S. paphiopedili* forms a cluster together with *Microstoma juglandis* (Berenger) Saccardo and may belong to the Microstromatales (Begerow *et al.*, 2000). *Fusidium*-like anamorphs belong to the Ascomycetes (Boekhout, 1995; Kirk *et al.*, 2001). Because of the observed differences, we propose to classify these mite-associated fungi in two anamorph genera, *Meira* and *Acaromyces*, belonging to the Exobasidiomycetidae (Ustilaginomycetes, Basidiomycota). From a morphological point of view, the mite-associated fungi cannot be differentiated easily from *Pseudozyma* spp.,

Table 1. Physiological characteristics of *Acaromyces ingoldii*, *Meira geulakonigii* and *Meira argovae*

Characteristics are scored as: +, growth; −, no growth; W, weak growth; D, delayed growth (after 2 or more weeks). Isolates AS 002, AS 003 and AS 006 gave identical results to AS 005^T. All three taxa are positive for assimilation of the carbon compounds D-glucose, D-xylose, sucrose, maltose, α,α-trehalose, raffinose, D-glucitol, D-mannitol and succinate and the nitrogen compound cadaverine. All three taxa are positive for the DBB reaction and growth at 25 and 30 °C. All three taxa show delayed growth on propane-1,2-diol. All three taxa are negative for fermentation of D-glucose, assimilation of the carbon compounds L-sorbose, D-glucosamine, L-rhamnose, methyl α-glucoside, 2-keto-D-gluconate, D-galacturonate, methanol, butane-2,3-diol, saccharate and galactonic acid and the nitrogen compounds creatinine, glucosamine and imidazole, growth on 50 % glucose, starch production and growth at 40 °C.

Characteristic	*Acaromyces ingoldii* AS 001^T	*Meira geulakonigii* AS 004^T	*Meira argovae* AS 005^T
Assimilation of carbon compounds:			
D-Galactose	D	+, D	+
D-Ribose	D	+	+
L-Arabinose	D	+	+
D-Arabinose	W	+	+
Cellobiose	+	W	+
Salicin	+	D	D
Arbutin	−, W	D	−, W, D
Melibiose	−, W	+	+, −
Lactose	D	D	−
Melezitose	+	−	+
Inulin	−, W	D	D, W
Soluble starch	−, W	D	D, +
Glycerol	−	D	D, W
meso-Erythritol	D, W	+	+
Ribitol	−, W	D, +	D, +
Xylitol	−, W	D	D, +
L-Arabinitol	−, W	+	D, +
Galactitol	−	D	D, +
myo-Inositol	+	−	−
Glucono-δ-lactone	−	D	+
D-Gluconate	D	D	−
D-Glucuronate	−, W	D	−
DL-Lactate	−	D	D, W
Citrate	−, W	D	+
Ethanol	D, W	D	D
Quinic acid	D	D	+
Assimilation of nitrogen compounds:			
Nitrate	+	−	+
Nitrite	+	−	+
Ethylamine	W	D	+
L-Lysine	−	+	−
Other tests:			
Growth without vitamins	+	+	+, −
Growth in 0·01 % cycloheximide	D	+	−
Growth in 0·1 % cycloheximide	−	+	−
Urease activity	+, W	+	+
Growth at 35 and 37 °C	−	+	−

because these species are also characterized by the presence of acropetal chains of fusiform conidia originating from sterigma-like structures occurring on narrow, hyaline and septate hyphae. However, as our sequence analysis clearly demonstrated that the mite-associated fungi do not belong to the Ustilaginomycetidae, we recognize them as separate genera.

Asexual smut fungi have been neglected because of taxonomic difficulties. Until the introduction of comparative sequencing of rDNA, it was almost impossible to appreciate the phylogenetic diversity of these fungi, since many isolates share a similar morphology and nutritional physiology (Boekhout, 1987, 1995; Boekhout *et al.*, 1995). Several anamorphic members of the Ustilaginales were originally

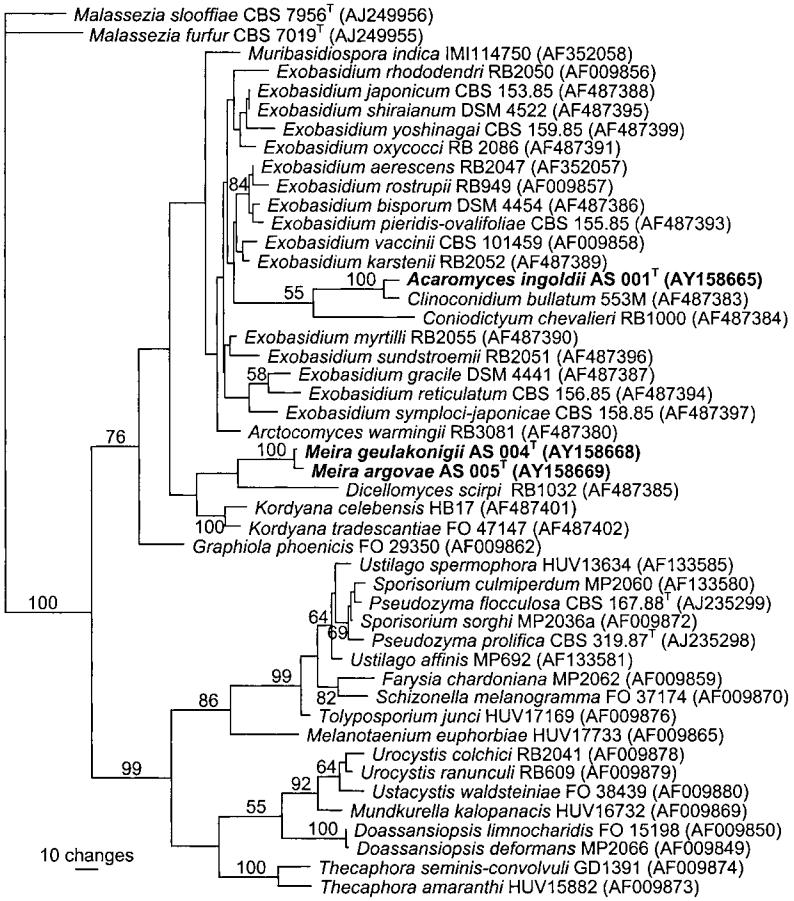

Fig. 4. Dendrogram based on sequences of the D1/D2 domains of the LSU rDNA demonstrating that isolates AS 001^T, AS 004^T and AS 005^T cluster within the Exobasidiomycetidae of the Ustilaginomycetes (Basidiomycota). Accession numbers are given in parentheses. Bar, 10 changes.

described in different genera, and some were even considered to belong to the Ascomycetes (Traquair *et al.*, 1988; Boekhout, 1987, 1995; Boekhout *et al.*, 1995). Sequence analysis of the LSU rDNA suggested that all *Pseudozyma* anamorphs belong to the Ustilaginales, which parasitize grasses (Ustilaginomycetidae, Ustilaginomycetes) (Begerow *et al.*, 2000; Fell *et al.*, 2000).

The life cycle of these mite-associated fungi, as well as those of most *Pseudozyma* species, is poorly known. Two alternatives seem possible. Either they represent the haplophase of otherwise sexual plant-pathogenic smuts, and the relationship between the anamorph and teleomorph fungi has not yet been established, or they are truly asexual fungi that originated from these plant pathogens.

Applied aspects

In a preliminary experiment, isolate AS 004^T reduced mite numbers by ~80 % within 1 week, thus demonstrating a high potential to control mites. We do not know whether the infected mites found in the field died as a result of fungal infection under natural conditions. However, the facts that our fungi were, in most cases, isolated from dead mites and that conidia from cultures of the very same fungi killed mites under laboratory conditions strongly suggest that mites may also be killed by the fungi under natural conditions.

A number of other smut anamorphs also have interesting biocontrol features. For example, *Pseudozyma flocculosa* (Traquair, L. A. Shaw & Jarvis) Boekhout & Traquair is a well-documented biocontrol agent of powdery mildew (Paulitz & Belangér, 2001). Other interesting applied features of smut anamorphs are the accumulation of lipids (~41 % of the dry weight) by *Candida* 107 (Gill *et al.*, 1977), which seems closely related to *Pseudozyma antarctica* (Goto *et al.*) Boekhout (T. Boekhout, unpublished), and the production of extracellular mannosylerythritol lipids and commercially exploited B-lipase by *P. antarctica* (Anderson *et al.*, 1998; Kitamoto *et al.*, 1990). We do not know whether the currently described fungi also share these properties, but it may be very interesting to explore this in the future.

Latin diagnosis of *Meira* Boekhout, Scorzetti, Gerson & Sztejnberg gen. nov.

Genus anamorphicum Exobasidiomycetidarum (*Ustilaginomycetes*, *Basidiomycota*). *Coloniis dimorphicis, primum zymoideis, cellulis fusiformibus, ad apicem e rachide acropetali proliferentibus; deinde hyphae septatae, hyalinae, vulgo cytoplasmate contracto, cellulis evacuatis separatae. Protuberantiae sterigmatoideae saepe prope septa formatae, e quibus catenae breves blastoconidiorum oriuntur. Mycelium aerium tenue e blastoconidiis constans coloniis aspectum velutinum praebens. Myo-inositolum haud assimilatur, nec amylum*

extracellulare formatum. Reactiones DBB *et ureasi positivae. Typus Meira geulakonigii* Boekhout, Scorzetti, Gerson & Sztejnberg.

Description of *Meira* Boekhout, Scorzetti, Gerson & Sztejnberg gen. nov.

Meira [Meir′a. Hebrew fem. n. *meira* light, to express the feeling of three of the authors (A. S., U. G., A. G.) after the phylogenetic position of the fungi was resolved, and also named after the wife of A. G.].

Anamorphic fungi, belonging phylogenetically to the Exobasidiomycetidae (Ustilaginomycetes, Basidiomycota), with dimorphic colonies. Initial yeast-like growth with fusiform cells showing polar budding on an acropetal rachis. Septate hyphae hyaline, usually with the cytoplasm retracted in cells, separated by lysed cells. Sterigma-like outgrowths, frequently occurring near the septa, give rise to short chains of fusiform blastoconidia. Thin aerial mycelium, made up of these blastoconidia, give the colony a somewhat velvety appearance. *myo*-Inositol is not assimilated and extracellular starch is not produced, DBB and urease reactions are positive.

The type species is *Meira geulakonigii* Boekhout, Scorzetti, Gerson & Sztejnberg. So far, the type species and *Meira argovae* are known only from mites, but it is not known whether their occurrence is limited to these animals.

Latin diagnosis of *Meira geulakonigii* Boekhout, Scorzetti, Gerson & Sztejnberg sp. nov.

Coloniae in agaro YPGA *dicto post* 4 *dies convexae, superficie venosa vel cerebriformi, marginem versus sulcatae, synnematibus obtectae, margine integra. In agaro morphologiae post* 48 *horas* ~6 mm diam., *convexae, paulatim conspicue sulcatae, partim synnematibus angustatis obtectae, sed etiam in sectoribus leves, haud lucidae, cremeo-albae; margo regularis. In agaro* YPGA *dicto post* 3 *hebdomades* ~30 mm diam., *albae, marginem versus pallide luteo-brunneae; superficies velutina pruinosa, partim arachnoidea; in medio plana vel modice elevata et verruculosa vel sulcata, marginem versus radiatim sulcata; margo integra vel modice erosa et sectoribus divisa. In agaro* YMA *dicto coloniae in medio synnematibus obtectae, marginem versus magis planae, cremeo-albae. In agaro* PDA *dicto post* 25 *hebdomades* 40 mm diam., *haud lucidae, obscure griseo-brunneae; in medio planae vel modice verrucosae et synnematibus angustatis obtectae, marginem versus sulcatae. Pigmentum brunneum in omnibus agaris diffundens. Primum cellulae zymosae fusiformes*, 7–17 × 2–3 μm, *utrinque sympodialiter proliferentes; hyphae* ~2–3 μm *latae, plerumque partim evacuatae, ad septa modice constrictae, nonnumquam in arthroconidia fragmentatae; catenae acropetales conidiorum ellipsoidalium vel fusiformium*, 5–17 × 2–4 μm *e protuberantiis sterigmatoideis et e latere et apice hypharum oriuntur. Proprietates physiologicae in Tabella* 1 *compositae. Typus* AS 004^T (=CBS 110052^T=NRRL Y-27483^T).

Description of *Meira geulakonigii* Boekhout, Scorzetti, Gerson & Sztejnberg sp. nov.

Meira geulakonigii (geu.la.kon.ig′i.i. N.L. adj. *geulakonigii* of Geula Konig, to commemorate the sister of A. G., who died during the course of the present study).

Colonies on YPGA after 4 days are highly convex, whitish, with the surface venose to cerebriform, radially furrowed near the margin, pruinose and covered with synnemata with entire margins. On morphology agar after 48 h, colonies are about 6 mm in diameter, convex, but becoming strongly furrowed, partly covered with tapered synnemata, but also with smooth sectors, dull, creamy white, with sharp margins. After 3 weeks on YPGA, colonies are about 30 mm in diameter, white, but pale greyish brown near the margin; finely velvety pruinose and, at places, somewhat arachnoid; centre about 15 mm in diameter, flat to somewhat elevated and somewhat warty to ridged, radially furrowed near the margin; margin entire or somewhat eroded and with sectors. On YMA, colonies are whitish pruinose and the centre is covered with synnemata, which become flattened towards the margin. On PDA, colonies are 40 mm in diameter, dull, dark greyish brown, with the centre 6 mm in diameter, flat to somewhat warty and with tapered synnemata and radially furrowed toward the marginal zone; the marginal zone is greyish brown, flat and outermost margin eroded; reverse dark brown. Brown pigment exudes on YPGA, YMA, MEA and PDA. Initial growth with ellipsoidal yeast cells, 7–17 × 2–3 μm, with polar sympodial budding; hyphae approximately 2–3 μm in diameter, usually partly lysed and somewhat constricted near the septa, may disarticulate into arthroconidia-like cells; acropetal chains of ellipsoidal to fusiform conidia, 5–17 × 2–4 μm in size, originate on sterigma-like structures laterally or terminally on the hyphae (Fig. 1b); short chains of conidia are also formed on mites (Fig. 2a, b). Physiological characteristics are presented in Table 1.

Meira geulakonigii differs from *Meira argovae* in that it does not assimilate nitrate and nitrite, it assimilates L-lysine, it shows cycloheximide resistance, it is able to grow at 35 and 37 °C and its hyphae are somewhat constricted near the septa. Moreover, the rDNA sequence of *Meira geulakonigii* differs from that of *Meira argovae* in 5 nt in the D1/D2 domain (LSU rDNA), 35 nt in the ITS1, 1 nt in the 5·8S rDNA and 10 nt in the ITS2 in addition to three deletions, of 6, 7 and 11 nt.

The type is isolate AS 004^T (=CBS 110052^T=NRRL as Y-27483^T), also preserved as a dried specimen in the herbarium CBS (Utrecht, The Netherlands), which was isolated from the citrus rust mite infesting grapefruit (*C. paradisi*) at Dan (Upper Galilee, Israel).

Latin diagnosis of *Meira argovae* Boekhout, Scorzetti, Gerson & Sztejnberg sp. nov.

Coloniae in agaro YPGA *dicto post* 48 *horas* ~4 mm diam., *puctiformes vel convexae; post* 3 *hebdomades* ~12 mm

diam., *in medio verrucosae, pulvinatae vel irregulariter rugosae, demum superficie valde irregulari, sed marginem versus planae; primum albidae, deinde pallide brunneo; superficies alba velutina vel pruinosa; margo erosa; reversum luteo-brunneum vel brunneum. Post* 3 *hebdomades in agaro* MEA *dicto ad* 30 mm diam., *in medio convexae; griseo-brunneae; superficies verrucosa vel sulcata vel reticulata, synnematibus sursum angustatis obtecta. In agaro* PDA *dicto post* 3 *hebdomades coloniae ad* ~30 mm diam., *in medio plus minusve planae vel irregulariter cristatae, leves, in medio lucide luteo-brunneae, marginem planam versus pallidiores. Pigmentum brunneum in omnes agaros diffundens. In coloniis juvenibus cellulae fusiformes zymoideae,* 7–20 × 1·5–2·5 μm, *ad apicem e rachide sympodiali proliferentes; in coloniis vetustioribus hyphae angustae,* ~1·5–2·0 μm *latae, ad septa haud constrictae, partim evacuatae; catenae acropetales conidiorum fusiformium e protuberantiis sterigmatoideis nonnumquam sympodialiter proliferentibus iuxta septa hyphalia oriuntur. Conidia basilaria* 8–25 × 1·0–2·5 μm, *apicem versus breviora,* 3–10 × 1–2 μm; *e rachide sympodiali germinantia. Proprietates physiologicae in Tabella* 1 *compositae. Typus* AS 005^T (=CBS 110053^T=NRRL Y-27482^T).

Description of *Meira argovae* Boekhout, Scorzetti, Gerson & Sztejnberg sp. nov.

Meira argovae (ar.go′va.e. N.L. adj. *argovae* of Argov, to recognize Y. Argov, who collected most of the infected mites).

Colonies on YPGA after 48 h are about 4 mm in diameter, punctiform to highly convex; after 3 weeks, about 12 mm in diameter, with the centre warty, pulvinate to irregularly ridged, finally becoming strongly uneven, but flat towards the margin; at first whitish, but soon becoming pale greyish brown; the surface is finely white velvety pruinose, with hairy synnemata; margin eroded; reverse brown. After 3 weeks on MEA, colonies are up to about 30 mm in diameter, with the centre convex; surface greyish brown, warty, ridged, furrowed to reticulate and covered with tapered synnemata that flatten towards the margin. After 3 weeks on PDA, colonies are up to about 30 mm in diameter, with the centre more or less flat or irregularly ridged, smooth, with the centre shiny yellowish brown (isabellina), gradually changing into a flat marginal zone; brown pigment exudes on YMA, YPGA, MEA and PDA. Isolate AS 003 differs by wider expansion of the colonies (e.g. 65 mm on YPGA after 3 weeks) and a more whitish colony with a pale reverse. Young colonies (after 48 h) consist of fusiform yeast cells, 7–20 × 1·5–2·5 μm, with polar budding on a sympodial rachis; in older colonies, slender, hyaline hyphae occur, approximately 1·5–2·0 μm wide, without constrictions near the septa and usually partly lysed; acropetal chains of fusiform conidia originate on sterigma-like structures, which may be sympodially branched and usually occur near the hyphal septa; conidia are 8–25 × 1·0–2·5 μm at the base of the chain and 3–10 × 1–2 μm near the apex (Fig. 1a); they germinate with a terminal sympodial rachis; short conidial chains also occur on mites (Fig. 2c, d). The physiological characteristics of the species are presented in Table 1.

The type, isolate AS 005^T (=CBS 110053^T=NRRL Y-27482^T), also preserved as a dried specimen in the herbarium CBS (Utrecht, The Netherlands), was isolated in autumn 1996 at Nes Ziona (coastal plain, Israel) from a carmine spider mite on leaves of castor bean (*Ricinus communis*). The other isolates reported here (AS 002, 003 and 006) are also from Israel.

Latin diagnosis of *Acaromyces* Boekhout, Scorzetti, Gerson & Sztejnberg gen. nov.

Fungi anamorphici Cryptobasidiaceae, Exobasidiomycetidarum, Exobasidio affines (*Ustilaginomycetes, Basidiomycota*). *Hyphae septatae, plerumque cytoplasmate contracto, cellulae alteris evacuatis separatae. Protuberantiae sterigmatoideae saepe iuxta septa oriundae, catenas blastoconidiorum proferentes. Mycelium aerium e blastoconidiis constans coloniis aspectum modice velutinum pruinosum praebens. Myo-inositolum assimilar, amylum extracellulare haud formatum. Reactiones* DBB *et ureasi positivae. Typus Acaromyces ingoldii* Boekhout, Scorzetti, Gerson & Sztejnberg.

Description of *Acaromyces* Boekhout, Scorzetti, Gerson & Sztejnberg gen. nov.

[non *Acaromyces* Lavie nomen nudum, no description, no Latin diagnosis and no type material indicated = *Kloeckera apiculata*) (Lavie, 1950)].

Acaromyces (A.ca.ro.my′ces. N.L. n. *acari* from Gr. n. *akari* mite; N.L. n. *myces* from Gr. n. *mukes* fungus; N.L. n. *Acaromyces* mite fungus).

Anamorphic fungus, belonging phylogenetically to the Cryptobasidiaceae, Exobasidiomycetidae (Ustilaginomycetes, Basidiomycota). Hyphae septate, usually with the cytoplasm retracted in cells separated by lysed cells. Sterigma-like outgrowths occur frequently near the septa, giving rise to chains of fusiform blastoconidia. Aerial mycelium thin and made up of blastoconidia, giving the colony a somewhat velvety-pruinose appearance. *myo*-Inositol is assimilated and extracellular starch is not produced. DBB and urease reactions are positive.

The type species is *Acaromyces ingoldii* Boekhout, Scorzetti, Gerson & Sztejnberg.

Latin diagnosis of *Acaromyces ingoldii* Boekhout, Scorzetti, Gerson & Sztejnberg sp. nov.

Coloniae in agaro YPGA *dicto post* 7 *dies* 8 mm diam., *e microcoloniis fusis constantes; haud lucidae, albidae, modice elevatae vel pulvinatae, tenaces, margine integra vel nonnullis hyphis radiantibus fimbriata; post* 3 *hebdomades coloniae* ~16 mm diam., *superficie sulcata, verrucosa vel cerebriformi, marginem versus radiatim sulcata, albidae; mycelio tenui*

pruinoso aerio obtectae; *margo erosa*; *reversum dilute luteo-brunneum*. *In agaro* PDA *post* 27 *hebdomades coloniae* ~25 mm diam., *in medio irregulariter sulcatae*, *margine plano*, *erosa*; *primum albidae*, *velutinae pruinosae*, *sed cito griseo-brunneae partim maculis albis velutinis obtectae*; *reversum brunneum*, *marginem versus partim crystallis cylindricis vel acuformibus aggregatis luteum*. *Hyphae septatae*, ~1–2 μm *latae*, *cytoplasmate contracto*, *cellulae alteris evacuatis separatae*. *Protuberantiae sterigmatoideae*, *sympodialiter proliferentes*, *iuxta septa formatae*, *catenas conidiorum fusiformium vel lanteolatorum proferentes*. *Blastoconidia basilaria* 20–35 × 23 μm, *apicem versus breviora*, ~6–15 × 1·5–3 μm. *Myo-inositolum assimalatur*, *amylum exctracellulare haud formatum*. *Reactiones* DBB *et ureasi positivae*. *Proprietates physiologicae in Tabella* 1 *compositae*. *Typus* AS 001^T (=CBS 110050^T=NRRL Y-27484^T).

Description of *Acaromyces ingoldii* Boekhout, Scorzetti, Gerson & Sztejnberg sp. nov.

Acaromyces ingoldii (in.gol′di.i. N.L. adj. *ingoldii* of Ingold, to recognize the contribution of T. C. Ingold to mycology).

Colonies on YPGA after 1 week are 8 mm in diameter, consisting of interconnected smaller colonies; dull, white, somewhat raised to pulvinate, tough, with the margin entire, but some radiating hyphae may occur on the surface of the agar plate. After 3 weeks, colonies are about 16 mm in diameter, with the surface furrowed, warty to cerebriform, and radially furrowed near the margin; dull, whitish; covered with thin pruinose aerial mycelium; margin somewhat eroded; reverse pale yellowish brown (isabella). On PDA, colonies are about 25 mm in diameter, with centre about 8 mm, irregularly ridged and furrowed, with flat marginal zone and eroded outermost margin; whitish at first, velvety pruinose, but soon becoming greyish brown and covered with white velvety patches; reverse brown; submerged yellowish patches of cylindrical to needle-shaped crystals near the margin; no exuding brown pigment. Hyphae septate, approximately 1–2 μm wide, with the cytoplasm retracted in cells separated by lysed cells; sterigma-like outgrowths, which may be sympodially branched, occur near the septa and give rise to chains of fusiform to lanceolate blastoconidia. Blastoconidia are 20–35 × 23 μm, shorter near the apex of the chain, 6–15 × 1·5–3 μm (Fig. 1c, d). *myo*-Inositol is assimilated and extracellular starch is not produced; DBB and urease reactions are positive. The physiological characteristics of the species are given in Table 1.

Can be differentiated from the two *Meira* species because it assimilates *myo*-inositol and does not utilize glucono-δ-lactone. More importantly, the rDNA sequence indicates that the species belongs to a different lineage within the Exobasidiomycetidae. The morphologically similar genus *Pseudozyma* belongs to a different clade in the Ustilaginomycetidae, namely the Ustilaginales.

The type and only strain, AS 001^T (=CBS 110050^T=NRRL Y-27484^T), also preserved in the herbarium CBS (Utrecht, The Netherlands), was isolated from a mite [citrus rust mite infesting grapefruit (*C. paradisi*) leaves, south of the Sea of Galilee, Israel], but it is not known whether the occurrence of the fungus is confined to mites.

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