Microbial Ecology

Oribatid Mites as Potential Vectors for Soil Microfungi: Study of Mite-Associated Fungal Species

C. Renker[1], P. Otto[2], K. Schneider[3], B. Zimdars[1], M. Maraun[3] and F. Buscot[1]

(1) Terrestrial Ecology, Institute of Botany, University of Leipzig, Johannisallee 21, D-04103, Leipzig, Germany
(2) Systematic Botany, Institute of Botany, University of Leipzig, Johannisallee 21, D-04103, Leipzig, Germany
(3) Institute of Zoology, Darmstadt University of Technology, Schnittspahnstraße 3, D-64287, Darmstadt, Germany

Received: 29 January 2005 / Accepted: 23 May 2005 / Online publication: 13 December 2005

Abstract

The ability of soil-living oribatid mites to disperse fungal propagules on their bodies was investigated. Classical plating methods were applied to cultivate these fungi and to study their morphology. Molecular markers were used for further determination. The nuclear ribosomal large subunit and the nuclear ribosomal internal transcribed spacer of DNA extracts of the cultured fungi as well as total DNA extracts of the mites themselves, also containing fungal DNA, were amplified and sequenced. Based on phylogenetic analysis, a total of 31 fungal species from major fungal groups were found to be associated with oribatid mites, indicating that mites do not selectively disperse specific species or species groups. The detected taxa were mainly saprobiontic, cosmopolitan (e.g., *Alternaria tenuissima*), but also parasitic fungi (*Beauveria bassiana*) for whose dispersal oribatid mites might play an important role. In contrast, no mycorrhizal fungi were detected in association with oribatid mites, indicating that their propagules are dispersed in a different way. In addition, fungi that are known to be a preferred food for oribatid mites such as the Dematiacea were not detected in high numbers. Results of this study point to the potential of oribatid mites to disperse fungal taxa in soil and indicate that co-evolutionary patterns between oribatid mites and their associated fungi might be rare or even missing in most cases, since we only detected ubiquitous taxa attached to the mites.

Introduction

Saprobiontic and mycorrhizal fungi are important organisms in the belowground system [20, 66]. The total length of fungal hyphae in soils is estimated to be about 67 km per gram dry weight [17]. Together with soil animals and bacteria, saprophytic fungi decompose the organic material in most ecosystems of the world [59], and fungi are the only organisms that are able to degrade highly resistant organic substrates such as lignin [19].

Dispersal of soil fungi has been little studied. For some fungal species, e.g., arbuscular mycorrhizal fungi, dispersal by water and wind was shown to be important [1, 77]. Besides this, dispersal by animals, vertebrates as well as invertebrates, was shown to be of great potential [1, 30, 44, 77]. Whereas vertebrates (e.g., ungulates) may provide long-distance dispersal, invertebrates may mainly influence short-distance dispersal. Especially, soil invertebrates such as earthworms, isopods, diplopods, mites, collembolans, enchytraeids, and nematodes may be important dispersers of fungal propagules [11, 42, 57, 58], e.g., 120 species of fungi were found on the surface of collembolan species in an aspen forest in Canada [72, 73]. Furthermore, Acari and Collembola are a main constituent of the biomass of Arthropoda in soils [27, 75] and oribatid mites are the most dominant group of Acari in soils [69], reaching abundance of approximately 30,000 individuals per square meter in mull forest soils [47, 49] and up to 160,000 individuals per square meter in spruce and moder forests [47]. Their ability to colonize available habitats quickly (and thereby disperse microbial propagules) has already been shown [60, 65]. Most species show distinct horizontal distribution [52], as they use specific microhabitats that could often be related to their feeding biology [54]. Much effort has been made to characterize the feeding habits of oribatid mites [6, 7, 25, 37, 38, 45, 48, 62, 64], but little is known about their dispersal capacities. In

Correspondence to: C. Renker; E-mail: crenker@uni-leipzig.de

 DOI: 10.1007/s00248-005-5017-8 • Volume 50, 518–528 (2005)

experiments, oribatid mites showed a maximum individual displacement of 42 cm/day. The daily average movement was generally of the order of a few centimeters, varying with the season and the water content of the litter and soils [9].

Many studies focused on the fungal profit from selective spore dispersal on the body surface or in the digestive tract of mites [39, 51, 67, 68]. On one hand, dispersal of spores on the animal surface generally appeared to be more important than via fecal pellets [53]; on the other hand, it was shown that numerous microbial propagules survive the gut passage and flourish in the fecal pellets shortly after deposition [24, 35]. As migrating soil arthropods transport fungal spores to new substrates, fungi can colonize these new resources [33, 34, 74]. However, the overall effect of oribatid mites and soil microarthropods on the soil fungal community in general is not well explored. The fungal community has been shown to recover faster in the presence of oribatid mites after a severe disturbance [46]; generally, however, the effect of oribatid mites on the structure of the fungal community appears to be limited [50].

The present study investigates the dispersal abilities of oribatid mites for fungal propagules. We isolated fungi from tracks of oribatid mites on agar plates and used the whole animal for DNA extraction. Fungal DNA amplified by polymerase chain reaction (PCR) originated from the surface of the mites. Fungal-specific primers for the nuclear ribosomal large subunit (LSU) and the nuclear ribosomal internal transcribed spacer (ITS) were used. In previous studies, fungi and other microorganisms were isolated from the body or the gut of the animal and were then cultivated for determination. In our study, the molecular analysis of fungi for the first time allowed the detection of uncultured fungi—which means fungi that were not isolated by our standardized procedure—associated with oribatid mites.

Materials and Methods

Study Site. In February 2004, oribatid mites were extracted from litter samples collected in an oak–beech forest (Kranichsteiner Wald). The site is located about 8 km northeast of Darmstadt (N49°54′, E8°44′) in Hesse (Germany) at 150–175 m above sea level. The parent rock is Rotliegendes covered with sand. The soil types are dystric gleysols and orthic luvisols (FAO–UNESCO classification); the humus form is a typical moder. The pH of the soil varies between 3.6 and 4.3. The tree layer is dominated by approximately 190-year-old oaks (*Quercus robur*) with interspersed beech (*Fagus sylvatica*) and hornbeam (*Carpinus betulus*). The field layer contains mainly *Luzula luzuloides*, *Milium effusum*, *Anemone nemorosa*, *Oxalis acetosella*, *Deschampsia cespitosa*, *Stellaria holostea*, *Melampyrum pratense*, and *Polytrichum formosum*. The fungal community of the Kranichsteiner Wald was characterized in the 1970s, and the presence of the lignicolous, the humicolous, and (ecto-)mycorrhizal fungi was discussed [31]. Species composition since this time has not changed (H. Große-Brauckmann in lit.). A short list of common soil microfungi isolated from soils of the Kranichsteiner Wald is given in Table 1.

Sampling of Oribatid Mites and Cultivation of Fungi. L/F layer material was collected and transferred to the laboratory where oribatid mites were extracted by heat [40, 43]. Oribatid mites were separated under a dissecting microscope and identified to species level. Four different species, *Paradamaeus clavipes* (Hermann, 1804), *Steganacarus magnus* (Nicolet, 1855), *Oribatella quadricornuta* (Michael, 1880), and *Oribatula tibialis* (Nicolet, 1855), were chosen for the experiments. These species are among the most abundant oribatid mites in the Kranichsteiner forest (K. Schneider, unpublished data) and can be identified easily under a stereomicroscope due to their comparable large body size. Regarding their ecology, *S. magnus* and *O. quadricornuta* represent primary decomposers, which predominantly

Table 1. List of common soil microfungi isolated from soils of the Kranichsteiner Wald near Darmstadt (Hesse, Germany)

Ascomycota
Alternaria alternata (Fr.) Keissl.;
  Anamorphic *Lewia* (Pleosporaceae)
Aureobasidium pullulans (de Bary) G. Arnaud;
  Anamorphic *Discosphaerina* (Dothioraceae)
Cladosporium sp.;
  Anamorphic *Mycosphaerella* (Mycosphaerellaceae)
Bipolaris spicifera (Bainier) Subram.;
  Anamorphic *Cochliobolus* (Pleosporaceae)
Dictyosphaeria sp.; Anamorphic Chaetosphaeriaceae
Cordana cf. *Pauciseptata* Preuss; Anamorphic Ascomycetes
Epicoccum nigrum Link 1815; Anamorphic Ascomycetes
  Syn.: *Epicoccum purpurascens* Ehrenb.
Geniculosporium sp.; Anamorphic Xylariaceae
Geomyces pannorum (Link) Sigler & J.W. Carmich.;
  Anamorphic *Pseudogymnoascus* (Myxotrichaceae)
  Syn.: *Chrysosporium pannorum* (Link) S. Hughes
Oidiodendron sp.; Anamorphic Myxotrichaceae
Paecilomyces sp.; Anamorphic *Byssochlamys* (Trichocomaceae)
Phialophora sp.; Anamorphic Ascomycetes
Scolecobasidium sp.; Anamorphic Ascomycetes
Torulomyces sp.; Anamorphic Ascomycetes
Ulocladium sp.; Anamorphic Pleosporaceae

Zygomycota
Absidia cylindrospora Hagem; Mucoraceae
A. glauca Hagem; Mucoraceae
Umbelopsis isabellina (Oudem.) W. Gams; Mortierellaceae
  Syn.: *Micromucor isabellinus* (Oudem.) Arx
    Mortierella isabellina Oudem.
Umbelopsis ramanniana (A. Møller) W. Gams; Mortierellaceae
  Syn.: *Micromucor ramannianus* (A. Møller) Arx
    Mortierella ramanniana (A. Møller) Linnem.

feed on litter, whereas *P. clavipes* and *O. tibialis* represent secondary decomposers, which prefer a fungal diet and only partially rely on litter [61]. Two individuals of each species were incubated on 1% Hagem medium (glucose 5 g L^{-1}, KH_2PO_4 0.5 g L^{-1}, NH_4Cl 0.5 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 0.5 g L^{-1}, malt extract 5.0 g L^{-1}, ferric EDTA 5 mL L^{-1}) at room temperature for 12 h. For animal pairs of the four species, this procedure was repeated five times at different concentrations of ampicillin to exclude bacteria, i.e., 250, 563, 1000, 1750, and 4000 μL L^{-1}. Thus, 10 animals per mite species were checked for their potential to act as fungal vector. After incubation, these mites were removed from the medium and directly used for DNA extraction (see below). Fungi growing along the routes of the mites were isolated on new petri dishes to obtain pure cultures of the fungi. However, especially on plates with low concentrations of ampicillin, there were numerous bacteria, making isolation of fungi difficult.

As an alternative medium for the isolation of fungi from mites, 1% MYP medium (malt extract 7.0 g L^{-1}, yeast extract 0.5 g L^{-1}, peptone 1.0 g L^{-1}) was used, but as the number of isolated fungi was below the one obtained with Hagem and no other species were detected, Hagem medium was used in all following experiments.

As some fungi did not develop conidiogenous cells and reproductive structures on Hagem medium, the mycelia of all fungi were transferred to Czapek–Dox medium (glucose 30 g L^{-1}, $NaNO_3$ 3 g L^{-1}, K_2HPO_4 1 g L^{-1}, KCl 0.5 g L^{-1}, $MgSO_4$ 0.5 g L^{-1}, $FeSO_4$ 0.01 g L^{-1}, tetracycline 0.1 g L^{-1}, chloramphenicol 0.1 g L^{-1}) to obtain characters suitable for species identification based on fungal morphology. The isolates were cultured at room temperature for 4 weeks and the plates were microscopically inspected at least twice a week.

Identification of Fungi based on Morphological Characters. Characters relevant for determination of fungal colonies growing on agar plates (Hagem medium and Czapek–Dox medium) were investigated by light microscopy. Apart from publications dealing with single genera (see the section "Remarks on morphology of selected taxa"), the following references were used for identification: Arx [4], Barnett and Hunter [5], Carmichael *et al.* [15], and Domsch *et al.* [21].

Nomenclature and systematic arrangement of fungi followed the British standards *Dictionary of the Fungi* [41] and *Index Fungorum* (http://www.indexfungorum.org). As we obtained only anamorphic states in culture, we used the names of the anamorphs and not those of the teleomorphs and holomorphs. Cultures of *Pseudozyma* sp. (CBS 10103) and *Rhodotorula* sp. (CBS 10104) were deposited at the Centraalbureau voor Schimmelcultures (CBS) in Utrecht.

DNA Extraction and PCR Conditions. To assess whether the fungi isolated from oribatid mites, using the approach described above, represent the whole spectrum of putative mite-associated fungi, fungal DNA was directly extracted from mites [22]. Genomic DNA from fungi obtained in culture was directly isolated from liquid-nitrogen-frozen hyphae after grinding using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) as recommended by the manufacturer.

Amplification of the ITS and LSU regions of the nuclear ribosomal DNA by PCR was performed on a Master cycler gradient system (Eppendorf, Hamburg, Germany) in a total volume of 50 μL containing 2 U *Taq* DNA polymerase (Promega, Heidelberg, Germany), 5 μL of 10× *Taq* polymerase reaction buffer (Promega), 4 μL 25 mM $MgCl_2$, 10 nmol of each dNTP (MBI-Fermentas, St. Leon-Rot, Germany), 50 pmol of each of the two primers and 1–3 μL of the DNA extract. The reactions were performed as hot-start PCR with 10 min initial denaturation at 94°C before adding the *Taq* polymerase at 80°C. The PCR program comprised 32 cycles (40 s at 94°C, 30 s at 54°C, 40 s at 72°C). A final elongation of 10 min at 72°C followed the last cycle.

To amplify fungal ITS regions, we used the primer set ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG) and ITS4 (5′-TCCTCCGCTTATTGATATGC). If no bands were detected in this PCR step, a nested PCR was performed with the primer set ITS1 (5′-TCCGTAGGTGAACCTGCGG) and ITS4 using 1 μL of the first PCR as DNA template. The LSU region was amplified using the primer pair LROR (5′-ACCCGCTGAACTTAAGC) and NL4 (5′-GGTCCGTGTTTCAAGACGG). These primer combinations were nearly specific for fungi. Even with the total DNA extracts derived from mites we only obtained seven clones containing mite amplicons (twice for primer set ITS5/ITS4, five times for primer set LROR/NL4.

Cloning, Sequencing, and Sequence Analyses. PCR products were directly cloned into the pCR 4-TOPO vector, using the TOPO TA Cloning kit for sequencing (Invitrogen Life Technologies, Karlsruhe, Germany) as described by the manufacturer. Clones were amplified and checked for their inserts with the primers mentioned above. Clones showing the same size in the PCR products were separately cut with restriction enzymes *Hin*fI and *Msp*I (MBI-Fermentas) to check their identity. All PCR products showing different product sizes, or the same product sizes but different restriction patterns, were further processed for sequencing.

The plasmid DNA containing the PCR product was extracted from 1.5 mL of *Escherichia coli* TOP10 chemically competent cells using the Perfectprep plasmid mini kit (Eppendorf). Cloned products were then sequenced in both directions with M13 reverse [5′-CAGGAAACAGCTATGAC] and M13 forward [5′-GTAAAACGACGGCCAG] on an ABI

PRISM 3100 Genetic Analyser (Applied Biosystems, Warrington, UK) using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) following the manufacturer's instructions.

DNA sequences of the D1/D2 region of the ribosomal large subunit as well as full nrITS were compared with GenBank sequences using the BLASTN program [2]. Our own sequences were submitted to the EMBL database under the accession numbers given in Tables 2 and 3 and Fig. 1. LSU sequences were manually aligned in Bioedit version 5.09 [32]. Ambiguously aligned positions were excluded from the phylogenetic analyses, resulting in an alignment of 409 putative homologous sites. Reference sequences from GenBank were also included to show the systematic position of the new sequences. Thus, the LSU data set we used consisted of sequences of 11 Basidiomycota (3 obtained in this study), 43 Ascomycota (17 obtained in this study), and 21 Zygomycota (11 obtained in this study). Trees were rooted with sequences of *Suberites ficus* (Porifera) and *Beroe ovata* (Ctenophora).

Phylogenetic trees were inferred using distance criteria as implemented in PAUP* 4.0b10 [70]. A neighbor-joining analysis was conducted based on the Kimura two-parameter model. The confidence of branching was assessed by using 1000 bootstrap resamplings.

Results

Altogether 31 fungal taxa were found in association with the investigated oribatid mites. Sixteen of these species were cultured from the body surface of the mites and determined to species or at least genus level combining molecular and morphological data (Table 2). Most of the detected species are common and widespread soil microfungi that were also isolated from soils of the field site (Table 1), but fungi frequently living in the phyllosphere (*Aureobasidium pullulans*, *Pseudozyma* sp.) or on plant material (especially *Alternaria tenuissima*) were also found. In the case of *Beauveria bassiana*, even a pathogenic fungus attacking mites was detected (Table 2). Not one of the known

Table 2. List of fungi cultivated from oribatid mites and identified to species or genus level, with respect to their ecology aspects and distribution

Fungi with corresponding sequence data of the ribosomal LSU/ITS	*Mode of life*	*Substratum, host*	*Distribution*	*Isolated from*
Absidia glauca Hagem (AJ876785/AJ876491)	S	Mainly soil	C	S
Alternaria tenuissima (Kunze) Wiltshire (AJ876766/AJ876483)	S	Mainly decaying plant material	C	P
Aureobasidium pullulans (de Bary) G. Arnaud (AJ876762, AJ876763/AJ876480, AJ876481)	S	Often inhabitant of the phyllosphere	C	S
Beauveria bassiana (Bals.–Criv.) Vuill. (AJ876771/AJ876485)	Ps (S)	Insects, spiders, and mites (also soil)	C	P
Cladosporium cladosporioides (Fresen.) G.A. de Vries (AJ876764/AJ876482)	S	Soil and plant material	C	O
Clonostachys rosea (Preuss) Mussat (AJ876769/AJ876484)	S	Soil and plant material	C (?)	O
Mucor hiemalis Wehmer (AJ876782, AJ876783/AJ876480, AJ876490)	S	Mainly soil	C	B
Penicillium subgen. *Penicillium* (AJ876775–AJ876776/n.d.)	S	Soil and plant material	C	B
Pseudozyma sp. (AJ876780/AJ876488)	S	Often inhabitant of the phyllosphere	T (?)	O
Rhodotorula sp. (AJ876779/AJ876487)	S	Mainly soil and water (?)	C (?)	S
Trichoderma koningii Oudem. (AJ876773/AJ876486)	S	Mainly soil	C (?)	B
Umbelopsis issabellina (Oudem.) W. Gams (AJ876788/AJ876493)	S	Mainly forest soil	T	B
Umbelopsis ramanniana (A. Möller) W. Gams (AJ876787/AJ876492)	S	Mainly forest soil (?)	T	B
Verticillium sp. (AJ876768/)	S (Pt)	Mainly soil (also plants)	C	O

Information on ecology summarized in the table represents condensed data extracted from the literature and only partially reveals autecological and chorological preferences. Question marks indicate uncertainties regarding the classification. We are aware of the fact that the meaningfulness of data for genera is very limited.

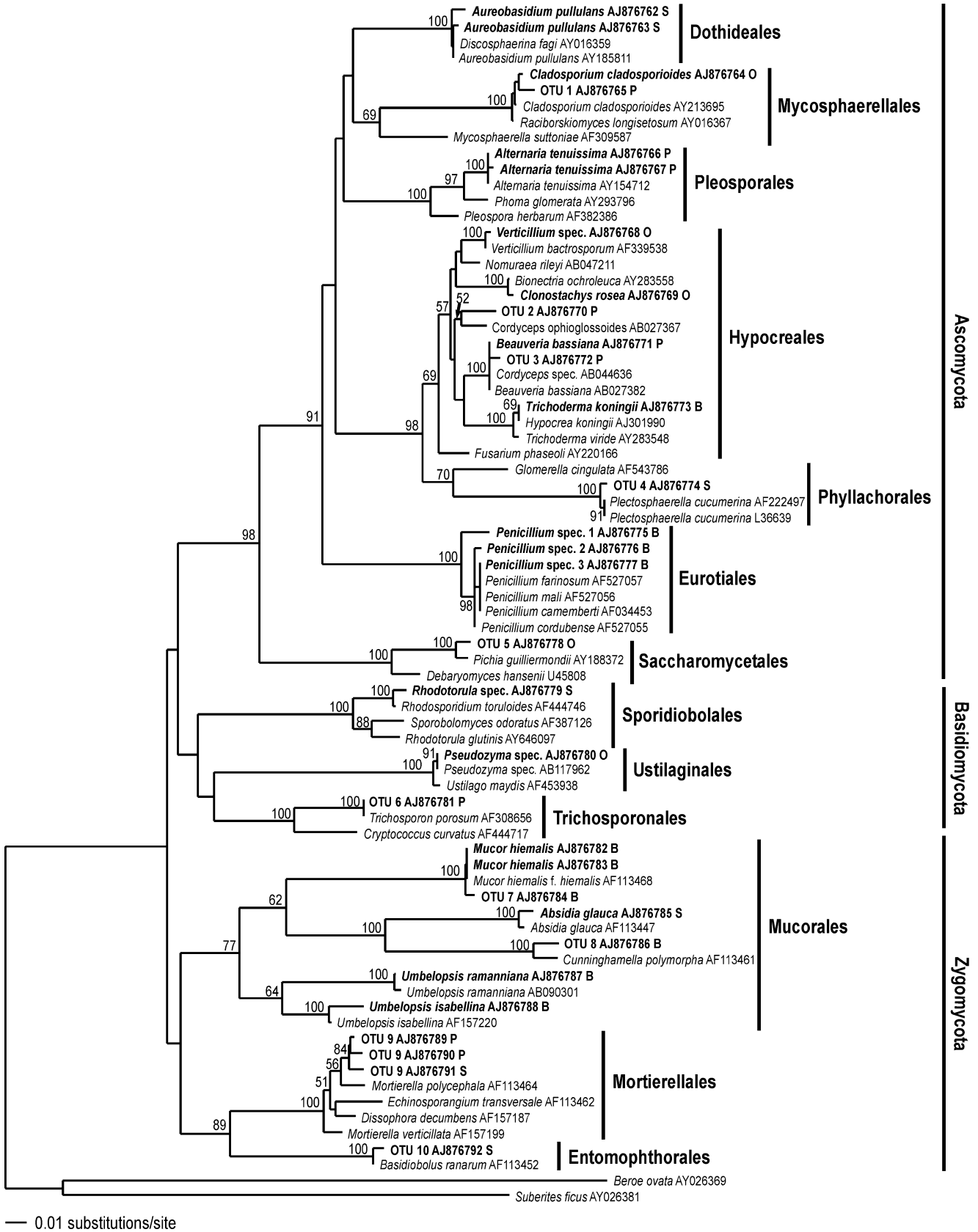
Three different species of *Penicillium* subgen. *Penicillium* were isolated from *O. quadricornuta*.

Mode of life: S, saprobiontic; Ps, parasitic; Pt, pathogenic. Distribution: C, cosmopolitic; T, mainly temperate regions. Isolated from: P, *Paradamaeus clavipes* (Hermann, 1804); S, *Steganacarus magnus* (Nicolet, 1855); B, *Oribatella quadricornuta* (Michael, 1880) and O, *Oribatula tibialis* (Nicolet, 1855). n.d., not done.

Table 3. List of fungi found in the total DNA extracts from mites with the primer sets ITS5/ITS4 for the ITS region of the ribosomal DNA and LROR/NL4 for the D1/D2 region of ribosomal LSU

		Oribatella quadricornuta		*Oribatula tibialis*		*Paradamaeus clavipes*		*Steganacarus magnus*	
Classified as	*Fungal species*	*ITS*	*LSU*	*ITS*	*LSU*	*ITS*	*LSU*	*ITS*	*LSU*
Basidiomycota									
OUT A (AJ876494/n.d.)	*Cryptococcus* sp.							AF444487 99%	
OUT B (AJ876495/n.d.)	*Erythrobasidium hasegawianum*					AF444522 98%			
	Sporobolomyces yunanensis					AB030353 99%			
OUT 6 AJ876496/ AJ876781)	*Trichosporon porosum*					AF414694 99%	AF308656 100%		
Ascomycota									
Alternaria tenuissima (n.d./AJ876767)							AY154712 99–100%		
OUT 3 (AJ876497/ AJ876772)	*Beauveria bassiana*					AB079609 100%	AB027382 99%		
	Cordyceps sp.						AB044636 99%		
OUT 2 (n.d./AJ876770)	*Cordyceps ophioglossiodes*						AB027367 96%		
	Nomuraea rileyi						AB047211 96%		
OUT C (AJ876498/n.d.)	*Cylindrocarpon* sp.							AY295332 98%	
OUT 5 (n.d./AJ876778)	*Pichia guilliermondii*				AY188372 99%				
OUT 4 (n.d./AJ876774)	*Plectosphaerella cucumerina*								AF222497 99%
OUT 1 (n.d./AJ876765)	*Raciborskiomyces longisetosum*						AY016367 100%		
Zygomycota									
OUT 10 (n.d./AJ876792)	*Basidiobolus ranarum*								AF113452 99%
OUT D (AJ876499, AJ876500/n.d.)	*Conidiobolus coronatus*					AJ345094 94%			
OUT 8 (AJ876501– AJ876503/AJ876786)	*Cunninghamella elegans*	AF346409 99%				AF346409 99%			
	Cunninghamella polymorpha		AF113461 96%						
OUT 9 (AJ876504–AJ876507/ AJ876789–AJ876791)	*Mortierella alpina*					AJ271630 94%		AJ271630 94–95%	
	Mortierella verticillata								AF157199 93%
	Mortierella polycephala						AF113464 94%		AF113464 92–94%
OUT 7 (AJ876508, AJ876509/AJ876784)	*Mucor hiemalis*	AY243950 98–99%	AF113468 99%				AF113468 99%		
OUT E (AJ876510/n.d.)	*Rhizopus oryzae*					AB097383 100%			
Umbelopsis ramanniana (n.d./AJ876787)			AB090301 98%						

Sequences identical to sequences of fungi cultured from the according mites were classified to the species level based on morphological survey. Sequences differing from cultivated fungi by more than 1% were classified as operational taxonomic units (OTU). OTUs that were only detected based on LSU or LSU and ITS sequences were numbered, whereas OTUs only detected based on ITS sequences were differentiated by letters. Column 2 gives the closest hit(s) of these OTUs to fungal species found by BLAST search. Accession numbers and sequence identities of the best hits are given for the according oribatid mite species
n.d., not detected.


Aureobasidium pullulans AJ876762 S
Aureobasidium pullulans AJ876763 S
Discosphaerina fagi AY016359
Aureobasidium pullulans AY185811
Dothideales
Cladosporium cladosporioides AJ876764 O
OTU 1 AJ876765 P
Cladosporium cladosporioides AY213695
Raciborskiomyces longisetosum AY016367
Mycosphaerella suttoniae AF309587
Mycosphaerellales
Alternaria tenuissima AJ876766 P
Alternaria tenuissima AJ876767 P
Alternaria tenuissima AY154712
Phoma glomerata AY293796
Pleospora herbarum AF382386
Pleosporales
Verticillium spec. AJ876768 O
Verticillium bactrosporum AF339538
Nomuraea rileyi AB047211
Bionectria ochroleuca AY283558
Clonostachys rosea AJ876769 O
OTU 2 AJ876770 P
Cordyceps ophioglossoides AB027367
Beauveria bassiana AJ876771 P
OTU 3 AJ876772 P
Cordyceps spec. AB044636
Beauveria bassiana AB027382
Trichoderma koningii AJ876773 B
Hypocrea koningii AJ301990
Trichoderma viride AY283548
Fusarium phaseoli AY220166
Hypocreales
Glomerella cingulata AF543786
OTU 4 AJ876774 S
Plectosphaerella cucumerina AF222497
Plectosphaerella cucumerina L36639
Phyllachorales
Penicillium spec. 1 AJ876775 B
Penicillium spec. 2 AJ876776 B
Penicillium spec. 3 AJ876777 B
Penicillium farinosum AF527057
Penicillium mali AF527056
Penicillium camemberti AF034453
Penicillium cordubense AF527055
Eurotiales
OTU 5 AJ876778 O
Pichia guilliermondii AY188372
Debaryomyces hansenii U45808
Saccharomycetales
Ascomycota
Rhodotorula spec. AJ876779 S
Rhodosporidium toruloides AF444746
Sporobolomyces odoratus AF387126
Rhodotorula glutinis AY646097
Sporidiobolales
Pseudozyma spec. AJ876780 O
Pseudozyma spec. AB117962
Ustilago maydis AF453938
Ustilaginales
OTU 6 AJ876781 P
Trichosporon porosum AF308656
Cryptococcus curvatus AF444717
Trichosporonales
Basidiomycota
Mucor hiemalis AJ876782 B
Mucor hiemalis AJ876783 B
Mucor hiemalis f. hiemalis AF113468
OTU 7 AJ876784 B
Absidia glauca AJ876785 S
Absidia glauca AF113447
OTU 8 AJ876786 B
Cunninghamella polymorpha AF113461
Umbelopsis ramanniana AJ876787 B
Umbelopsis ramanniana AB090301
Umbelopsis isabellina AJ876788 B
Umbelopsis isabellina AF157220
Mucorales
OTU 9 AJ876789 P
OTU 9 AJ876790 P
OTU 9 AJ876791 S
Mortierella polycephala AF113464
Echinosporangium transversale AF113462
Dissophora decumbens AF157187
Mortierella verticillata AF157199
Mortierellales
OTU 10 AJ876792 S
Basidiobolus ranarum AF113452
Entomophthorales
Zygomycota
Beroe ovata AY026369
Suberites ficus AY026381
0.01 substitutions/site

macromycetes in the Kranichsteiner Wald [31] was detected in association with oribatid mites. For all species, ITS and LSU sequences were generated, classified by BLASTN and compared with morphological data, which led to an undoubted classification. Only in the case of the three *Penicillium* subgen. *Penicillium* species were we not able to gather ITS sequences. Having used the primer set ITS5/ITS4, which failed to amplify DNA of these fungi, we tried the primer set ITS1/ITS4, which was not successful either.

Further 15 fungal taxa were only detected in total DNA extracts from the oribatid mite specimens and, without any morphological data, they were classified as operational taxonomic units (OTU) (Table 3). Five of these OTUs were only detected based on ITS sequences, five based on LSU sequences, and the remaining five based on ITS and LSU sequences. All these OTUs showed sequence identities of at least 94% with fungal sequences in GenBank using the BLASTN program. Most OTUs had sequence identities of 99–100%, which allows one to assume a close relationship between the taxa represented by our sequences and these best hits deposited in GenBank. This assumption was also confirmed by the phylogenetic tree (Fig. 1) in which most OTUs fall in close vicinity to their best hits found by BLASTN.

Ten (3 in culture, 7 in total DNA extract from mites), 5 (1/4), 13 (11/2), and 9 (5/4) fungal species were found in association with *O. quadricornuta*, *Or. tibialis*, *P. clavipes*, and *S. magnus*, respectively. Only three zygomycetous species, i.e., OTU 8 (closely related to *Cunninghamella* sp.), OTU 9 (closely related to *Mortierella* sp.), and OTU 7 (cf. *Mucor hiemalis*) were found in association with more than one mite species (Table 3).

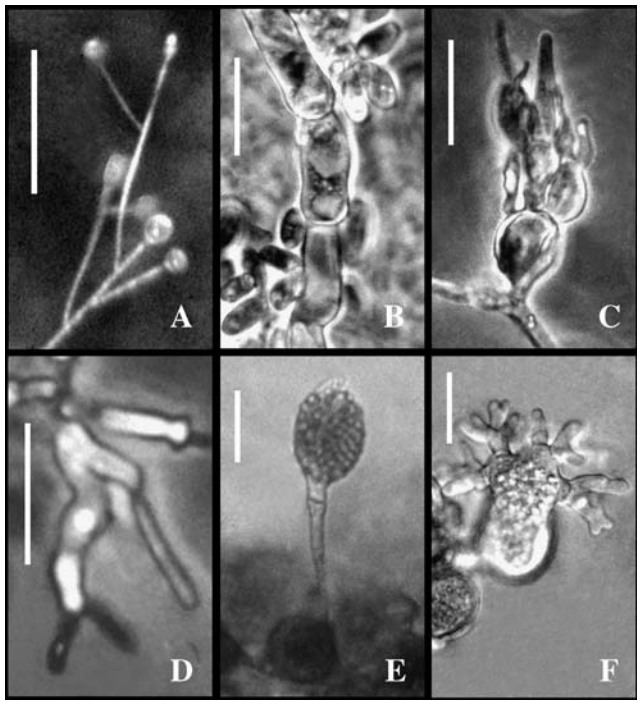


Figure 2. Microphotographs of selected fungi isolated from oribatid mites. (A) *Absidia glauca*, sporangiophores always arising singly at young hyphae. Bar = 300 μm. (B) *Aureobasidium pullulans*, hyphae and conidia with characteristic guttulae. Bar = 20 μm. (C) *Clonostachys rosea*, old conidiophore and conidiogenous cells changing into chlamydospore-like swollen cells. Bar = 20 μm. (D) *Pseudozyma* sp., hypha with sterigma-like structure and elongated conidia. Bar = 10 μm. (E) *Umbelopsis issabellina*, sporangiophore and sporangium, basal hyphae bearing a dark chlamydospore. Bar = 20 μm. (F) *U. ramanniana*, chlamydospore germinating with lateral granulous outgrowth and radiating divergent hyphae. Bar = 20 μm.

Remarks on Morphology of Selected Taxa. For the majority of cultivated fungi, a reliable determination of species on morphological characters was possible. In some cases, as listed below, a species-level identification failed because relevant features were lacking. The following list also contains fungi determined to species level but showing aberrant or noteworthy features.

Absidia glauca. On all culture media the fungus mainly produced sporangiophores arising singly from stolons, bearing branches as typical of sect. *Repens*. Only on older hyphae, a few sporangiophores also occurred in whorls of two, rarely three, as described for sect. *Glauca* (e.g., [18]; see Fig. 2). Our isolate showed an intermediate position between both taxa regarding this character. All other features corresponded to *A. glauca*, and molecular data confirmed this determination.

Clonostachys rosea. The cultured fungus belongs to an anamorph complex commonly described as *Gliocladium roseum* Bain. According to the determination based on molecular results, it corresponds to *Bionectria ochroleuca* (see Table 2) and, following *Index Fungorum*, the isolate has to be named *Clonostachys* (*Gliocladium* is an anamorph of *Sphaerostilbella*).

Figure 1. Phylogenetic tree of fungi found in association with oribatid mites in an oak–beech forest near Darmstadt (Hesse, Germany). The tree was obtained by distance analysis with the neighbor-joining method (Kimura two-parameter model) based on sequences of the D1/D2 region of the ribosomal large subunit. Sequences of *Suberites ficus* (Porifera) and *Beroe ovata* (Ctenophora) served as outgroup. Bootstrap values (>50%) were determined for neighbor-joining (1000 resamplings). Sequences obtained in this study are shown in bold face. Sequences with fungal names were from cultured fungi, whereas sequences from total DNA extracts of oribatid mites are given as operational taxonomic units (OTU). Letters indicate oribatid mite species from which sequences were derived (B, *Oribatella quadricornuta*; O, *Oribatula tibialis*; P, *Paradamaeus clavipes*; S., *Steganacarus magnus*).

Mucor hiemalis. For a reliable identification of *Mucor* species the sexual compatibility should be considered [63]. The nonproduction of zygospores indicates a heterothallic species. Morphological and genetic data supported the determination as *M. hiemalis*.

Beauveria bassiana. In cultures at least 2 weeks old, apart from characteristic clustered conidiogenous cells divided into venter and rhachis (up to 25 μm long), another type of conidiogenous cells occurred that is not mentioned in literature [18]. Their size was dominantly subulate and the cells reached a length up to 50 μm.

Penicillium spp. All of the isolates obtained from mites belong to the subgenus *Penicillium*. Within the frame of this study, an application of methods to determine species of *Penicillium* (e.g., scanning electron microscopy, infection tests with plants [29]) was not intended.

Pseudozyma sp. The characters of hyphae and budding as well as the formation of sterigma-like outgrowths giving rise to blastoconidia indicate that the fungus belongs to anamorphic Ustilaginales [12] (see Fig. 2). Biochemical tests were not carried out. The affiliation to the genus *Pseudozyma* is based on molecular data.

Rhodotorula sp. In culture, the fungus produced budding cell colonies with or without a pink color. This is in correspondence with characters of several yeastlike genera including *Rhodotorula*. No morphological features suitable for genus determination were detected. *Rhodotorula* (anamorph of *Rhodosporidium*) was identified by molecular analysis.

Verticillium sp. The fungus developed only one type of conidiogenous cell and conidiophore, and all revealed morphological characters are in accordance with *Verticillium* (and not with another anamorph possessing a *Verticillium* state). The molecular results suggest a close relation to the nematophagous *V. bactrosporum* Drechsler, but especially due to the size of conidiogenous cells (subulate instead of narrowly lageniform) and spores (more or less ellipsoid instead of bacilliform) this species must be excluded [23]. The gathered morphological data are not sufficient to determine the isolate to species level.

Discussion

Fungal species from a wide range of taxa (Basidiomycota, Ascomycota and Zygomycota, see Fig. 1) were found to be associated with oribatid mites, indicating the important role of these animals for the dispersal of several fungal taxa in soil [9]. Differences in the observed fungal spectrum associated with different mites might just coincide with low numbers of individuals studied. However, antifungal and antibacterial activities were detected in some mite species [26] and one might think about a different potential of fungi to become attached to surfaces. Attachment of yeasts (i.e., for *Rhodosporidium toruloides*, which is closely related to our *Rhodotorula* sp.) was shown to be mediated by adhesives [14]. All these features might influence the observed "fungus–mite associations."

There is evidence that perhaps one key function of soil invertebrates is the dispersal of microbial propagules [72]. Although the range of dispersal by mites might be limited, one has to consider that in a patchy environment, such as soils, favorable and habitable sites are islands with heterogeneous spatiotemporal distribution. These sites have to be reached by each specialized fungus before establishment and reproduction can occur [3, 28, 36]. Hence, dispersal, even over a limited distance, might facilitate the establishment of fungi in favorable patches. Therefore, even tiny members of the soil mesofauna might act as suitable vectors. Although, for instance *S. magnus* shows an average daily movement of only 4.11 cm in summer, with a maximum individual movement of 21.5 cm [9], this species is well known to enter layers of fresh leaf litter for oviposition, which provides direct access to an open patch for fungi.

Presumably, oribatid mites and other soil animals influence the distribution, dispersal, and growth of soil fungi during their feeding activity and movement. Most studies indicate that most individuals of mites and collembolans are associated with approximately 20 fungal species [6, 16, 55]. The species number associated with each animal appears to be related to body surface area [55]. This might be an explanation for the relatively large number of fungal species (120) detected in association with Collembola [72, 73] compared to that found on the smaller oribatid mites in our study (31). Note, however, that the extraction of oribatid mites in our study was done once in February, when fungal activity in the field might have been low.

Concerning the origin of fungal DNA in our study, one has to consider two possibilities: (1) the mite body surface and (2) the gut of the mite. Although large parts of fungal material, even the most recalcitrant fungal spores, are destroyed during gut passage [56, 71], approximately 50% of fungal spores seem to be able to sprout afterward [73]. There are two good reasons to assume that the fungal DNA amplified in this study was mainly derived from the body surface of the respective mites. First, we never found fecal pellets in petri dishes with mites directly isolated from soil. This might be due to the extended handling time during animal extraction from soil when animals seem to deflate their gut content. Therefore, especially in the case of fungal isolates, all fungi very likely derived from the mite body surfaces. Second, the chosen target regions are too large to be amplified from fungi in gut contents. Due to DNA degradation during gut passage, target regions for food

analyses in animals should not be larger than 200 to 300 bp [78]. As ITS sequences were in a range between 552 bp for OTU 6 and 801 bp for *Pseudozyma* sp. and between 616 bp for *C. rosea* and 795 bp for OTU 10 in LSU sequences, target regions in our study were at least two times larger than recommended for amplification of gut contents.

Whereas we detected several saprophytic and parasitic fungi, we found no mycorrhizal fungi associated with oribatid mites, suggesting that dispersal by mites is of little importance for mycorrhizal fungi. The reasons for this phenomenon are not known. Possibly, mycorrhizal spores, especially those of arbuscular mycorrhizal fungi, are too large to be carried on the surface of oribatid mites. Ectomycorrhizal fungi that produce hypogeous sporocarps (such as *Tuber* or *Rhizopogon*), which are often dispersed by soil-dwelling animals—however, even this was mainly shown for vertebrates [e.g., 10, 18]—are quite rare in Germany. Thus, spores of most ectomycorrhizal fungi are lifted above ground in fruiting bodies and thereby are dispersed by wind. However, at least other propagules such as mycelia should have the chance of being dispersed by mites, as both can be found in the same habitat.

Only a few dark pigmented fungi (Dematiacea) were found to be associated with oribatid mites. Oribatid mites (and other soil animals) are known to preferentially feed on Dematiacea, especially on the genera *Ulocladium* and *Alternaria* [45, 48, 62], and it was speculated that oribatid mites preferentially disperse fungal species that they feed on, which would be an indication for a mutualistic relationship between oribatid mites and some fungal taxa [8, 76]. However, in contrast to such a hypothesis, the results of our study suggest that there might be no mutual relationships or co-evolutionary process between oribatid mites and soil fungi. The only fungal taxon that is often associated with oribatid mites and other soil microarthropods is *Beauveria*, simply because this fungus is able to degrade chitin and therefore it is often found attached to bodies of microarthropods where it degrades the chitin of the living and later that of dead animals. The finding that some fungi (Ustilaginales) are specifically dispersed by soil mites (Eriophyidae and Tetranychidae) [13] probably might be an exception or might be related to these specific mite taxa. However, in our study, *A. glauca* and *B. bassiana*, with their briefly annotated aberrations from the typical characters (see "Results") could give cause to assume a specificity that should encourage further research.

Acknowledgment

We thank Claudia Krüger for her help with the cultivation of the fungi isolated from the oribatid mites.

References

1. Allen, MF (1987) Re-establishment of mycorrhizas on Mount St Helens: migration vectors. Trans Br Mycol Soc 88: 413–417
2. Altschul, SF, Madden, TL, Schäffer, AA, Zhang, J, Zhang, Z, Miller, W, Lipman, DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389–3402
3. Andrews, JH (1991) Comparative Ecology of Microorganisms and Macroorganisms. Springer, Berlin, Heidelberg, New York
4. von Arx, JA (1981) The Genera of Fungi Sporulating in Pure Culture, 3rd ed. J. Cramer, Vaduz.
5. Barnett, HL, Hunter, BB (1998) Illustrated Genera of Imperfect Fungi. 4th ed. APS, St. Paul.
6. Behan, VM, Hill, SB (1978) Feeding habits and spore dispersal of oribatid mites in the North American arctic. Rev Ecol Biol Sol 15: 497–516
7. Behan-Pelletier, VM, Hill, SB (1983) Feeding habits of sixteen species of Oribatei (Acari) from an acid peat bog, Glenamoy, Ireland. Rev Ecol Biol Sol 20: 221–267
8. Bernini, F (1986) Current ideas on the phylogeny and adaptive radiation of Acarida. Boll Zool 53: 279–313
9. Berthet, PL (1964) Field study of the mobility of Oribatei (Acari), using radioactive tagging. J Anim Ecol 33: 443–449
10. Bertolino, S, Vizzini, A, Wauters, LA, Tosi, G (2004) Consumption of hypogeous and epigeous fungi by red squirrel (*Sciurus vulgaris*) in subalpine conifer forests. Forest Ecol Manag 202: 227–233
11. Blackwell, M, Malloch, D (1991) Life-history and arthropod dispersal of a coprophilous-stylophage. Mycologia 83: 360–366
12. Boeckhout, T (1995) *Pseudozyma* Bandoni emend. Boekhout, a genus for yeast-like anamorphs of Ustilaginales. J Gen Appl Microbiol 41: 359–366
13. Boeckhout, T, Theelen, B, Houbraken, J, Robert, V, Scorzetti, G, Gafni, A, Gerson, U, Sztejnberg, A (2003) 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. Int J Syst Evol Microbiol 53: 1655–1664
14. Buck, JW, Andrews, JH (1999) Attachment of the yeast *Rhodosporidium toruloides* is mediated by adhesives localized at sites of bud cell development. Appl Environ Microb 65: 465–471
15. Carmichael, JW, Kendrick, WB, Conners, IL, Sigler, L (1980) Genera of Hyphomycetes. University of Alberta Press, Edmonton
16. Christen, AA (1975) Some fungi associated with Collembola. Rev Ecol Biol Sol 12: 723–728
17. Christensen, M (1989) A view of fungal ecology. Mycologia 81: 1–19
18. Colgan, W, Claridge, AW (2002) Mycorrhizal effectiveness of *Rhizopogon* spores recovered from faecal pellets of small forest-dwelling mammals. Mycol Res 106: 314–320
19. Dijkstra, EF, Boon, JJ, Van Mourik, JM (1998) Analytical pyrolysis of a soil profile under Scots pine. Eur J Soil Sci 49: 295–304
20. Dix, NJ, Webster, J (1995) Fungal Ecology. Chapman and Hall, London
21. Domsch, KH, Gams, W, Anderson, TH (1993) Compendium of soil fungi, Vols. 1 and 2. IHW, Eching
22. Doyle, JJ, Doyle, JL (1990) Isolation of plant DNA from fresh tissue. Focus 12: 13–15
23. Drechsler, C (1941) Some hyphomycetes parasitic on free-living terricolous nematodes. Phytopathology 31: 773–802
24. Dromph, KM (2001) Dispersal of entomopathogenic fungi by colembolans. Soil Biol Biochem 33: 2047–2051
25. Farahat, AZ (1966) Studies on the influence of some fungi on Collembola and Acari. Pedobiologia 6: 258–268
26. Fogaça, AC, da Silva, PI, Miranda, MTM, Bianchi, AG Miranda, A, Ribolla, PEM, Daffre, S (1999) Antimicrobial activity of a bovine

hemoglobin fragment in the tick *Boophilus microplus*. J Biol Chem 274: 25330–25334
27. Fourman, KL (1936) Kleintierwelt, Kleinklima und Mikroklima in Beziehung zur Kennzeichnung des forstlichen Standorts und der Bestandesabfallzersetzung auf bodenbiologischer Grundlage. Mitt Forstwirtsch Forstwiss 1936: 596–615
28. Gadgil, M (1971) Dispersal: population consequences to evolution. Ecology 52: 253–261
29. Gams, W (1993) Supplement and Corrigendum to the Compendium of Soil Fungi. IHW, Eching
30. Gehring, CA, Wolf, JE, Theimer, TC (2002) Terrestrial vertebrates promote arbuscular mycorrhizal fungal diversity and inoculum potential in a rain forest soil. Ecol Lett 5: 540–548
31. Große-Brauckmann, H, Große-Brauckmann, G (1977) Makromyzeten des vorderen und hinteren Odenwaldes (ein floristischer Vergleich). Z Pilzkunde 43: 59–74
32. Hall, TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41: 95–98
33. Hanlon, RDG (1981) Influence of grazing by Collembola on the activity of senescent fungal colonies grown on media of different nutrient concentrations. Oikos 36: 362–367
34. Hanlon, RDG, Anderson, JM (1979) The effects of Collembola grazing on microbial activity in decomposing leaf litter. Oecologia 38: 93–99
35. Harinikumar, KM, Bagyaraj, DJ (1994) Potential of earthworms, ants, millipedes, and termites for dissemination of vesicular–arbuscular mycorrhizal fungi in soil. Biol Fertil Soils 18: 115–118
36. Harper, JL (1981) The meanings of rarity. In: Synge, H (Ed.) The Biological Aspects of Rare Plant Conservation. Wiley, New York, pp 189–203
37. Hubert, J, Šustr, V, Smrž, J (1999) Feeding of the oribatid mite *Scheloribates laevigatus* (Acari: Oribatida) in laboratory experiments. Pedobiologia 43: 328–339
38. Hubert, J, Žilová, M, Pekár, S (2001) Feeding preferences and gut contents of three panphytophagous oribatid mites (Acari: Oribatida). Eur J Soil Biol 37: 197–208
39. Jacot, AP (1930) Moss-mites as spore-bearers. Mycologia 22: 94–96
40. Kempson, D, Lloyd, M, Ghelardi, R (1963) A new extractor for woodland litter. Pedobiologia 3: 1–21
41. Kirk, PM, Cannon, PF, David, JC, Stalpers, JA (2001) Ainsworth & Bisby's Dictionary of the Fungi, 9th ed. CABI Publishing, Oxon
42. Klironomos, JN, Moutoglis, P (1999) Colonization of non-mycorrhizal plants by mycorrhizal neighbours as influenced by the collembolan, *Folsomia candida*. Biol Fertil Soils 29: 277–281
43. Macfayden, A (1961) Improved funnel-type extractors for soil arthropods. J Anim Ecol 30: 171–184
44. Mangan, SA, Adler, GH (2002) Seasonal dispersal of arbuscular mycorrhizal fungi by spiny rats in a neotropical forest. Oecologia 131: 587–597
45. Maraun, M, Migge, S, Schaefer, M, Scheu, S (1998) Selection of microfungal food by six oribatid mite species (Oribatida, Acari) from two different beech forests. Pedobiologia 42: 232–240
46. Maraun, M, Visser, S, Scheu, S (1998) Oribatid mites enhance the recovery of the microbial community after a strong disturbance. Appl Soil Ecol 9: 175–181
47. Maraun, M, Scheu, S (2000) The structure of oribatid mite communities (Acari, Oribatida): patterns, mechanisms and implications for future research. Ecography 23: 374–383
48. Maraun, M, Martens, H, Migge, S, Theenhaus, A, Scheu, S (2003) Adding to 'the enigma of soil animal diversity': fungal feeders and saprophagous soil invertebrates prefer similar food substrates. Eur J Soil Biol 39: 85–95
49. Maraun, M, Salamon, J-A, Schneider, K, Schaefer, M, Scheu, S (2003) Oribatid mite and collembolan diversity, density and community structure in a moder beech forest (*Fagus sylvatica*): effects of mechanical perturbations. Soil Biol Biochem 35: 1387–1394
50. McLean, MA, Kaneko, N, Parkinson, D (1996) Does selective grazing by mites and collembolan affect litter fungal community structure? Pedobiologia 40: 97–105
51. Meier, FA, Scherrer, S, Honegger, R (2002) Faecal pellets of lichenivorous mites contain viable cells of the lichen-forming ascomycete *Xanthoria parietina* and its green algal photobiont, *Trebouxia arboricola*. Biol J Linn Soc 76: 259–268
52. Mitchell, MJ (1978) Vertical and horizontal distributions of oribatid mites (Acari: Cryptostigmata) in an aspen woodland soil. Ecology 59: 516–525
53. Paine, TD, Raffa, KF, Harrington, TC (1997) Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annu Rev Entomol 42: 179–206
54. Pande, YD, Berthet, P (1973) Studies on the food and feeding habits of soil Oribatei in a black pine plantation. Oecologia 12: 413–426
55. Pherson, DA, Beattie, AJ (1979) Fungal loads of invertebrates in beech leaf litter. Rev Ecol Biol Sol 16: 325–335
56. Ponge, JF, Charpentie, MJ (1981) Étude des relations microflore–microfaune: expériences sur *Pseudosinella alba* (Packard), Collembole mycophage. Rev Ecol Biol Sol 18: 291–303
57. Rantalainen, ML, Fritze, H, Haimi, J, Kiikkilä, O, Pennanen, T, Setälä, H (2004) Do enchytraeid worms and habitat corridors facilitate the colonisation of habitat patches by soil microbes. Biol Fertil Soils 39: 200–208
58. Renker, C, Alphei, J, Buscot, F (2003) Soil nematodes associated with the mammal pathogenic fungal genus *Malassezia* (Basidiomycota: Ustilaginomycetes) in Central European forests. Biol Fertil Soils 37: 70–72
59. Scheu, S (1993) Litter microflora—soil macrofauna interactions in lignin decomposition: a laboratory experiment with ^{14}C-labelled lignin. Soil Biol Biochem 25: 1703–1711
60. Scheu, S, Schulz, E (1996) Secondary succession, soil formation and development of a diverse community of oribatids and saprophagous soil macro-invertebrates. Biodivers Conserv 5: 235–250
61. Schneider, K, Migge, S, Norton, RA, Scheu, S, Langel, R, Reineking, A, Maraun, M (2004) Trophic niche differentiation in soil microarthropods (Oribatida, Acari): evidence from stable isotope ratios (^{15}N/^{14}N). Soil Biol Biochem 36: 1769–1774
62. Schneider, K, Maraun, M (2005) Feeding preferences among dark pigmented fungal taxa (''Dematiacea'') indicate limited trophic niche differentiation of oribatid mites (Oribatida, Acari). Pedobiologia 49: 61–67
63. Schipper, MAA (1978) 1. On certain species of *Mucor* with a key to all accepted species. 2. On the genera *Rhizomucor* and *Parasitella*. Stud Mycol 17: 1–71
64. Schuster, R (1956) Der Anteil der Oribatiden an den Zersetzungsvorgängen im Boden. Z Morph Ökol Tiere 45: 1–33
65. Skubala, P (1999) Colonization of a dolomitic dump by oribatid mites (Acari, Oribatida). Pedobiologia 43: 145–159
66. Smith, SE, Read, DJ (1997) Mycorrhizal Symbiosis, 2nd ed. Academic Press, San Diego
67. Stefaniak, O, Seniczak, S (1976) The microflora of the alimentary canal of *Achipteria coleoptrata* (Acarina, Oribatei). Pedobiologia 16: 185–194
68. Stefaniak, O, Seniczak, S (1981) The effect of fungal diet on the development of *Oppia nitens* (Acari, Oribatei) and on the microflora of its alimentary tract. Pedobiologia 21: 202–210
69. Strenzke, K (1952) Untersuchungen über die Tiergemeinschaften des Bodens: Die Oribatiden und ihre Synusien in den Böden Norddeutschlands. Zoologica 37: 1–137

70. Swofford, DL (2003) Paup*. Phylogenetic Analysis Using Parsimony (*and other Methods), Ver. 4.0b10. Sinauer Associates, Sunderland, MA
71. Van der Drift, J (1965) The effect of animal activity in the litter. In: Hallsworth, EG, Crawford, DV (Eds.) Experimental Pedology, Butterworth, London, pp 227–235
72. Visser, S (1985) Role of soil invertebrates in determining the composition of soil microbial communities. In: Fitter, AH, Atkinson, D, Read, DJ, Usher, MB (Eds.) Ecological Interactions in Soil. Plants, Microbes and Animals. Blackwell, Oxford, pp 297–317
73. Visser, S, Parkinson, D, Hassall, M (1987) Fungi associated with *Onychiurus subtenuis* (Collembola) in an aspen woodland. Can J Bot 65: 635–642
74. Visser, S, Whittaker, JB, Parkinson, D (1981) Effects of collembolan grazing on nutrient release and respiration of a leaf litter inhabiting fungus. Soil Biol Biochem 13: 215–218
75. Volz, P (1935) Untersuchungen über Mikroschichtung der Fauna von Waldböden. Zool Jahrb Abt Syst Ökol Geogr 66: 153–210
76. Wallwork, JA (1983) Oribatids in forest ecosystems. Annu Rev Entomol 28: 109–130
77. Warner, NJ, Allen, MF, MacMahon, JA (1987) Dispersal agents of vesicular–arbuscular mycorrhizal fungi in a disturbed arid ecosystem. Mycologia 79: 721–730
78. Zaidi, RH, Jaal, Z, Hawkes, NJ, Hemingway, J, Symondson, WOC (1999) Can multiple-copy sequences of prey DNA be detected amongst the gut contents of invertebrate predators? Mol Ecol 8: 2081–2087