Three new genera of fungi from extremely acidic soils

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Abstract Extremely acidic soils (pH<3) harbour poorly diversified mycobiota that are very different from less acidic habitats. During investigations of the mycobiota from several highly acidic soils in the Czech Republic and a coastal site in the Antarctic Peninsula, a group of hyaline fungal isolates was obtained. Based on phenotype and nuclear ribosomal DNA sequences (ITS region, SSU, LSU), the isolates belonged to three phylogenetic lineages within two different classes, Sordariomycetes and Leotiomycetes (Pezizomycotina, Ascomycota). The first lineage is described here as a new genus and species *Acidothrix acidophila* gen. nov. et sp. nov. (Amplistromataceae, Sordariomycetes, Ascomycota). The most closely related species to this new clade are wood-inhabiting fungi. The isolates belonging to the second and the third lineages are also described as two new genera and species *Acidea extrema* gen. nov. et sp. nov. and *Soosiella minima* gen. nov. et sp. nov. (Helotiales, Leotiomycetes, Ascomycota). Their position and the relationships within Helotiales are discussed. *Soosiella minima* was acidotolerant, *Acidothrix acidophila* and *Acidea extrema* exhibited both acidotolerant and acidophilic characteristics. All the species were slightly halophilic. The adaptation of hyaline fungi from mesophilic lineages to highly acidic environments has been revealed. The association between highly acidic and Antarctic habitats is discussed.

Keywords *Amplistromataceae* · Micromycetes · Acidophilic · *Acidomyces* · *Acidiella* · Helotiales

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Introduction

Highly acidic habitats (pH<3) represent some of the most extreme environments for microbial growth. Despite the extreme conditions, these habitats harbour highly diversified microbial communities in which fungi represent an abundant and important component (Amaral Zettler et al. 2002, 2003, 2013; Baker et al. 2004, 2009; López-Archilla and Amils 1999; López-Archilla et al. 2001, 2004). Only fragmentary data are available on fungal diversity and their role within acidophilic microbial communities, but it is apparent that mycobiota of highly acidic substrates are different from less acidic habitats and are dominated by a small number of mainly dematiaceous fungal species (Amaral Zettler et al. 2002, 2003; Baker et al. 2004, 2009; Hujslová et al. 2010, 2013; López-Archilla et al. 2004). To date, only three strictly acidophilic fungi *Acidomyces acidophilus* (Selbmann et al. 2008), *Hortaea acidophila* (Hölker et al. 2004) and *Acidomyces acidothermus* (Yamazaki et al. 2010; Hujslová et al. 2013) have been identified. Taxonomically, all these black meristematic fungal species, together with the acidotolerant fungus *Acidiella bohemica* (Hujslová et al. 2013), another species

typical of extremely acidic soils, belong to the family Teratosphaeriaceae (Capnodiales, Dothideomycetes, Ascomycota).

In the present study, we were interested in the comparative analysis of the mycobiota inhabiting extremely acidic soils (pH <3) primarily in geographically isolated localities of small areas in the Czech Republic. During our investigation a group of hyaline isolates were obtained. Based on phenotype and nuclear ribosomal DNA (ITS region, SSU, LSU) sequences, the isolates were placed in Amplistromataceae (Sordariomycetidae, Sordariomycetes) and in two phylogenetically isolated positions within Helotiales (Leotiomycetes).

The family Amplistromataceae has been established for two genera, *Amplistroma* and *Wallrothiella*, of exclusively wood-inhabiting fungi with similar morphological characteristics and acrodontium-like asexual morphs (Huhndorf et al. 2009). Based on molecular data (LSU rDNA sequences) the family was found to be monophyletic; nevertheless, its position within Sordariomycetidae was not resolved, so it was referred to as *incertae sedis* (Huhndorf et al. 2009).

The order Helotiales represents the largest and the most diverse group in the Leotiomycetes encompassing plant pathogens, endophytes, nematode-trapping fungi, mycorrhizae, ectomycorrhizal parasites, fungal parasites, terrestrial saprobes, aquatic saprobes, root symbionts and wood rot fungi (Wang et al. 2006a, b). Because of the limited knowledge about interconnections between asexual and sexual morphs, the systematics of the Helotiales is complicated (Wang et al. 2006a, b). Based on rDNA sequences, some clades were recognized with substantial support within the Helotiales but the monophyly of the Helotiales as well as the most helotialean families has not been (Wang et al. 2006a, b). Thus, more data from the rDNA regions and protein-coding genes, wider sampling from all families recognized in the Helotiales and the Leotiomycetes, as well as molecular data from environmental samples are needed for a more comprehensive view within the Helotiales (Wang et al. 2006a, b).

In the present paper, one new fungal genus within the family Amplistromataceae and two new genera within the order Helotiales are described and their growth responses to different pH values and salt concentrations are determined.

Materials and methods

Sampling, isolation, morphological and cultural characterization

Sixteen samples of extremely acidic soil (pH<3) were collected from four sampling sites in the Czech Republic in May and November 2007 (Fig. 1, Table 1). The samples were processed using two methods and three types of isolation media. The methods were direct inoculation of soil (M1) (Fassatiová 1986) and the soil washing technique (M2) (von Kreisel and Schauer 1987), and the media were 2 % malt agar (MA2), acidified 2 % malt extract agar (MEA-pH2) and acidified soil agar with rose Bengal and glucose (SEA-pH2) (Pitt 1980; Fassatiová 1986). The pH of the MEA and SEA was adjusted to 2 with concentrated H_2SO_4. SEA was prepared from the substrata of the respective sampling site. Streptomycin was added to all media (0.1 g/l) to suppress bacterial growth. The plates were incubated at 5 °C, 24 °C and 37 °C. After 7–14 days, the emerging colonies were transferred to identification media.

All measurements and observations were performed using fungal structures grown for 14 days on MEA and incubated in the dark at 24 °C. Other media used for colony description were malt extract agar (MEA-pH2) and potato carrot agar (PCA) (Fassatiová 1986). Colour codes were determined

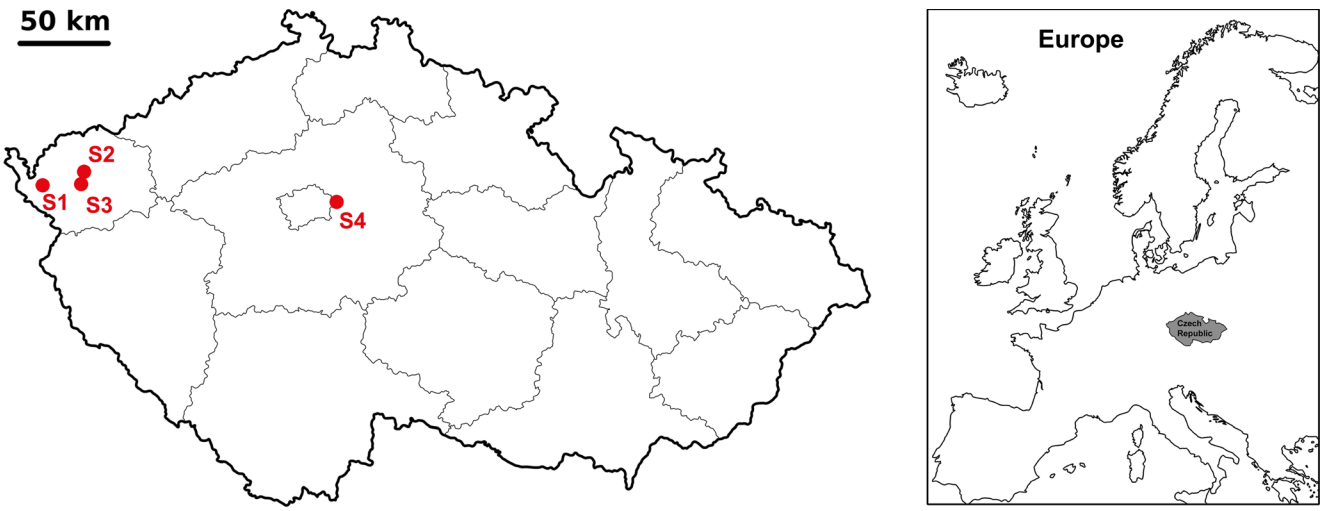


Fig. 1 Map of the Czech Republic showing the four sampling sites: **S1**—50°08′60″ N, 12°24′00″ E, **S2**—50°15′00″ N, 12°46′12″ E, **S3**—50°15′00″ N, 12°46′48″ E, **S4**—50°06′36″ N, 14°31′48″ E

Table 1 Characterization of four sampling sites. Frequency of isolated fungi was calculated as percentage of positive samples. Four samples were analyzed from each site

Site code	Sampling site	Characterization	Soil pH	Frequency [%]		
				Acidothrix acidophila	*Acidea extrema*	*Soosiella minima*
S1	Soos National Natural Reserve, Czech Republic	Area including peat bogs, mineral fens, salt marshes and highly acidic places with bare soil	1–2	–	25	25
S2	Mírová, Czech Republic	Kaolin quarry with exposed sulfur rich brown coal beds	1.5–2.5	75	100	25
S3	Jimlíkov, Czech Republic	Kaolin quarry with exposed sulfur rich brown coal beds	2–4	–	75	–
S4	Cihelna v Bažantnici National Monument, Czech Republic	Clay quarry with exposed sulfur rich brown coal beds	1–2	–	25	–

according to the Munsell System (1966). Slides were mounted in water and observed using light microscopy.

Strains examined

Cultures from 84 hyaline fungal isolates obtained from highly acidic soil (pH<3) outlined above, four strains previously isolated from the same substrate and reported by Hujslová et al. (2010) and a strain SH26-1 isolated from alkaline coastal soil on Snow Hill Island, Antarctica, were studied in the present paper. Culturing procedures for the isolate from Antarctica were previously reported (Arenz and Blanchette 2009). The ex-type and other representative strains have been deposited in the Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands (CBS), Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University in Prague, Czech Republic or in the personal collection of the first author (code MH) (Table 2).

DNA and molecular phylogenetic analyses

Genomic DNA was isolated from 14- to 28-day-old cultures using a Microbial DNA isolation kit (MoBio Laboratories Inc., Carlsbad, CA, USA). RAPD fingerprinting was performed with primers 8F and 10R as described in Hujslová et al. (2010). The ITS1, 5.8S, and ITS2 regions of the rDNA, together with partial LSU rDNA, were amplified using primer pairs ITS1, ITS5 (White et al. 1990) or ITS1F (Gardes and Bruns 1993) and NL4 (O'Donnell 1993) or LR6 (White et al. 1990). The SSU rDNA gene was amplified using primers NS1, NS17, NS4 and NS24 (White et al. 1990), NSSU1088R and NSSU1088 (Kauff and Lutzoni 2002). The amplification protocol was the same as in Pažoutová et al. (2012). Custom purification of the PCR products and sequencing of the DNA was performed at Macrogen (Seoul, Korea) using the same primers listed above together with NS2 (White et al. 1990) and NL1 (O'Donnell 1993). GenBank accession numbers are provided in Table 2. Sequence manipulations were done in BioEdit v. 7.0.4.1 software (Hall 1999). A BlastN similarity search (Altschul et al. 1997) was used to find similar sequences in the GenBank database. Two DNA sequence datasets were prepared. The first consisted of LSU rDNA sequences and was used to resolve the placement and phylogenetic relationships of the first group of our isolates within the Sordariomycetes. Taxon selection was patterned on the dataset of Huhndorf et al. (2009). The second dataset consisted of SSU and LSU sequences of Helotiales and other major groups in the Leotiomycetes and was based on the dataset published by Wang et al. (2006a) (TreeBase No. M2570). Both alignments were combined with the closest matches from GenBank. DNA sequences were aligned using the T-coffee web server, and ambiguous positions were subsequently aligned based on Core analysis of local reliability (Notredame et al. 2000; Poirot et al. 2003). The first LSU dataset consisted of 100 sequences, 1,333 positions (567 variable and 404 parsimony-informative sites), and the second dataset consisted of concatenated LSU rDNA (116 sequences) and SSU (107 sequences), with 1,520 positions (752 variable, and 487 parsimony-informative sites).

Phylogenetic relationships were inferred from the maximum likelihood and Bayesian methods. *Scutellinia scutellata* and *Neolecta irregularis* were used as outgroups. For both LSU rDNA and a dataset containing concatenated LSU and SSU rDNA, the model of molecular evolution for each separate alignment was assessed using jModelTest (Guindon and Gascuel 2003; Posada 2008). This analysis showed GTR to be the most relevant model, with site-to-site rate variation approximated with a gamma distribution and an estimated proportion of invariable sites. For the likelihood analysis, we used a fast bootstrapping algorithm (Stamatakis 2006) in RAxML (version 7.2.7) conducted on the CIPRES Science gateway Web server (RAxML-HPC2 on TG) (Miller et al. 2010). For the Bayesian analysis, we used MrBayes (version 3.1.2) (Huelsenbeck and Ronquist 2001) run on the same server used above (MrBayes on TG) with 10 million generations, sampling trees every 1,000 generations and discarding the first

Table 2 List of studied isolates and their GenBank accession numbers

Species	Strain no. and reference	Sampling site	Isolation conditions			GenBank accession no. (ITS, LSU, SSU)
			Method	Medium	t (°C)	
Acidothrix acidophila	**CBS 136259 (=CCF 3799[a]=MH 560)**	S1	–	–	–	FJ430781
	CCF 4344 (=MH 1205)	S2	M2	SEA (pH2)	24	KF286988
	CCF 3800[a] (=MH 664)	S1	–	–	–	
	CCF 4565 (=MH 1237)	S2	M2	MEA (pH2)	24	
	MH 566[a]	S1	–	–	–	FJ430780
	MH 1036	S2	M2	MEA (pH2)	24	
Acidea extrema	**CBS 136258 (=CCF 4345=MH 1180)**	S2	M1	SEA (pH2)	24	JX124323
	CCF 3830[a] (=MH 72)	S1				FJ430779
	CCF 4346 (=MH 1246)	S3	M2	MA2	5	
	CCF 4348(=MH 1264)	S2	M2	MA2	5	
	MH 1185	S2	M1	MEA (pH2)	24	JX124324
	MH 1125	S2	M2	MA2	24	JX124325
	MH 1288	S2	M2	MEA (pH2)	5	JX124326
	MH 903	S3	M1	MEA (pH2)	5	
	MH 977	S2	M1	MA2	24	
	MH 1277	S3	M2	MEA (pH2)	5	
	MH 1191	S2	M2	MEA (pH2)	24	
	MH 951	S2	M1	MEA (pH2)	5	
	MH 1255	S2	M1	SEA (pH2)	5	
	CCF 4566 (=SH26-1)	Snow Hill Island, Antarctica	Alkaline coastal soils	JX124322		
Soosiella minima	**CBS 136257 (=CCF 4350=MH 1230)**	S1	M1	MA2	24	JX124327
	CCF 4575 (=MH 1236)	S2	M1	MEA (pH2)	24	
	MH 1318	S2	M1	SEA (pH2)	5	

Ten strains used for the pH growth test are in bold; eight strains used for the salinity growth tests are underlined

Abbreviations: *S1 – S4* codes of the sampling sites (see Table 1), *M1* direct inoculation of soil, *M2* soil washing technique, *MA2* 2 % malt agar, *MEA (pH2)* acidified 2 % malt extract agar, *SEA (pH2)* acidified soil agar with rose Bengal and glucose, *MH* personal culture collection of M. Hujslová, *CCF* culture collection of fungi, Prague

[a] Hujslová et al. (2010)

half of the trees as a burn-in. The convergence of two runs with four chains was evaluated by Tracer v. 1.5.0 (Rambaut and Drummond 2003). The resulting files were then combined and a 50 % majority-rule consensus tree was computed.

Growth at different pH levels

The effect of pH on the growth of *Acidothrix acidophila* (four isolates), *Acidea extrema* (five isolates) and *Soosiella minima* (one isolate) was determined by measuring colony diameter on MEA (Table 2). Eight different pH values ranging from 1 to 8 were used. To permit polymerisation of the agar at pH 1, twice the amount of agar was added. The pH of the medium was adjusted with concentrated H_2SO_4 or NaOH after sterilisation. The triplicate plates were inoculated with mycelial segments, incubated at 24 °C and measured after 14 days.

Growth at different NaCl concentrations

The effect of NaCl on the growth of *Acidothrix acidophila* (two isolates), *Acidea extrema* (five isolates) and *Soosiella minima* (one isolate) was determined by measuring colony diameter on MEA (Table 2). Four NaCl concentrations 0.2 M (12 g NaCl/l), 0.5 M (29 g NaCl/l), 2.5 M (146 g NaCl/l) and 5 M (303 g NaCl/l) were used according to the scale describe by Kushner (1978). The triplicate plates were inoculated with mycelial segments, incubated at 24 °C and measured after 14 days.

Results

The 89 hyaline fungal isolates studied were divided into three groups using phenotype and RAPD fingerprinting. Selected isolates from each group were characterised by analysis of

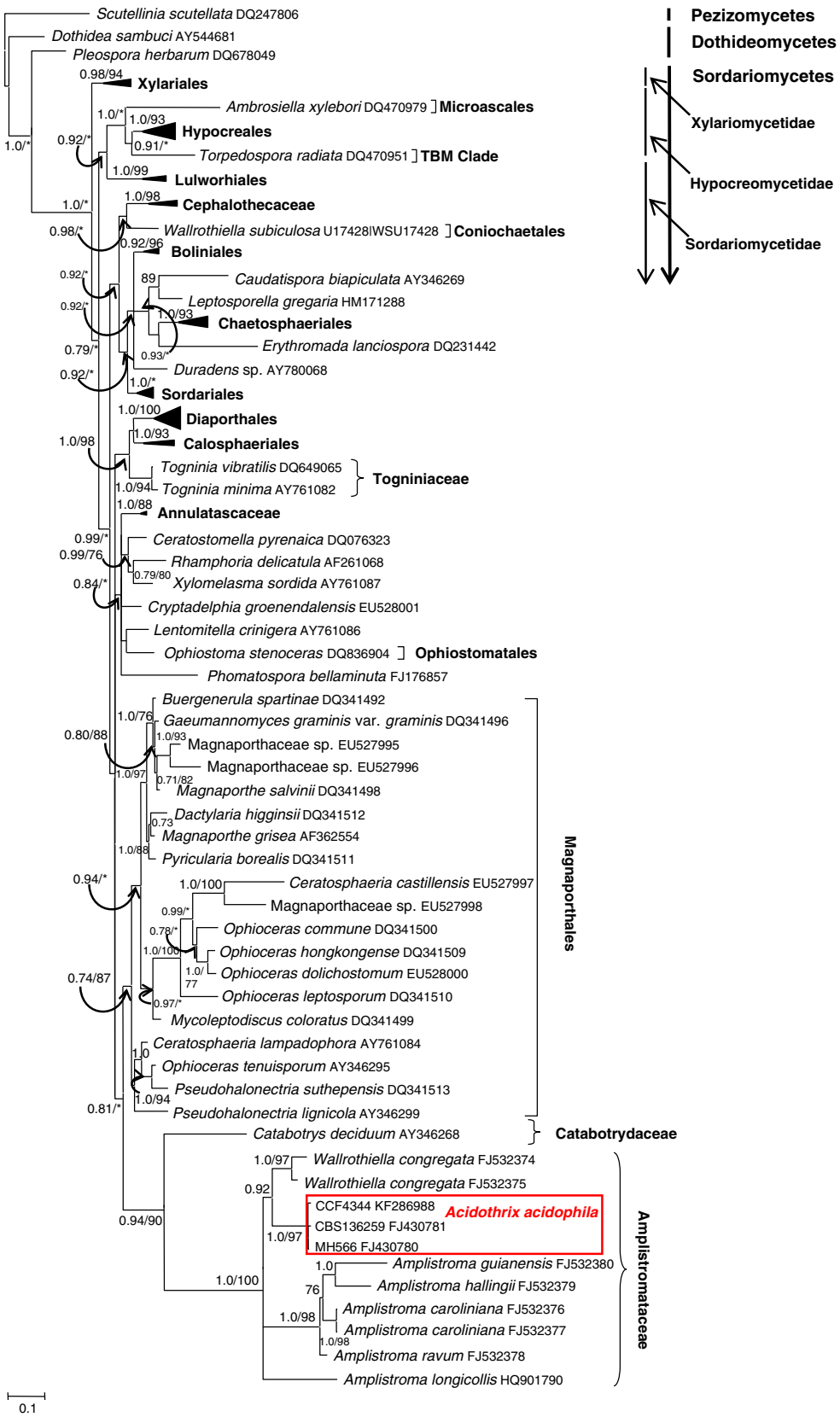


Fig. 2 Phylogenetic tree of Sordariomycetes based on the LSU rDNA sequences. Phylogeny reconstructed using Bayesian inference with Bayesian posterior probabilities (>0.7) noted above individual branches

rDNA sequences (Table 2). Based on the phylogeny data, the isolates were placed in three phylogenetically isolated lineages within two different fungal classes, the Sordariomycetes and Leotiomycetes.

The first lineage represented by the first group (nine isolates) was accommodated within the family Amplistromataceae (Fig. 2). The ITS and LSU rDNA sequences showed the closest similarity to an isolate of *Amplistroma ravum* Huhndorf, A.N. Mill., M. Greif & Samuels (ITS rDNA 95 % and LSU rDNA 93 % similarity; FJ532378). Based on SSU rDNA sequences our isolates were closely related to *Ascitendus austriacus* (Réblová, Winka & Jaklitsch) J. Campb. & Shearer (GQ996542) and one unidentified sordariomycetaean isolate (EF622536) (96 % similarity).

The second and the third lineage (77 and three isolates, respectively) were found to be in two distinct phylogenetic lineages within the order Helotiales (Fig. 3). The second lineage contained ITS rDNA sequences most closely related to helotialean isolate from the Antarctic Peninsula region (99 % similarity; FJ235962). LSU rDNA sequences of our isolates were closely related to several isolates of *Articulospora tetracladia* Ingold (96 % similarity; EU998922, etc.). Based on SSU rDNA sequences, our isolates were similar to two uncultured clones (RT5in6 and RT3n5) from highly acidic river samples in Spain (99 % similarity; AY082984, AY082969 Amaral Zettler et al. 2002). The same similarity (99 %) was found with isolates of several bryosymbionts and aquatic helotialean fungi—*Hymenoscyphus* sp. (EU940026, EU940027, EU940025), *Discinella schimperi* (Navashin) Redhead & K.W. Spicer (EU940043, EU940054), *Tricladium patulum* Marvanová (AY357285), *Tetrachaetum elegans* Ingold (EU357280), *Anguillospora filiformis* Greath. (AY178825) and several isolates of *Articulospora tetracladia* (EU998927, etc.).

The third lineage represented by three isolates had ITS rDNA sequences most closely related to endophytic leotiomycetaean isolates (98 % similarity; JQ759534, HQ207068, HQ207059). Sequences of LSU rDNA show 98 % similarity with the same three isolates (JQ759534, HQ207068 and HQ207059) and one unidentified mycorrhizal isolate (AY394892). The SSU rDNA sequences from this lineage were nearly identical to one of the published *Hyphodiscus hymeniophilus* sequences (99 % similarity; DQ227258) and 96 % similar to other GenBank entries from this species (GU727555, DQ227263, GU727551).

Both the MB and ML analyses of the first LSU dataset revealed phylogenetic trees that strongly support the placement of the first phylogenetic lineage, described here as a new genus and species *Acidothrix acidophila* Hujslová & M. Kolařík, forming a group sister to *Wallrothiella congregata* (FJ532374, FJ532375) within the family Amplistromataceae (Fig. 2). Among the nearest neighbours were species of *Amplistroma* (Fig. 2).

The second lineage, forming a separated group in the LSU-SSU rDNA phylogenetical trees, here described as a new genus and species *Acidea extrema* Hujslová & M. Kolařík, clustered in a group with two uncultured clones RT5in6 and RT3n5 (AY082984, AY082969). The closest neighbours were *Articulospora tetracladia* (EU998927 EU998922), *Fontanospora fusiramosa* Marvanová, Peter J. Fisher & Descals (GQ411265), *Varicosporium elodeae* W. Kegel (AY425613), *Tricladium patulum* (AY357285) and *Tetrachaetum elegans* (AY357280) (Fig. 3).

Based on the analysis of the same dataset, the third lineage formed a separate group (Fig. 3) here described as a new genus and species *Soosiella minima* Hujslová & M. Kolařík. Relationships among *Soosiella minima* and other species in the tree were not well resolved (Fig. 3).

Fig. 3 Phylogenetic tree of Leotiomycetes based on the combined LSU and SSU rDNA sequences. Phylogeny reconstructed using Bayesian inference with Bayesian posterior probabilities (>0.7) noted above individual branches. The blue boxes marked taxa of Helotiales sensu Wang et al. (2006a)

Taxonomy

Acidothrix Hujslová & M. Kolařík, **gen. nov.** MB 805194

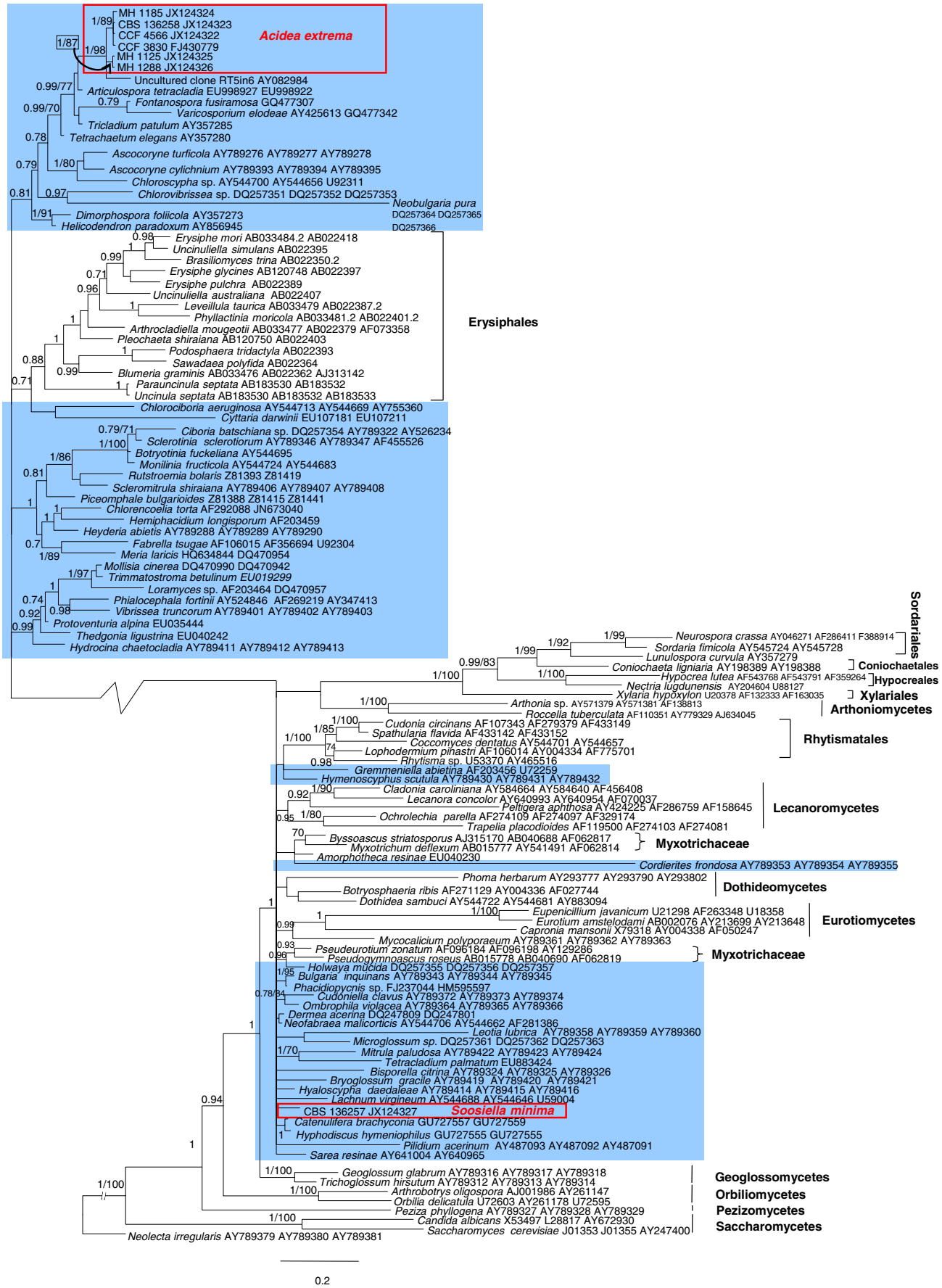
Asexual morph, hyphomycetes. Colonies plane, with abundant aerial mycelium forming floccules and funicules, sporulation abundant, white to slightly salmon (5YR8/2); on acidic medium compact, centrally forming funicules, powdery, white. Conidiophores acrodontium–like, semimacronematous or macronematous. Conidia single, globose, or ellipsoidal to lacrimose, with hilum. Sexual morph unknown, phylogenetic placement in Amplistromataceae.

Etymology: from *acidus* (Latin) "acidic" and *thrix* (Greek) "hair", refers to the occurrence in acidic substrata and its morphological resemblance to the genus *Sporothrix*.

Type species: ***Acidothrix acidophila*** Hujslová & M. Kolařík, sp. nov.

Acidothrix acidophila Hujslová & M. Kolařík, **sp. nov.** MB 805424 (Fig. 4)

Colonies on MEA (pH 5.5) at 24 °C, 21 days reaching a diameter of 76–77 mm; spreading, with abundant aerial mycelium forming floccules and funicules, sporulation abundant, white to slightly salmon (5YR8/2), reverse honey to ochre (5YR5/10). Colonies on acidic medium (MEA pH 2) achieving diameters of 19–33 mm in 21 days at 24 °C; compact, centrally forming funicules, with ruffled margin, powdery, coloured white, reverse cream to beige (7.5YR6/10). On PCA at 24 °C in 21 days colonies compact, centrally heaped with flat margin, without aerial mycelium, yeast-like; reaching 15–20 mm in diam. Conidiophores semimacronematous consisting of a single phialide only, or macronematous consisting of stipe bearing two to six phialides, sometimes in verticilate arrangement (prostrate). Stipe 10–20×2.5–3.0 μm.


Acidea extrema
MH 1185 JX124324
CBS 136258 JX124323
CCF 4566 JX124322
CCF 3830 FJ430779
MH 1125 JX124325
MH 1288 JX124326
Uncultured clone RT5in6 AY082984
Articulospora tetracladia EU998927 EU998922
Fontanospora fusiramosa GQ477307
Varicosporium elodeae AY425613 GQ477342
Tricladium patulum AY357285
Tetrachaetum elegans AY357280
Ascocoryne turficola AY789276 AY789277 AY789278
Ascocoryne cylichnium AY789393 AY789394 AY789395
Chloroscypha sp. AY544700 AY544656 U92311
Chlorovibrissea sp. DQ257351 DQ257352 DQ257353
Neobulgaria pura DQ257364 DQ257365 DQ257366
Dimorphospora foliicola AY357273
Helicodendron paradoxum AY856945
Erysiphe mori AB033484.2 AB022418
Uncinuliella simulans AB022395
Brasiliomyces trina AB022350.2
Erysiphe glycines AB120748 AB022397
Erysiphe pulchra AB022389
Uncinuliella australiana AB022407
Leveillula taurica AB033479 AB022387.2
Phyllactinia moricola AB033481.2 AB022401.2
Arthrocladiella mougeotii AB033477 AB022379 AF073358
Pleochaeta shiraiana AB120750 AB022403
Podosphaera tridactyla AB022393
Sawadaea polyfida AB022364
Blumeria graminis AB033476 AB022362 AJ313142
Parauncinula septata AB183530 AB183532
Uncinula septata AB183530 AB183532 AB183533
Erysiphales
Chlorociboria aeruginosa AY544713 AY544669 AY755360
Cyttaria darwinii EU107181 EU107211
Ciboria batschiana sp. DQ257354 AY789322 AY526234
Sclerotinia sclerotiorum AY789346 AY789347 AF455526
Botryotinia fuckeliana AY544695
Monilinia fructicola AY544724 AY544683
Rutstroemia bolaris Z81393 Z81419
Scleromitrula shiraiana AY789406 AY789407 AY789408
Piceomphale bulgarioides Z81388 Z81415 Z81441
Chlorencoelia torta AF292088 JN673040
Hemiphacidium longisporum AF203459
Heyderia abietis AY789288 AY789289 AY789290
Fabrella tsugae AF106015 AF356694 U92304
Meria laricis HQ634844 DQ470954
Mollisia cinerea DQ470990 DQ470942
Trimmatostroma betulinum EU019299
Loramyces sp. AF203464 DQ470957
Phialocephala fortinii AY524846 AF269219 AY347413
Vibrissea truncorum AY789401 AY789402 AY789403
Protoventuria alpina EU035444
Thedgonia ligustrina EU040242
Hydrocina chaetocladia AY789411 AY789412 AY789413
Neurospora crassa AY046271 AF286411 F388914
Sordaria fimicola AY545724 AY545728
Lunulospora curvula AY357279
Sordariales
Coniochaeta ligniaria AY198389 AY198388
Coniochaetales
Hypocrea lutea AF543768 AF543791 AF359264
Nectria lugdunensis AY204604 U88127
Hypocreales
Xylaria hypoxylon U20378 AF132333 AF163035
Xylariales
Arthonia sp. AY571379 AY571381 AF138813
Roccella tuberculata AF110351 AY779329 AJ634045
Arthoniomycetes
Cudonia circinans AF107343 AF279379 AF433149
Spathularia flavida AF433142 AF433152
Coccomyces dentatus AY544701 AY544657
Lophodermium pinastri AF106014 AY004334 AF775701
Rhytisma sp. U53370 AY465516
Rhytismatales
Gremmeniella abietina AF203456 U72259
Hymenoscyphus scutula AY789430 AY789431 AY789432
Cladonia caroliniana AY584664 AY584640 AF456408
Lecanora concolor AY640993 AY640954 AF070037
Peltigera aphthosa AY424225 AF286759 AF158645
Ochrolechia parella AF274109 AF274097 AF329174
Trapelia placodioides AF119500 AF274103 AF274081
Lecanoromycetes
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Myxotrichum deflexum AB015777 AY541491 AF062814
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Phoma herbarum AY293777 AY293790 AY293802
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Pseudeurotium zonatum AF096184 AF096198 AY129286
Pseudogymnoascus roseus AB015778 AB040690 AF062819
Myxotrichaceae
Holwaya mucida DQ257355 DQ257356 DQ257357
Bulgaria inquinans AY789343 AY789344 AY789345
Phacidiopycnis sp. FJ237044 HM595597
Cudoniella clavus AY789372 AY789373 AY789374
Ombrophila violacea AY789364 AY789365 AY789366
Dermea acerina DQ247809 DQ247801
Neofabraea malicorticis AY544706 AY544662 AF281386
Leotia lubrica AY789358 AY789359 AY789360
Microglossum sp. DQ257361 DQ257362 DQ257363
Mitrula paludosa AY789422 AY789423 AY789424
Tetracladium palmatum EU883424
Bisporella citrina AY789324 AY789325 AY789326
Bryoglossum gracile AY789419 AY789420 AY789421
Hyaloscypha daedaleae AY789414 AY789415 AY789416
Lachnum virgineum AY544688 AY544646 U59004
CBS 136257 JX124327
Soosiella minima
Catenulifera brachyconia GU727557 GU727559
Hyphodiscus hymeniophilus GU727555 GU727555
Pilidium acerinum AY487093 AY487092 AY487091
Sarea resinae AY641004 AY640965
Geoglossum glabrum AY789316 AY789317 AY789318
Trichoglossum hirsutum AY789312 AY789313 AY789314
Geoglossomycetes
Arthrobotrys oligospora AJ001986 AY261147
Orbilia delicatula U72603 AY261178 U72595
Orbiliomycetes
Peziza phyllogena AY789327 AY789328 AY789329
Pezizomycetes
Candida albicans X53497 L28817 AY672930
Saccharomyces cerevisiae J01353 J01355 AY247400
Saccharomycetes
Neolecta irregularis AY789379 AY789380 AY789381
0.2

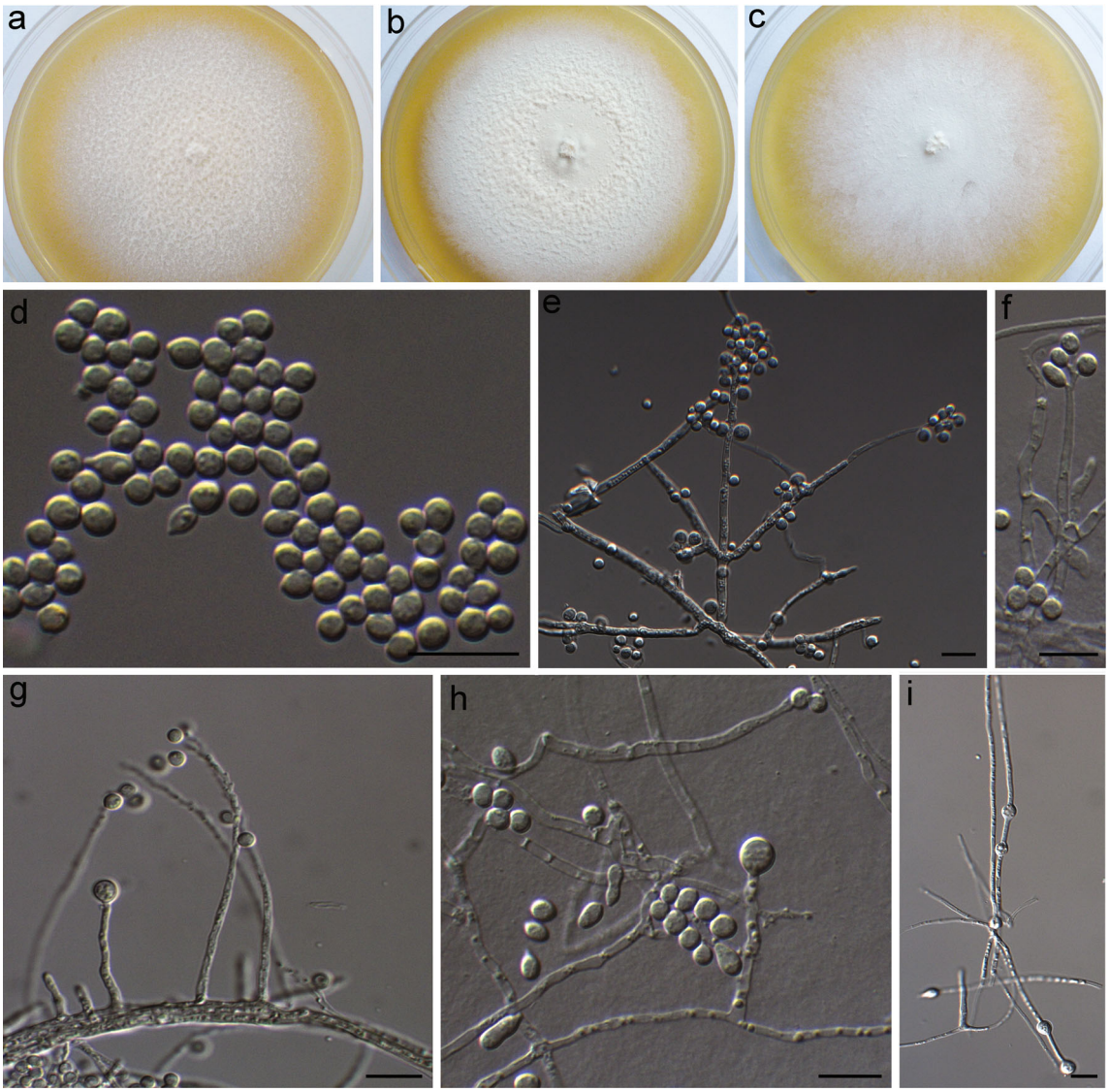

Fig. 4 *Acidothrix acidophila*. **a, b** Colony on MEA pH 5.5 at 24 °C, 21 days; **c** Colony on MEA pH 2 at 24 °C, 21 days; **d** Conidia globose, elipsoidal or lacrimose with hilum; **e-h** Conidiophores and conidia; **i** Conidia proliferating by hyphae and bearing other conidia. Scale bars = 10 μm

Phialides proliferating sympodially forming a long rachis, 35–100 × 1.0–1.5 μm. The first conidium on the phialide is larger, (3.7-) 4.3 (-6.1), sometimes proliferating by hyphae and bearing other conidia. Subsequent conidia, formed on the proliferating conidiogenous cells are single, globose, or ellipsoidal to lacrimose, with hilum, sometimes budding, giving rise to another conidium (2.8-) 3.5–4.0 (-4.5). Vegetative hyphae 2–3 μm wide, non granular. Sexual morph unknown.

Etymology: from Latin *acidus* "acidic" + Greek *philos* "loving", refers to its physiological abilities

Habitat: highly acidic soil (pH < 3)

Distribution: Czech Republic

Holotype: Czech Republic, Western Bohemia, The Soos National Natural Reserve, 50°08′60″ N, 12°24′00″ E, alt. 437, from soil (pH 1.8), August 2005, izol. M. Hujslová, holotype PRM 922615 (dried ex-type culture CBS 136259); isotype PRM 922616

Other specimens examined: The above description is based on ex-type strain. Other isolates CCF 3800, CCF 4344, CCF 4565, MH 1036 had the same morphology.

Acidea Hujslová & M. Kolařík, **gen. nov.** MB 805195

Asexual morph, hyphomycetes. Colonies compact, in some isolates with ruffled margin, centrally heaped to cerebriiform, wrinkled, funiculose or yeast-like, white to beige (10YR7/4). Mycelium sterile, 2.2–0.5.5 μm wide, sparsely branched, fully filled with single line of granules, often fragmenting. Sexual morph unknown, phylogenetic placement in Leotiomycetes.

Etymology: Acidea refers to the occurrence in acidic substrata.

Type species: ***Acidea extrema*** Hujslová & M. Kolařík, sp. nov.

Acidea extrema Hujslová & M. Kolařík, **sp. nov.** MB 805425 (Fig. 5)

Colonies on MEA (pH 5.5) at 24 °C, 21 days reaching a diameter of 17–36 mm, on acidic medium (MEA pH 2) achieving diameters of 17.5–23 mm in 21 days at 24 °C. On both media colonies compact, in some isolates with ruffled margin, centrally heaped to cerebriform, wrinkled, funiculose or yeast-like, white to beige (10YR7/4), reverse honey to ochre (7.5YR5/10). On PCA at 24 °C in 21 days colonies compact, centrally heaped with flat margin, without aerial mycelium, yeast-like; 25 mm in diam. Mycelium sterile, 2.2–0.5.5 μm wide, sparsely branched, fully filled with single line of granules, often fragmenting. Sexual morph unknown.

Etymology: from the adjective *extremus* (Latin) "extreme", refers to the extreme character of the substrate of origin.

Habitat: highly acidic soil (pH<3)

Distribution: Czech Republic

Holotype: Czech Republic, Western Bohemia, kaolin quarry Mírová, 50°15′00″ N, 12°46′12″ E, alt. 414 m, from soil (pH 2), May 2007, izol. M. Hujslová, holotype PRM 922617 (dried ex-type culture CBS 136258); isotype PRM 922618

Other specimens examined: The above description is based on ex-type strain. Other isolates CCF 4346, CCF 4348, MH 1185, MH 1288 had the same morphology.

Soosiella Hujslová & M. Kolařík, **gen. nov.** MB 805196

Asexual morph, hyphomycetes. Colonies slow growing, compact, heaped, spiny-like, white, reverse beige (10YR4/4). Mycelium sterile, 2.5–4.0 um wide, sparsely branched, irregularly granular. Sexual morph unknown, phylogenetic placement in Leotiomycetes.

Etymology: Soosiella refers to the locality from which the first isolate was obtained (Soos National Natural Reserve, Czech Republic)

Type species: ***Soosiella minima*** Hujslová & M. Kolařík, sp. nov.

Soosiella minima Hujslová & M. Kolařík, **sp. nov.** MB 805447 (Fig. 5)

Colonies on MEA (pH 5.5) at 24 °C, 21 days reaching a diameter of 8 mm; slow growing, compact, heaped, spiny-like, white, reverse beige (10YR4/4). On acidic medium

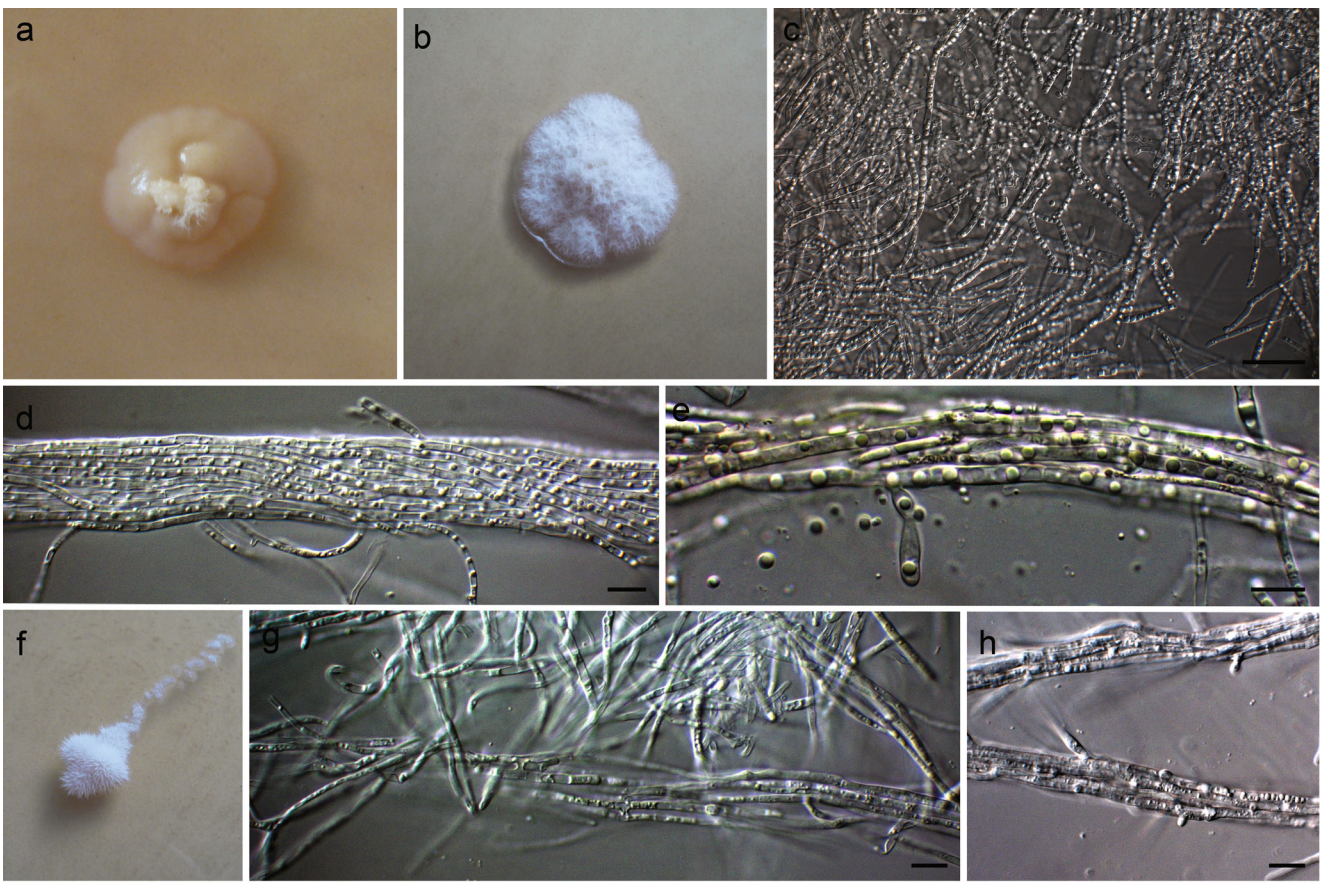

Fig. 5 *Acidea extrema*. **a** Colony on MEA pH 2 at 24 °C, 21 days; **b** Colony on MEA pH 5.5 at 24 °C, 21 days; **c**, **d**, **e** Sterile mycelium fully filled with granules; *Soosiella minima*. **f** Colony on MEA pH 5.5 at 24 °C, 21 days; **g**, **h** Sterile mycelium irregularly granular. Scale bars=10 μm

(MEA pH2) at 24 °C in 21 days no growth of colonies; on PCA at 24 °C colonies yeast-like, achieving diameters of 2 mm. Mycelium sterile, 2.5–4.0 μm wide, sparsely branched, irregularly granular. Sexual morph unknown.

Etymology: from *minimus* (Latin) "extremely small" refers to the poor growth abilities

Habitat: highly acidic soil (pH<3)

Distribution: Czech Republic

Holotype: Czech Republic, Western Bohemia, Soos National Natural Reserve, 50°08′60″ N, 12°24′00″ E, alt. 437 m, from soil (pH 2), November 2007, izol. M. Hujslová, holotype PRM 922619 (dried ex-type culture CBS 136257); isotype PRM 922620

Growth at different pH levels

The four isolates of *Acidothrix acidophila* were capable of growing over a pH range from 2 to 8 and two isolates (MH 1205 and MH 1036) showed limited growth at pH 1 (Fig. 6). One isolate (MH 560) showed unimodal growth with optimum at pH 3 and three isolates (MH 1036, MH 1205 and MH 1237) exhibit the bimodal growth response with two distinct peaks at pH 3 and 6 (Fig. 6).

One isolate (SH26-1) of *Acidea extrema* was capable of growing over a pH range from 2 to 7 (Fig. 6). Remaining four *Acidea* isolates were able to grow at pH range from 2 to 8 and three of them (MH 1180, MH 1125 and MH 1288) grew at pH 1 (Fig. 6). Three isolates (MH 1180, MH 1288, SH26-1) showed unimodal growth with optimum at pH 6, pH 3 and pH5 respectively and two isolates exhibited bimodal growth curves with two optima at pH 2 and 6 (MH 1125, MH 1185) (Fig. 6).

The tested isolate of *Soosiella minima* grew over a pH range from 3 to 6 with optimum at pH 4 (Fig. 6). No growth was recorded at pH 1, 2, 7 and 8 (Fig. 6).

Growth at different NaCl concentrations

All tested isolates of all three species were able to grow over a range of NaCl concentration from 0 to 0.5 M (Fig. 7). None of the isolates were capable of growing at concentrations of 2.5 M and 5 M (Fig. 7). *Acidothrix acidophila* isolates showed optimum growth in MEA without salt. The isolates of *Acidea extrema* showed different growth optima, ranging from MEA without salt (MH 1125, MH 1288 and SH26-1) to 0.2 M MEA (MH 1180, MH 1185). *Soosiella minima* exhibited optimum growth at 0.5 M MEA (Fig. 7).

Discussion

Phylogenetic analysis showed placement of *Acidothrix acidophila* in a group of wood-inhabiting fungi that includes *Wallrothiella congregata* and species of *Amplistroma* (Checa et al. 2012, 2013; Huhndorf et al. 2009) (Fig. 2). Delimitation of both genera is based on the morphology of the sexual stage, the asexual stages are not distinctive. The acrodontium-like asexual morphs occurring in *Amplistroma carolinianum*, *A. erinaceum*, *A. longicollis*, *A. ravum* and *Wallrothiella congregata* (Checa et al. 2012, 2013; Huhndorf et al. 2009) are closely similar to the morphology of *Acidothrix acidophila* (Fig. 4). *A. acidophila* is an acidophilic soil fungus and this characteristic is unique among members of the family. This fact, together with its phylogenetic position outside both mentioned genera, warranted placement in a new genus.

The closest relatives of *Acidea extrema* are two uncultured clones (RT5in6 and RT3n5) isolated from highly acidic samples obtained from the Tinto River in Spain (AY082984, AY082969) (Amaral Zettler et al. 2002) (Fig. 3). Other closely related species belong to aquatic fungi, including *Articulospora tetracladia*, *Fontanospora fusiramosa*, *Varicosporium elodeae*, *Tricladium patulum* and *Tetrachaetum elegans* (Fig. 3). Some aquatic hyphomycetes are able to inhabit various extreme environments such as

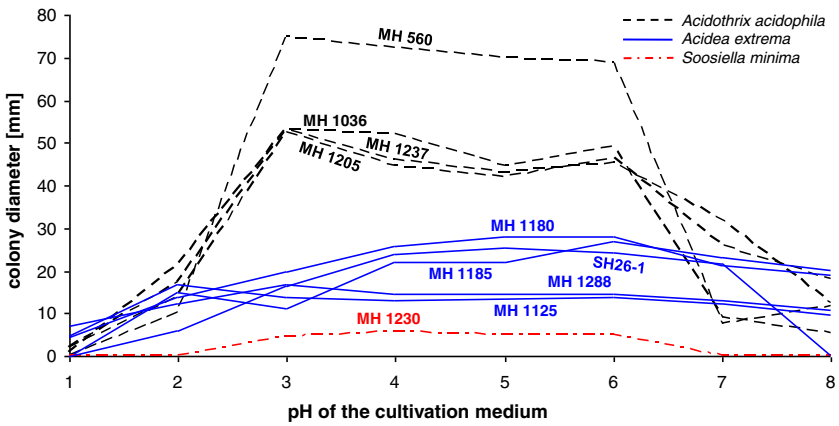

Fig. 6 Growth of the nine strains of *Acidothrix acidophila*, *Acidea extrema* and *Soosiella minima* (Table 2) on MEA at different pH values after 14 days at 24 °C

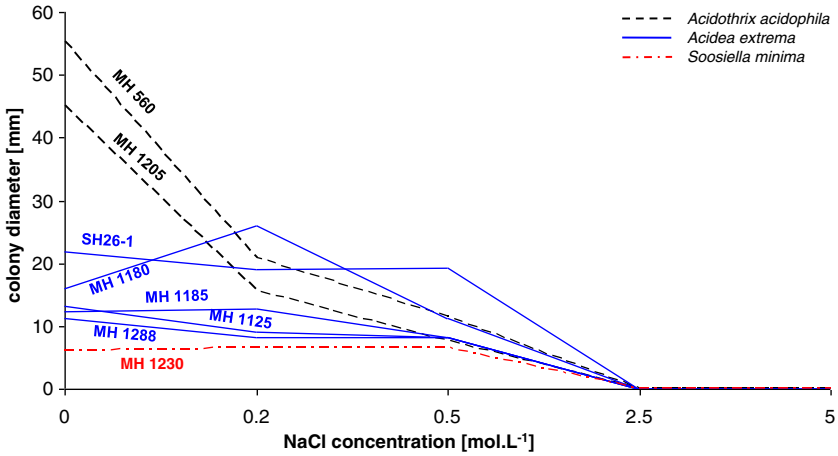

Fig. 7 Growth of the eight strains of *Acidothrix acidophila*, *Acidea extrema* and *Soosiella minima* (Table 2) on MEA with different NaCl concentrations after 14 days at 24 °C

Arctic and subarctic streams, warm sulphur springs or substrates polluted by heavy metals. Nevertheless, more data are needed to elucidate the extent of adaptation of these various aquatic fungi to stress factors (Krauss et al. 2011). Although our fungus is sterile and cannot be morphologically compared to these other related fungi, we decided to place it in a new genus because of its different phylogenetic position, ecology and physiology from other related genera.

Based on the results of the same dataset, the third newly described species *Soosiella minima* occurred as an unsupported sister clade to taxa with various morphologies and ecological preferences (Fig. 3). Placement within the Helotiales remains unclear. In general, the tree was not well resolved, support for the backbone was weak and the monophyly of the Helotiales was not resolved (Fig. 3). These results coincide with Wang et al. (2006a, b) who concluded that more molecular data and wider sampling are needed to elucidate the relationships within the Helotiales as well as Leotiomycetes.

The results of the growth test confirmed high adaptability of all three species to extreme pH. All described species were capable of growth at a pH of 3 or lower (Fig. 6); therefore, they may be classified as acidotolerant (Zak and Wildman 2004). From the studied species, *A. acidophila* and *A. extrema* were capable of growing at pH 2, which coincides with the pH values of the soil from which they were isolated except the *Acidea* isolate SH26-1 originating from alkaline soil (Table 1 and 2). Although this isolate was from a coastal Antarctic site where the soil pH was 8.1 (Arenz and Blanchette 2011), it was not able to grow at pH 8 in the laboratory study (Fig. 6). Since this fungus can grow at pH 2 to about pH 8, it appears that other factor(s) or a combination of factors are involved in the ability of this fungus to grow in non-hyperacid Antarctic soils as well as highly acidic soils. It is unclear which factors are most important to influence fungi inhabiting highly acidic substrates. However, two important factors affecting fungal growth in Antarctic soils are carbon and nitrogen content (Arenz and Blanchette 2011). In Antarctic soils, carbon and nitrogen content is minimal (Arenz and Blanchette 2011), and thus limiting for fungi, and it seems that in highly acidic soils, where the lack of vegetation cover results in low organic matter, this factor might also be important. However, detailed ecological and physiological studies are needed to confirm this assumption.

Three of four *Acidea* isolates and two of four *Acidothrix* isolates were unique among described taxa by their growth at pH 1 (Fig. 6). Only a few fungal species like *Acidomyces acidophilus*, *A. acidothermus* and *Hortaea acidophila* and two unidentified species *Paecilomyces* sp. and *Penicillium* sp. 4 were previously reported to grow at pH 1 (Gimmler et al. 2001; Hölker et al. 2004; Hujslová et al. 2010; Yamazaki et al. 2010).

Most of the isolates of *A. acidophila* and *A. extrema* exhibited bimodal growth curves which coincide with typical acidotolerant characteristics (Gimmler et al. 2001). Two isolates of each species exhibited a unimodal growth curve where the growth optimum shifted to pH 3 (Fig. 6), hence it should be classified as acidophilic (Cavicchioli and Torsten 2000). This phenomenon was also found in *Acidomyces acidophilus* which is classified as strictly acidophilic (Selbmann et al. 2008); however, the isolate exhibited a bimodal growth curve and thus is considered acidotolerant (Gimmler et al. 2001). The phenomenon of bimodal growth curves as the result of hydrogen ion impact was also recorded in several previous studies (Corum 1941; Mehrotra 1964; Verma 1969; Zabel and Morrell 1992; Griffin 1994).

Concerning the salt tolerance, all species tested were able to grow on salinities from 0 to 0.5 M MEA (Fig. 7); therefore, they may be classified as slight halophiles which is how many marine fungi have been classified (Kushner 1978). Despite the isolate SH26-1 originating from soil in Antarctica where high salinity (pH 8.1) represents a significant stress factor, no difference in tolerance to NaCl was found among it and the isolates from acidic soils (Fig. 7). This finding coincides with results from Arenz and Blanchette (2011) who confirmed that salinity affects fungi indirectly through its influence on primary producer presence.

All three newly described species were found in two or more highly acidic locations (Table 1 and 2) and all of them showed high adaptability to extreme conditions of the studied substrate (Figs. 6 and 7), but only two species (*Acidothrix acidophila* and *Soosiella minima*) may be considered exclusive inhabitants of highly acidic environments. The third one, *Acidea extrema*, was also isolated from a non-acidic environment in the Antarctic (Table 2), which indicates that this fungus can cope with a wider spectrum of extreme factors than other acidophilic and acidotolerant fungi. Moreover, these results show that two seemingly very different environments such as those found in Antarctic soils and extremely acidic soils probably share some factor(s) that allow fungal growth to occur under these unusual conditions. The close connection of these two extreme environments was found also by Hujslová et al. (2013) where an exclusive fungal inhabitant of highly acidic soils, *Acidiella bohemica*, was found to be a close relative of fungi isolated from rocks in Antarctica.

Conclusions

Despite the extreme conditions for life found in highly acidic habitats (pH<3), these environments harbour a fungal community that is different from less acidic habitats and dominated by a small number of fungal species (Amaral Zettler et al. 2002, 2003; Baker et al. 2004, 2009; Hujslová et al. 2010, 2013; López-Archilla et al. 2004). To date, only four meristematic fungal species were known exclusively from extremely acidic habitats, *Acidomyces acidophilus* (Selbmann et al. 2008), *Hortaea acidophila* (Hölker et al. 2004), *Acidomyces acidothermus* (Yamazaki et al. 2010; Hujslová et al. 2013) and *Acidiella bohemica* (Hujslová et al. 2013). In the present study, three new genera and species *Acidothrix acidophila*, *Acidea extrema* and *Soosiella minima* with high adaptability to extreme conditions were described. All of these fungi inhabit extreme acidic habitats in geographically distant sites, and together with the four meristematic fungi a core assemblage of the acidophilic fungal community is being elucidated. All but one species within this community are known exclusively from highly acidic substrates. *Acidea extrema* represents the exception showing ability to also populate non-acidic extreme environments and thus has a wider adaptability to extreme conditions. Previously reported acidophilic fungi are typically dematiaceous and belong to the Teratosphaeriaceae, a family comprising a diverse collection of stress-tolerant fungi. In the present work we have revealed the adaptation of hyaline fungi in mesophilic lineages to highly acidic environments.

Acknowledgments This work was supported by the Grant Agency of the Charles University in Prague (project No. 63009), by Czech Institutional Research Concept (No. AV0Z5020903), and by the institutional resources of the Ministry of Education, Youth and Sports of the Czech Republic. We thank the staff of Soos National Natural Reserve and Sedlecky kaolin a. s. for the permission to sample. We are grateful to Ota Rauch for the selection of localities and Radek Pelc for technical assistance. Research in Antarctica was supported by National Science Foundation Grant No. 0537143 to RAB. We would like to thank the British Antarctic Survey (BAS) and the crew of the HMS Endurance for facilitating travel to sites on the Antarctic Peninsula and Dr. Brett Arenz for his work to collect isolates on Snow Hill Island. The senior author also acknowledges the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, South Africa, for support during a sabbatical visit to the Institute.

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