RESEARCH ARTICLE

Sodiomyces alkalinus, a new holomorphic alkaliphilic ascomycete within the *Plectosphaerellaceae*

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Abstract   In this study we reassess the taxonomic reference of the previously described holomorphic alkaliphilic fungus *Heleococcum alkalinum* isolated from soda soils in Russia, Mongolia and Tanzania. We show that it is not an actual member of the genus *Heleococcum* (order *Hypocreales*) as stated before and should, therefore, be excluded from it and renamed. Multi-locus gene phylogeny analyses (based on nuclear ITS, 5.8S rDNA, 28S rDNA, 18S rDNA, RPB2 and TEF1-alpha) have displayed this fungus as a new taxon at the genus level within the family *Plectosphaerellaceae, Hypocreomycetidae, Ascomycota*. The reference species of actual *Heleococcum* members showed clear divergence from the strongly supported *Heleococcum alkalinum* position within the *Plectosphaerellaceae*, sister to the family *Glomerellaceae*. Eighteen strains isolated from soda lakes around the world show remarkable genetic similarity promoting speculations on their possible evolution in harsh alkaline environments. We established the pH growth optimum of this alkaliphilic fungus at c. pH 10 and tested growth on 30 carbon sources at pH 7 and 10. The new genus and species, *Sodiomyces alkalinus* gen. nov. comb. nov., is the second holomorphic fungus known within the family, the first one being *Plectosphaerella* – some members of this genus are known to be alkalitolerant. We propose the *Plectosphaerellaceae* family to be the source of alkaliphilic filamentous fungi as also the species known as *Acremonium alcalophilum* belongs to this group.

INTRODUCTION

The fungal kingdom is highly diverse and versatile, with members able to grow under various environmental conditions. Although the majority of fungi are considered as neutrophiles, showing optimal growth in moderate habitats (e.g. 25–30 °C, pH 5–7), some of them have adapted to thrive in extreme environments where abiotic conditions are so harsh that most organisms cannot survive. One such stressful condition is high alkalinity, to which some fungi have become adapted. Alkaliphilic fungi, i.e. fungi that are capable of growing at high pH, above pH 9 (Horikoshi 1999), have been little studied. Only a handful of filamentous alkaliphilic fungi have been reported to date (Nagai et al. 1995, 1998). The natural habitats for this kind of fungi are believed to be soda soils and soda lakes, as are often encountered in arid and semi-arid areas. These sites represent an unusual, naturally occurring stable ecotope where the overall biodiversity is compromised due to significant ambient stress, namely, high salt-osmotic pressure and high pH. Besides these natural sites, there are also sites created by human industrial processes like concrete and paper manufacture. These industries are known to create alkaline wastes that are potential habitats for alkaliphilic fungi (Mueller et al. 2004). Already in 1923, Johnson (1923) showed the ability of *Fusarium oxysporum*, *F. bullatum* and *Penicillium variabile* to grow at the extremely high pH of 11. Probably the most notable study on alkaliphilic fungi was the isolation and description of mitosporic *Acremonium alcalophilum*, which has a growth optimum at around pH 9, by Okada et al. (1993). More recently, Kladwang et al. (2003) isolated a number of alkali-tolerant species in Thailand and Elíades et al. (2006) reported eight species of alkaliphilic and alkalitolerant soil fungi from Argentina, taxonomically distributed through *Bionectriaceae*, *Trichocomaceae*, *Sporormiaceae*, *Ceratostomataceae* and *Sordariaceae*. However, overall, information on the biodiversity of alkaliphilic filamentous fungi is scarce.

Fungi growing at extreme pH values are of scientific interest for the general study of fungal adaptive evolution as well as for the evaluation of their potential in producing commercially valuable substances. Obviously, the fungi adapting to alkalinity must have metabolic pathways that have become modified with respect to those seen in related neutrophilic fungi. For instance, enzymes that are being secreted into the ambient environment should work optimally in alkalinity in order to provide sufficient amounts of nutrients (Kladwang et al. 2003). In addition, adaptations to alkaline environments are required for structures involved in exporting metabolites like toxins and antibiotics, for domains of the membrane transporters exposed to ambient environment and for regulation of gene expression by ambient pH. Alkaliphilic fungi are likely to possess unique properties that have not been well elucidated so far.

In 2005, a new alkaliphilic holomorphic fungus from hyper-saline soda soils (pH around 10) was isolated, described and placed among members of the genus *Heleococcum* as *H. alkalinum*. The genus *Heleococcum* (order *Hypocreales*) seemed appropriate based on morphological and ecological features (Bilanenko et al. 2005). *Heleococcum* species are known to be soil saprobes, all producing *Acremonium*-like anamorphs (except *H. aurantiacum*) along with bright-coloured cleistothecial ascomata (Jørgensen 1922, Tubaki 1967, Udagawa et al. 1995).

Upon considering the ecological distinctiveness of *H. alkalinum* and also some differences in morphological features compared to other *Heleococcum* species, we decided to investigate the taxonomic position of this species with the help of molecular phylogeny. We used multiple molecular phylogenetic markers including ribosomal rDNA (18S, 28S, ITS and 5.8S) and protein-coding genes for the second largest subunit of RNA polymerase II (RPB2) and for transcriptional elongation factor 1-alpha (TEF1-alpha). Results demonstrated that this fungus does not belong in *Heleococcum* but instead belongs to a new genus within the family of the *Plectosphaerellaceae*. We confirm its alkaliphilic nature and speculate on its possible role in alkaline ecotopes, based on growth experiments carried out on media containing various carbon sources at different pH levels. We also discuss the significance of the low levels of genetic variation observed among strains isolated from soda lakes located thousands of kilometres apart.

MATERIALS AND METHODS

Strains and media

In this study we used 18 strains of *Heleococcum alkalinum* (Bilanenko et al. 2005) isolated from soils near soda lakes in Russia, Mongolia and Tanzania (Table 1). All strains were grown at 27 °C on alkaline agar medium (AA) containing per litre: 1) Na_2CO_3 – 24 g, $NaHCO_3$ – 6 g, NaCl – 5 g, KNO_3 – 1 g, K_2HPO_4 – 1 g; 2) malt extract (Merck) – 17 g, yeast extract (Difco) – 1 g, agar (Difco) – 20 g. Components 1 and 2 were autoclaved separately for 20 min at 120 °C and mixed together after cooling which resulted in a final pH of c. 10. Reference strains were members of other species placed in the genus *Heleococcum*, including *H. japonense* CBS 397.67 and *H. aurantiacum* CBS 201.35. In addition, we included some pertinent *Acremonium* cultures, *A. alcalophilum* CBS 114.92 and *A. antarcticum* CBS 987.87, that were obtained from the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands). The *Acremonium* and *Heleococcum* isolates were maintained on standard malt extract agar (MEA), oat meal agar (OA) (Mueller et al. 2004) or our own AA medium at 27 °C.

Growth at different pH

To elucidate the pH growth optimum of the strains we used malt extract-based medium buffered at pH levels ranging from 4 to 11.4. Acetic acid buffers were used to create pH 4 and 5.2, while phosphate buffers were used for pH 5.9, 7 and 7.8. Carbonate buffers were employed to set pH 8.7 and 9.8. Finally, a Na_2HPO_4/NaOH buffer system was used to generate pH 10.5 and 11.4. The final buffer concentration in all media was set to 0.1 M. The core nutrient component of media contained per litre: malt extract (Merck) – 17 g, yeast extract (Difco) – 1 g, agar (Difco) – 20 g. Buffers and nutrient components were autoclaved separately for 20 min at 120 °C and mixed afterwards, making up complete media. Strains were inoculated in so-called race tubes (Perkins & Pollard 1986). Four tubes were inoculated per strain in media at each of these pH values: 4, 5.2, 5.9, 7, 7.8, 8.7, 9.8, 10.5 and 11.4. Linear growth was measured once or twice a week for c. 3 mo.

Growth on different carbon sources

To analyse the capacity of *H. alkalinum* to use diverse carbon sources at neutral and alkaline conditions, we employed 31 media buffered at pH 7 and 10 based on various carbon sources ranging in complexity (including no-C source as a control). The final buffer concentration (phosphate buffer for pH 7 and carbonate buffer for pH 10) was chosen to be 0.1 M. All media had final salts concentrations per litre of NaCl – 5 g, KNO_3 – 1 g, K_2HPO_4 – 1 g. The salt component was autoclaved separately. Simple soluble sugars, namely, D(+)glucose, D(-)fructose, D(+)galactose, D(+)mannose, D(+)xylose, L(+)arabinose, L-rhamnose, D(+)glucuronic acid, D(+)cellobiose, D(+)maltose, D(+)lactose, D(+)raffinose, sucrose, were used at 25 mM final concentration. They were gently (15 min at 110 °C) autoclaved separately from salt and agar solutions and were subsequently added to the final media after cooling. A 1 % concentration was chosen for the following complex carbon sources: arabinogalactan, beechwood xylan, birchwood xylan, oat spelt xylan, guar gum, soluble starch, apple pectin, inulin, hydrolytic lignin, alpha-cellulose and chitin. A 3 % concentration was used for: sugar beet pulp, citrus pulp, soybean hulls, cotton seed hulls, alfalfa meal and corn gluten. All media were supplemented with solution of metal traces (van Diepeningen et al. 2008). Complex substrates were autoclaved together with agar. Growth experiments on carbon sources were conducted at 27 °C in six replicates with initial inoculation of c. 1 400 spores per replicate. Growth patterns were recorded through 12 d.

Morphological studies with cryo-SEM and SEM

A piece (0.5 × 0.5 cm) of mycelium with agar was cut out from the plate directly and glued on a brass Leica sample holder with carbon paste (Leit-C, Neubauer Chemikalien, Germany). It was further frozen in liquid nitrogen and simultaneously fitted into the cryo-sample loading (VCT 100) system. The Leica sample holder was transferred to a non-dedicated cryo-preparation

Table 1 Strains of *Sodiomyces alkalinus* (former *Heleococcum alkalinum*) used in the study. Locations, characteristics of soil samples and isolation date are indicated.

Strain	CBS no.	Location	Place	Soil pH	Total salts (g/kg)	Depth (cm)	Saltification type	Date
F7	CBS 132729	Kunkur steppe, Chitinskaya area, Russia	Low-salt soda lake	–	–	0 to 5	Soda	1999
F8	CBS 133680	Kunkur steppe, Chitinskaya area, Russia	Low-salt soda lake	–	–	0 to 5	Soda	1999
F9	CBS 133681	Kunkur steppe, Chitinskaya area, Russia	Low-salt soda lake	–	–	0 to 5	Soda	1999
F10	CBS 132730	Tanzania	Natron lake	10 (water)	–	0 to 5	Soda	1999
F11	CBS 110278	Choibalsan area, North-East Mongolia	Shar-Burdiyn lake	10.7	49	0 to 5	Soda	1999
F12	CBS 132731	North-East Mongolia	Barun-Undziyn lake	10.5	82	0 to 5	Soda	1999
F13	CBS 132732	Kulunda steppe, Altai, Russia	Solyonoe lake	10	187	0 to 5	Chloride	Aug. 2002
F14	CBS 133682	Kulunda steppe, Altai, Russia	Karakul' lake	9.8	144	0 to 5	Soda	Aug. 2002
F15	CBS 133683	Kulunda steppe, Altai, Russia	Mirabilit lake	9.7	165	0 to 5	Soda-chloride-sulfate	Aug. 2002
F16	CBS 133684	Kulunda steppe, Altai, Russia	Petuhovskoe lake	10.2	163	0 to 5	Soda	Aug. 2002
F17	CBS 133685	Kulunda steppe, Altai, Russia	Bezimyannoe lake	9.9	310	0 to 5	Soda	Aug. 2002
F18	CBS 132733	Kulunda steppe, Altai, Russia	Tanatar lake	10.2	73	0 to 5	Soda	Aug. 2002
F19	CBS 133686	Kulunda steppe, Altai, Russia	Mirabilit lake	9.6	100	0 to 5	Soda-chloride-sulfate	Aug. 2002
F20	CBS 133687	North Gobi, Mongolia	Bayan-Dzag area	9.3	43	1 to 2	Sulfate-soda	Aug. 2003
F21	CBS 133688	North Gobi, Mongolia	Bayan-Dzag area	9.2	6	10 to 18	Sulfate-soda	Aug. 2003
F22	CBS 133689	Kulunda steppe, Altai, Russia	North	10	22	0 to 5	Soda	Aug. 2005
F23	CBS 133690	Kulunda steppe, Altai, Russia	Karagay lake	9.9	43	0 to 5	Soda	Aug. 2005
F24	CBS 133691	Kulunda steppe, Altai, Russia	Gor'koye lake	10.4	30	0 to 5	Soda	Aug. 2005

system (MED 020/VCT 100, Leica, Vienna, Austria) onto a sample stage at -93 °C. In this cryo-preparation chamber, the samples were freeze dried for 3 min at -93 °C at 1.3×10^{-6} mbar to remove water vapour contamination from the surface. The sample was sputter-coated with a layer of 10 nm tungsten at the same temperature and transferred into the field emission scanning microscope (Magellan 400, FEI, Eindhoven, The Netherlands) on the sample stage at -122 °C at 4×10^{-7} mbar. The analysis was performed with SE at 1 and 2 kV, 13 pA. All images were recorded digitally.

We also examined fungal samples by scanning electron microscopy after 1 h of 2.5 % glutaraldehyde fixation and series of 0.1 M phosphate buffer (pH 7.2) washing steps (20 min each) followed by dehydration through an ethanol series (30 %, 50 %, 70 % and 96 % concentration) and acetone before critical point drying in CO_2, carbon and metal coating. Specimens were observed under a JEOL (Japan) scanning electron microscope. Light microscopic studies were done with a Nikon Eclipse 80i. Taxonomic novelties were deposited in MycoBank (www.MycoBank.org; Crous et al. 2004).

DNA extraction

All strains used in the study were grown in Petri dishes containing either AA or MEA depending on which yielded better growth performance for the specific strain. Plates were incubated at 27 °C for c. 5 d on a cellophane membrane. Total genomic DNA (free of RNA) was extracted from mycelium harvested from the cellophane surface with DNeasy Plant Mini kit (Qiagen Inc., Chatsworth, California, USA). DNA quality and concentration was verified with a NanoDrop 2000.

Polymerase chain reaction and sequencing

Amplification and sequencing were performed for five nuclear loci: ITS1–ITS4 region including 5.8S rDNA, LR0R–LR9 region of 28S rDNA, NS1–NS8 of 18S rDNA, 5–7 region of RPB2 and 983–2218 region of TEF1-alpha. The final volume of the PCR mix was 25 µL which contained 5× GoTaq Green buffer (Promega, USA), 400 µM dNTP, 0.4 µM of each primer, 0.02 U GoTaq polymerase (Promega, USA), 5–100 ng template genomic DNA, 1 mM $MgCl_2$. PCR conditions were as follows: for ITS 5 min at 94 °C; 33 cycles of 1 min at 94 °C, 1 min at 51 °C, 1 min at 72 °C; for 28S rDNA 5 min at 94 °C; 33 cycles of 1 min at 94 °C, 1 min at 49 °C, 2 min at 72 °C; for 18S rDNA 5 min at 94 °C; 33 cycles of 1 min at 94 °C, 1 min at 52 °C, 2 min at 72 °C; for 5–7 region of RPB2 gene 5 min at 94 °C; 9 cycles of 1 min at 94 °C, 1 min at 60 °C to 50 °C (with 1 degree decrement each cycle), 1.5 min at 72 °C followed by 32 cycles of 1 min at 94 °C, 1.5 min at 50 °C, 1.5 min at 72 °C; for TEF1-alpha gene 5 min at 94 °C; 9 cycles of 1 min at 94 °C, 1 min at 66 °C to 56 °C (with 1 degree decrement each cycle), 1.5 min at 72 °C followed by 32 cycles of 1 min at 94 °C, 1.5 min at 56 °C, 1.5 min at 72 °C. All reactions were kept 7 min at 72 °C for final extension step. Standard fungal primers were used for PCR listed in the overview by Binder & Hibbett (2003) available at http://www.clarku.edu/faculty/dhibbett/Protocols_Folder/Primers/Primers.pdf. The amplification products were visualized on a 1 % agarose gel stained with ethidium bromide, purified using GeneElute PCR Clean-Up Kit (Sigma) according to the manufacturer instructions and subsequently sequenced by the chain termination method at Eurofins MWG Operon (Germany) service. LR7; NS4 and NS6 primers for 28S rDNA and 18S rDNA regions, respectively, were supplemented for sequencing in addition to original PCR primers. Raw sequence chromatograms were viewed and edited using CodonCodeAligner v. 3.7.1 (CodonCode Corporation, Dedham, MA, USA) and DNAStar Lasergene EditSeq v. 7.1.0 (DNASTAR Inc., Madison, WI, USA).

Phylogenetic analyses

Newly generated sequences were deposited in GenBank with accession numbers listed in Table 2. Sequences of ITS1 & 2 (including 5.8S rDNA gene), 28S rDNA gene (LSU), 18S rDNA gene (SSU) and protein coding genes RPB2 and TEF1-alpha were used for phylogenetic analysis. Reference sequences of *Sordariomycetes* members needed for phylogenetic reconstruction were obtained from GenBank along with our new data.

Separate datasets of each gene were constructed with a multiple sequence alignment online service MAFFT v. 6 (Katoh & Toh 2008) and were further reviewed and edited manually in BioEdit v. 7.1.3.0 (Hall 1999). Ambiguously aligned regions

Table 2 Taxa used in the phylogenetic analyses with their GenBank accession numbers. Newly generated accessions marked in **bold**.

Species	Voucher	Appearance in tree*	SSU	LSU	RPB2	TEF1-alpha	ITS
Acanthonitschkea argentinensis	SMH 1395	1	_	AY695259	FJ968943	FJ969042	_
Acremonium alcalophilum	CBS 114.92	1, 2	**JX158486**	**JX158443**	**JX158465**	**JX158399**	**JX158421**
Acremonium antarcticum	CBS 987.87	1, 2	**JX158487**	**JX158444**	**JX158466**	**JX158400**	**JX158422**
Acremonium brunnescens	CBS 559.73	2		HQ231966			_
Acremonium cucurbitacearum	CBS 683.88	2		HQ231968			
Acremonium furcatum	CBS 122.42	2		EF543831			AY378154
Acremonium murorum	CBS 154.25	1	FJ176824	FJ176880	FJ238363	_	_
Acremonium nepalense	CBS 971.72	2		HQ231970			DQ825971
Acremonium persicinum	CBS 310.59	1	FJ176822	FJ176878	FJ238361	_	_
Acremonium restrictum	CBS 178.40	2		HQ232119			_
Acremonium strictum	CBS 346.70	1	FJ176823	FJ176879	FJ238362	_	GQ376096
Acremonium stromaticum	CBS 863.73	2		HQ232143			DQ825969
Acrostalagmus annulatus	DAOM 212126	2		GU180646			GU180632
Acrostalagmus luteoalbus	CBS 194.87	2		EF543826			
Apiognomonia errabunda	AR 2813	1	DQ862045	AF408334	DQ862014	DQ862030	DQ313525
Bionectria ochroleuca		1	DQ862044	DQ862027	DQ862013	DQ862029	_
Camarops microspora	CBS 649.92	1	DQ471036	AY083821	DQ470937	DQ471108	_
Camarops ustulinoides	DEH 2164	1	DQ470989	DQ470941	DQ470882	DQ471050	
Cercophora caudata	CBS 606.72	1	DQ368659	AY999113	DQ368646	_	AY999135
Chaetosphaerella phaeostroma	SMH 4257	1	_	AY695264	FJ968940	FJ969004	_
Colletotrichum boninense	CMT74	2		JQ754138			_
Colletotrichum gloeosporioides	CMT32	2		JQ754114			_
Coniochaeta ostrea	CBS 507.70	1	DQ471007	DQ470959	DQ470909	DQ471078	_
Cordyceps cardinalis	OSC 93609	1	AY184973	AY184962	DQ522422	DQ522325	_
Cryphonectria parasitica	ATCC 38755	1	DQ862048	EU199123	DQ862017	DQ862033	AY141856
Cryptosporella hypodermia	CBS 171.69	1	DQ862049	DQ862028	DQ862018	DQ862034	EU199225

Table 2 (cont.)

Species	Voucher	Appearance in tree*	SSU	LSU	RPB2	TEF1-alpha	ITS
Diaporthe eres	CBS 109767	1	DQ471015	_	DQ470919	DQ479931	DQ491514
Diatrype disciformis	CBS 197.49	1	DQ471012	DQ470964	DQ470915	DQ471085	_
Didymostilbe echinofibrosa	AR 2824	1	AY489674	AY489706	_	AY489601	DQ135999
Epichloe typhina	ATCC 56429	1	U32405	_	DQ522440	AF543777	JN049832
Eutypa lata	CBS 208.87	1	DQ836896	DQ836903	DQ836889	DQ836909	DQ006927
Fracchiaea broomeana	SMH 2809	1	_	AY695268	FJ968942	FJ969039	
Gelasinospora tetrasperma	CBS 178.33	1	DQ471032	DQ470980	DQ470932	DQ471103	AY681178
Gibellulopsis nigrescens	DAOM 226890	2		GU180648			GU180631
Gibellulopsis piscis	CBS 892.70	2		EF543835			DQ825985
Glomerella acutata	ICMP4850	2		JN939938			_
Glomerella cingulata	FAU 553	1, 2	AF543762	AF543786	_	AF543773	_
Gnomonia gnomon	CBS 199.53	1	DQ471019	AF408361	DQ470922	DQ471094	DQ491518
Graphostroma platystoma	CBS 270.87	1	DQ836900	DQ836906	DQ836893	DQ836915	_
Heleococcum aurantiacum	CBS 201.35	1	_	**JX158442**	**JX158464**	**JX158397**	**JX158419**
Heleococcum japonense	CBS 397.67	1	**JX158485**	**JX158441**	**JX158463**	**JX158398**	**JX158420**
Hydropisphaera erubescens	ATCC 36093	1	AY545722	AY545726	AY545731	DQ518174	_
Hypocrea americana	OSC 100005	1	AY544693	AY544649	_	DQ471043	DQ491488
Hypocrea lutea	ATCC 208838	1	AF543768	AF543791	DQ522446	AF543781	_
Lasiosphaeria ovina	SMH 4605	1	DQ836894	AY436413	AY600284	DQ836908	AY587923
Leucostoma niveum	AR 3413	1	DQ862050	AF362558	DQ862019	DQ862035	_
Lignincola laevis	JK 5180A	1	U46873	U46890	DQ836886	_	_
Lindra thalassiae	JK 5090A	1	DQ470994	DQ470947	DQ470897	DQ471065	DQ491508
Lulworthia grandispora	JK 4686	1	DQ522855	DQ522856	DQ518181	DQ497608	_
Melanconis alni	AR 3500	1	DQ862052	AF408371	DQ862021	DQ862037	_
Melanconis marginalis	AR 3442	1	DQ862053	AF408373	DQ862022	DQ862038	_
Melanconis stilbostoma	AR 3501	1	DQ862054	AF408374	DQ862023	DQ862039	_
Melanopsamma pomiformis	ATCC 18873	1	AY489677	AY489709	EF692511	AY489604	AF081478
Melanospora tiffanii	ATCC 15515	1	AY015619	AY015630	AY015637	_	_
Melanospora zamiae	ATCC 12340	1	AY046578	U17405	AY046580	_	_
Microascus trigonosporus	CBS 218.31	1	DQ471006	DQ470958	DQ470908	DQ471077	DQ491513
Musicillium theobromae (Verticillium theobromae) 1	CBS 968.72	2		EF543838			EF543859
Musicillium theobromae (Verticillium theobromae) 2	CBS 458.51	2		EF543837			EF543858
Peethambara spirostriata	CBS 110115	1	AY489692	AY489724	EF692516	AY489619	_
Petriella setifera	CBS 437.75	1	DQ471020	DQ470969	DQ836883	DQ836911	_
Plectosphaerella alismatis	CBS 113362	2		JF780521			JF780523
Plectosphaerella citrullae 1	Plect 189	2		HQ239050			HQ238964
Plectosphaerella citrullae 2	Plect 157	2		HQ239048			HQ238962
Plectosphaerella cucumerina	DAOM 226828	1, 2	GU180612	GU180647	GU180663	_	GU180630
Plectosphaerella melonis	Plect 148	2		HQ239007			HQ238968
Plectosphaerella pauciseptata	Plect 186	2		HQ239012			HQ238971
Plectosphaerella plurivora	Plect 329	2		HQ239017			HQ238972
Plectosphaerella ramiseptata	Plect 158	2		HQ239049			HQ238963
Pleospora herbarum	CBS 191.86	1	DQ247812	DQ247804	DQ247794	DQ471090	DQ491516
Podospora fimiseda	CBS 990.96	1	_	AY346296	AY780190	_	AY515361
Roumegueriella rufula	CBS 346.85	1	DQ522561	DQ518776	DQ522461	DQ522355	_
Selinia pulchra	AR 2812	1	_	GQ505992	_	HM484841	HM484859
Sodiomyces alkalinus F7	**CBS 132729**	1, 2	**JX158467**	**JX158423**	**JX158445**	**JX158379**	**JX158401**
Sodiomyces alkalinus F8	**CBS 133680**	1, 2	**JX158468**	**JX158424**	**JX158446**	**JX158380**	**JX158402**
Sodiomyces alkalinus F9	**CBS 133681**	1, 2	**JX158469**	**JX158425**	**JX158447**	**JX158381**	**JX158403**
Sodiomyces alkalinus F10	**CBS 132730**	1, 2	**JX158470**	**JX158426**	**JX158448**	**JX158382**	**JX158404**
Sodiomyces alkalinus F11	**CBS 110278**	1, 2	**JX158471**	**JX158427**	**JX158449**	**JX158383**	**JX158405**
Sodiomyces alkalinus F12	**CBS 132731**	1, 2	**JX158472**	**JX158428**	**JX158450**	**JX158384**	**JX158406**
Sodiomyces alkalinus F13	**CBS 132732**	1, 2	**JX158473**	**JX158429**	**JX158451**	**JX158385**	**JX158407**
Sodiomyces alkalinus F14	**CBS 133682**	1, 2	**JX158474**	**JX158430**	**JX158452**	**JX158386**	**JX158408**
Sodiomyces alkalinus F15	**CBS 133683**	1, 2	**JX158475**	**JX158431**	**JX158453**	**JX158387**	**JX158409**
Sodiomyces alkalinus F16	**CBS 133684**	1, 2	**JX158476**	**JX158432**	**JX158454**	**JX158388**	**JX158410**
Sodiomyces alkalinus F17	**CBS 133685**	1, 2	**JX158477**	**JX158433**	**JX158455**	**JX158389**	**JX158411**
Sodiomyces alkalinus F18	**CBS 132733**	1, 2	**JX158478**	**JX158434**	**JX158456**	**JX158390**	**JX158412**
Sodiomyces alkalinus F19	**CBS 133686**	1, 2	**JX158479**	**JX158435**	**JX158457**	**JX158391**	**JX158413**
Sodiomyces alkalinus F20	**CBS 133687**	1, 2	**JX158480**	**JX158436**	**JX158458**	**JX158392**	**JX158414**
Sodiomyces alkalinus F21	**CBS 133688**	1, 2	**JX158481**	**JX158437**	**JX158459**	**JX158393**	**JX158415**
Sodiomyces alkalinus F22	**CBS 133689**	1, 2	**JX158482**	**JX158438**	**JX158460**	**JX158394**	**JX158416**
Sodiomyces alkalinus F23	**CBS 133690**	1, 2	**JX158483**	**JX158439**	**JX158461**	**JX158395**	**JX158417**
Sodiomyces alkalinus F24	**CBS 133691**	1, 2	**JX158484**	**JX158440**	**JX158462**	**JX158396**	**JX158418**
Stachylidium bicolor	DAOM 226658	2		GU180651			
Torrubiella alba (Lecanicillium alba)	CBS 726.73a	1	AF339586	AF339537	EF468934	EF468781	AJ292464
Varicosporina ramulosa	RVG 113	1	U43846	U44092	DQ836888	_	
Verticillium albo-atrum	CBS 130.51	2		HQ231976			DQ825977
Verticillium alfalfae	PD683	2	_				JN187991
Verticillium dahliae	ATCC 16535	1, 2	AY489705	DQ470945	DQ522468	AY489632	_
Verticillium isaacii	PD752	2	_				JN188018
Verticillium zaregamsianum	PD739	2	_				JN188008
Xylaria acuta	ATCC 56487	1	AY544719	AY544676	DQ247797	DQ471048	AF163026
Xylaria hypoxylon	OSC 100004	1	AY544692	AY544648	DQ470878	DQ471042	DQ491487

* Appears in large scale tree (1), small scale tree (2) or both

Table 3 Loci and substitution models used in the phylogenetic analyses. Information on included base pairs is provided.

Phylogenetic analysis	Locus	Nucleotide substitution model	Characters	Phylogenetically informative characters	Uninformative variable characters	Invariable characters
1	18S rDNA	TIM+I+G (or GTR+I+G for MrBayes)	1652	328	185	1139
	28S rDNA	GTR+I+G	1381	421	158	801
	RPB2	GTR+I+G	954	571	43	340
	TEF1alpha	GTR+I+G	867	310	70	487
	5.8S rDNA	GTR+G	159	29	9	124
	total		**5013**	**1659 (33 %)**	**465 (9 %)**	**2891 (58 %)**
2	ITS with 5.8S rDNA	HKY+I+G	472	135	21	316
	28S rDNA	GTR+G	843	154	48	641
	total		**1315**	**289 (22 %)**	**69 (5 %)**	**957 (73 %)**

were removed from the data matrix. For better illustration of phylogenetic relations among taxa we generated two matrices with different datasets and resolution power: 1) we concatenated five gene alignments (SSU, LSU, RPB2, TEF1-alpha and 5.8S rDNA) using Mesquite 2.74 (Maddison & Maddison 2010) for a combined large scale taxa phylogenetic analysis showing subphyla and orders of *Sordariomycetes*. The matrix was subdivided into nine partitions (three for ribosomal genes and six for each codon position in two protein-coding genes). 2) For the second, low-taxon level phylogeny, we combined ITS regions including 5.8S rDNA and 28S rDNA on another taxa set for a detailed resolution of *Plectosphaerellaceae* family members within *Sordariomycetes* clade. Both analyses are deposited in TreeBase (submission ID 12948).

Nucleotide substitution models for partitions were tested in the jModelTest v. 0.1.1 (Rannala & Yang 1996, Posada & Buckley 2004, Posada 2008) software package. The Akaike Information Criterion (AIC) implemented in jModelTest was used to select for best fit models after likelihood score calculations were done. Evolutionary models listed in Table 3 were further used for ML and BI analyses.

Maximum likelihood (ML) and Bayesian Inference (BI) analyses were used for phylogeny estimation. ML evaluation was conducted in GARLI v. 2.0 (Zwickl 2006) with random starting trees through partitions. The number of search runs was set to 5 for a large scale taxa matrix and 10 for low-taxon level taxon matrix. Bootstrap analyses were replicated 200 and 100 times, respectively. A 50 % majority-rule consensus tree was generated in the SumTrees v. 3.3.1 application in the DenroPy v. 3.11.0 package (Sukumaran & Holder 2010) running under Python 2.6 platform. Bayesian (BI) analysis was performed as implemented in MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001). The first partition in the large scale taxon matrix was fitted into GTR+I+G, since that was the parameter closest to best-fit TIM+I+G, which is not included in MrBayes. Metropolis-coupled Markov chain Monte Carlo (MCMCMC) searches were run for 5M generations sampling every 100th tree. Two independent analyses with four chains each (one cold and three heated) were run until stationary distribution was achieved. Convergence of the run logs was analysed in TRACER v. 1.5 (http://beast.bio.ed.ac.uk/Tracer). The first 15 000 (30 %) 'burn-in' trees were excluded from further analysis. The rest was summarized to produce a 50 % majority-rule consensus tree with recovery of posterior probabilities (PP).

RESULTS

Isolation of strains and genetic identity

Eighteen strains were isolated from soda-soil samples collected at different sites in Russia, Mongolia and Tanzania. They have been deposited in the CBS collection (Utrecht, The Netherlands) (Table 1). All of them readily produce abundant ascomata and show intense conidiation on an alkaline agar medium of pH 10

and all share the same morphological characteristics. In addition, all strains have nearly identical sequences for the five loci studied: SSU, LSU, RPB2, TEF1-alpha and 5.8S rDNA. Even the ITS regions and third codon positions of protein-coding genes (RPB2 and TEF1-alpha), known to have relatively high rate of mutation accumulation, have minor or no nucleotide differences. We found nucleotide substitution mutations in 18 sites across a 6189-base alignment derived from the studied loci. Most of the substitutions were at 3rd codon positions in the RPB2 gene.

Phylogenetic analyses

In the first analysis, the dataset for the five-gene phylogeny consisted of 70 taxa within the *Sordariomycetes*. The data matrix comprised 5 013 characters, of which 1 659 (33 %) were phylogenetically informative, 2 891 invariable and 465 noninformatively variable. The negative log likelihoods (-Ln) for ML BP and BI were 54607.294 and 55042.994. MCMCMC runs converged and had a deviation of around 0.008 at the end of the run. Maximum likelihood bootstrap and posterior probabilities (PP) are provided on the corresponding internodes on the 50 % majority rule Bayesian tree shown (Fig. 1). In both our phylogenetic analyses, clades supported with ML BP/BI PP exceeding 90/0.95 were considered very strong and are displayed as thickened braches. Our five-locus phylogeny provides firm topological support for three major monophyletic subphylum-level clades of *Sordariomycetes* outlined previously (Eriksson 2006, Zhang et al. 2006), namely, *Xylariomycetidae* (100/1.0), *Sordariomycetidae* (79/1.0) and *Hypocreomycetidae* (97/1.0). The marine fungi order *Lulworthiales* displayed a strong supported clade (100/1.0) with no clear assessment to any of the other subphyla within the *Sordariomycetes*. The taxonomic position of the *Lulworthiales* has been a matter of discussion since the description of the order, and it currently has *incertae sedis* status (Kohlmeyer et al. 2000, Hibbett et al. 2007). The monophyletic order *Hypocreales* within the *Hypocreomycetidae* forms a well-supported clade (92/1.0) in which the genus *Heleococcum* is located. We used a representative isolate of the type species of *Heleococcum*, *H. aurantiacum* (CBS 201.35) as well as an isolate of *H. japonense* (CBS 397.67) as references to compare with the taxonomic position of *H. alkalinum*. *Heleococcum aurantiacum* and *H. japonense* stand close together as members of the family *Bionectriaceae* within the *Hypocreales* as stated previously (Rossman et al. 2001). Surprisingly, however, all *H. alkalinum* strains clustered as a distinct clade within the highly supported (100/1.0) broader monophyletic clade comprising the family *Plectosphaerellaceae*. A novel genus name was therefore needed. We propose *Sodiomyces alkalinus* gen. & comb. nov. as the new name for this fungus. This name will be used in the remainder of this paper.

Our second, two-locus (ITS including 5.8S rDNA and 28S rDNA) phylogenetic analysis was done to further resolve the *Plectosphaerellaceae* clade and place *Sodiomyces alkalinus* strains among the other members of the family (Fig. 2). The data
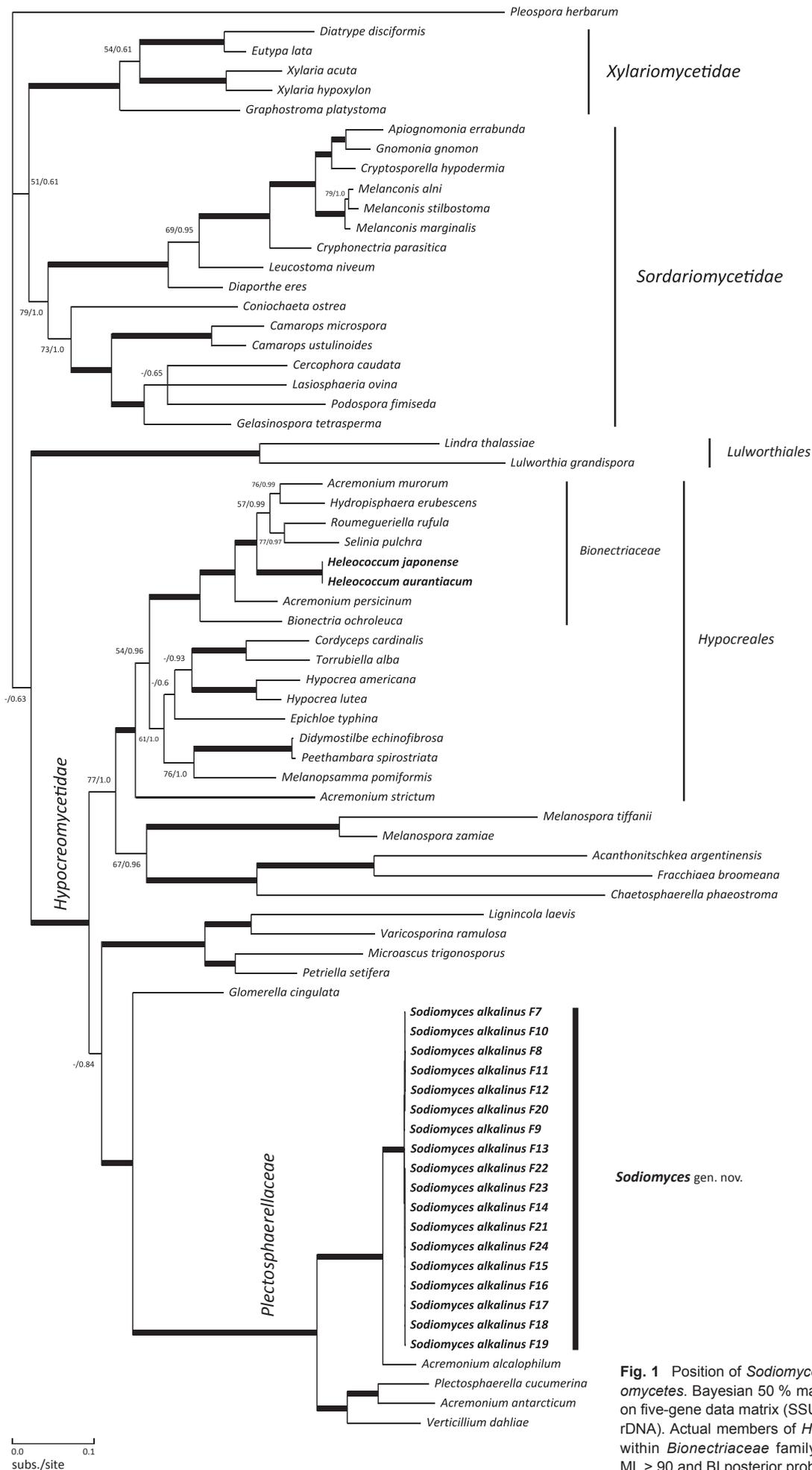
Fig. 1 Position of *Sodiomyces alkalinus* clade within *Sordariomycetes*. Bayesian 50 % majority-rule consensus tree based on five-gene data matrix (SSU+LSU+RPB2+TEF1-alpha+5.8S rDNA). Actual members of *Heleococcum* genus marked **bold** within *Bionectriaceae* family. Thickened branches indicate ML > 90 and BI posterior probability (PP) > 0.95.
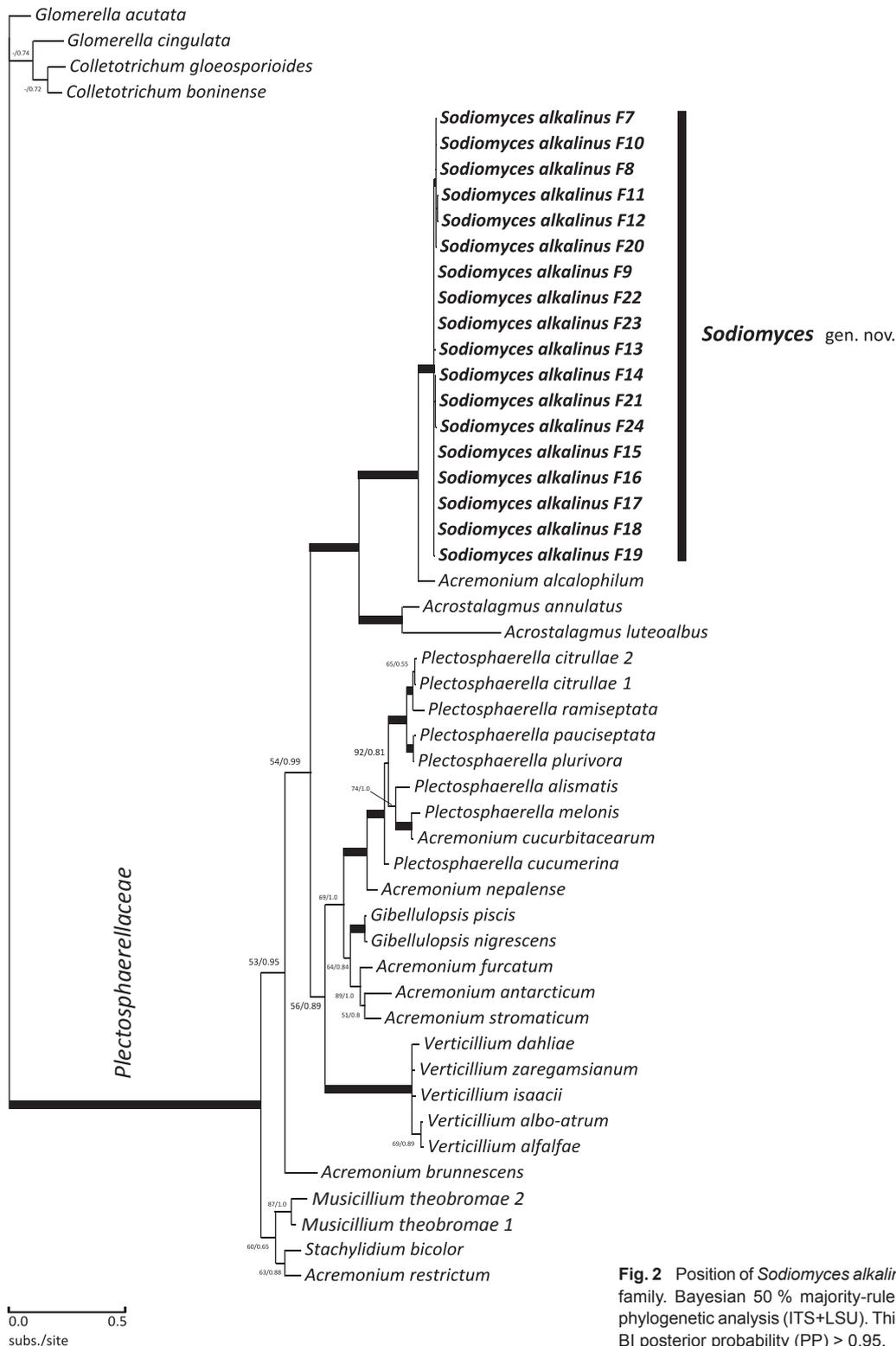
Fig. 2   Position of *Sodiomyces alkalinus* strains within *Plectosphaerellaceae* family. Bayesian 50 % majority-rule consensus tree based on two-locus phylogenetic analysis (ITS+LSU). Thickened branches indicate ML > 90 and BI posterior probability (PP) > 0.95.

matrix consisted of 50 taxa and 1 315 characters: 289 (22 %) phylogenetically informative, 957 invariable and 69 variable non-informative. Four members of the sister family *Glomerellaceae* were used as outgroup. ML bootstrap and BI analyses had likelihoods (-Ln) of 5501.97 and 5721.683, respectively. The deviation between the Markov chain runs was around 0.004 at the end. Members of the holomorphic genus *Plectosphaerella* make up a highly supported clade (92/1.0) as does the asexual plant pathogen *Verticillium* s.str. clade (97/1.0). *Verticillium theobromae* appears closer to the root of the tree, lending some doubt to the monophyly of *Verticillium*. It has been renamed *Musicillium theobromae* by Zare et al. (2007). The two species of the genus *Acrostalagmus* form a long branched clade with

strong 99/1.0 support values. Anamorphic *Acremonium* species known to be highly polyphyletic, occupy various separate branches in our phylogenetic tree. The overall topology is highly consistent with that seen in previous phylogenetic studies of the *Plectosphaerellaceae* (Zare et al. 2007, Weisenborn et al. 2010, Reblova et al. 2011, Carlucci et al. 2012). Here again, our *Sodiomyces alkalinus* strains group together and show a highly supported (100/1.0) clade close to *A. alcalophilum*.

Growth patterns on different substrates

Sodiomyces alkalinus is an alkaliphilic fungus clearly showing a characteristic growth pattern at different pH levels on MEA-based media (Fig. 3). The maximum growth rate of ex-type
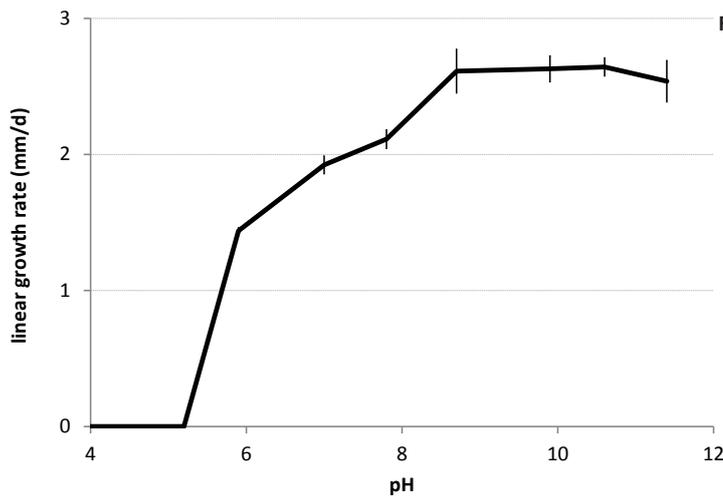
Fig. 3 Growth pattern of *Sodiomyces alkalinus* (CBS 110278) at different pH.

strain F11, 2.6 mm/d, was recorded at pH from 8.7 to 10.5, with only a small reduction in growth rate seen at pH 11.4. On the other side of the pH scale, the fungus was still able to grow at pH 6 with an almost halved growth rate (1.4 mm/d). At pH 5.2 and lower, no growth was observed.

The growth rates of strain F11 on 30 carbon sources at pH 7 and 10 varied (Fig. 4, 5). The graphs display, from left to right, carbon sources ranging from simple to complex. Control plates with no carbon source still showed faint growth, presumably, due to the traces of nutrients in the agar. As can be seen in Fig. 5, simple sugars do not provide sufficient nutrients for good growth, resulting in poorly formed colonies with thin sterile hyphae. Some substrates such as D(+)galactose inhibited growth significantly and resulted in an irregular colony shape. More complex substrates like rhamnose, maltose, cellobiose, raffinose, arabinogalactan, beechwood xylan, and oat-spelt xylan provided the nutrients for a richer morphology and facilitated initiation of conidiation. All complex media made with raw plant materials as carbon sources yielded rich colony morphology with developed aerial mycelium, abundant conidia and formation of ascomata. Apple pectin did not promote rich colony formation, but rather, initiated the formation of ascomata towards the end of the incubation period. The fungus did not produce discernible colonies on pure cellulose or chitin. In all cases, growth on media at pH 10 was better than at pH 7, again showing the adaptation of *Sodiomyces alkalinus* to alkaline environments. The species could not initiate growth on lignin

and sugar beet pulp-based media at pH 7, but showed good growth at pH 10 on those substrates. On lignin at pH 10, the fungus showed morphology similar to that seen on polysaccharides, developing only asexual sporulation.

Taxonomy

Sodiomyces A.A. Grum-Grzhim., A.J.M. Debets & Bilanenko, *gen. nov.* — MycoBank MB801368

Etymology. From the English *soda* and Latin *mycetes*, referring to the ability of filamentous fungus grow at high ambient pH and salts.

Type species. *Sodiomyces alkalinus* (Bilanenko & M. Ivanova) A.A. Grum-Grzhim., A.J.M. Debets & Bilanenko.

A genus of the family *Plectosphaerellaceae* in *Ascomycota*.

Asexual morph. Acremonium-like.

Sexual morph. *Cleistothecia* dark-brown, 120–150 µm diam, peridium multi-layered, pseudoparenchymatous, with folded surface, exoperidium composed of 3–5 layers of angular cells. *Paraphyses* absent. *Asci* thin-walled, without apical apparatus, saccate, unitunicate, scattered irregularly in the ascocarp, embedded in a gelatinous matrix. *Ascospores* released by dissolution of the ascus wall before maturity, accumulating within the ascocarp, released in a slimy mass, liberated by pressure within the ascocarp. *Ascospores* ellipsoidal or ovoid, 12–15 × 5–7 µm, medially 1-septate, not constricted at the septum, thick-walled, pale brown, smooth.
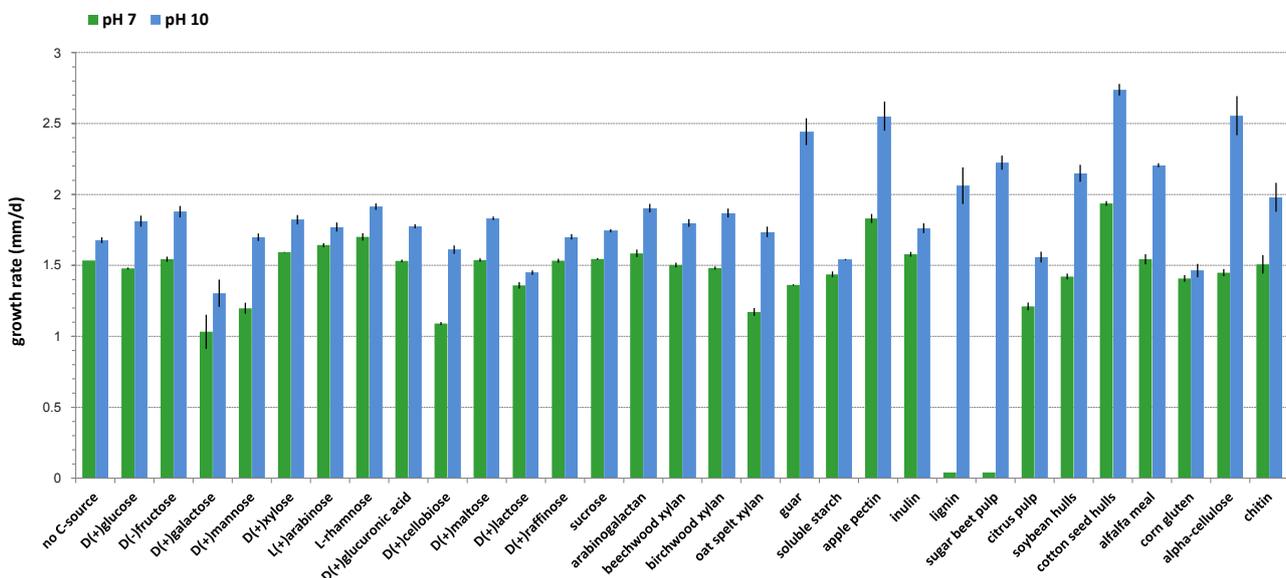

**Fig. 4** Growth rate on different carbon sources of *Sodiomyces alkalinus* (CBS 110278).
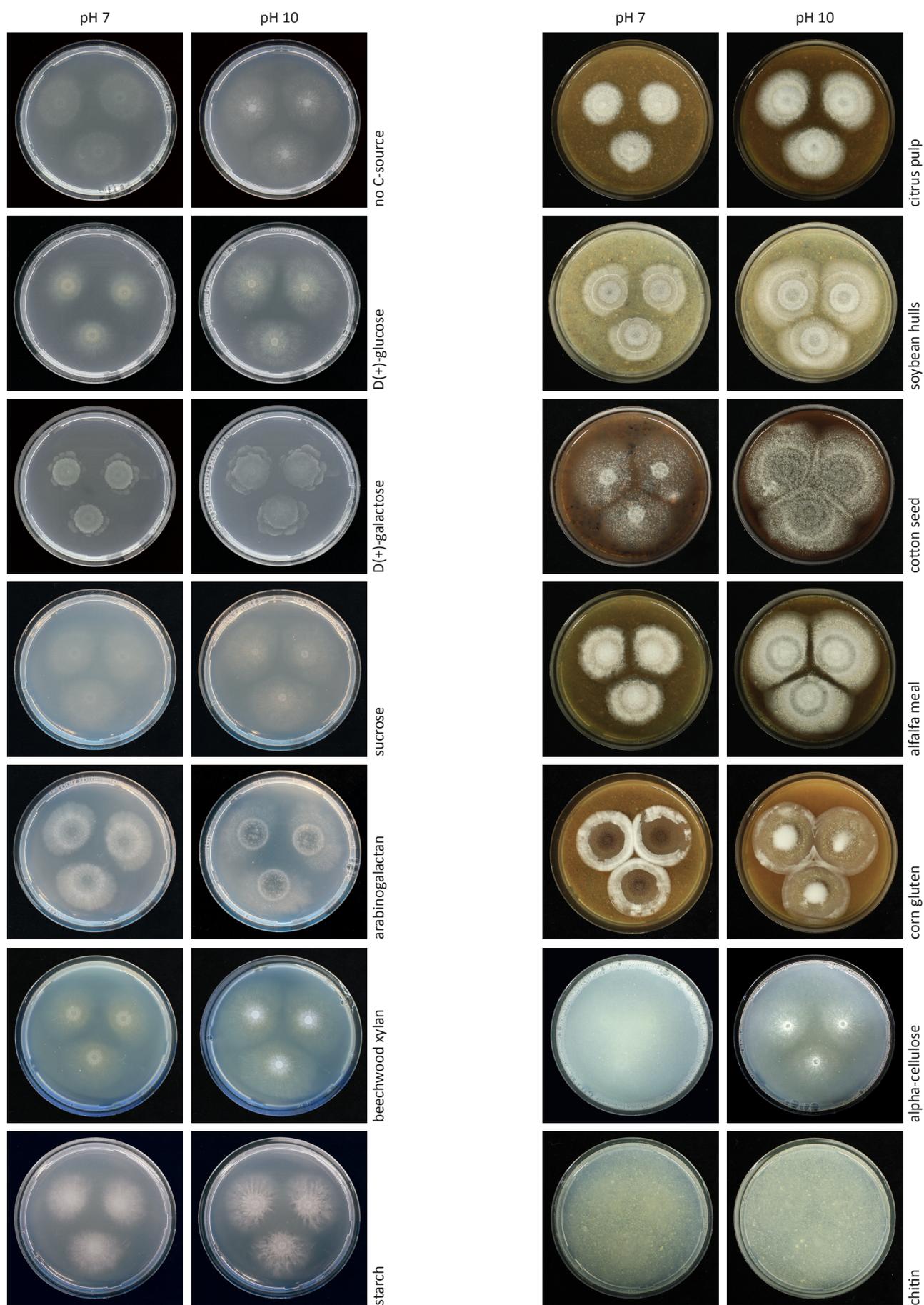
Fig. 5 Plates with *Sodiomyces alkalinus* (CBS 110278) 12 d old colonies on different carbon sources at pH 7 and 10.
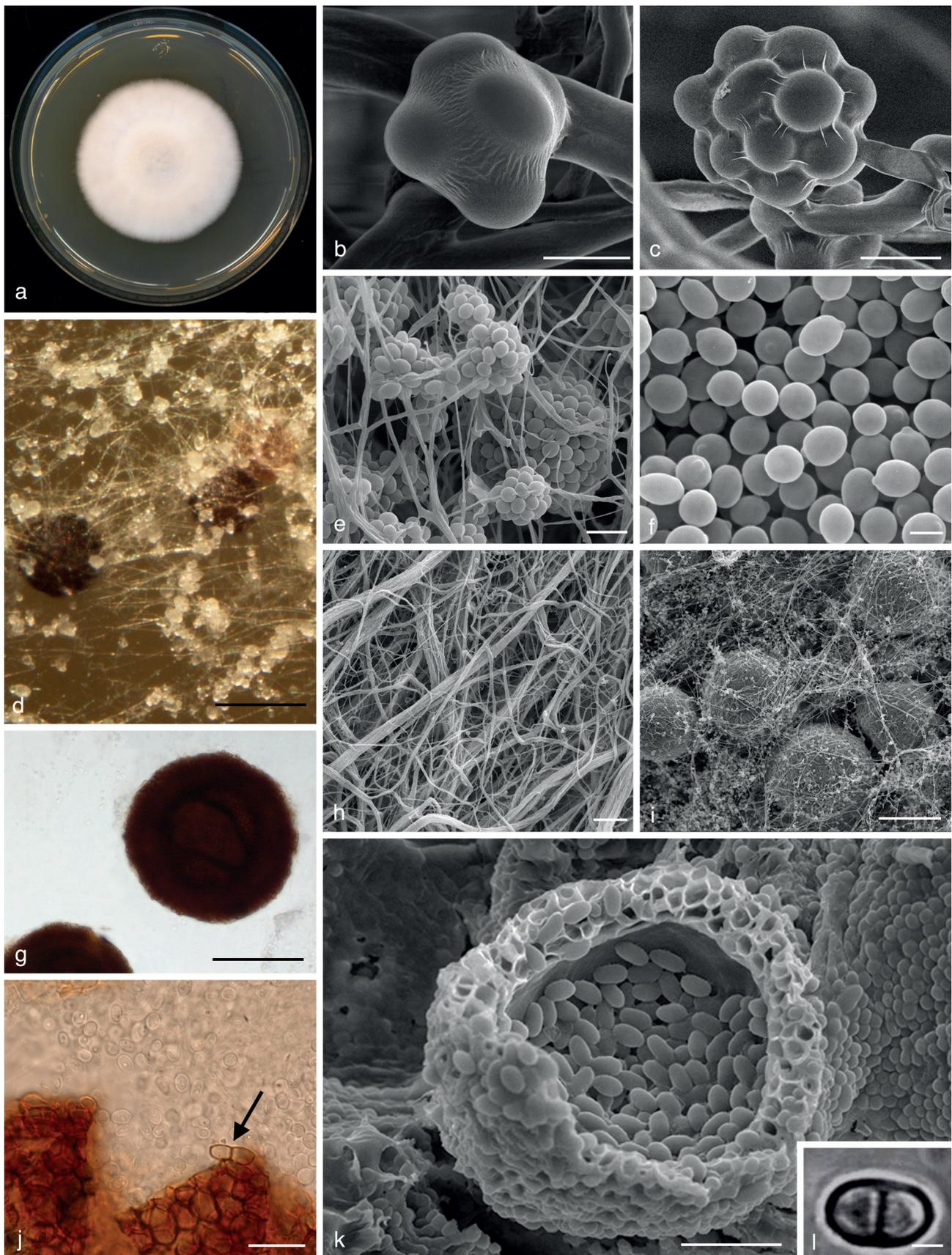
Fig. 6 *Sodiomyces alkalinus* (CBS 110278). a. Ten-day-old colony on alkaline agar (9 cm Petri dish); b. young conidial head on monophialide (cryoSEM); c. matured conidial head on (branched) monophialide (cryoSEM); d. colony overview (LM); e. acremonium-like conidial heads (SEM); f. conidia (SEM); g. cleistothecia; h. hyphal cords (SEM); i. colony overview on alkaline agar (SEM); j. open cleistothecium, surrounded by conidia, at the arrowhead a two-celled ascospore; k. multilayered exoperidium of ascoma (SEM); l. two-celled ascospore (LM). — Scale bars: b = 4 µm; c = 5 µm; d, i = 100 µm; e = 10 µm; f, l = 3 µm; g = 50 µm; h, k = 30 µm; j = 15 µm

Sodiomyces alkalinus (Bilanenko & M. Ivanova) A.A. Grum-Grzhim., A.J.M. Debets & Bilanenko, *comb. nov.* — MycoBank MB801369; Fig. 6

Basionym. *Heleococcum alkalinum* Bilanenko & M. Ivanova, Mycotaxon 91: 501. 2005.

Ascomata dark brown, superficial on the substratum, globose, 120–250 µm diam, non-ostiolate, cleistothecial, not changing colour in 3 % KOH and lactic acid. *Peridium* multi-layered, pseudoparenchymatous, with folded surface; exoperidium composed of 3–5 layers of angular cells. *Paraphyses* absent. *Asci* thin-walled, without apical apparatus, saccate, unitunicate, scattered irregularly in the ascocarp, embedded in a gelatinous matrix. *Ascospores* released by dissolution of the ascus wall before maturity; ascospores accumulating within the ascocarp, released in a slimy mass, liberated by pressure within the ascocarp, ellipsoidal or ovoid, 12–15 × 5–7 µm, medially 1-septate, not constricted at the septum, thick-walled, pale brown, smooth. *Asexual morph* in *Acremonium* sect. *Nectrioidea*. *Conidiation* abundant, mostly nematogenous, partially plectonematogenous. *Conidiophores* predominantly basitonously verticillate, rarely with solitary branches. *Phialides* variable, 15–60 µm long, gradually tapering towards the apex, rather thin-walled. *Conidia* aseptate, aggregated in spherical slimy masses, rarely in short columns, lemon-shaped at first, becoming subglobose or ellipsoidal at maturity, 4.5–5.5 × 4.0–4.5 µm, smooth as observed by SEM, hyaline. *Chlamydospores* absent.

Culture characteristics — Colonies on alkaline agar (AA, pH 10–10.2) rather fast-growing, reaching 38–40 mm diam in 10 d at 25 °C. On MEA (pH 6.5) growing more slowly, reaching 5.5 mm diam in 10 d. Young colonies white; later, black concentric zones appearing as a result of formation of abundant ascomata, velvety to woolly. Reverse colourless. Odour pleasant. Exudate absent. Decumbent vegetative hyphae thin-walled, hyaline, 0.5–2.0 µm wide. Mycelium consisting of hyaline, smooth-walled, septate hyphae, 1–3 µm wide, often fasciculate.

Specimen examined. Mongolia, Choibalsan area, the soda soil (pH 10.7) on the edge of Shar-Burdiyn lake, 1999, *D. Sorokin*, culture ex-type F11 = CBS 110278 = VKM F-3762.

Notes — Previously described as *Heleococcum alkalinum* (*Bionectriaceae*, *Hypocreales*). Based on the results of ITS, LSU, SSU, 5.8S rDNA, RPB2, TEF1-alpha analyses, it was, however, shown to be a new genus and species in the *Plectosphaerellaceae*, with maximal support in ML and BI analyses.

DISCUSSION

General knowledge of alkaliphilic filamentous fungi is extremely poor. Here we contribute an assessment of the taxonomic position of all 18 known isolates of *Sodiomyces alkalinus* (formerly *Heleococcum alkalinum*), a new placement for a species of holomorphic filamentous *Ascomycota* from soda soils. All isolates of *Sodiomyces alkalinus* were found, with maximal support, to belong to a new genus within the *Plectosphaerellaceae* (Zare et al. 2007). Although the description of this family includes fungi with perithecial ascomata only, we are obliged to place the cleistothecial *Sodiomyces alkalinus* in this family. More new taxa are needed to clarify the taxonomic situation of the *Plectosphaerellaceae*.

The multi-locus phylogenies placed *Sodiomyces alkalinus* close to another alkaliphilic ascomycete – *Acremonium alcalophilum*, described by Okada et al. in 1993. Despite its genetic proximity, *A. alcalophilum* has significant morphological differences, leading us to decide not to transfer it into *Sodiomyces*. These differences are the following: *Acremonium alcalophilum* shows pleomorphism in conidium ontogeny among phialidic, sympo-

dial, arthric, blastic and retrogressive modes (cladobotryum-, trichothecium- and basipetospora-like), especially on alkaline media, whereas *S. alkalinus* only has phialidic conidiogenesis. The phialoconidia and phialides of *A. alcalophilum* are widely variable in morphology, while *S. alkalinus* has uniform conidial morphology and consistently shaped phialides ranging from single to branched. *Sodiomyces alkalinus* is holomorphic unlike *A. alcalophilum*, which is only known as a mitosporic fungus. The pH growth optimum of *A. alcalophilum* is 9.0–9.2 and in *Sodiomyces* it is 9.5–10.5.

Nagai et al. (1995, 1998) proposed that hypocrealean hyphomycetes show a particular tendency to develop the capacity for alkaliphilic growth. In addition, he showed that some species that we now know belong to the *Plectosphaerellaceae*, including *Stachylidium bicolor*, *Acremonium furcatum*, unidentified *Acremonium* species and some *Verticillium* species, displayed alkaliphilic abilities. *Acremonium alcalophilum* belongs to the same clade. Another study cited a *Plectosporium* species (*Plectosphaerellaceae*), isolated from mantis shrimp in seawater, that grew abundantly at pH 10 (Duc et al. 2009). Our phylogenetic data confirm that the family *Plectosphaerellaceae* constitutes an important reservoir of alkaliphilic filamentous fungi.

All 18 strains of *Sodiomyces alkalinus* display remarkable genetic similarity at the studied loci, even though the sites of isolation lay thousands of kilometres apart. This consistency might reflect the possible evolutionary constraints occurring in harsh natural environments such as soda soils. Several relevant scenarios for the distribution of genetically similar fungi among these sites may be proposed. Firstly, the origin and worldwide dissemination of the organism might be an evolutionarily recent event. Dispersal by airborne conidia or migrant birds could be responsible. A second reason could be strong selection pressure in alkaline habitats leaving little opportunity for inhabitants to develop evolutionary variation. In other words, alkaliphiles could be evolutionary constrained by their adaption to those particular ambient conditions. The fact that non-functional or highly variable regions (ITS1, ITS2 regions and 3rd codon positions) have not accumulated mutations suggests the first scenario is more likely.

Sodiomyces alkalinus shows abundant growth and good morphological development on alkaline agar medium at pH 10; it also grows most rapidly under the same conditions. A growth pattern like the one shown in Fig. 3 is rare among filamentous fungi. Known alkaliphilic filamentous fungi often develop only the anamorphic stage in their life cycles. *Sodiomyces alkalinus* is capable of developing teleomorphic as well as anamorphic states. This ability might be adaptive to harsh alkaline environment and may be linked to particular morphological features. As can be seen in Fig. 6 (b, c), for example, conidial heads are embedded into a mucous matrix. It seems to have been altered somewhat by cryo-SEM preparation and it appears as a membranous sheath rather than as a slimy matrix. Phialidic conidiogenesis offers no mechanism through which an extra membrane can form that envelops the entire conidial head, making the presence of a mucous non-cellular substance the most likely interpretation of the structure seen. A slimy matrix might prevent conidia from suffering excessive evaporation; this would enhance their viability in the dry, saline conditions often encountered in alkaline soil areas. However, some neutrophilic fungi such as *Acremonium macroclavatum* (Watanabe et al. 2001), *Stachybotrys chartarum* and others (Schroers et al. 2005) are known to produce similar mucous substances in conidial heads. In *S. alkalinus*, ascospores also become embedded in a slimy matrix during early lysis of the ascus wall, a process that is complete by the time the fruiting body is mature. In addition, cleistothecia possess several layers of cells (Fig. 6k) in the wall that may provide protection against pH stress.

Our growth experiments on different carbon sources might offer a clue as to the possible ecological role of *Sodiomyces alkalinus* in alkaline soils. On purified sugars only thin faint mycelium was produced even with trace metals present in the media. These results indicate that the fungus is not prototrophic and requires growth supplements for optimal growth. Fungal growth was somewhat better on di- and tri-saccharides than on mono-saccharides, and the former compounds stimulated relatively vigorous mycelial growth as well as the formation of small numbers of conidial heads. The inability to form perceptible colonies on cellulose and chitin at both pH values is unexpected, as these polymers seem to be the abundantly available substrates in soda soils and lakes. Small crustaceans with chitinous shells, such as *Artemia salina*, often are abundant in these habitats (Browne & MacDonald 1982). The intense sporulation and well-developed morphology seen on media containing complex plant materials suggests that *S. alkalinus* is saprobic on decaying plant material in alkaline soil ecosystems. Vegetation in study sites mainly consists of halophyte grasses, *Anabasis salsa*, *Atriplex verrucifera*, *Halocnemum strobilaceum*, *Salicornia europaea*, *Suaeda acuminata*, *S. corniculata*, *S. prostrata* and *S. salsa*.

A further systematic study of fungal biodiversity in alkaline soils would help revealing the ecology and possible evolutionary trends pertinent to the alkaliphilic trait. Elucidating how alkaliphilic fungi respond to external pH and why specifically high ambient pH is needed for optimal growth might help in unravelling the adaptation hallmarks allowing access to alkaline habitats. Such studies will surely add to the general picture of signal transduction pathway mediated by ambient pH in fungi.

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