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An unusual sexual stage in the alkalophilic ascomycete *Sodiomyces alkalinus*

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ABSTRACT

Exploring life cycles of fungi is insightful for understanding their basic biology and can highlight their ecology. Here, we dissected the sexual and asexual life cycles of the obligate alkalophilic ascomycete *Sodiomyces alkalinus* that thrives at extremely high pH of soda lakes. *S. alkalinus* develops acremonium-type asexual sporulation, commonly found in ascomycetous fungi. However, the sexual stage was unusual, featuring very early lysis of asci which release young ascospores inside a fruit body long before its maturation. In a young fruit body, a slimy matrix which originates from the combined epiplasm of asci and united cytoplasm of the pseudoparenchymal cells, surrounds pooled maturing ascospores. Upon maturity, the ascospores are forcibly released through a crack in the fruit body, presumably due to an increased turgor pressure. These features of the sexual stage development resemble the ones found in unrelated marine fungi, indicating convergent evolution of the trait. We hypothesise these developmental features of *S. alkalinus* to be adaptive in the conditions of periodically inundated rims of soda lakes where the fungus thrives.

1. Introduction

Life history traits and developmental details of fungi throughout the life cycle can highlight their ecology. The developmental features are of particular interest in fungi which display unique physiology and inhabit narrow ecological niches, as they may provide insights on the adaptation hallmarks to a given environment, as it was addressed in a halophilic fungus *Wallemia* (Kunčič et al., 2010). With this approach in mind, we studied the life cycle and developmental stages of the obligately alkalophilic fungus *S. alkalinus*.

S. alkalinus shows an *in vitro* growth optimum at high pH of 8–10, a very unusual trait in filamentous fungi. This species was isolated from the rims of soda lakes (pH 9.2–11) in Mongolia, Tanzania and Western Siberia, and placed within the *Plectosphaerellaceae* (*Ascomycota*) using DNA-based information (Grum-Grzhimaylo et al., 2013a). Members of the *Plectosphaerellaceae* include mostly asexual genera, such as *Acrostalagmus*, *Chordomyces*, *Gibbellulopsis*, *Lectera*, *Musicillium*, *Verticillium*, and *Brunneomyces*, which develop acremonium/verticillium-like sporulation. A few species of the family (e.g. *Lectera* spp.) are known to develop conidiomata, structures intermediate between sporodochia and acervuli (Cannon et al., 2012; Giraldo et al., 2017; Grum-Grzhimaylo et al., 2016; Hirooka et al., 2014; Palm et al., 1995). Notably, *Plectosphaerella* and *Sodiomyces* are the only genera where a sexual stage was observed. Perithecial fruit bodies of *Plectosphaerella* were classified as "*Sordaria*"-type based on the similar developmental details found in an unrelated saprobic ascomycete *Sordaria* (Uecker, 1993). However, the *Plectosphaerella* species are usually asexual as pathogens or endophytes of vascular plants and brown algae (Bubnova et al., 2014; Carlucci et al., 2012; Su et al., 2017). Interestingly, all three *Sodiomyces* species develop cleistothecial (enclosed) fruit bodies, as opposed to the perithecia that are typical for the closely related *Plectosphaerella* (Grum-Grzhimaylo et al., 2016).

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The difference in fruit body morphology between the related fungi, coupled with the unique physiology of *S. alkalinus*, led us to initiate a detailed study of its life cycle. Using various microscopy and staining methods, we followed the development of the asexual and sexual stages in *S. alkalinus*.

2. Materials and methods

2.1. Strains and media

The studied strains of the soda-lake fungus *S. alkalinus* were F7–F18 (CBS132729, CBS133680, CBS133681, CBS132730, CBS110278^{type}, CBS132731, CBS132732, CBS133682, CBS133683, CBS133684, CBS133685, CBS132733, respectively). The details on the locations, soil samples and dates can be found in Grum-Grzhimaylo et al. (2013a). We used 12 monosporic (obtained from a single uninucleate conidium) cultures derived from each of the above mentioned strains to ensure they were homokaryotic. Routine sub-culturing was performed on alkaline agar medium (AA, pH ca. 10) at 25 °C (Grum-Grzhimaylo et al., 2013a).

2.2. Cytological techniques and sample preparations for microscopy

For DAPI (4′,6-diamidino-2-phenylindole, Sigma) staining, cells were fixed in Carnoy's fluid (Evans, 1959), stained (0,5 μg/ml), and washed with distilled water. Toluidine blue staining was performed according to Brachet (1957). The development of the fruit bodies was followed in time, by observations both *in situ* and on the microscope slides. Older fruit bodies (>4–7 d old) were embedded into Paraplast Plus medium (Sigma) or Epon resin (Fluka). Cross-sections of the material embedded into Paraplast Plus (about 10–30 μm thick) were made by the microtome. After removing the Paraplast, the cross-sections were examined under the microscope. Cross-sections of the material embedded into Epon (Fluka) were 10–30 nm thick and prepared using the LKB 8800 Ultratome III (LKB, Sweden).

2.3. Light microscopy (LM)

Light microscopy was performed using the Carl Zeiss Axioskop 40 FL (Zeiss, Jena, Germany). Images were recorded by the AxioCam MRc (Zeiss, Jena, Germany) and processed with Zeiss AxioVision v.3.1 software. Fluorescent microscopy was performed using the light filter set #01 with maximum excitation at 365 nm, and emission at 397 nm.

2.4. Transmission electron microscopy (TEM)

Colonies grown on AA for 4–10 d were fixed in 3 % glutaraldehyde (Merck) in 0.1 M Na-phosphate buffer (pH 7.2) for 2 h at room temperature. The samples were washed three times in 0.1 M Na-phosphate buffer and treated with 1 % osmium tetroxide in 0.1 M Na-phosphate buffer for 60 min at room temperature. In some cases, cells were fixed with 4 % $KMnO_4$. Mycelium with the fruit bodies was then washed three times with 0.1 M Na-phosphate buffer, dehydrated in ethanol series (30 %, 50 %, 70 %, 96 %), exchanged to acetone, and embedded into the Epon resin (Fluka). 40–60 nm thick cross-sections were cut from blocks using the LKB 8800 Ultratome III (LKB, Sweden) and post-stained with 4 % (w/v) uranyl acetate solution for 60 min, followed by the 15–20 min exposure to 3 % (w/v) Reynolds lead citrate stain (Reynolds, 1963). After staining, the cross-sections were examined with the JEM-100B (Jeol, Japan) transmission electron microscope.

3. Results

3.1. Asexual stage

On the surface of alkaline agar medium (AA), both conidia (uninucleate) and ascospores (two-celled with a single nucleus in each cell) usually started germination by one or several germination vesicles – spherical cells that later transformed into hyphae. However, if incubated in a buffer solution, both types of spores first formed an apical germination tube which quickly elongated, became septate, and later developed into typical hyphae (Fig. 1). Vegetative mycelial cells in *S. alkalinus* were uninucleate, with a spherical, ellipsoid, or filiform nucleus, which often crossed the septum and moved to the adjacent cell (Fig. 2). On AA, 4–7-d-old mycelium started to form simple non-branched conidiophores or single phialides (Fig. 3). Subsequently conidiophores started branching (often multiple times) and finally developed uninucleate enteroblastic phialoconidia, closely resembling the conidiation type observed in the *Acremonium* sect. *Nectrioidea* (Bilanenko et al., 2005).

During the conidium development, we observed a nucleus located in the conidiogenous cell (phialide) migrating towards the cell apex, where it underwent mitosis. Occasionally we found mitoses occurring in the central part of a phialide. The bud of a developing conidium started to form before the mitosis in the phialide was complete. One of the daughter nuclei subsequently migrated towards the phialide apex, into a developing conidium. A similar type of conidiogenesis was documented in other ascomycetes, such as *Aspergillus* and *Sclerotinia* (Fischer and Timberlake, 1995; Schroers, 2001; Willetts and Calonge, 1969). All 12 studied isolates of *S. alkalinus* produced slime, which facilitates the aggregation of vegetative hyphae into mycelial chords and sticks conidia together into heads, similar to that found before in the ex-type isolate F11 (Grum-Grzhimaylo et al., 2013a).

In summary, we observed acremonium-like conidiogenesis in *S. alkalinus*, with somewhat different developmental timing across the isolates, at first represented by single phialides and unbranched conidiophores emerging from single hyphae or mycelial chords. Later they transformed into branched conidiophores with a terminal verticillium-like bundle of several phialides.

3.2. Sexual stage

All 12 homokaryotic strains of *S. alkalinus* that we studied were self-fertile and developed sexual stage, indicating a homothallic mating system. Below, we describe the generalized sexual development of *S. alkalinus* combined from the observations of multiple studied isolates.

3.2.1. Ascomal initials

The *S. alkalinus* fruit bodies originated from the lateral coiled branches of aerial mycelium, called ascogonia, which separated from the parental cell by the basal septum (Fig. 4A). We observed ascogonia with up to three coils, similar to what was found in the related fungus *Plectosphaerella cucumerina* (Uecker, 1993). On the contrary, ascogonia in other cleistothecial fungi, such as *Talaromyces* and perithecial *Chaetomium* often develop multiple coils (Stolk and Samson, 1972; Whiteside, 1961). Apart from spirally-coiled ascogonia, we found some coiled around itself in various planes forming the glomeruli, which were also observed in *P. cucumerina* (Uecker, 1993). Then, ascogonia became septate, and consisted of uninucleate cells, which were sometimes vacuolated (Fig. 4B–E). The development proceeded with the formation of lateral branches on the basal hypha, called investing hyphae, which later gave rise to the peridium (fruit body wall). Occasionally, we observed the ascogonia originated from those lateral

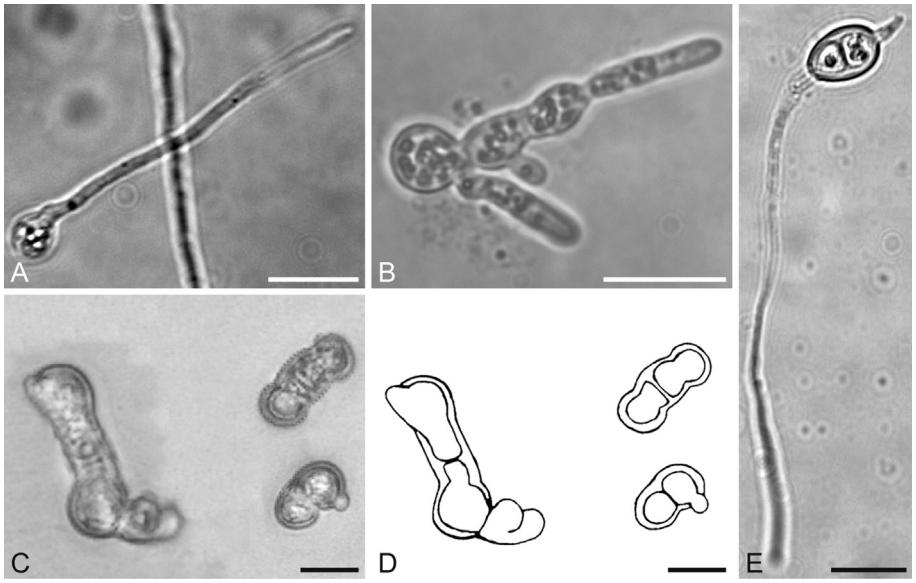

Fig. 1. Spore germination in *S. alkalinus* (LM). (A) Conidium germination in buffer (pH 10.5). (B) Conidium germination on alkaline agar medium. (C, D) Ascospore germination on alkaline agar. (E) Ascospore germination in buffer (pH 10.5). Size bars, 10 μm.

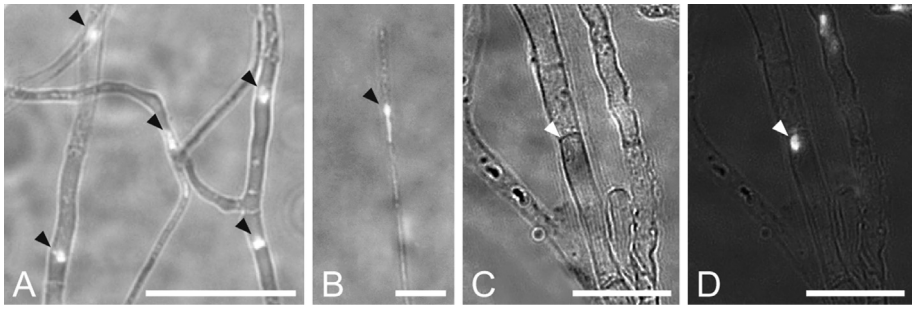

Fig. 2. Nuclei in vegetative structures of *S. alkalinus* (LM + DAPI). (A) Mycelium with uninucleate cells (nuclei are arrowed). (B) Filiform nucleus (arrowed) in a thin hypha. (C, D) Migration of a nucleus (arrowed) through the septal pore. Size bars, A = 10 μm, B–D = 5 μm.

branches, or from the apexes of the hyphae. In the latter case, the investing hyphae developed from the subjacent cells. Branched septate investing hyphae eventually enveloped the ascogonium to form a 2–3 cell-layered peridium of a young fruit body and an ascomal centrum pseudoparenchyma inside of it. Similar development of a young ascoma has been demonstrated in many fungi, e. g. in *Melanospora zamiae* and *P. cucumerina* (Goh and Hanlin, 1998; Uecker, 1993).

After the peridium development initiation, the ascogonium consisted of 5–6 cells, which had an optically- and electron-dense cytoplasm with the affinity to toluidine blue, $KMnO_4$, and OsO_4 (Fig. 5). We found the cytoplasm of the ascogonial cells to be very rich in ribosomes – similarly to other cleistothecial or perithecial species, such as in *P. cucumerina* and members of *Chaetomium*, *Talaromyces* (Kurylowich et al., 1980; Uecker, 1993; Whiteside, 1961).

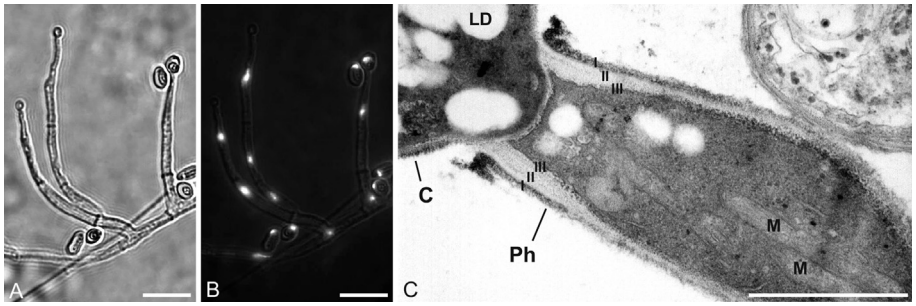

Fig. 3. Nuclei in asexual structures of *S. alkalinus*. (A–B) Nuclei in conidiophores, phialides and conidia (LM + DAPI). (C) Phialide with conidium being formed (TEM). I, III – outer and inner wall layers with an additional wall layer (II) at the collar region. C – conidium, LD – lipid droplets, M – mitochondria, Ph – phialide. Size bars, A, B = 5 μm, C = 1 μm.

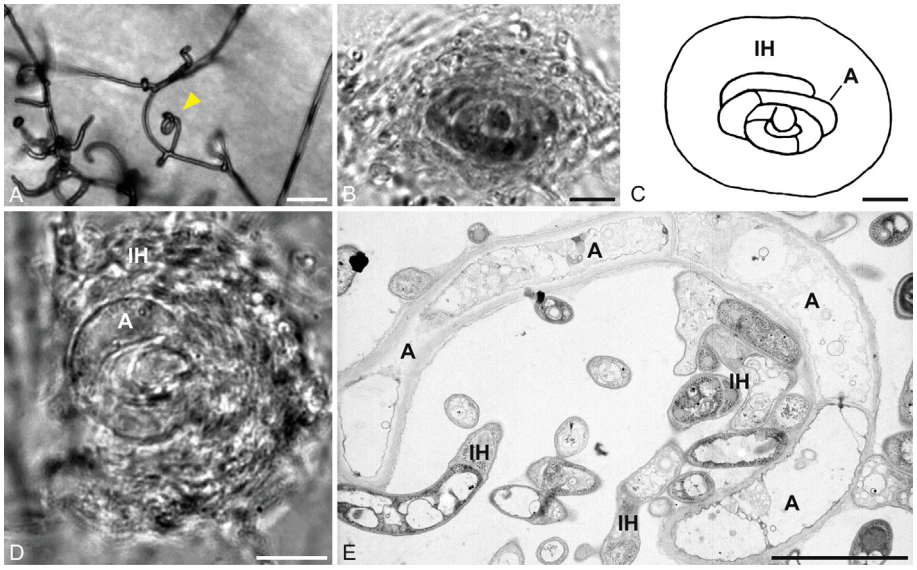

Fig. 4. Fruit body initiation in *S. alkalinus*. (A) Coiled ascogonium (arrowed, LM). (B, C) Septate ascogonium surrounded by investing hyphae (LM). (D, E) Vacuolization of ascogonial cells (LM, TEM). A – ascogonium, IH – investing hyphae. Size bars, A = 10 μm, B–D = 5 μm, E = 2 μm.

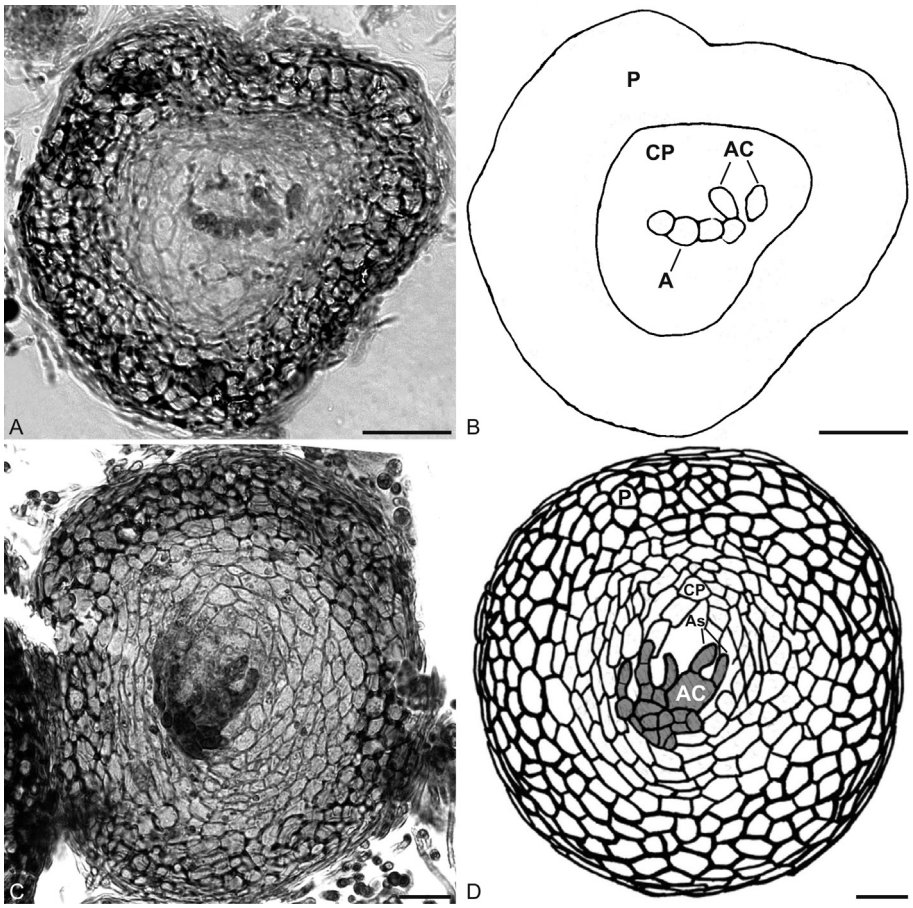

Fig. 5. Fruit body development in *S. alkalinus* (LM). (A, B) Formation of ascogenous cells by branching off from ascogonium. (C, D) Development of asci from large ascogenous cells and initiation of pseudoparenchyma cells lysis in the fruit body centrum. A – ascogonium, AC – ascogenous cells, As – asci, CP – centrum pseudoparenchyma, P – peridium. Size bars, A–D = 10 μm.

3.2.2. Ascomal centrum development

The inner part of the young fruit body comprises the ascomal centrum, which gives rise to the ascogenous cells that produce asci. Similarly to other ascomycetous fungi, the cells of a young ascomal centrum in *S. alkalinus* started to differentiate. A fraction of the ascomal centrum cells became angular and transformed into a supporting tissue, called pseudoparenchyma. Its uninucleate polygonal cells contained large glycogen deposits, while the cells of the young peridium were rich in both glycogen and lipid droplets. The ascogonial cells produced ascogenous cells as the lateral thin-walled branches of the ascogonium, enclosed within the centrum pseudoparenchyma (Fig. 5). Initially these ascogenous cells were uninucleate, but later they grew larger and became binucleate (Figs. 6 and 7A). Interestingly, upon further development of the ascogenous cells, the surrounding pseudoparenchymal cell walls started to degrade. Its cell walls gradually disappeared leading to the pooling of their cytoplasms where the most of nuclei eventually deteriorated. This unified cytoplasm contained endoplasmic reticulum membranes, microbodies, small vacuoles, mitochondria, lipid droplets, and glycogen deposits. The ascogenous cells and the young asci originated from the binucleate ascogenous cells grew into this united cytoplasm. Notably, the inner cell layer of the peridium also contributed to the formation of the united cytoplasm (Fig. 6). The degradation of the cell walls, suggests an activation of lytic enzymes within the common cytoplasm.

3.2.3. Ascus development

As the terminal ascogenous cells grew, their nuclei divided to form a dikaryon. We did not observe croziers, a hook-like structures typically found in many ascomycetes upon the formation of asci (Raju, 1980; Read and Beckett, 1996). Asci started to develop by branching of the dikaryotic ascogenous cells (Fig. 7B). Thus, our results indicate that dikaryotization occurs in the ascogenous cells, similarly to the unrelated species of *Chaetomium* and *Sordaria* (Corlett, 1966; Engh et al., 2010; Whiteside, 1961). In *S. alkalinus*, the young asci were binucleate, however later they became uninucleate, with a large (2.53 ± 0.17 μm x 2.03 ± 0.07 μm) nucleus, roughly twice the size of the haploid nuclei (1.01–1.47 ± 0.1 μm x 0.89–1.18 ± 0.1 μm) found in the ascogenous cells. Evidently, in a young ascus, two homokaryotic nuclei fused to produce a diploid nucleus, and thus formed a meiocyte (Fig. 7B), indicative of homomixis and the homothallic mating system in *S. alkalinus*.

3.2.4. Meiosis in asci

Our results indicate that diploid nucleus in the young ascus subsequently undergoes meiosis as follows. At prophase I, the ascus grew and became subellipsoid, elongate, or broadly clavate, and its single nucleus enlarged becoming diffuse (Fig. 7C). Later, DNA grouped into bivalents, a stage indicative of metaphase I (Fig. 7D). At anaphase I, chromosomes migrated to the ascus poles. Eventually DNA grouped in two nuclei indicative of entering the telophase I stage (Fig. 7E). We observed that during the meiosis I the spindle

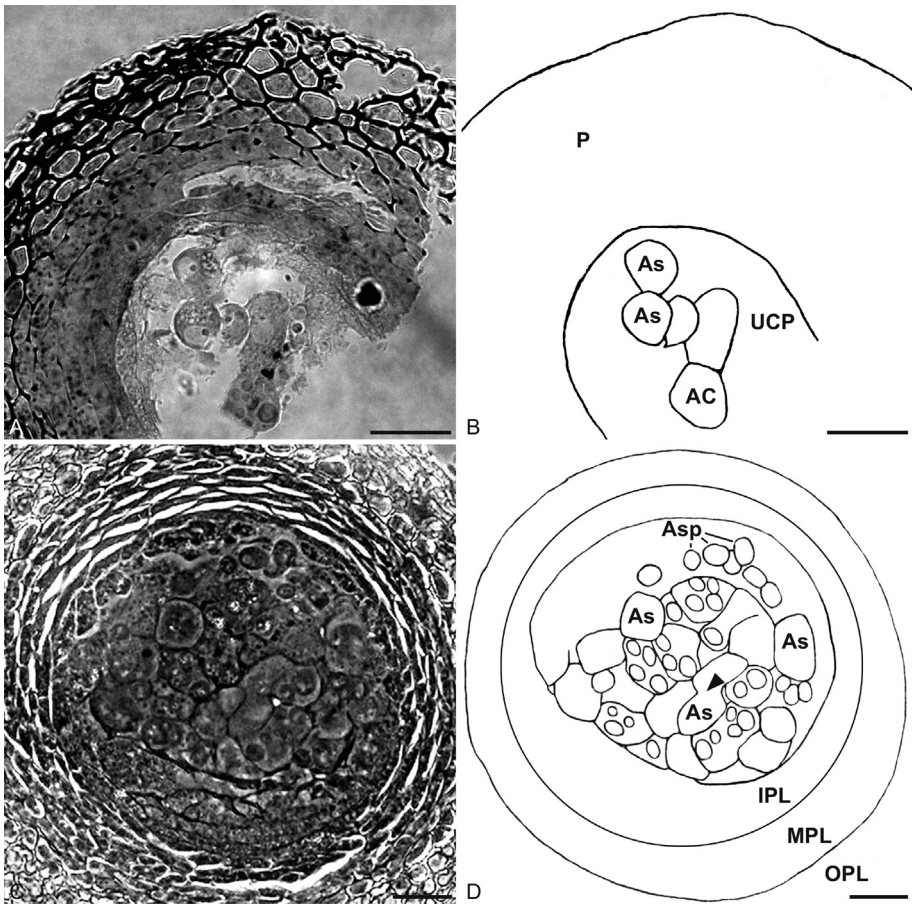

Fig. 6. Fruit body development in *S. alkalinus* (LM). (A, B) Formation of asci from ascogenous cells and unified centrum pseudoparenchyma in the ascoma centrum. (C, D) Ascospores delimitation within the asci and lysis of the asci walls. Some ascospores are in the asci, while others lie free in the combined epiplasm. Note two asci being fused (arrowed). AC – ascogenous cells, As – asci, Asp – ascospores, IPL – inner peridium layer, MPL – middle peridium layer, OPL – outer peridium layer. UCP – unified centrum pseudoparenchyma. Size bars, A–D = 10 μm.

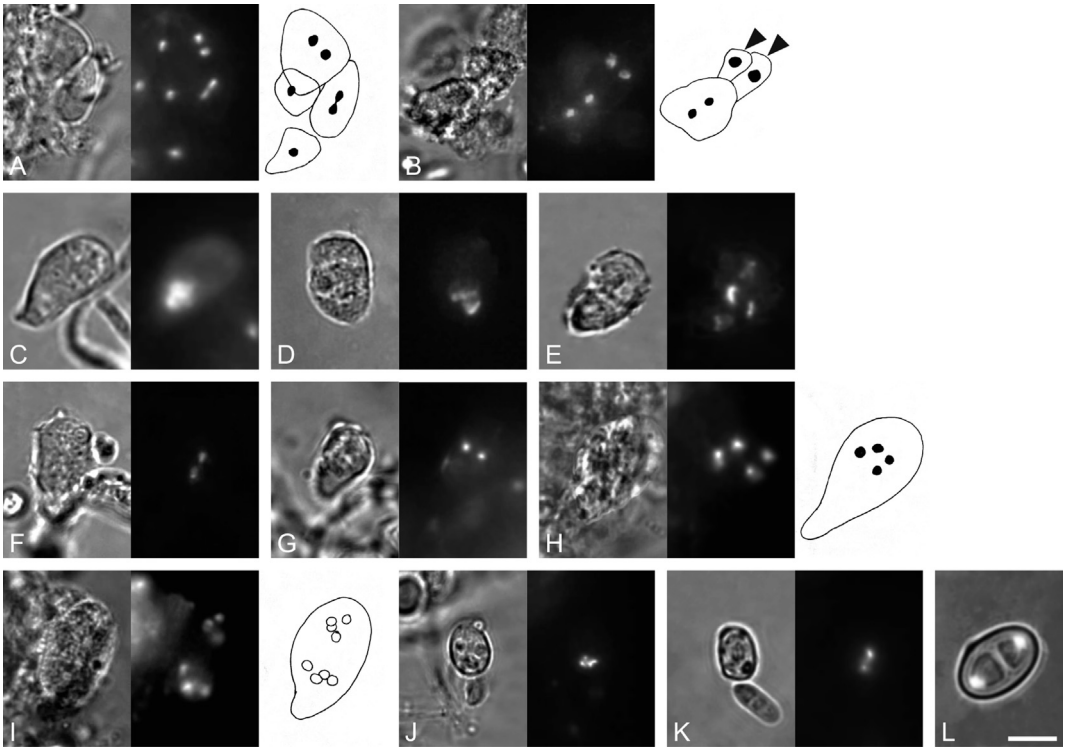

Fig. 7. Nuclear behaviour during asci development in *S. alkalinus* (LM + DAPI). (A) Large ascogenous cells with mitotically dividing nuclei. (B) Large ascogenous cell producing two young asci (arrowed). Each young ascus fused both nuclei to establish a diploid nucleus. (C–E) Meiotic prophase I, metaphase I, telophase I in a young ascus, respectively. (F, G) Pair of nuclei in a young ascus after meiosis I. (H) Telophase II (nuclear tetrad formation), (I) 8-nucleate ascus after postmeiotic mitosis. (J, K) Mitosis in young ascospores: at mitotic prophase (J) and early anaphase (K). (L) Mature two-celled ascospore with a single nucleus in each cell. Size bars, A–L = 5 μm.

orientation was parallel to the longitudinal axis of an ascus. Often after the first meiotic division a large basal vacuole appeared in the ascus. Such vacuoles have also been observed in the basidia of *Agaricus bisporus*, and were proposed to provide an additional positioning control of nuclei along with the cytoskeleton elements (Kamzolkina et al., 2006).

Right after the meiosis I, both newly formed nuclei migrated towards the apex of the ascus and underwent a second meiotic division (meiosis II). Meiosis II progressed asynchronously between the two nuclei and resulted in the formation of a tetrad (Fig. 7F–H). The spindle orientation at meiosis II could vary. Typically during the meiosis II (more rarely at meiosis I) the asci gradually became polygonate, which evidently was a consequence of their dense packing and squeezing within the ascomal centum.

3.2.5. Mitosis in asci

Four post-meiotic nuclei in a young ascus migrated in pairs in the opposite directions – one pair towards the base of the ascus, and the other one towards its apex. Subsequently, each nucleus underwent mitosis that occurred asynchronously between the four nuclei, and resulted in the formation of 8 nuclei (Fig. 7I).

3.2.6. Ascospore development

Shortly after the meiosis and subsequent mitosis, the walls of the ascospores started to form (Fig. 7J–L). Surprisingly, as soon as the young ascospores became delimited, we observed the lysis of the walls of asci (Figs. 8 and 9A, B). Therefore, in *S. alkalinus* the ascus wall lysis occurs long before the ascospores are fully developed. This strongly contrasts to other ascomycetous fungi, in which the walls of asci degrade after the ascospore maturation (Glass et al., 1990; Maharachchikumbura et al., 2016; Raju, 2002). The ascospores of *S. alkalinus* matured in the combined cytoplasm, which was released from asci and pseudoparenchymal cells upon the degradation of their walls, as opposed to other fungi, including closely related *P. cucumerina*, in which ascospores develop in the individual asci located in the cavity within the ascoma (Uecker, 1993). Due to these early lysis events within the *S. alkalinus* fruit bodies, determination of the ascospore number per ascus proved to be challenging. We nonetheless hypothesize that each early ascus harbours 8 ascospores disorderly arranged within the ascus.

Young ascospores remained uninucleate until their outer wall layer started to form. Then the single nucleus underwent mitosis, after which the daughter nuclei migrated to the opposite poles of the ascospore (Fig. 7J, K). At mitotic prophase, the spots of chromosomes were often observed. Such chromosome appearance was also demonstrated in other ascomycetes (El-Ani, 1959, 1971; Rogers, 1965). Mitotic spindle oriented parallel to the longitudinal axis of the ascospore. Walls of mature ascospores were bi-layered with a thicker and relatively electron-dense outer layer, and a thinner, more electron-transparent inner layer. After mitosis, the inner wall of the ascospore formed protrusions which grew towards the centre to build a septum with a central pore (Fig. 9C and D). At this stage, the outer layer of the ascospore became significantly thicker, completing the development of a two-celled ascospore.

3.2.7. Other features

We observed only limited size increase in asci during their development (by 1.5–2 times at maximum), contrary to other fungi, especially those having perithecial multi-spored eutunicate asci, which display a substantial extension during meiosis (Read and Beckett, 1996).

Curiously, when the majority of *S. alkalinus* ascospores matured, the combined epiplasm of the fused asci and the united cytoplasm of the pseudoparenchymal cells formed a slimy matrix inside the

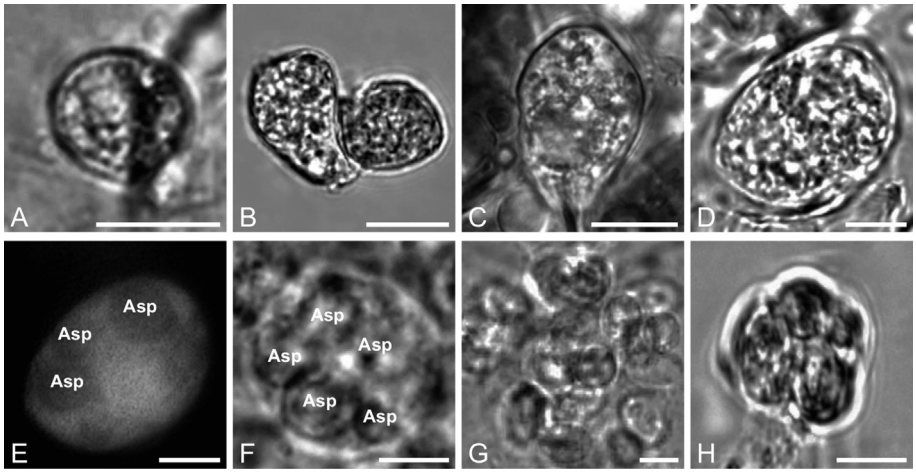

Fig. 8. Ascus development in *S. alkalinus* (LM). (A–D) Young asci. (E) Rhodamine staining of the young ascus to highlight the ascospores being delimited within the young ascus. (F) Ascus with young ascospores. (G) Lysis of an ascus wall followed by the ascospores release. (H) A group of ascospores after the ascus wall lysis. Asp – ascospores. Size bars, 5 μm.

ascoma. Thus, the mature fruit body of *S. alkalinus* contained a mass of ascospores submerged into the slimy matrix (Fig. 10A). Probably an increase of turgor pressure due to the accumulation of simple sugars processed from the more complex slime polysaccharides, results in the ascoma cracking and forcible release of the ascospores in a slime plume (Fig. 10B).

In *S. alkalinus*, we did not observe any subhymenial disk, nor suprahymenial bell, the structures often found in many perithecial and some cleistothecial fungi (Luttrell, 1951; Luttrell and Rogerson, 1959; Malloch, 1981; Uecker, 1993; Uecker and Staley, 1973). A clearly defined hymenium was not detected either, although this structure was found in both perithecial and cleistothecial species, such as *Thielavia*, *Petromyces alliaceus*, *Emericellopsis minima*, *P. cucumerina*, and *Westerdykella ornata* (Benjamin, 1955; Fennel and Warcup, 1959; Malloh and Cain, 1973; Stolk, 1955; Uecker, 1993). However, we observed some sterile paraphyse-like elements in the developing ascomal centrum of *S. alkalinus*. Asci were arranged disorderly within the ascoma of *S. alkalinus*, which is typical among the cleistothecial ascomycetes, although a few exceptions exist (Malloh and Cain, 1973).

The described life cycle of *S. alkalinus* with cytological details is summarized in Figs. 11 and 12.

4. Discussion

Our results demonstrated a homothallic mating system in *S. alkalinus*, as all 12 studied isolates were self-fertile and produced fruit bodies with viable ascospores. A quick screen of publicly available genome of *S. alkalinus* (JGI Mycocosm genome portal; https://genome.jgi.doe.gov/Sodal1/Sodal1; Grigoriev et al., 2014) revealed the presence of both MAT1-1-1 and MAT1-2-1 idiomorphs (JGI protein IDs 349317 and 331470, respectively) required for sexual reproduction in ascomycetes, confirming homothallism in *S. alkalinus* (Wilken et al., 2017). Although this does not exclude the potential for outcrossing in *S. alkalinus*. Homothallic species are known to have a clonal population structure strongly affected by geographical features (Dyakov, 1999), and often found in habitats with extreme environmental conditions. In support, the genetic analysis of fungi isolated from fresh-water, saline, and hyper-saline habitats in and around the Dead Sea revealed that genetic diversity and sex rate declines in hyper-stress environment (Kis-Papo et al., 2003). In line with this notion, our previous phylogenetic analysis of *S. alkalinus* showed highly similar genetic loci even between the strains collected thousands kilometres apart (Grum-Grzhimaylo et al., 2013a). These data indicate a predominant clonal population structure in *S. alkalinus*, due to asexual reproduction and/or a homothallic lifestyle at its natural habitat of soda lakes.

A notable feature of the vegetative and asexual stage of *S. alkalinus* is the production of slime around hyphae and conidia, which aggregates them into chords and spore heads. This slime is supposedly provides a protective barrier against harsh salty and alkaline environment, and probably helps dispersing spores by water in a natural habitat, as these spores are hydrophilic and easily scatter in aqueous solutions (Grum-Grzhimaylo et al., 2013a, 2016). It should be noted, however, that such acremonium-type asexual morphology is not restricted to alkalophiles and is common in fungi within the *Sordariomycetes*, especially in the *Bionectriaceae* (Maharachchikumbura et al., 2016; Summerbell et al., 2011).

Among fungi within the *Plectosphaerellaceae*, the sexual stage has been observed only in two genera – *Plectosphaerella* and *Sodiomyces*. Curiously, despite being closely related, these two genera develop dissimilar sexual stages. Uecker (1993) extensively studied the life cycle of *P. cucumerina*, which develops perithecia containing unitunicate asci with an apical ring-like discharge apparatus. Such type of sexual stage is commonly found in the *Hypocreomycetidae*, where the *Plectosphaerellaceae* is placed (Zhang et al., 2006). However, perithecia of *P. cucumerina* are rarely observed. This fungus is usually found as an asexual inhabitant of the rhizosphere, or as a pathogen or endophyte of plants and brown algae (Bubnova et al., 2014; Carlucci et al., 2012). The current study describes the development of a distinct type of fruit body in *S. alkalinus*, a species that thrives in a drastically different environment than the *Plectosphaerella* species, yet genetically closely related to them. The presence of morphologically dissimilar fruit bodies within the related groups of fungi is not uncommon, and usually found in groups, members of which exhibit wide ecological distribution. For instance, the species of the *Bionectriaceae, Chaetomiaceae, Coniochaetaceae, Lasiosphaeriaceae, Microascales, Niessliaceae* can be found in various habitats, including terrestrial, such as decaying matter or dung, but also in freshwater and marine habitats. Some of them are opportunistic pathogens of animals and immunocompromised humans (Maharachchikumbura et al., 2016).

Fungi which develop cleistothecia are often found in both terrestrial and periodically inundated habitats. For example, the species of *Batista*, commonly found as saprobes on wood, develop cleistothecia with 8-spored asci that retain its walls until the

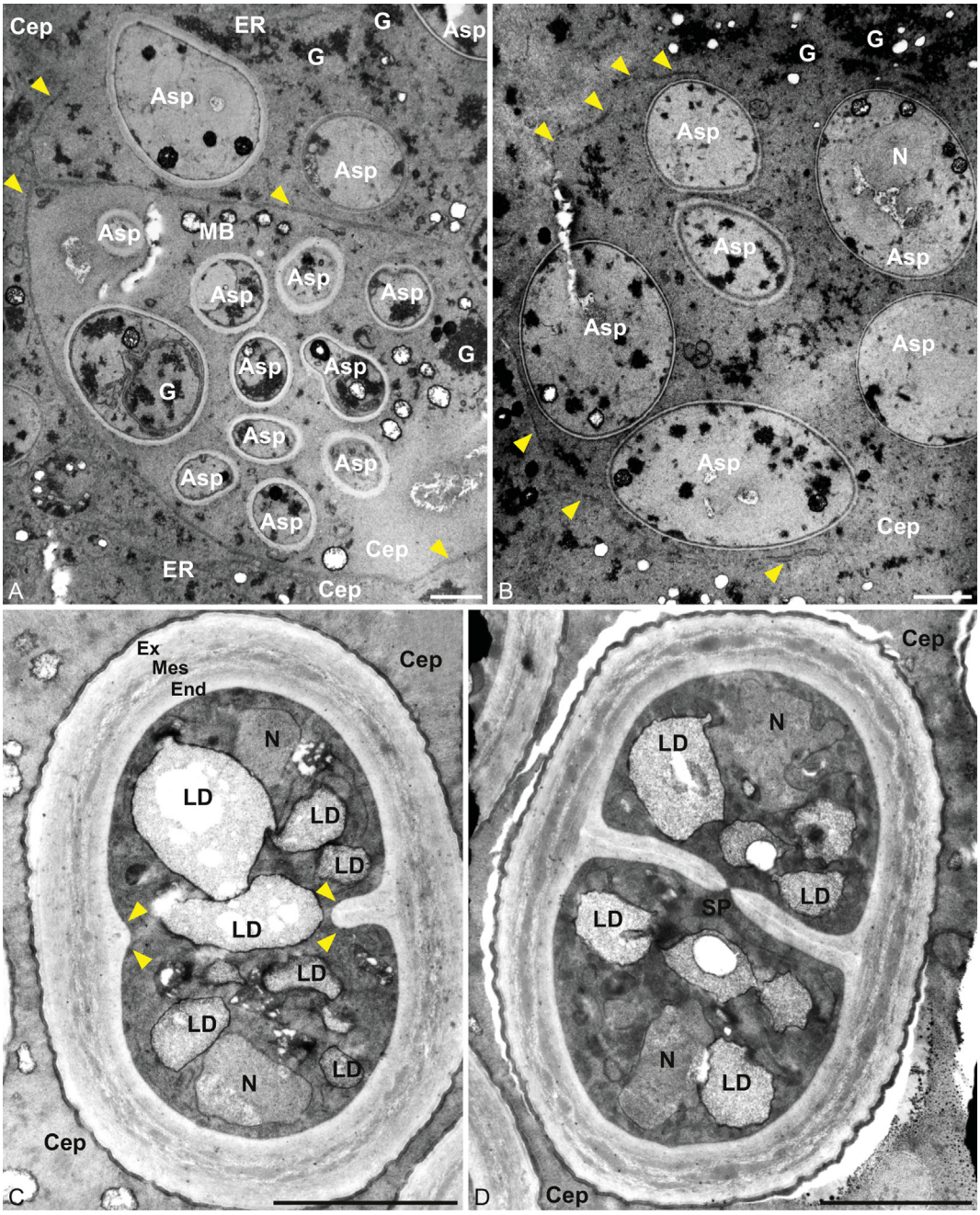

Fig. 9. Asci maturation and ascospores development in *S. alkalinus* (TEM). (A) Initial stage of ascus wall lysis (arrowed). (B) Almost complete lysis of ascus wall (arrowed). (C) Young ascospore. Note the septum formation initiation (arrowed). (D) Mature two-celled ascospore with a fully developed septum. Asp – ascospores, Cep – combined epiplasm, ER – endoplasmic reticulum, End – endosporium, Ex – exosporium, G – glycogen, LD – lipid droplets, MB – microbodies, Mes – mesosporium, N – nucleus. SP – septal pore. Size bars, A–B = 1 μm, C–D = 2 μm.

complete ascospore maturation (Maharachchikumbura et al., 2016). The species of *Emericellopsis* and *Heleococcum*, which are often isolated from wetlands, periodically inundated soils, and marine environments, have similar sexual stage (Rossman et al., 1999). Perithecial ascomata, however, are usually found in fungi from marine environments. For example, the *Halosphaeriaceae* is a large group of marine-bourne fungi that form typical necked perithecia fitted with sterile elements, such as pseudoparenchymatous polygonal cells developing into periphyses and evanescent catenophyses, which may completely disappear in the mature perithecium (Spatafora et al., 1998). Walls of asci in marine fungi are often thin and evanescent, releasing its ascospores into the cavity of a fruit body before their maturation (Kohlmeyer and Kohlmeyer, 1979). The released ascospores are gradually pushed up by the young asci that are growing underneath. In this study, we demonstrated the unique development of asci in *S. alkalinus*, which is distinct from the one found in closely related terrestrial fungi, and have similarities with the unrelated marine fungi. This indicates that soda lakes and marine environments may impose similar selective pressures resulting in the convergent evolution of the traits governing sexual development of these fungi. In *S. alkalinus*, we showed that despite the formation of cleistothecial fruit body, the walls of its asci, pseudoparenchymal and peridium cells degrade long before the ascospores maturation. The

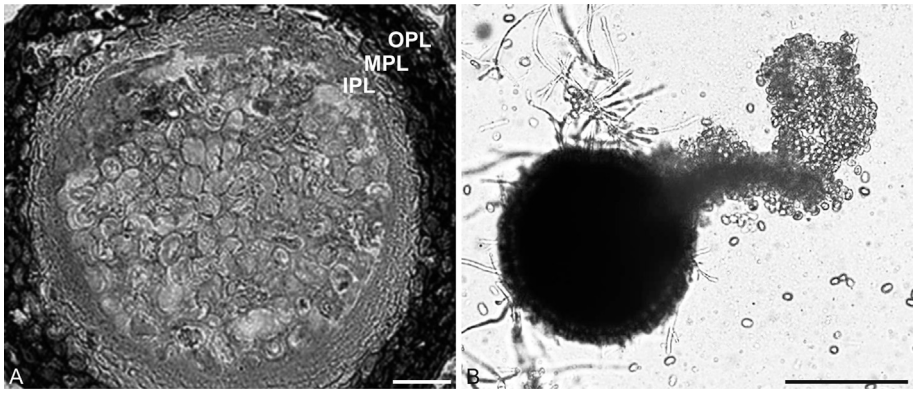

Fig. 10. Mature ascoma (cleistothecium) of *S. alkalinus* (LM). (A) Mass of ascospores enclosed within the ascoma. (B) Opening of the ascoma by a crack liberating ascospores embedded in slime. IPL – inner peridium layer, MPL – middle peridium layer, OPL – outer peridium layer. Size bars, A = 10 μm, B = 100 μm.

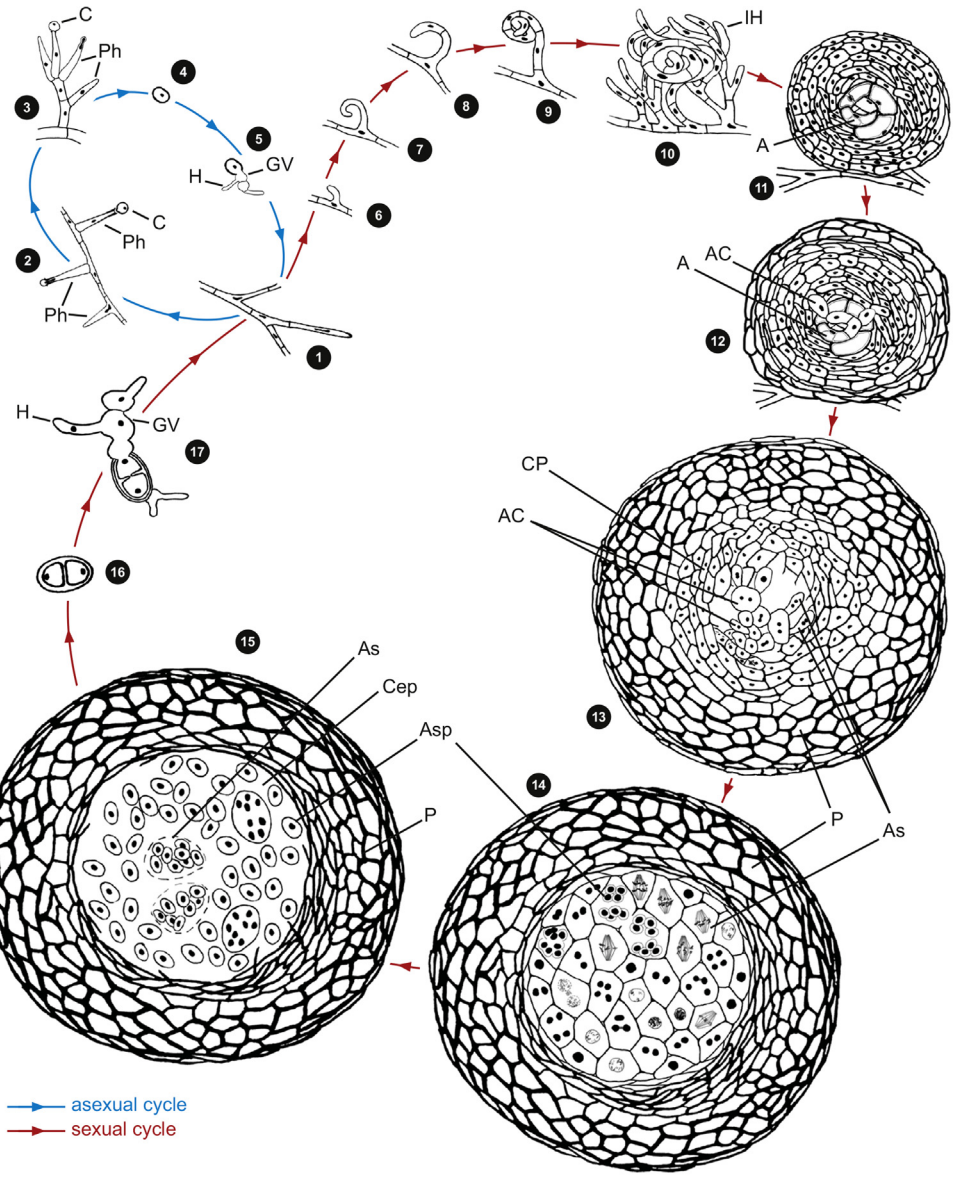

Fig. 11. Summarized life cycle of *S. alkalinus*. 1–5 – asexual propagation: 1 – vegetative mycelium, 2 – simple conidiophores (single philalides), 3 – branched conidiophore with phialides and a developing conidium. 4 – uninucleate conidium. 5 – conidium germination by vesicles and hyphae. 6–17 – sexual propagation: 6–9 – ascogonium development. 10–11 – investing hyphae covering ascogonium. 12 – young ascoma forming ascogenous cells, which originate by branching from ascogonium. 13 – young ascoma with young asci which develop from the ascogenous cells within the centrum pseudoparenchyma. 14 – ascoma with asci at different development stages: from meiosis to ascospore delimitation. 15 – ascoma with young ascospores maturing in combined epiplasm. 16 – mature ascospore. 17 – germinating ascospore. A – ascogonium, AC – ascogenous cells, As – asci, Asp – ascospores, C – conidium, Cep – combined epiplasm, CP – centrum pseudoparenchyma, GV – germination vesicle, H – hyphae, IH – investing hyphae, P – peridium, Ph – phialides.

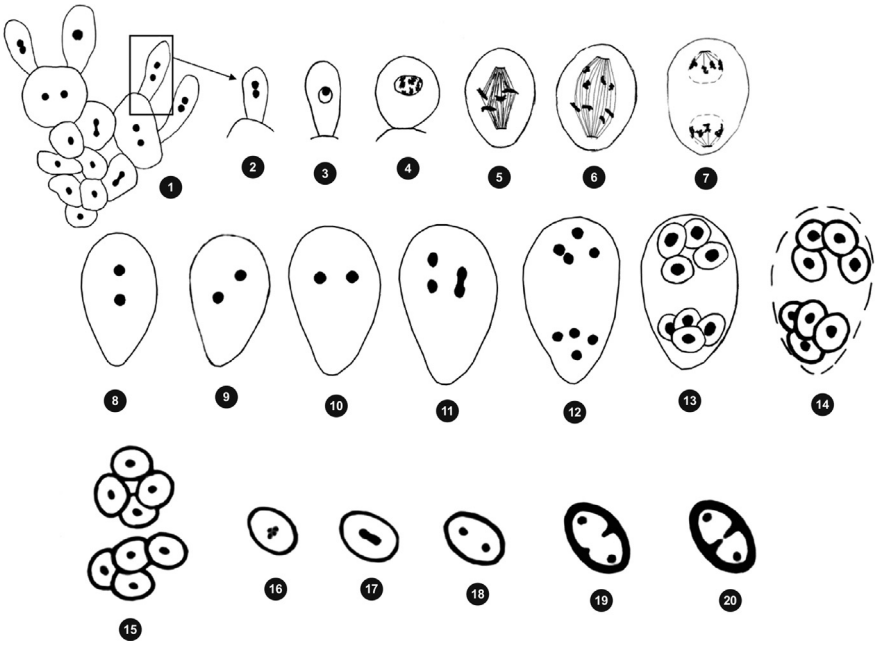

Fig. 12. Asci and ascospores development in *S. alkalinus*. 1 – ascogenous system and a young ascus (boxed). 2 – fusion of haploid nuclei within a young ascus. 3 – ascus with a diploid nucleus. 4 – ascus during meiosis, prophase I. 5 – metaphase I. 6 – anaphase I. 7–10 – telophase I. 11 – anaphase II & telophase II. 12 – postmeiotic mitosis. 13 – ascospores delimitation. 14, 15 – ascus tunic lysis and formation of ascospores walls. 16 – pre-mitotic nucleus in the ascospore. 17, 18 – mitosis. 19, 20 – septum formation.

developing ascospores are thus submerged in the combined cytoplasmic matrix consisting of fused epiplasm of asci, and combined cytoplasm of pseudoparenchymal and inner peridium cells. Active cell wall lysis within the ascomata of *S. alkalinus* supposedly creates a favourable milieu for the ascospores maturation in the extreme environment of soda lakes. We suspect simple (mono-/oligo-) sugars, which are likely present in this cytoplasmic matrix, to be responsible for the protectant functions for the maturing ascospores. Protective role of these sugars against osmotic and pH stresses have been demonstrated before (Iturriaga et al., 2009). In support, a closely-related alkalophilic species *Sodiomyces tronii* accumulated a considerable amount of trehalose upon pH stress (Bondarenko et al., 2017). We are currently addressing this hypothesis by investigating the contents of the combined epiplasm in *S. alkalinus*.

Ascospore appendages found in marine fungi, and fungi inhabiting the periodically inundated territories, presumably are an important dispersal mechanism that keeps the ascospores afloat (Grum-Grzhimaylo et al., 2013b; Koch and Jones, 1989; Kohlmeyer and Kohlmeyer, 1979; Zhang et al., 2006). Interestingly, unlike in marine fungi, the ascospores of *S. alkalinus* lack appendages. We hypothesise that extreme mineralization of soda lake water coupled with seasonal inundation abolishes the need for appendages as means for improving the spores' buoyancy. However, we did find that the release of *S. alkalinus* ascospores occurs forcibly through a crack in the ascoma, which should facilitate the dispersal of ascospores. Similar dispersal mode was shown for the most marine fungi and is achieved likely as a result of an increased turgor pressure (Kohlmeyer and Kohlmeyer, 1979).

In summary, we followed the life cycle of the alkalophilic fungus *S. alkalinus* which inhabits the rims of soda lakes. The fungus develops an acremonium-like asexual sporulation found in many species throughout the *Ascomycota*. We found that sexual stage in *S. alkalinus*, i.e. cleistothecial fruit bodies with early-deteriorating asci walls, is markedly different from what was observed in its terrestrial relatives. The sexual stage of *S. alkalinus* in many ways resembles the ones observed in distantly related marine species, indicating convergent evolution of sexual development in these fungi. Slimy conidial heads and bulk release of ascospores embedded in common slime indicates that water is an important vector for the fungus' dispersal. We treat the described developmental hallmarks as adaptive to the harsh conditions of soda lakes.

Conflict of interest

The authors declare no conflict of interest.

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References

Benjamin, C.R., 1955. Ascocarps of *Aspergillus* and *Penicillium*. Mycologia 47, 669–687.

Bilanenko, E., Sorokin, D., Ivanova, M., Kozlova, M., 2005. *Heleococcum alkalinum*, a new alkali-tolerant ascomycete from saline soda soils. Mycotaxon 91, 497–507.

Bondarenko, S.A., Ianutsevich, E.A., Danilova, O.A., Grum-Grzhimaylo, A.A., Kotlova, E.R., Kamzolkina, O.V., Bilanenko, E.N., Tereshina, V.M., 2017. Membrane lipids and soluble sugars dynamics of the alkaliphilic fungus *Sodiomyces tronii* in response to ambient pH. Extremophiles 21, 743–754.

Brachet, J., 1957. Biochemical Cytology. Academic Press, New York.

Bubnova, E.N., Grum-Grzhimaylo, O.A., Konovalova, O.P., Marfenina, O.E., 2014. Fifty years of mycological studies at the white Sea biological station of Moscow state University: challenges, results, and outlook. Moscow Univ. Biol. Sci. Bull. 69, 23–39.

Cannon, P., Buddie, A., Bridge, P., de Neergaard, E., Lübeck, M., Askar, M., 2012. *Lectera*, a new genus of the *Plectosphaerellaceae* for the legume pathogen *Volutella colletotrichoides*. MycoKeys 3, 23–36.

Carlucci, A., Raimondo, M.L., Santos, J., Phillips, A.J.L., 2012. *Plectosphaerella* species associated with root and collar rots of horticultural crops in southern Italy. Persoonia 28, 34–38.

Corlett, M., 1966. Perithecium development in *Chaetomium trigonosporum*. Can. J. Bot. 44, 155–162.
Dyakov, Y.T., 1999. Sistemi razmnozheniya gribov i ich evolutsia. Mikologia i Fitopatologia 33, 137–149 (In Russian).
El-Ani, A.S., 1959. Chromosome numbers in the *Hypocreales* 1. Nuclear division in the ascus of *Nectria peziza*. Am. J. Bot. 46, 412–417.
El-Ani, A.S., 1971. Chromosome numbers in the *Hypocreales*. II. Ascus development in *Nectria cinnabarina*. Am. J. Bot. 58, 56–60.
Engh, I., Nowrousian, M., Kück, U., 2010. *Sordaria macrospora*, a model organism to study fungal cellular development. Eur. J. Cell Biol. 89, 864–872.
Evans, H.J., 1959. Nuclear behaviour in the cultivated mushroom. Chromosoma 10, 115–135.
Fennell, D.I., Warcup, J.H., 1959. The ascocarps of *Aspergillus alliaceus*. Mycologia 51, 409–415.
Fischer, R., Timberlake, W.E., 1995. *Aspergillus nidulans apsA* (anucleate primary sterigmata) encodes a coiled-coil protein required for nuclear positioning and completion of asexual development. J. Cell Biol. 128, 485–498.
Giraldo, A., Gené, J., Sutton, D.A., Wiederhold, N., Guarro, J., 2017. New acremonium-like species in the *Bionectriaceae* and *Plectosphaerellaceae*. Mycol. Prog. 16, 349–368.
Glass, N.L., Metzenberg, R.L., Raju, N.B., 1990. Homothallic *Sordariaceae* from nature: the absence of strains containing only the *a* mating type sequence. Exp. Mycol. 14, 274–289.
Goh, T.K., Hanlin, R.T., 1998. Ultrastructural observations of ascomal development in *Melanospora zamiae*. Mycologia 90, 655–666.
Grigoriev, I.V., Nikitin, R., Haridas, S., Kuo, A., Ohm, R., Otillar, R., Riley, R., Salamov, A., Zhao, X., Korzeniewski, F., Smirnova, T., Nordberg, H., Dubchak, I., Shabalov, I., 2014. MycoCosm portal: gearing up for 1000 fungal genomes. Nucleic Acids Res. 42 (D1).
Grum-Grzhimaylo, A.A., Debets, A.J.M., van Diepeningen, A.D., Georgieva, M.L., Bilanenko, E.N., 2013a. *Sodiomyces alkalinus*, a new holomorphic alkaliphilic ascomycete within the *Plectosphaerellaceae*. Persoonia 31, 147–158.
Grum-Grzhimaylo, A.A., Georgieva, M.L., Bondarenko, S.A., Debets, A.J.M., Bilanenko, E.N., 2016. On the diversity of fungi from soda soils. Fungal Divers. 76, 27–74.
Grum-Grzhimaylo, A.A., Georgieva, M.L., Debets, A.J.M., Bilanenko, E.N., 2013b. Are alkalitolerant fungi of the *Emericellopsis* lineage (*Bionectriaceae*) of marine origin? IMA Fungus 4, 213–228.
Hirooka, Y., Kawaradani, M., Sato, T., 2014. Description of *Gibellulopsis chrysanthemi* sp. nov. from leaves of garland chrysanthemum. Mycol. Prog. 13, 13–19.
Iturriaga, G., Suárez, R., Nova-Franco, B., 2009. Trehalose metabolism: from osmoprotection to signaling//International journal of molecular sciences. Vol. 10. N 9. P. 3793–3810.Johnson, T.W., 1956. Marine fungi. II. *Ascomycetes* and *Deuteromycetes* from submerged wood. Mycologia 48, 841–851.
Kamzolkina, O.V., Volkova, V.N., Kozlova, M.V., Pancheva, E.V., Dyakov, Y.T., Callac, P., 2006. Karyological evidence for meiosis in the three different types of life cycles existing in *Agaricus bisporus*. Mycologia 98, 763–770.
Kis-Papo, T., Kirzhner, V., Wasser, S.P., Nevo, E., 2003. Evolution of genomic diversity and sex at extreme environments: fungal life under hypersaline Dead Sea stress. Proc. Natl. Acad. Sci USA 100, 14970–14975.
Koch, J., Jones, E.G., 1989. The identity of *Crinigera maritima* and three new genera of marine cleistothecial ascomycetes. Can. J. Bot. 67, 1183–1197.
Kohlmeyer, J., Kohlmeyer, E., 1979. Marine Mycology. The Higher Fungi. Academic Press, New York.
Kunčič, M.K., Kogej, T., Drobne, D., Gunde-Cimerman, N., 2010. Morphological response of the halophilic fungal genus Wallemia to high salinity. Appl. Environ. Microbiol. 76 (1), 329–337.
Kuryłowicz, W., Kurząkowski, W., Woźnicka, W., Połowniak-Pracka, H., Paszkiewicz, A., Luba, J., Piorunowski, J., 1980. Atlas of Ultrastructure of *Penicillium chrysogenum* in Course of Biosynthesis of Penicillin. Chemia Publishing Office, Warsaw.
Luttrell, E.S., 1951. Taxonomy of pyrenomycetes. Univ. Mo. Stud. 24, 1–120.
Luttrell, E.S., Rogerson, C.T., 1959. Homothallism in an undescribed species of *Cochliobolus* and in *Cochliobolus kusanoi*. Mycologia 51, 195–202.
Maharachchikumbura, S.S.N., Hyde, K.D., Jones, E.B.G., Mckenzie, E.H.C., Bhat, J.D., Dayarathne, M.C., Huang, S.-K., Norphanphoun, C., Senanayake, I.C., Perera, R.H., Shang, Q.-J., Xiao, Y., D'Souza, M.J., Hongsanan, S., Jayawardena, R.S., Daranagama, D.A., Konta, S., Goonasekara, I.D., Zhuang, W.-Y., Jeewon, R., Phillips, A.J.L., Abdel-Wahab, M.A., Al-Sadi, A.M., Bahkali, A.H., Boonmee, S., Boonyuen, N., Cheewangkoon, R., Dissanayake, A.J., Kang, J., Li, Q.-R., Liu, J.K., Liu, X.Z., Liu, Z.-Y., Luangsa-Ard, J.J., Pang, K.-L., Phookamsak, R., Promputtha, I., Suetrong, S., Stadler, M., Wen, T., Wijayawardene, N.N., 2016. Families of *Sordariomycetes*. Fungal Divers. 79, 1–317.
Malloch, D., 1981. The plectomycete centrum. In: Ascomycete Systematics. Springer, New York, NY, pp. 73–91.
Malloch, D., Cain, R.F., 1973. The genus *Thielavia*. Mycologia 65, 1055–1077.
Palm, M.E., Gams, W., Nirenberg, H.I., 1995. *Plectosporium*, a new genus for *Fusarium tabacinum*, the anamorph of *Plectosphaerella cucumerina*. Mycologia 87, 397–406.
Raju, N.B., 1980. Meiosis and ascospore genesis in *Neurospora*. Eur. J. Cell Biol. 23, 208–223.
Raju, N.B., 2002. Meiosis and ascospore development in nonlinear asci of *Neurospora pannonica*. Mycologia 94, 99–104.
Read, N.D., Beckett, A., 1996. Ascus and ascospore morphogenesis. Mycol. Res. 100, 1281–1314.
Reynolds, E.S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17, 208–212.
Rogers, J.D., 1965. *Hypoxylon fuscum*. I. Cytology of the ascus. Mycologia 57, 789–803.
Rossman, A.Y., Samuels, G.J., Rogerson, C.T., Lowen, R., 1999. Genera of Bionectriaceae, Hypocreaceae and Nectriaceae (Hypocreales, ascomycetes). Stud. Mycol. 42, 3–238.
Schroers, H.-J., 2001. A monograph of *bionectria* (*Ascomycota, Hypocreales, Bionectriaceae*) and its *Clonostachys* anamorphs. Stud. Mycol. 46, 1–214.
Spatafora, J.W., Volkmann-Kohlmeyer, B., Kohlmeyer, J., 1998. Independent terrestrial origins of the *Halosphaeriales* (marine *Ascomycota*). Am. J. Bot. 85, 1569–1580.
Stolk, A.C., 1955. *Emericellopsis minima* sp. nov. and *Westerdykella ornata* gen. nov., sp. nov. Trans. Br. Mycol. Soc. 38, 419–424.
Stolk, A.C., Samson, R.A., 1972. The genus Talaromyces: studies on Talaromyces and related genera II. Stud. Mycol. 2, 1–65.
Su, L., Deng, H., Niu, Y.C., 2017. Phylogenetic analysis of *Plectosphaerella* species based on multi-locus DNA sequences and description of *P. sinensis* sp. nov. Mycol. Prog. 16, 823–829.
Summerbell, R.C., Gueidan, C., Schroers, H.-J., De Hoog, G.S., Starink, M., Rosete, Y.A., Guarro, J., Scott, J.A., 2011. *Acremonium* phylogenetic overview and revision of *Gliomastix, Sarocladium*, and *Trichothecium*. Stud. Mycol. 68, 139–162.
Uecker, F.A., 1993. Development and cytology of *Plectosphaerella cucumerina*. Mycologia 85, 470–479.
Uecker, F.A., Staley, J.M., 1973. Development of the ascocarp and cytology of *Lophodermella morbida*. Mycologia 65, 1015–1027.
Whiteside, W.C., 1961. Morphological studies in the *Chaetomiaceae*. I. Mycologia 53, 512–523.
Wilken, P.M., Steenkamp, E.T., Wingfield, M.J., De Beer, Z.W., Wingfield, B.D., 2017. Which MAT gene? *Pezizomycotina* (*Ascomycota*) mating-type gene nomenclature reconsidered. Fungal Biol. Rev. 31, 199–211.
Willetts, H.J., Calonge, F.D., 1969. Spore development in the brown rot fungi (*Sclerotinia* spp.). New Phytol. 68, 123–131.
Zhang, N., Castlebury, L.A., Miller, A.N., Huhndorf, S.M., Schoch, C.L., Seifert, K.A., Rossman, A.Y., Rogers, J.D., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Sung, G.-H., 2006. An overview of the systematics of the *Sordariomycetes* based on a four-gene phylogeny. Mycologia 98, 1076–1087.