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Saksenaea vasiformis revisited after 64 years

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ABSTRACT

The genus *Saksenaea* was first discovered from India in 1953 from the forest soils of Sagar (Madhya Pradesh) by Late Professor S.B.Saksena of Dr. Hari Singh Gour University, Sagar (M.P.) and named after his Ph.D. supervisor, Prof. R.K.Saksena of Allahabad University, Allahabad. Incidentally the same fungus was discovered by Farrow around this time in 1954 from Barro Colorado Island from the soils of Panama region. The fungus was once thought to be limited in distribution has now been reported from various places from India and abroad. Because of its unique sporangial characters and non-motile spores in a columellate sporangium it was placed in the order *Mucorales* of the Division *Zygomycota*. Ellis and Hesseltine (1974) created a new family *Saksenaeaceae* for the genus. However, Kirk *et al*. (2008) have placed the genus in the family *Radiomycetaceae*. The taxonomic status of the family has been critically evaluated. In 1976, Azello *et al* published an important paper in which the fungus was found to be serious human pathogen. From 1976 to 2017, more than 50 research papers have been published on the pathogenic nature of the fungus causing severe cutaneous, sub-cutaneous and rhino-orbital-cerebral infections on humans as well as animals. In 2010, Alvarej *et al.* published a paper based on molecular phylogeny and proposed two more species of this genus. The paper closes with a discussion on the origin of *Zygomycetes* and the possible origin of *Zygomycetes* from Chytridiaceous ancestors such as *Nowakowskiella* whose sporangia look very similar to *Saksenaea* and also based on biochemical parameters of *Chytridiomycetes* and *Zygomycetes*.

KEY WORDS : *Saksenaea*, Taxonomy, pathogenic aspects and phylogeny.

INTRODUCTION

Saksenaea vasiformis Saksena was first isolated and described from forest soils, in Sagar, Madhya Pradesh in India by Saksena (1953). Since its first discovery it has been isolated from widespread tropical sources and appears to be world wide in distribution (Ellis and Hesseltine, 1974). Farrow (1954) found it in mixed clay and humus soil of Barro Colorado Island. Goos (1963) reported it from Honduras, Joffe and Borut (1966) from Israel. Hodges (1962) was the first to note its occurrence in the United States and found it in the nursery soil collected in Georgia. The fungus which was earlier supposed to be rare was found not to be so scare. It has been discovered several times at Sagar and its vicinity and also from other places in India such as Allahabad, Varanasi, Jabalpur and South India (Baijal, 1967; Dwivedi, 1961; Pillai and Ahmed, 1993). The fungus is now believed to be worldwide in its distribution (Chien *et al.,* 1992; Vega *et al.,* 2006; Alvarej *et al*., 2010).

TAXONOMY AND MOLECULAR PHYLOGENY

With the publication of very important paper by Ajello *et al* (1976) on the fungus *Saksenaea vasiformis* Saksena as human pathogen made the discoverer (S.B.Saksena) and the fungus (*S. vasiformis*) internationally known. Another landmark was the year 1974 when *Saksenaea* genus was placed in a separate family *Saksenaeaceae* by Ellis and Hesseltine in the year 1974 when two monotypic genera *Saksenaea vasiformis* (Saksena,1953) and *Echinosporangium transversale* Malloch (Malloch, 1967) were placed in a separate family *Saksenaeaceae*. Zygospores have not been observed in either of the two species. Ellis and Hesseltine (1974) admitted an uncertainty regarding a close relationship of *Echinosporangium* and *Saksenaea* and justified the placement of the two genera in a single family "*Saksenaceae*" because neither can possibly be placed in the recognized families of *Mucorales* as they currently exit. Seven reasons were given in support of grouping them together but all of them except one "anomalous sporangia" could apply to many of the *Mucorales*. The columellate sporangia of *S. vasiformis* vs the acolumellate sporangia of *E. transversale* were not stressed as an important feature separating the two taxa; *Echinosporangium* [now renamed as *Lobosporangiun* M. Blackwell & Benny (Benny and Blackwell, 2004)] is currently a member of *Mortierellaceae* (*Mortierellales*) whereas here *Saksenaea* is in the *Saksenaeaceae* (*Mucorales*).

Hesseltine and Ellis (1973) in an article in the Volume IV B "**The Fungi- An Advanced Treatise"** edited by Anisworth *et al.* (1973) Chapter 11 *Mucorales* announced the creation of the family *Saksenaeaceae* with a foot note in the article, the creation of the family *Saksenaeaceae* that this family is to be published in the journal Mycologia Volume 66 in the year 1974. The article published in the journal Mycologia was authored by the two authors in reverse order i.e. Ellis, J.J. and C.W. Hesseltine (1974) entitled; "Two new families of *Mucorales*" wherein the two authors have validly published the two families i.e., *Saksenaeaceae* and *Radiomycetaceae* (containing two genera *Radiomyces* and *Hesseltinella*) with Latin diagnosis.

Thus the family *Saksenaeaceae* Hesseltine and Ellis 1973 [(Sparrow and Sussman (Eds.) The Fungi Volume IVB, p.202 (*nomen nudum*, without a Lain diagnosis, Art.36.1 of the ICBN, McNeill *et al.* 2006)] should not apply to the family *Saksenaeaceae* as that was only a reporting of the new family in the edited volume as the two families were infact actually created when the article was published by Ellis and Hesseltine in the journal Mycologia Volume 66 (1) pp 87-95, 1974 where the Latin diagnosis of the two families *Saksenaeaceae* with the type genus *Saksenaea* and *Echinosporangium* and the family *Radiomycetaceae* with the two genera *Radiomyces* Embree and *Hesseltinella* represented by the type *H. vesiculosa* Upadhyay. Canon and Kirk (2007) in the fungal families of the world, Wallingford, United Kingdom CAB International, 456p have not recognized the family *Saksenaeaceae* and so is the case by Kirk *et al.* (2008) in "**Dictionary of the Fungi**" 10th edition but is considered a

synonym of *Radiomycetaceae* in both references.

Placing the genus *Saksenaea* in the family *Radiomycetaceae* does not seem justified due to the reason that in the family *Radiomycetaceae* there is the production of sporangioles, which does not occur in *Saksenaea*.

A new dimension was given to the taxonomic aspect of *Saksenaea* by the interesting paper of Alvarej *et al*. (2010) entitled, "Molecular Phylogeny and proposal of two new species of the emerging pathogenic fungus *Saksenaea*. They included eleven isolates of *Saksenaea* from different culture collections of the world including ATCC (American Type Culture Collection, Manassas, VA), Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; CNRMA, Centre National de Référence Mycologie Paris, France; FMR, Facultat de Medicina i Ciències de la Salut., Spain; NRRL ARS culture collection(also known as NRRL collections) Peoria, IL; UTHSC, Fungus Testing Laboratory , University of Texas Health Science Center, San Antonio.

This work was done by a polyphasic study based on analysis of the sequences of the Internal Transcribed Spacer (ITS) region, domains D_1 and D_2 of the 28SrRNA gene and the elongation factor 1α (EF-1α) genes as well as by evaluation of the relevant morphological and physiological characteristics of a set of clinical and environmental strains. Alvarej *et al*. (2010) demonstrated that *S. vasiformis* is a complex of species. They proposed two more species of *Saksenaea* i.e. *S. oblongispora*, characterized by oblong sporangiophores and unable to grow at 42°C and *S. erythrospora* characterized by large sporangiophores and sporangia and by the ellipsoid sporangiophores, concave in the lateral view. The characteristic features of three species of *Saksenaea* are given below in a tabular form (**Table**) and in **Fig. 1**.

Table: Characteristic features of three species of *Saksenaea*

Saksenaea vasiformis	*S. oblongispora*	*S. erythrospora*
Optimum temperature for growth was 25°C to 37°C and the minimum temperature was 15°C. The fungus grew at 42°C but did not grow at 50°C.	Optimum temperature for growth was 25°C. The minimum temperature was 15°C. The fungus did not grow at 42°C.	Optimum temperature for growth was 25°C and the minimum temperature was 15°C. The fungus grew at 42°C but did not grow at 50°C.
Sporangiophores generally arising singly 65 - 100 µm long, 6 -10 µm wide.	Sporangiophores generally arising singly, hyaline, unbranched 80 -100 µm long, 6-10 µm wide with profuse dichotomous rhizoidal complex. Sporangiophores mainly oblong.	Sporangiophores erect, generally arising singly at first hyaline soon become light brown, 100 -150 µm long, 7-11 µm wide.
Sporangia produced terminally, flask shaped, asperulate at low magnification, ornamented with many irregular bacilliform protuberances under SEM. Sporangia measuring up to 110 µm long with a long neck (60-90 µm).	Sporangia terminal multispored hyaline, flask shaped asperulate at low magnification, ornamented with many irregular bacilliform protuberances under SEM, measuring 70-110 µm long with along neck (60-90 µm).	Sporangia terminal multispored, flask shaped, asperulate, ornamented with bacilliform protuberances under SEM, measuring 100-220 µm long with a long neck (80-200 µm).
Sporangiospores mainly cylindrical with rounded ends, hyaline 5-7 x 2-3 µm.	Sporangiospores mainly oblong, 5 - 6.5 x 3-4.5 µm in size.	Sporangiospores mainly ellipsoidal biconcave on lateral view, 5-5.5 x 2.5-3 µm in size.

THE PATHOGENIC ASPECT

Coming to the pathogenic aspect of *Saksenaea* since the first report in 1976 by Ajello *et al*. in the journal Mycologia, Ellis and Hesseltine (1974) in their paper entitled "Two new species of *Mucorales*" mention that recently two different isolates were sent to them for identification which came from burn patients. This observation of Ellis and Hesseltine in the year 1974 seems to be the first indication that the fungus *Saksenaea* came from patients with burn wounds. From 1976 to 2016 there are perhaps nearly 75 or more cases of pathogenic infections due to *Saksenaea* species.

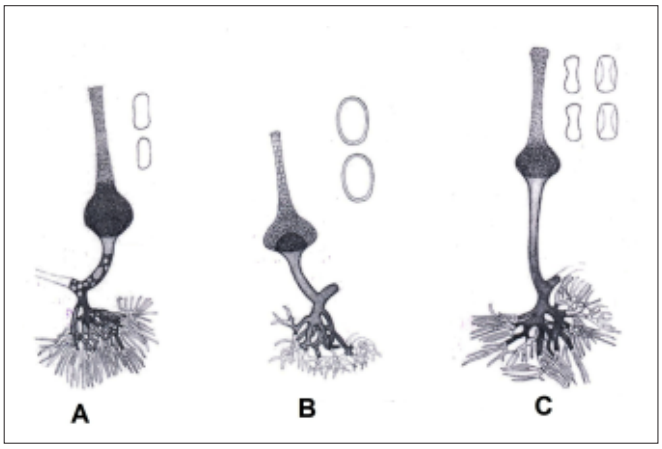

Fig. 1. Sporangiophores and sporangiospores of: (A) *Saksenaea vasiformis* (B) *S. oblongispora* and (C) *S. erythrospora*

In the first cutaneous zygomycosis due to *S. vasiformis* was reported by Padhye *et al*. (1988) from India in a rice mill worker. The infection involved the foot with multiple sinuses. Amputation of the toe part of foot followed by a split thickness of graft treatment with potassium iodide cured the infection.

The second report of *S. vasiformis* cutaneous zygomycosis came form Chakrabarti *et al.* (1997) from Chandigarh. In 2006, Padmaja *et al.* from the Dept. of Microbiology, Andhra Medical College, Visakhapatnam reported the cutaneous zygomycosis-Necrotizing fasciitis due to *S. vasiformis.* Through an early diagnosis was made and debridement carried out Amphotericin could not be administered in full dose because of interstitial nephritis and the patient was lost. It may be mentioned that the male patient aged 35 years was working as constable in the tribal area of Visakhapatnam. The infection developed after appendisectomy. The patient later died in a private hospital.

Cutaneous zygomycosis due to *S. vasiformis* reported by Baradkar and Kumar (2009) at Lokmanya Tilak Municipal Medical College and General Hospital, Mumbai where a 54 year old female presented ulcerative wound over left cheek. Since, an early diagnosis was done; the patient was successfully treated with intravenous Amphotericin B.

Fatal primary subcutaneous zygomycosis caused by *S. vasiformis* was reported by Kaushik *et al.* in 2012 from the Department of General Surgery, Microbiology and Pathology from Govt. Medical College and Hospital, Chandigarh where a 60 year old female patient developed ulceration and pain in the right gluteal region following an intra muscular multivitamin injection at that site one week previously. The infection remained undiagnosed for nearly two weeks, leading to a fatal outcome.

Vega *et al.* (2006) published a review paper of about 26 cases of *S. vasiformis* infection since 1976 when Ajello *et al*. (1976) first reported the case of human infection by *S. vasiformis.* Vega *et al*. (2006) compiled information on 26 cases of *S. vasiformis* infection and a case report of an Equadorian

adolescent who suffered serious burns after a car accident. It developed as a localized cutaneous infection which was successfully treated with surgical debridement and Amphotericin B. In 2012, Robin Kaushik *et al.* (2012) gave information of 13 cases from the year 2000 on a world wide basis where country, predisposing cause/s, treatment given and the outcome of the efforts of the treatment were given in a tabular form. In the 13th case which they reported from Chandigarh, the patient died due to infection remaining undiagnosed for nearly 2 weeks leading to fatal outcome.

Another interesting aspect was the report of Chander *et al*. (2015) where in they reported that *S. erythrospora* as an emerging mucoralean pathogen causing necrotizing fasciitis- a series of 5 cases from India in the 19th Congress of International Society for Human and Animal Mycology. Rhinosinusitis caused by *S. erythrospora* was reported by Uma Tendolkar *et al.* (2015) from the Department of Microbiology, Tilak Municipal Medical College and General Hospital, Sion, Mumbai where a 44 year old woman was diagnosed with a pre-septal cellulitis and pansinusitis and they claimed that it is the first report of its isolation of *S. erythrospora* from India. Treatment with Amphotericin B was successful in this case. In one recent report by Rodriguez *et al.* (2016) mycomycosis caused by *S. erythrospora* has been reported following esthetic breast augmentation surgery associated with medical tourism in Colombia. Three previous cases have been reported in literature, two associated with trauma (a sailing accident in Argentina and a combat trauma in Iraq) and one as a case of Rhino sinusitis in India. The organism was identified phenotypically and confirmed biologically after rDNA amplification and sequencing. According to Rodriguez *et al*. (2016) two months later the patient remained hospitalized awaiting reconstructive surgeries. According to Rodriguez *et al*. (2016) mycromycosis should be considered in the differential diagnosis of necrotizing infections of the skin and soft tissues that evolve repeatedly after cosmetic surgery performed in the tropical and subtropical countries.

ORIGIN OF ZYGOMYCETES

Before we close this article it will be worthwhile to discuss the possible origin of *Zygomycetes* or *Zygomycota* which is one of the five divisions or phyla of Kingdom Fungi and the true fungi include the divisions *Chytridiomycota*, *Zygomycota*, *Glomeromycota*, *Ascomycota* and *Basidiomycota*. The origin of *Zygomycota* is explained by two different views. According to Bessey (1950) and Hesseltine (1952), they have evolved from *Saprolegniales* from genera like *Geolegnia* and *Aplanes*. However, this long held view of some mycologists that *Ascomycota* evolved from red algae can be put to rest with studies on the sequencing of ribosomal RNA (Bhattacharya *et al.,* 1992; Hendriks *et al.,* 1991). Apart from the biochemical and molecular evidence there are filamentous species among *Chytridiomycota* to justify relatedness to higher fungi on morphological basis. Nevertheless it is very uncertain weather any extant species of *Chytridiomycota* is close to the ancestral form for the *Zygomycota* and higher fungi.

According to Gaumann and Dodge (1928), Jaczewski (1929), and Gaumann (1952) believed that *Mucorales* or *Zygomycetes* have been directly derived from the *Chytridiomycetes*. The presence of chitin in the cell wall suggests that *Mucorales* and *Zygomycetes* are derived from *Chytridiales*. The discovery of the fungus *Saksenaea vasiformis* as a member of *Mucorales* has strengthened this view. Saksena in his presidential address to the botany section of the Indian science congress held at the Osmania University Hyderabad in 1979 hypothesized that *Zygomycetes* have arisen from chytridiomycetous forms. There is a striking resemblance between the fungus *Nowakowskiella* (*Chytridiomycetes*) and *Saksenaea* (*Zygomycetes*). Saksena has weighted the arguments for and against the two hypotheses more closely.

When Saksena in 1979 gave his Presidential Address, he did not have the realization that Oomycetous fungi (to which *Saprolegnia* or *Phytophthora* belong) are not true fungi and the division *Straminopila* (or *Stramenophila*) will be recognized by D.N. Patterson in 1989 from organisms based on unique flagellum hair structure that is bearing mastigonemes and other biochemical characters. Chromista and heterokont is the earlier equivalent kingdom terms used in which heterokont zoosporic organisms have been used. The oomycetous fungi which include eight orders might have given rise to the zygomycetous fungi are completely ruled out.

There is no denying the fact that *Oomycetes* and *Zygomycetes* have γ linolenic acid in their lipids whereas all higher fungi have α linolenic acid (Shaw 1965). However, in view of the enormous differences in the cell wall structure and drastic differences in the organization of tryptophan biosynthesis enzymes as well as dissimilar lysine pathways, it seems improbable that *Zygomycetes* and *Oomycetes* are phylogenetically related. The common occurrence of γ linolenic acid in their lipids in these two groups of organisms may be considered as a case of convergence in evolution (Aneja and Mehrotra, 2015). According to Blackwell and Spatafora (2004) *Zygomycetes* comprise a monophyletic group if the *Glomales* and perhaps *Basidiobolous ranarum* are excluded. Although, it is not flagellated, this species has been placed within the core chytrid group in analysis based on r DNA evidence (Nagahama *et al*., 1995; Jenson *et al* 1998).

In trees based on α and β tubulin genes, however *B. ranarum* falls within the *Zygomycetes*. The theory though not original by Saksena put forth in 1979 is fully justified that origin of *Zygomycetes* is from chytridiaceous ancestors.

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