Taxonomic notes on *Allomyces neomoniliformis* (Blastocladiaceae) isolated from Nanital lake, Uttarakhand, India

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**Abstract**

During a survey of zoosporic fungi in the Nanital lake in 2014, a species of *Allomyces* was recorded. It was found in the lake soil which was rich in organic matter (leaves, roots and twigs) by using snake skin as a substrate through baiting technique. This specimen was distinctly different in having different life cycle involving cyst formation and its subsequent reduction divisions. The resistant sporangia zoospores were markedly smaller and characteristically biflagellate rather than uniflagellate as reported in other species of *Allomyces*. After comparison with type material and a survey of the literature, this species was identified as *Allomyces neomoniliformis*, known and documented only from one collection in the past from India but with no proper description. A complete morphological description, a description of the holotype, cystogenes type of life cycle, illustrations, and photographs are presented in this study. Thus, results have increased our knowledge of the occurrence and distribution of the *Allomyces* in India, complementing with the previous studies.

**Keywords**

*Allomyces*; Blastocladiomycota; Cystogenes; India; Taxonomy

**Introduction**

Blastocladiomycota is one of the seven currently recognized phyla within the kingdom Mycota [1]. Some species of Chytridiomycota, which were traditionally considered in one of its orders Blastocladiales [2,3] have been recently reclassified, redefined, described and separated into a new distinct phylum Blastocladiomycota on the basis of molecular phylogeny using ribosomal RNA genes and zoospore ultrastructural characters [1,4]. Blastocladiomycota is comprised of ecologically diverse zoospore-producing true fungi with a global distribution, characterized by reproducing asexually by means of posteriorly whiplash-like uniflagellate zoospore with a single insertion scourge [5]. Blastocladiomycota presently contains approximately 180 species within the order Blastocladiales and its five recognized families [6]: (i) Blastocladiaceae (2); (ii) Catenaariaceae [7]; (iii) Coelomomyctaceae [8]; (iv) Physodermataceae [9]; and (v) Sorochytriaceae [10]. These families consist of fourteen genera namely *Allomyces*, Microallomyces, Blastocladia, Blastocladiella, Blastocladiopsis, Catenomyces, Catenophlyctis, Caternaria, Coelomomyces, Coelomomyctidium, Paraphysoderma, Physoderma, Sorochytrium and Urophlyctis [11,12].

*Allomyces* is unique and best known among all these genera in that the thallus is either gametophytic (haploidy) or sporophytic (diploid) and thus exhibits a definite isomorphic alternation of generations [11,12]. There are three recognized subgenera according to the mode of life cycle exhibited by *Allomyces* [13]: Brachiallomyces (with only sporophytic phase) - including *A. anomalus* and all other forms having a similar life cycle; Cystogenes (with sporophytic phase dominant, the gametophyte represented by cyst and gametes) - including *A. moniliformis* Coker and Braxton and *A. neo-moniliformis* Indoh; and Euallomyces (with isomorphic alternation of sporophytic and gametophytic generations) - including *A. javanicus* Kniep. *A. arbuscula* Butler, and all other forms having a similar life cycle. Along with these differences the species in this genus may vary markedly in size, organization, and type of development, but they have several common characteristics. The genus *Allomyces* inhabit aquatic and terrestrial ecosystems that can be found from tropics to arctic regions, where moisture content and temperature vary considerably. They have frequently been observed mostly growing as saprobes on wide variety of substrates (especially of animal and plant origin) in soil and water to help in recycling of organic materials, maintenance of energy budget, food chain and productivity in terrestrial and aquatic ecosystems [14]. Hence, distinct life cycles, diverse ecological roles and phylogenetics suggest that the group is ancient [12]. This ancient divergence is also corroborated by fossil evidence demonstrating an alteration of generations in *Allomyces* like resting sporangia from Devonian which are remarkably similar to those of extent species [15,16].


In India, the real start of systematic study and research on Blastocladiomyota is credited to Butler [17], who first described and discovered two species of *Allomyces: A. arbuscula* and *A. moniliformis*. Later, other species (*A. neo-moniliformis*, *A. javanicus*, *A. macrogyrus* and *A. anomalus*) were reported by Ramakrishnan and Subramanian [18], Dayal [19], Bhargava and Singh [20], Srivastava [21], Thakurji [22], Rama Rao [23], Prabhujii and Srivastava [24], Khulbe [25] and Prabhujii [26,27]. As for the *Allomyces* recovered from various habitats, some studies were also carried out by Sati and Khulbe [28], Chowdhry and Agrawal [29], Sati [30,31], Khulbe [32] and Kaur [33]. Prabhujii and Sinha [34] described a new species of *A. recurvus* which has been recently reported by Singh [35] again. However, still the available taxonomic information on *Allomyces* from Indian sub-continent is far from satisfaction, especially considering scientific information on their identification, taxonomy, phenology,
distribution and complexity of the life cycle. Therefore, a lot of work in this field is required to have a full spectrum of systematics and distribution, particularly the biodiversity of Allomyces in the country.

Keeping this goal in mind, during aquatic fungal diversity survey of the Nanital lake, Uttarakhand, India, a distinct Allomyces specimen was found with a dark brown coloration. After further study, the identity of the species was determined to be *A. neomoniliformis*, originally published from a collection made in Gorakhpur, Uttar Pradesh, India [20]. To our knowledge, this fungus has not been reported since its original publication in India. Hence, the objective of this study was to contribute to the knowledge of Allomyces by giving particular emphasis on taxonomical/morphological features of special interest on *A. neomoniliformis*. Therefore, the current study was undertaken on the snake skin bait to re-evaluate this species using morphological characters to provide a key for its identification and taxonomy.

**Materials and Methods**

**Isolation and identification of Allomyces**

Baiting technique was used for the recovery of Allomyces [36,37]. The samples of water and soil were collected at random from the Nanital lake (29° 40’ N, 79° 47’ E), Uttarakhand, India at seasonal intervals, in 2014, with the aid of sterile plastic bags and taken to the laboratory. Each sample was processed into triplicates, which were introduced in separate Petri dishes and flooded with 40 mL of sterile deionized water. Each triplicate was multiply baited with boiled hemp seeds (*Cannabis sativa*), sesame seeds and keratin (purified snake skin), specifically for the isolation of *Allomyces*. All triplicates were incubated at a temperature of 20°C, controlled by air conditioning for two weeks.

The baits were periodically examined under a microscope for about two weeks and when the growth of the *Allomyces* on the baits had reached a minimum length of 4 to 5 mm, hyphae bearing zoosporangia were removed by using very fine scissors and forceps. These excised hyphae were then washed thoroughly with sterile distilled water. Afterwards, they were then subjected to morphological and microscopical observations by hanging drop mounts made for observation of zoospore germination, gamete formation, fusion and their different growth stages etc.

For identification, the water cultures of the isolate were examined for morphological features of thallus and its developmental pattern on keratin baits using a light microscope. The isolate was examined to assess the range and variation in thallus structural features, including types of sporangia, their morphometric characterization such as shape, size, pits over the walls, discharge apparatus, number of discharge pores/tubes, types of zoospores discharge, flagellation of the zoospore, presence or absence of a conspicuous gametophytic phase, type of branching, possession of rhizoids and morphology of rhizoidal system of the thallus. Identification and characterization of the isolate were done in accordance with Sparrow [36] 'Aquatic Phycomycetes', Karling [14] 'Chytridiomyctetarum Iconographia', original descriptions of the species and other relevant taxonomic literatures containing original descriptions of taxa.

After identification, the isolate was documented by means of image captured through Dewinter microscope and specimen was deposited at the Laboratory of Mycopathology and Microbial Technology, Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi, India.

**Results**

**Description of the species**


*Allomyces cystogenus* R. Emers., Lloydia. 4: 136. 1941; Mycologia 30: 120. 1938.

*Allomyces cystogenus var. elongatus* R. Emers., Lloydia 4: 136. 1941.

**Morphology**

The main thallus was composed of rhizoids, a trunk and successive subdichotomous branched principal hyphae, which bore the reproductive structures (thin walled sporangia and resistant sporangia) at their tips (Figure 1A and B). The basal portion or trunk was about 20-45 μm in diameter at base, anchored to the substrate by rhizoids, with numerous slender branches, which were separated into segments by pseudosepta. The hyphae were indeterminate in length, coarse, cylindrical, or blunt, branched straight, lacked walls, contents colorless which divided in a characteristic dichotomous pattern on the keratin bait (Figure 1). The reproductive structures were borne abundantly on the branches as thin walled sporangia (primary and secondary/mitosporangia) and resistant sporangia (meiosporangia). Primary sporangia (P) were terminal and clavate to cylindrical, 25-40 x 48-90 μm in diameter, with an apical papillum. The secondary sporangia (S) were produced beneath the primary ones, which were smaller, barrel shaped, sometimes catenulate, mostly in short linear chains with the younger ones becoming ovoid to spherical with truncate ends and a lateral discharge papillum (Figure 1E). Zoosporangia (mitosporangia) diploid, ovoid, 10-12 x 8-9 μm in size, with a long posterior flagellum (monoplasitic) and were amoeboid before encystment, which on germination forms sporophytic plant again (Figure 1D and E). Resistant sporangia (resting sporangium or resting spores in which meiosis normally occurs to renew the gametophyte generation) serve as a means of sustaining unfavorable conditions, abundant, terminals or cimosis, melanized dark brown color, oval to elongate, thick-walled with truncate base, 40-65 x 24-35 μm, with broadly rounded apex and conspicuous scattered pits, deciduous at maturity from thin clasping hyphal membrane (Figure 1D, F, J and K). Resistant sporangial zoosporangia (meiospores) discharge after the resting period, through slit in the wall, mostly bearing a pair of posterior flagella (characteristically biflagellate), 9-12 μm in diam. with sluggish movement typically amoeboidly, rarely moving far from their parent resistant sporangia, quickly encysting to produce single-celled cyst (C) which represents the gametophyte thallus (Figure 1C). Cysts divide mitotically to form four colorless, markedly smaller, uninflagellate isogametes (haploid), 8-10 μm in diam., which fuse in pairs producing zygote. On germination, they reestablishes the sporophytic generation of Allomyces (diploid thallus). This type of life cycle is normally associated with some algae and plants, but known with respect to fungi only known in this genus.

**Specimen examined**

Many isolate were isolated using snake skin as a substrate through baiting technique from lake soil, rich in organic matter (leaves, roots and twigs). (January, 31, 2014 Nanital, Uttarakhand, India; Nanital lake soil), No. 52 culture.
Figure 1A: Young developing sporophytic thallus.

Figure 1B: Mature thallus with some empty sporangia and resting sporangia.

Figure 1C: Primary sporangia (P) with short linear chain of secondary sporangia (S); Empty sporangia with apical papillum (pointed by arrow) and a single-celled cyst (C) representing gametophyte thallus.

Figure 1D: Dark brown color resistant sporangia (R) with thin clasping hyphal membrane.

Figure 1E: Encysted zoospores (pointed by arrow).

Figure 1F: Melanized dark brown color resistant sporangia deciduous at maturity.
Figure 1G: Branch with developing primary sporangia, secondary sporangia and resistant sporangia.

Figure 1H: Branch with developing primary sporangia and resistant sporangia.

Figure 1I: Branch with a deciduous resistant sporangia.

Figure 1J: Thallus with successive subdichotomous branched hyphae, which bore the reproductive structures.

Figure 1K: Resistant sporangia with pits around its wall.

Figure 1L: Empty cysts (E) which helps in reestablishing the sporophytic generation of Allomyces. Bars = 60 µm for A and B; 40 µm for C-L.
Ecology and distribution

Similar to most species of Allomyces, *A. neomoniliformis* is known from studies in which water or soil is baited with appropriate substrates such as hemp seeds, rosaceous fruits, pollens or insect body parts. *A. neomoniliformis* is thus likely performs an active role in decomposition of cellulosic and keratinic substrates within the ecosystem. *A. neomoniliformis* like its close relative *A. moniliformis* is commonly found in humid soil in the tropics, although it has been identified from many other regions of the world [38]. It grows commonly on organic debris in soil as well as water bodies and has been also isolated often from moist garden soils. It is mostly recovered in comparatively lower temperature months by using boiled hemp seeds as bait. The richest seasons in *A. neomoniliformis* were spring and winter, whereas the poorest was summer. The resting spore of *A. neomoniliformis* can germinated after incubation for half year, and formed thallus on bait in water to initiate a new life cycle.

Geographical distribution

USA, India, Japan, China, Taiwan, Mexico, Argentina, Brazil, Burma, Venezuela, Indonesia and Netherland.

Key for identification

*A. neomoniliformis* has a strikingly very different type of life cycle (involving cyst formation and subsequent reduction divisions) which distinguishes it markedly from other species of *Allomyces* such as *A. javanicus* and *A. arbuscula*, possessing a conspicuous alternation of sporophyte and gametophyte generations. Zoospores of resistant sporangia are markedly smaller and characteristically biflagellate rather than uninflagellate as occurring in the other forms.

Discussion

Since the inception of the studies on mycology the investigations on higher fungi have, undoubtedly, moved forward at a constant pace and are touching new heights. However, unfortunately the graph of studies on scientific explorations and advanced studies in the field of Blastocladiomycota, similar to as in Chytridiomycota, is continuously going down as compared to other groups of aquatic fungi as far as the Indian scenario is concerned which has arrived at the verge of extinction [39]. For a long time in India, the studies of Blastocladiomycota were focused on the morphology and taxonomy while ecological, physiological and molecular data were scarce. Other aquatic fungi of the order Saprolegniales and Peronosporales have, however, received a greater attention and several studies have been conducted on them [40,41]. Therefore, reliable quantitative and qualitative information are essential to fill up the lacunae in the existing knowledge. Extensive surveys have to be made in order to understand the diversity and distribution patterns of this group of fungi growing under Indian conditions. Aiming this goal in mind, the taxonomy of *A. neomoniliformis*, isolated from the Nainital, has been studied together with their features of special interest. Although, several other members of Blastocladiaceae have also been isolated, but, taking the specificity of work those have not been taken into account in this study. To date, characterization of *A. neomoniliformis* has been ambiguous in India, often resulting in confusion with other species of *Allomyces*. Also, *A. neomoniliformis* has so far been reported once in India [20], and we compared our isolates with the previous descriptions. We present more detailed descriptions of *A. neomoniliformis* and showing as how they are distinct from other species of this genus.

Emerson [42] first erected and identified this species with a life cycle similar to that of *A. moniliformis* and described its sexual life cycle. In *A. moniliformis* and *A. neomoniliformis*, the wide pits in the wall of the resistant sporangia and the presence of the characteristic cysts as described by Emerson [42] offer taxonomic characters of such distinctiveness in determining species. Later, Mccurrie [43] and Teter [44] confirmed it with some deviations in the life history of this organism. Later, many studies provided the cytological information over the various historical inconsistencies, cytogenetic events and zoospore ultrastructure during its life cycle [45, 46, 47] while its comparative physiology was studied by Ingraham [48], Ingraham and Emerson [49], Nolan [50] and Nielsen [51]. The taxonomy of *A. neomoniliformis* has been traditionally based on many characters mentioned by these investigators. However, these phenotypic characteristics are extremely variable within different strains of the same species of *Allomyces* and are morphologically difficult to distinguish. These facts, together with the formation of natural hybrids (e.g. *Allomyces x javanicus*), makes the delimitation and putative identification of *Allomyces* challenging. In addition, some studies have shown two clades within *Allomyces* with strains identified as *A. arbuscula* in both clades, suggesting that species concept in this genus needs revision [5]. Also, most of what is known of the distribution and diversity of *Allomyces* is known from cultural studies using baiting rather than direct observation of samples. Therefore, the investigation of environmental DNA has great potential to enlighten our understanding of the suitable habitat and diversity of *Allomyces* as it has been done in other fungal lineages in soil and plant roots [52,53,54].

Further, the result from present study may help to extend the knowledge of the occurrence of *A. neomoniliformis* in India by complementing previous study. In spite of the encouraging results of the present study more effort is needed for better understanding the members of the Blastocladiomycota. Therefore, isolation of this taxon from multiple disparate geographic locations and habitats will further provide better understanding of microhabitat, distribution, frequency of occurrence or relative abundance, and genetic divergence of the members of ancient lineage of this true fungus.

Conclusion

The results of the present study showed an exhaustive taxonomic description and ecological/distributional data of *A. neomoniliformis*. It has been studied second time in India, which is also the first record of it on keratin bait. This study is also a first attempt to provide a taxonomic key for their identification using solely morphological and developmental characters on keratin bait through colored illustrations for most of the different stages associated with their life cycle. Most of the monographs related to the *A. neomoniliformis* are outdated, as they lacked information on many pertinent microscopic details. These illustrations and descriptions can be used as a reference for the proper taxonomical description of this species.

Acknowledgements

The authors gratefully acknowledge Serena Rasconi, Oslo University, Norway along with S.K. Prabhuji, M.G. Post Graduate College, Gorakhpur, India for invaluable assistance in providing taxonomic literatures. The authors express their appreciation to F.H. Gleason, University of Sydney, Australia and Ram Dayal, Varanasi, India for their helpful suggestions and encouragement.
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