

# The genus *Allomyces* (Blastocladiomycota) in the State of Piauí, Brazil

José de Ribamar de Sousa Rocha<sup>1,2,3</sup>, Laércio de Sousa Saraiva<sup>1</sup>, Janete Barros da Silva<sup>1</sup>  
and Maria do Amparo de Moura Macêdo<sup>1</sup>

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**ABSTRACT** - (The genus *Allomyces* (Blastocladiomycota) in the State of Piauí, Brazil). Brazilian ecosystems have been intensively exploited for agricultural expansion, however, the diversity of zoosporic organisms in such biomes remains little known. Therefore, further research is required to better understand their role within these ecosystems. Studies with zoosporic fungi were carried out and 22 *Allomyces* isolates were obtained from soil samples collected at six municipalities from Piauí State. After identification procedures, the taxa were grouped into four species: *A. anomalus* R. Emers., *A. arbusculus* E.J. Butler, *A. moniliformis* Coker & Braxton, and *A. neomoniliformis* Indoh. *A. arbusculus* had the highest rate of resistant sporangia viability (10%) and the largest geographical distribution in Piauí, occurring in seven out of ten sites studied. Countrywide, they occur within 14 municipalities from three states. Greater knowledge about the geographical distribution of *Allomyces* in Brazil is being pioneered in the State of Piauí. Novel information regarding the diversity and occurrence, as well as taxonomic characteristics of the isolates is presented herein.

**Keywords:** biodiversity, geographical distribution, zoosporic organisms

**RESUMO** - (O gênero *Allomyces* (Blastocladiomycota) no Estado do Piauí, Brasil). Os biomas no Brasil estão sendo intensamente explorados para expansão das fronteiras agrícolas e a diversidade de organismos zoospóricos ainda é pouco explorada. O papel desses organismos não é totalmente conhecido nos ecossistemas e estudos iniciais para conhecimento da diversidade dos mesmos se fazem necessários. Em estudos com fungos zoospóricos foram obtidos 22 isolados de *Allomyces* de amostras de solo coletadas em seis municípios do Piauí. Após a identificação, os táxons foram agrupados em quatro espécies: *A. anomalus* R. Emers., *A. arbusculus* E. J. Butler, *A. moniliformis* Coker & Braxton e *A. neomoniliformis* Indoh. *A. arbusculus* apresentou a mais alta taxa de viabilidade de zoosporângios de resistência (10%) e a maior distribuição geográfica no Piauí, ocorrendo em sete dos dez locais estudados, e no Brasil, ocorrendo em 14 municípios de três Estados. O Piauí é o Estado com o melhor conhecimento da distribuição geográfica de *Allomyces* no Brasil. São apresentadas informações da distribuição geográfica no Piauí e comentários sobre as características dos isolados.

**Palavras-chave:** biodiversidade, distribuição geográfica, organismos zoospóricos

## Introduction

*Allomyces* is a genus characterized by filamentous mycelium and dichotomous branching. It presents planogametes and zoospores with a single posterior flagellum. The genus was established by Butler (1911) based upon the species *A. arbusculus*, in India. It was reclassified from Chytridiomycota to a new phylum Blastocladiomycota (Fungi) (James *et al.* 2006).

The genus *Allomyces* (Blastocladiales) comprises nine species and has very wide geographical distribution, occurring in five continents (Kirk *et al.*

2008). Such genus is represented by several species in the United States, Mexico, Costa Rica, Brazil and Argentina, which indicates a wide geographic distribution in both temperate and tropical regions of the Western Hemisphere. So reasonably one would assume that future research on this taxon could also find a similar distribution in the hottest regions of the Eastern Hemisphere (Hawkesworth *et al.* 1996, Stecio & Eliades 2001).

Biodiversity studies of zoosporic organisms, including Blastocladiomycota, in all Brazilian biomes are still very scarce, and the current limited knowledge

1. Universidade Federal do Piauí, Campus Ministro Petrônio Portella, Mestrado em Desenvolvimento e Meio Ambiente, PRODEMA, Núcleo de Pesquisa no Trópico Ecotonal do Nordeste - TROPEN, Av. Universitária, 1310, 64049-550 Teresina, Piauí, Brasil
2. Universidade Federal do Piauí, Campus Ministro Petrônio Portella, Centro de Ciências da Natureza, Departamento de Biologia, s/n, Ininga, 64049-550 Teresina, Piauí, Brasil
3. Corresponding author: ribamar10@hotmail.com

about these organisms is also due to the very few research specialists in Brazil (Jerônimo *et al.* 2015). As a matter of fact, these organisms play a major role in the balance of ecosystems and soil fertilization process, since they are found in different ecosystems where they act as organic matter decomposers, by reducing the various types of substrates to simple elements, then ready to be mineralized by subsequent decomposers (Powell 1993, Barr 2001, Shearer *et al.* 2007).

It has been already known that most Brazilian ecosystems are being over-exploited for agricultural expansion. Conversely, the diversity of this group of organisms within the biomes remains little known, and therefore further studies are needed in order to enhance the knowledge about their diversity. Although *Allomyces* is the first genus within the Blastocladiomycota phylum to be reported in Brazil (Wolf 1941), there is lack of information about the diversity and geographic distribution of such taxon (Pires-Zottarelli & Rocha 2010).

However, recent studies have contributed to better understanding of the biodiversity and distribution of zoosporic organisms in the State of Piauí (Rocha *et al.* 2001, Rocha 2006, Pereira & Rocha 2008, Rocha *et al.* 2010a, 2010b, 2014, Trindade-Junior & Rocha 2013, Rocha & Macêdo 2015). The present study brings new information regarding the genus *Allomyces* in Piauí. The characteristics of the isolates are described herewith.

## Material and methods

The studies were carried out between 1998 and 2015 within ten areas of six municipalities in the State of Piauí. From 1998 to 2000, the municipality of Piripiri within Parque Nacional de Sete Cidades ( $4^{\circ}5'59''S$  and  $41^{\circ}42'50''W$ ); in 2007, the municipality of Caracol within Parque Nacional Serra das Confusões ( $9^{\circ}7'30''S$  and  $43^{\circ}48'11''W$ ) as well as the municipality of Teresina, within the urban area of rio Poti and Parque Zoobotânico; in 2009, the municipality of Floriano within the urban area of rio Parnaíba ( $06^{\circ}46'01''S$  and  $43^{\circ}01'22''W$ ); in 2010, the municipality of Campo Maior within Açude Grande ( $4^{\circ}50'6''S$  and  $42^{\circ}10'13''W$ ); in 2011, the municipality of Teresina within Parque Lagoas do Norte ( $5^{\circ}5'21''S$  and  $42^{\circ}48'6''W$ ); in 2014, the municipality of Teresina within the urban area of rio Parnaíba as well as Caju-Açú Settlement ( $5^{\circ}5'21''S$  and  $42^{\circ}48'6''W$ ); and in 2015, the municipality of Demerval Lobão within Povoado Mutum ( $05^{\circ}21'97''S$  and  $42^{\circ}45'23''W$ ).

Samples were collected from surface water samplings (100 ml) in sterilized glass jars; and topsoil (200 g) was collected at 10 cm depth in the soil profile, with the aid of a sterile spatula, in polyethylene bag labeled with information on the collection site. The samples were transported to the zoosporic fungi Laboratory at the Universidade Federal do Piauí and prepared as per multiple-baiting (adapted) method (Emerson 1941, Sparrow 1960, Barr 1987, Steciow & Eliades 2001, Marano *et al.* 2008). The water samples were placed in Petri dishes (100 mm diameter), and then cellulosic organic substrates (corn straw, sorghum seeds, onion cataphylls, cellophane and filter paper) were added to the media; the substrates were previously prepared for colonization by zoosporic organisms. From soil samples, aliquots of 20 g were placed in Petri dishes (100 mm diameter) filled with sterile distilled water (30 ml), and after a brief agitation of soil, it was decanted for 20 minutes and then the same substrates were added. The Petri dishes were incubated at room temperature ( $30-32^{\circ}C$ ) under light and dark photoperiod for seven days. And then after that, the substrates were mounted on glass slides and examined under a microscope (Olympus BX 41, Tokyo, Japan). The colonized substrates were then transferred to Petri dishes filled with sorghum seeds and sterilized distilled water for colony replication.

In order to study the life cycle and description of *Allomyces* species, the isolates were dried on filter paper at room temperature ( $30-32^{\circ}C$ ) for eight weeks. Also, to reestablish *Allomyces* life cycle, the filter paper containing the resistant structures was placed into a Petri dish with sterile distilled water and sorghum seeds. After the incubation at room temperature ( $30-32^{\circ}C$ ) for 24 hours, the resistant structures germinated and released the zoospores, reestablishing the life cycle.

The viability rate is calculated in order to establish the germination percentage of resistant structures (Emerson & Wolfson 1954). Furthermore, we mounted three Petri dishes with filter paper and filled them with dehydrated resistant zoosporangia as well as sterile distilled water, and then after incubation for 24 h, a 100 resistance zoosporangia were accounted for, and from these, we quantified the zoospores released. After germination and colony growth, 20 measurements were taken to assess the vegetative and reproductive structures as well as the upper and lower values of size-variation interval. The species have been identified within the literature, such as Emerson (1941), Sparrow

(1960) and Wolf (1941). After identification, the species were then described. These species were incorporated into the Culture Collection, Laboratory of Zoosporic Fungi, Universidade Federal do Piauí (Zoosporic Fungi of Brazil, ZFBR).

## Results and Discussion

The species of the genus *Allomyces* found in the State of Piauí were the following: *A. arbusculus* E. J. Butler, *A. anomalus* R. Emers., *A. moniliformis* Coker & Braxton and *A. neomoniliformis* Indoh. The species were recorded for the municipalities of Campo Maior, Caracol, Demerval Lobão, Piripiri and Teresina (figure 1).

Moreover, five species have been recorded for five Brazilian States: *A. macrogynus* (R. Emers.) R. Emers., in São Paulo; *A. anomalus*, in São Paulo, Rio Grande do Sul and Piauí; *A. arbusculus*, in Pernambuco, São Paulo, Rio Grande do Sul and Piauí; *A. moniliformis*, in Piauí, and *A. neomoniliformis*, in Piauí (Pires-Zottarelli & Rocha 2010) (figure 1).

In South America, besides Brazil, *Allomyces* was also recorded for Argentina, where *A. arbusculus*, *A. macrogynus* and *A. neomoniliformis* occur (Steciow et al. 2012). The life cycles of *Allomyces*, according to Emerson (1941), are represented by three subgenera. The subgenus *Eualloomyces* develops gametophytes, which produce mobile gametes fusing and forming planozygotes. The latter develop into sporophytes producing thin-walled zoosporangia and resistance zoosporangia. The zoospores formed in the thin-walled zoosporangia are haploid and develop into sporophytes, whereas the zoospores formed in the resistance zoosporangia are diploid and develop into gametophytes.

In the subgenus *Cystogenes*, resistance zoosporangia produce diploid zoospores, which encyst, undergo meiosis and produce gametes that fuse and develop into sporophyte. Unlike the subgenus *Brachyallomyces* which produces diploid zoospores, but they not develop into gametophytes.

Based upon our findings, the species are divided into three subgenera as proposed by Emerson (1941): *A. arbusculus* belongs to the subgenus *Eualloomyces*; *A. anomalus* belongs to the subgenus *Brachyallomyces*, while *A. moniliformis* and *A. neo-moniliformis* belong to the subgenus *Cystogenes*.

***Allomyces anomalus*** R. Emers. Lloydia, 4: 133.1941. Figures 2-5, 17-19

Cylindrical basal cell, 60-180 × 60-80 µm. Dichotomous or sympodial branching hyphae,

pseudocells up to 320 × 18-25 µm, occasionally producing rhizoids. Sporophytes with thin-walled zoosporangia, terminals, ellipsoid or ovoid, with round apex, or catenulate with truncate apex, 38-92 × 25-45 µm, with 1-4 releasing papillae. Zoospores ovoid, oblong or ellipsoid, 8-10 µm, with a long posterior flagellum. Resistance zoosporangia terminal or sympodial, ovoid, with round apex and truncate base, 35-62 × 25-35 µm, exospore thick, yellowish-brown, very punctuated; zoospores with a long posterior flagellum; the cysts germinate forming sporophytes.

Resistance zoosporangium viability: 5%.

Material examined: BRAZIL. PIAUÍ: Piripiri, Parque Nacional de Sete Cidades, soil sample, 23-II-1999, J.R.S. Rocha 132 (ZFBR006).

Supplementary Material: BRAZIL. PIAUÍ: Campo Maior, Açude Grande, soil sample, 23-VIII-2010, L. Cronemberger 69 (ZFBR163); Caracol, Parque Nacional Serra das Confusões, soil sample, 5-IV-2007, J.R.S. Rocha, (ZFBR 20); Teresina, Parque Lagoas do Norte, soil sample, 25-V-2011, O. Trindade Jr. 120 (ZFBR161).

Geographical distribution countrywide: Pernambuco: Recife (Cavalcanti, 2001); Rio Grande do Sul: Santa Cruz (Wolf 1941); São Paulo: Assis and Santo Anastácio (Milanez, 1984); Cubatão (Milanez et al. 2007); São Paulo (Milanez et al. 2007).

Emerson (1941) placed this type of life-cycle fungi lineage in a species-form with the binomial *A. anomalus*. The asexual phase is similar to *A. arbusculus* and *A. javanicus*. This author also observed that some lineages showed sexual failure after a 2-year period and no longer produced gametophytes, suggesting that this type of short life cycle (Brachy) might be a way in which only the asexual phase of full life-span lineages is produced. The cell division of resistance zoosporangia is mitotic and the formed zoospores are diploid, yielding thereby sporophytes. The studied isolates showed no significant difference in comparison with the original description. Nevertheless, the formation of rhizoids in pseudocells other than basal is reported to the genus for the first time (figures 5 and 17). The dense distribution of pores on the resistance zoosporangia is in accordance with Emerson's observations (1941).

*Allomyces arbusculus* E. J. Butler. Ann. Bot. London, 25: 1027. 1911. Emend. Hatch, J. Elisha Mitchell Sci. Soc., 49(1): 163. 1933.

Figures 6-9, 20-22

Cylindrical basal cell, 80-160 × 50-85 µm. Branched hyphae, dichotomous or sympodial, pseudocells up to 350 × 15-25 µm. Sporophytes with thin-walled zoosporangia, terminals, ellipsoid or ovoid, with round apex, or catenulate with truncate apex, 35-70 × 23-45 µm, with 1-4 releasing papillae. Zoospores ovoid, oblong or ellipsoid, 8-12 × 4-6 µm, with a long posterior flagellum. Resistance zoosporangia terminal or sympodial, ovoid, with round apex and truncate base, 32-60 × 23-32 µm, exospore thick, yellowish-brown, very punctuated. Zoospores with a long posterior flagellum; the cysts germinate forming the gametophyte similar to sporophyte.

Gametangia terminals and pairing during early development of the gametophyte, then sympodial or catenulate, alternating the female gametangium terminal, globose, ovoid, clavate, 40-60 × 20-38 µm, with grayish or colorless content, supported on a male gametangium, short or long, cylindrical or barrel-shaped, 45-60 × 32-38 µm, with light-yellow or salmon-colored content, occasionally colorless. Colorless female gametes, ovoid or subspherical, 10-13 × 8-9 µm. Male gametes, ovoid or subspherical,

6-8 × 4-7 µm. After germination, the zygote forms the sporophyte.

Resistance zoosporangium viability: 10%.

Material examined: BRAZIL. PIAUÍ: Piripiri, Parque Nacional de Sete Cidades, soil sample, 26-VIII-1998, J.R.S. Rocha 32 (ZFBR005); soil sample, 24-XI-1998, J.R.S. Rocha (ZFBR 007); soil sample, 26-VII-1999, J.R.S. Rocha (ZFBR008).

Supplementary material: BRAZIL. PIAUÍ: Campo maior, Açude Grande, soil sample, 21-VI-2010, L. Cronemberger 32 (ZFBR167); Demerval Lobão, riacho Mutum, soil sample, 2-III-2015, A. Macedo 46 (ZFBR169); Floriano, rio Parnaíba, soil sample, 21-VI-2009, N.C. Negreiros 123 (ZFBR162); Teresina, Parque Ambiental Lagoas do Norte, soil sample, 23-IX-2011, O.C. Trindade Jr. 147 (ZFBR172); rio Poti, soil sample, 24-V-2007, J.R.S. Rocha 152 (ZFBR08W); rio Parnaíba, soil sample, 20-IV-20014, N. Sousa 133 (ZFBR1Y2); Parque Zoobotânico de Teresina, soil sample, 18-VIII-2007, L. Sousa 147 (ZFBR12ZB).

Geographical distribution countrywide: Paraíba: Areias (Joffily 1947); Rio Grande do Sul: Santa Cruz (Wolf 1941); São Paulo: Campinas (Joffily 1947); Assis, Caiuá, Cândido Mota, Cruzália Paulista, Fartura, Marabá Paulista, Palmital, Presidente Epitácio, Presidente Prudente, Salto Grande and Teodoro Sampaio (Milanez 1984).

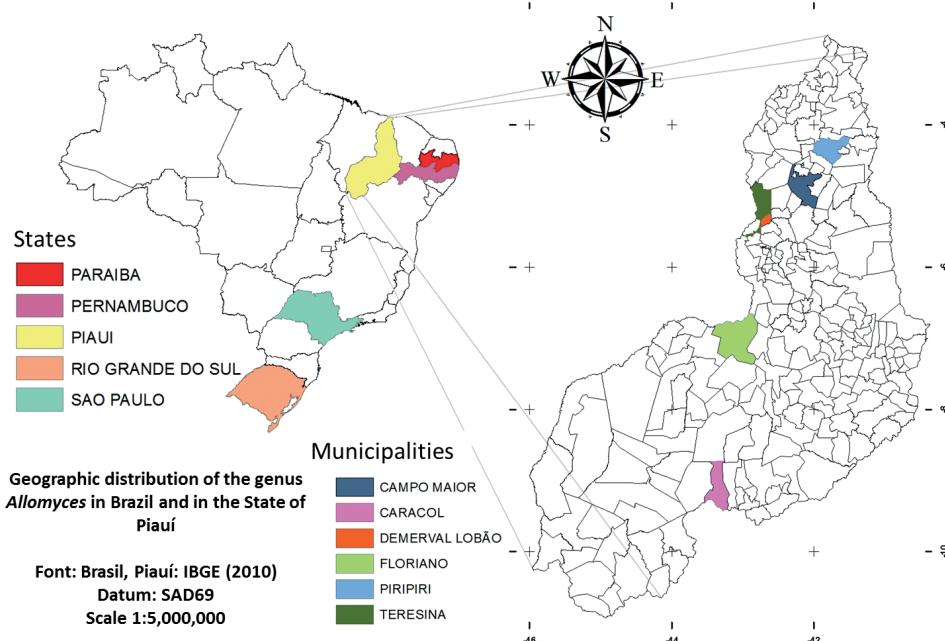
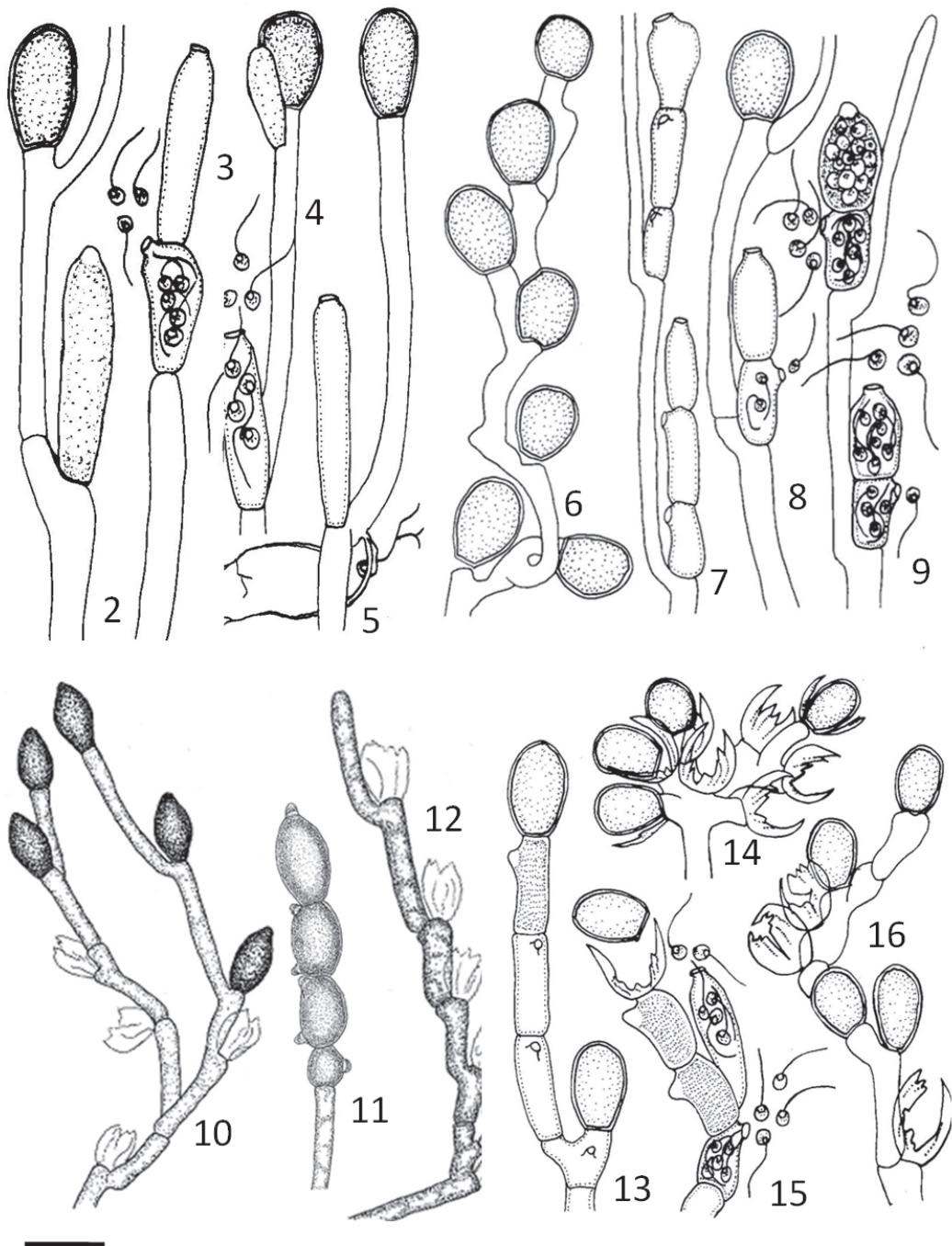
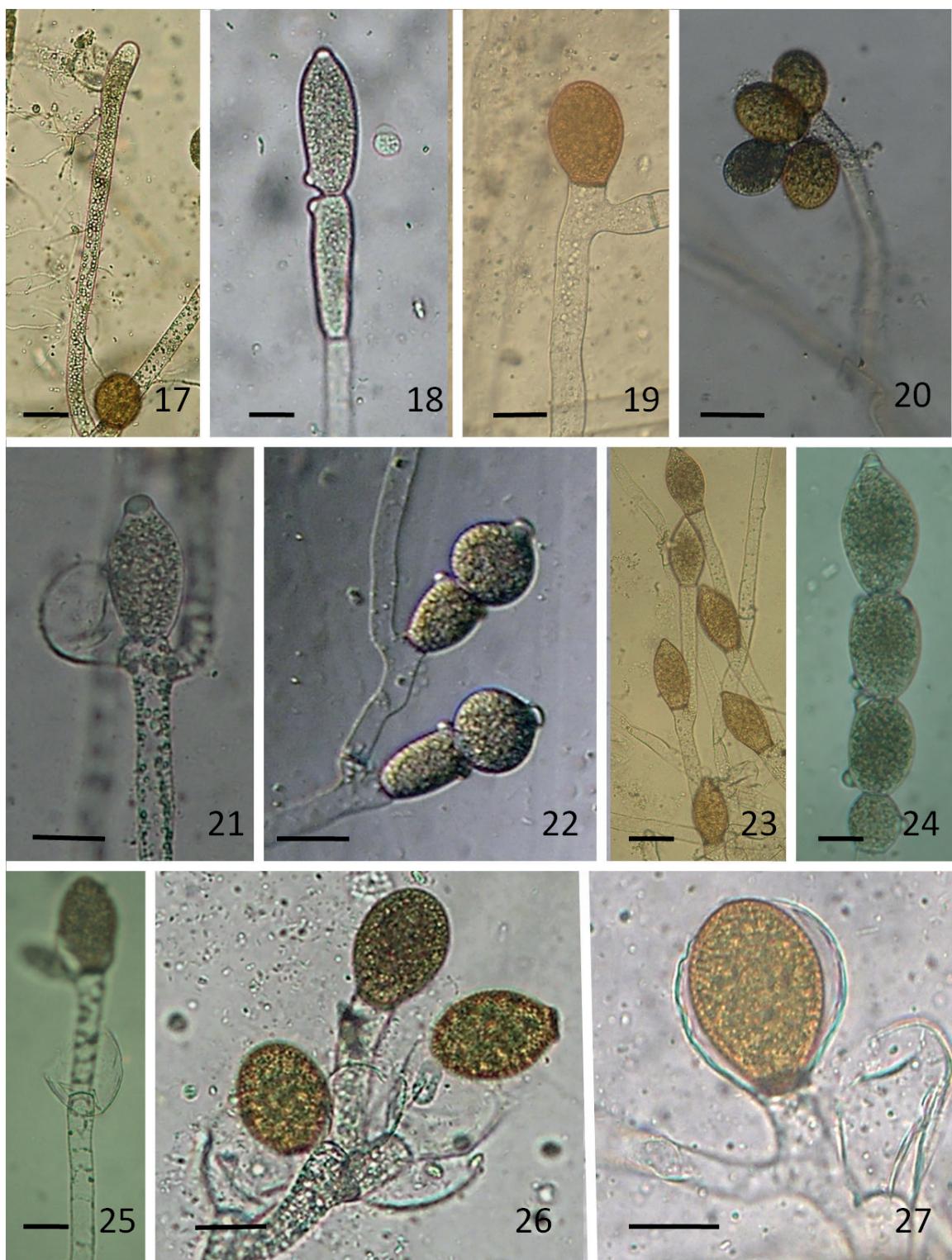


Figure 1. Geographic distribution of the genus *Allomyces* in Brazil and in the State of Piauí.



Figures 2-16. *Allomyces* species in the State of Piauí, Brazil. 2-5. *A. anomalus*. 2. Hypha with thick-walled resistant zoosporangium and thin-walled zoosporangium. 3. Thin-walled zoosporangia, upper one empty, lower one releasing zoospores. 4. Resistant zoosporangium in terminal position and thin-walled zoosporangium releasing zoospores. 5. Hypha with rhizoids, thin-walled zoosporangium and resistant zoosporangium in terminal position. 6-9. *A. arbusculus*. 6. Resistant zoosporangia, sympodial. 7. Thin-walled zoosporangia, empty and catenulate. 8. Resistant zoosporangia in terminal position, pair of lateral gametangia, the female in upper position, empty, and the male in lower position releasing gametes. 9. Two pairs of gametangia, the female in upper position, and the male in lower position, both releasing gametes. 10-12. *A. moniliformis*. 10. Empty sheaths and resistant zoosporangia with tapered apex. 11. Thin-walled zoosporangia, moniliform. 12. Empty Sheaths. 13-16. *A. neomoniliformis*. 13. Resistant zoosporangia terminal over catenulated thin-walled zoosporangia. 14. Resistant zoosporangia cymose, with empty sheaths. 15. Resistant zoosporangia terminal, sheath, thin-walled catenulated zoosporangia, both releasing zoospores. 16. Resistant zoosporangia and empty sheaths. Bar: 45 µm.



Figures 17-27. *Allomyces* species in the State of Piauí, Brazil. 17-19. *A. anomalus*. 17. Hypha with rhizoids. 18. Thin-walled zoosporangia with papillae, prior to releasing zoospores. 19. Lateral hypha with pseudo-septum, resistant zoosporangia terminal. 20-22. *A. arbusculus*. 20. Resistant zoosporangia, sympodial. 21. Thin-walled zoosporangia, one empty and the other with apical papilla. 22. Gametangia pairs with apical papillae. The female gametangium positioned above the male gametangium. 23-25. *A. moniliformis*. 23. Resistant zoosporangia with tapered apex. 24. Thin-walled zoosporangia, moniliform. 25. Empty sheath and resistant zoosporangium terminal. 26-27. *A. neomoniliformis*. 26. Resistant zoosporangia and empty sheaths. 27. Thick-walled resistant zoosporangium, spaced out pores. Bar = 45 µm.

Hyphae may show more constriction in the forming region of the pseudo-septa. Some gametes showed amoeboid movement. Male gametangium often occurs with little pigmentation or completely colorless (figure 22). We observed pigmentation of some hyphae from aged culture collections. The gametophyte producing resistance zoosporangia concomitant with gametangia has been observed in several cultures. The formation of resistance zoosporangia in sexual mycelia of Euallomyces occurs regularly under certain conditions (Sparrow 1960). The viability of resistance zoosporangium (10%) was the highest among the other isolates studied.

*Allomyces moniliformis* Coker & Braxton. J. Elisha Mitchell Sci. Soc., 42: 139. 1926. Emend. Emerson, Mycology, 30: 127. 1938.

Figures 10-12, 23-25

Cylindrical basal cell,  $38-130 \times 12-36 \mu\text{m}$ . Dichotomically branched hyphae, pseudocells up to  $320 \times 10-30 \mu\text{m}$ , slightly constricted in the pseudo-septum, hyaline, but in aged cultures they may become light-pink. Sporophytes with thin-walled zoosporangium, terminals, cylindrical or clavate,  $30-65 \times 18-25 \mu\text{m}$ , with an apical papilla, or catenulate, ovoid or globose, with truncate apex,  $12-50 \times 17-25 \mu\text{m}$ , 1(-4) lateral release papilla(-ae), often moniliformly displayed, successively decreasing in size with the smallest measuring  $20 \mu\text{m}$  in diameter; zoospores ovoid, oblong or ellipsoid,  $10-14 \times 9-14 \mu\text{m}$ , with a long posterior flagellum, amoeboid prior to cysts. Resistance zoosporangia terminal, elongated, ovoid or ellipsoid, with tapered (acute) apex, truncate base,  $32-45 \times 15-25 \mu\text{m}$ , usually deciduous while leaving the sheath or capsule (surrounding membrane) at maturity, exospore thick, yellowish-brown or reddish brown, with spaced out pores; zoospores with a long posterior flagellum. Cysts (reduced gametophytes)  $8-12 \mu\text{m}$ , occasionally up to  $20 \mu\text{m}$ , possibly due to the non-occurrence of cleavage, releasing up to four isogamic gametes, with a long posterior flagellum,  $6-8 \times 4-7 \mu\text{m}$ . Germinating zygote to form the sporophyte.

Resistance zoosporangium viability: 5%.

Material examined: BRAZIL. PIAUÍ: Piripiri, Parque Nacional de Sete Cidades, soil sample, 26-VIII-1998, J.R.S. Rocha (ZFBR 147); Parque Nacional de Sete Cidades, soil sample, 26-XI-1998, J.R.S. Rocha (ZFBR 148).

Supplementary Material: BRAZIL. PIAUÍ: Teresina, Cajú-Açu Settlement, soil sample, 22-V-2014, *L. Saraiva 111* (ZFBR34W); Povoado Baixão dos Afonsinhos, soil sample, 22-X-2014, *L. Saraiva 121* (ZFBR12TG); rio Poti, soil sample, 23-X-2007, J.R.S. Rocha 169 (ZFBR56MY).

Geographical distribution countrywide: Piauí (Pires-Zottarelli & Rocha 2010, Steciow et al. 2012, Maia & Carvalho 2015).

The moniliform display of zoosporangia is an evident feature of the taxon (figures 11 and 12). The resistance zoosporangia of this species are the most elongated within the genus, with tapered apex (figures 10 and 23) in contrast with the round apex of *A. arbusculus* (resistance zoosporangia) (Coker & Braxton 1926). The pores within the thick-walled resistance zoosporangia are distinctly larger and more visible than the other species (Wolf 1939, Indoh 1940, Emerson 1941). However, the pore distribution, within the thick-walled resistance zoosporangia, was not considered as a major taxonomic feature by Emerson (1941), since it was not indicated in his description of *Allomyces* species. Furthermore, Teter (1944) cited a lineage of *A. moniliformis* solely producing non-flagellated spores and Sparrow (1960) attributed this phenomenon to some environmental factor that suppressed the flagellum. The lineage studied by Teter (1944) often repeated the zoospore emergence. Coker and Braxton (1926) had already described similar process in *A. moniliformis*, however, Emerson (1941) likely supposed that the cited authors might have confused the phenomenon with the early cyst emergence, without repetition of emergence behavior. In the present survey, neither non-flagellated zoospores nor emergence repetition were observed in the isolates studied.

*Allomyces neomoniliformis* Indoh. Sci. Rept. Tokyo Bunrika Daigaku, 4: 271. 1940.

Figures 13-16, 26-27

Cylindrical basal cell,  $60-150 \times 23-45 \mu\text{m}$ . Hyphae subdichotomously branched, pseudocells up to  $280 \times 15-25 \mu\text{m}$ , constricted in the pseudo-septum, hyaline. Sporophytes with thin-walled zoosporangia, terminals or cymose, cylindrical or clavate,  $70-112 \times 18-23 \mu\text{m}$ , with an apical papilla or catenulate, barrel-shaped, with truncate apex,  $18-23 \times 20-56 \mu\text{m}$ , with lateral papilla. Zoospores ovoid, oblong or ellipsoid,  $10-12 \times 4-8 \mu\text{m}$ , with a long posterior flagellum. Resistance zoosporangia terminal,

sympodial or cymose, ovoid, globose or ellipsoid, with round apex, truncate base,  $35-60 \times 23-30 \mu\text{m}$ , rarely epigyne on a zoosporangium, usually deciduous while leaving the capsule (surrounding membrane) at maturity, exospore thick, yellowish-brown, with spaced out pores up to  $3 \mu\text{m}$ ; zoospores with a long posterior flagellum. Cysts (reduced gametophytes)  $8-12 \mu\text{m}$ , occasionally reaching up to  $20 \mu\text{m}$ , possibly due to the non-occurrence of cleavage, releasing up to four isogamic gametes, with a long posterior flagellum, ellipsoid,  $6-8 \times 4-7 \mu\text{m}$ . Germinating zygote to form the sporophyte.

Resistance zoosporangium viability: 8%.

Material examined: BRAZIL. PIAUÍ: Piripiri, Parque Nacional de Sete Cidades, soil sample, 26-VIII-1998, J.R.S. Rocha (ZFBR 017); Parque Nacional de Sete Cidades, soil sample, 28-V-1999, J.R.S. Rocha (ZFBR 019).

Supplementary Material: BRAZIL. PIAUÍ: Teresina, Parque Lagoas do Norte, soil sample, 22-VI-2011, O.C. Trindade Jr. 92 (ZFBR23OZ).

Geographical distribution countrywide: Piauí (Pires-Zottarelli & Rocha 2010, Steciow *et al.* 2012, Maia & Carvalho 2015).

The pores within the thick-walled resistance zoosporangium are more spaced out than other species (figure 27). They were also deciduous while leaving the capsule at maturity (figures 26 and 27). We observed the growing of thin-walled zoosporangia as well as resistance zoosporangia in a cymose arrangement (figure 26). As a matter of fact, there were no significant differences in comparison to the original description of the species.

The resistance zoosporangia are remarkably resistant structures to environmental conditions and may remain viable for long periods. Emerson (1941) observed that, when completely dried, the resistance zoosporangia can remain viable for several hours at  $100^\circ\text{C}$  or a number of years at ordinary temperatures. There have been reports on their conservation viability of nearly ten years. The viability rate, observed in this study, was generally lower for all species, which reached at most 10%. *A. arbusculus* (resistance zoosporangium) showed the highest viability rate (10%) and the largest geographical distribution in the State of Piauí, occurring within seven out of ten studied sites. Its occurrence was also found within 14 municipalities in three Brazilian States.

All isolates of the species reported for Piauí were obtained from soil samples.

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