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Oomycetes (Oomycota) from Maranhão State, Brazil[1]

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ABSTRACT - (Oomycetes (Oomycota) from Maranhão State, Brazil). This study aimed at enhancing the knowledge on the diversity and distribution of Oomycetes within Parque Natural Municipal Lagoa do Sambico, in Timon municipality, Maranhão State, Brazil as well as their geographical distribution in Brazil as a whole. Of the 98 isolates, 16 were identified as Oomycetes, which belong to six families: Achlyaceae, Leptolegniellaceae s. lat., Pythiaceae s. lat., Olpidiopsidaceae s. lat., Saprolegniaceae s. str. and Verrucalvaceae. We report herewith the first records of *Olpidiopsis aphanomycis* Cornu and *Saprolegnia luxurians* (Bhargava & G.C. Srivast) R.L. Seym. for Brazil, as well as *Achlya* aff. *diffusa* J.V. Harv. *ex* T.W. Johnson, *Achlya proliferoides* Coker, *Aphanomyces helicoides* Minden, *Aplanopsis terrestris* Höhnk, *Dictyuchus monosporus* Leitg., *Globisporangium proliferum* (Cornu) P.M. Kirk, and *Pythiogeton uniforme* A. Lund for the State of Maranhão.
Keywords: diversity, environment, geographic distribution, zoosporic organisms

RESUMO - (Oomicetos (Oomycota) do Estado do Maranhão, Brasil). Este estudo foi realizado a fim de contribuir para o conhecimento da diversidade e distribuição geográfica de oomicetos no Parque Natural Municipal Lagoa do Sambico, na cidade de Timon, Estado do Maranhão, Brasil; bem como a sua distribuição geográfica no Brasil como um todo. De um total de 98 isolamentos, 16 táxons foram identificadas como espécies de oomicetos, pertencentes a seis famílias: Achlyaceae, Leptolegniellaceae s. lat., Pythiaceae s. lat., Olpidiopsidaceae s. lat., Saprolegniaceae s. str. e Verrucalvaceae. *Olpidiopsis aphanomycis* Cornu e *Saprolegnia luxurians* (Bhargava & G.C. Srivast) R.L. Seym. são relatadas pela primeira vez para o Brasil e *Achlya* aff. *diffusa* J.V. Harv. *ex* T.W. Johnson, *Achlya proliferoides* Coker, *Aphanomyces helicoides* Minden, *Aplanopsis terrestris* Höhnk, *Dictyuchus monosporus* Leitg, *Globisporangium proliferum* (Cornu) P.M. Kirk e *Pythiogeton uniforme* A. Lund, para o Estado do Maranhão.
Palavras-chave: ambiente, distribuição geográfica, diversidade, organismos zoospóricos

Introduction

The phylum Oomycota (Kingdom Straminipila), has two classes (Saprolegniomycetes and Peronosporomycetes). Saprolegniomycetes includes three orders, seven families and 33 genera, whereas Peronosporomycetes has three orders, five families and 40 genera. Further several classes, five orders, ten families and 13 genera as classified as *incertae sedis* (Beakes *et al.* 2014). The phylum comprises saprobes and parasites found in aquatic and terrestrial ecosystems; its members play an important role in the decomposition of organic matter and participate in the cycling of nutrients. Important parasites attack plants, algae, fish, crustaceans, fungi, mosquito larvae, nematodes, rotifers, mammals, and even humans (Marano *et al.* 2008, Beakes *et al.* 2014).

Oomycota are widely distributed and ubiquitous. Saprobic species have extensive enzymatic capacity, with the ability to degrade a wide range of vegetal and animal substrates such as cellulose (algae and plant debris), keratin (snake skin, hair and feather), chitin (exoskeleton of crustaceans and insects), lignin (dead tissue of woody plants) and sporopollenin (pollen grains). Saprotrophic species are abundantly found in decaying plant material, but their occurrence and frequency are underestimated unless specific techniques are applied for their study (Hawksworth 2004, Marano *et al.* 2011).

The genera *Saprolegnia*, *Achlya* and *Aphanomyces* are commonly found parasitizing fish as well as their

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eggs. *Pythium* and *Phytophthora* species are highly pathogenic in plants of economic interest (Moore-Landecker 1996, Alexopoulos *et al.* 1996). *Pythium insidiosum* stands out as equine parasite, but also parasites other animals such as cattle, goats, dogs, cats, as well as human. Subcutaneus ulcerative lesions in animals caused by this species have been consistently reported (Santurio *et al.* 1998, Leal *et al.* 2001, Sallis *et al*. 2003, Rech *et al.* 2004), including in humans for the first time in Brazil (Bosco *et al*. 2008) and in horses in Piauí State (Rocha *et al.* 2010). However, there have been very few reports of Oomycetes in Maranhão State. A single study by Sales (2009), surveyed species of Oomycetes from groundwater wells in three villages within Timon municipality. The present study aimed at increasing the knowledge on the diversity of Oomycetes within Parque Natural Municipal Lagoa do Sambico, municipality Timon, in Maranhão State, Brazil.

Materials and methods

Study area - The municipality of Timon is located on the left bank of Parnaíba River, which borders Piauí State. Timon comprises an area of 1,743,246 km², with a population density of 89,18 individuals per km² and an estimated population of 161,721 inhabitants. The municipality has a warm tropical dry climate with little temperature variation, at times exceeding 40 °C, the minimum being rarely less than 22 °C. Centroid coordinates are 5º05'S latitude and 42º50'W longitude, with an elevation of at 69 m.a.s.l., situated 426 km far from the capital of Maranhão State, São Luís (IBGE 2013) (figure 1). The Parque Natural Municipal Lagoa do Sambico was created by Municipal Decree-Law 1754/2012 (Timon 2012). The park comprises an area of 80,502.25 m^2. Plant communities are dominated by herbaceous vegetation. It has a natural lagoon called Sambico (Rima 2011). Five water and soil samples were obtained every two months from August 2014 to May 2015, at four different points of the lagoon, for a total of 40 samples.

Isolation technique - The multiple baiting technique with cellulosic, chitinous and keratinous substrates was applied to isolate Oomycetes from the samples, following Milanez (1989), according to organic material is used as bait for Oomycetes colonization and culture. Water samples were collected from the lake surface with the aid of a plastic cup and then stored in wide-mouth sterile glass flasks (100 ml Wheaton vials), with perforated plastic cover to allow oxygenation of the water. Prior to collection, nine baits were added to each flask: cellulosic substrates (corn straw, sorghum seeds, onion skin, cellophane and paper filter) and chitinous (termite wings) as well as keratinous (snake skin and human hair) items, which served as organic colonization substrates. Soil samples were collected with the aid of a sterile metal spatula, and the surface layers were removed and about 250 g of soil were collected at depth of approximately 20 cm, then packed in 500 g polyethylene bags properly labeled with the respective collection points. The collected material was transported to the Mycology

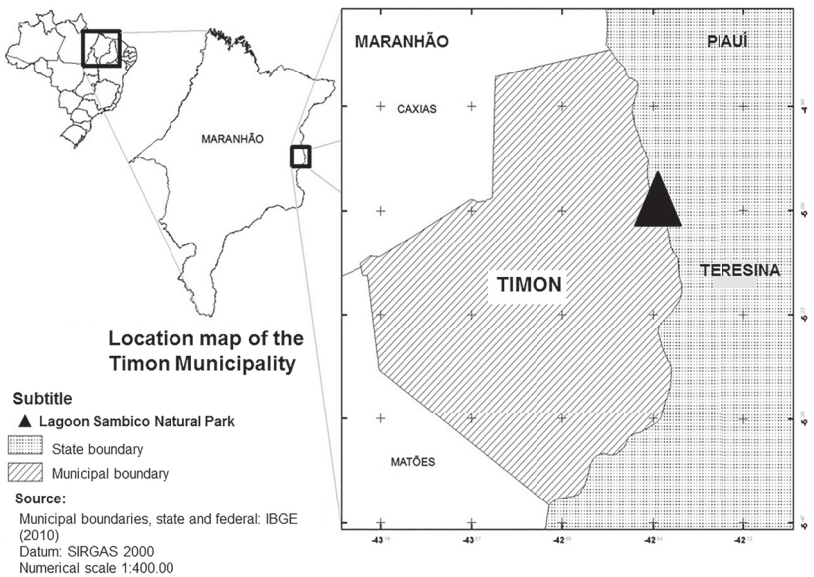


Figure 1. Geographical location of Parque Natural Municipal Lagoa do Sambico, Timon municipality, Maranhão State, Brazil.

Laboratory of Universidade Federal do Piauí (UFPI) for analysis. 30 ml from water samples were placed into Petri dishes (100 × 20 mm) with organic substrates. Approximately, 20 g from the soil samples were placed into in Petri dishes (100 × 20 mm) with sterile distilled water to decant. After decanting, cellulosic, chitinous and keratinous substrates were added to the plates. Then the plates were incubated with the samples at room temperature (30-32 °C) for a 7-day period.

We performed microscopic analysis of the samplings in order to assess the presence of Oomycetes, which were then placed into new Petri plates with the respective substrates to be colonized (cellulosic, chitinous or keratinous). Subsequently, sterile distilled water was added to each plate. The plates were incubated for seven days for the identification of developing fungal structures. The maintenance of the strains was done with the exchange of water and adding new substrates to each plate.

Vegetative and reproductive structures were recorded by using an optical microscope (Olympus BX41 model, Tokyo, Japan), and photographed with a Nikon digital camera (Coolpix - S4100). Taxonomic descriptions of isolates were done in accordance with pertinent publications (Sparrow 1956, Johnson Jr. 1960, Scott 1961, Dick 1990, Johnson *et al*. 2002). Selected strains were deposited in the mycology culture collection at Universidade Federal do Piauí (UFPI).

Results and Discussion

Beakes *et al.* (2014) carried out an up-to-date phylogenetic and taxonomic review of the Straminipila (Labyrinthulomycota, Hyphochytridiomycota, and Oomycota) based upon molecular sequence data, biology and evolutionary history. This taxonomic revision was adopted in the present study. We obtained 98 isolates of Oomycota, including 16 species, which belong to Achlyaceae (*Achlya aff. diffusa* J.V. Harv. *ex* T.W. Johnson, *A. proliferoides* Coker, *A. orion* Coker & Couch, *Brevilegnia linearis* Coker, and *Dictyuchus monosporus* Leitg.), Leptolegniellaceae s. lat. (*Leptolegniella keratinophila* Huneycutt), Pythiaceae s. lat. (*Globisporangium proliferum* (Cornu) P.M. Kirk, *Pythiogeton ramosum* Minden, *P. uniforme* A. Lund, *P. utriforme* Minden, and *Pythium graminicola* Subram.), Olpidiopsidaceae s. lat. (*Olpidiopsis aphanomycis* Cornu) Saprolegniaceae s. str. (*Aplanopsis terrestris* Höhnk, and *Saprolegnia luxurians* (Bhargava & G.C. Srivast) R.L. Seym.), Verrucalvaceae (*Aphanomyces helicoides* Minden, and *A. keratinophilus* Ôokubo & Kobayasi) (table 1).

Achlyaceae

Achlya aff. ***diffusa*** J.V. Harv. *ex* T.W. Johnson, The Genus *Achlya*: Morphology and Taxonomy: 64. 1956.
Figures 2-3

Zoosporangia abundant, 220-320 × 18-38 µm, filiform, irregular, sometimes curved. Gemmae abundant. Dilatations spherical or globular, 63-137 µm diâm., large, disposed between the oogonia. Oogonia abundant, 34-53 µm diâm., sessile, lateral, occasionally intercalary, spherical, terminal, globular, smooth-walled, usually with pores. Peduncle simple, 14-32 × 11-19 µm, straight, curved, or bent, not tapering towards the base. Oospores 19-25 µm diâm., eccentric, usually 2-6 per oogonium, spherical. Antheridia frequent, monoclinous and diclinous, persistent and long. Antheridial cells branched, not persistent, attached by projections.

Material examined: BRAZIL. Maranhão: Timon, Parque Natural Municipal Lagoa do Sambico, soil and water samples in the respective collection spots, 20-X-2014, *J.B. Silva* (A2/2-S2/2-S3/2-S4/2); 18-XII-2014, J. B. Silva (A2/3-S3/3-S4/3); 23-II-2015, *J.B. Silva* (S3/4-S4/4).

Geographical distribution in Brazil: Piauí: Parque Nacional de Sete Cidades (Rocha 2002).

Oogonia were abundant in the material, but not observed proliferating oogonia (figure 2), and the dilatations were very large (63-137 µm diâm.). These two characteristics are not in accordance with the findings by Rocha (2002), which found abundant proliferating oogonia, and dilatations with measurement lower (47-98 µm diâm.) (figure 3).

Achlya orion Coker & Couch, J. Elisha Mitchell Scient. Soc. 36: 100. 1920.
Figure 4

Zoosporangia 235-294 × 20-34 µm, filiform, fusiform, sympodial renewal. Zoospores encysted 10-12 µm diâm. Gemmae present. Oogonia 34-49 µm diâm., lateral or terminal, spherical, proliferating, smooth-walled; twisted stalk, some being straight. Oospheres 29-39 µm diâm. Oospores 19-24 µm diâm., eccentric, spherical, some oval, 1-4 per oogonium.

Table 1. Oomycetes families and species, according Beakes *et al*. (2014), found within Parque Natural Municipal Lagoa do Sambico, Timon municipality, Maranhão State, Brazil. (*) First record for Brazil. (**) First record for Maranhão State. Source: Direct research (2015).

Family/Species
Achlyaceae
Achlya aff. *diffusa* J.V. Harv. *ex* T.W. Johnson**
Achlya orion Coker & Couch
Achlya proliferoides Coker**
Brevilegnia linearis Coker
Dictyuchus monosporus Leitg.**
Leptolegniellaceae s. lat.
Leptolegniella keratinophila Huneycutt
Pythiaceae s. lat.
Globisporangium proliferum (Cornu) P.M. Kirk**
Pythiogeton ramosum Minden
Pythiogeton uniforme A. Lund**
Pythiogeton utriforme Minden
Pythium graminicola Subram.
Olpidiopsidaceae s.lat.
Olpidiopsis aphanomycis Cornu*
Saprolegniaceae s. str.
Aplanopsis terrestris Höhnk**
Saprolegnia luxurians (Bhargava & G.C. Srivast) R.L. Seym.*
Verrucalvaceae
Aphanomyces helicoides Minden**
Aphanomyces keratinophilus Ôokubo & Kobayasi

Antheridia androgynous and monoclinous, rarely diclinous; simple, some branched. Antheridial cells tubular, simple or branched.

Material examined: BRAZIL. Maranhão: Timon, Parque Natural Municipal Lagoa Sambico, soil and water samples at collection spots, 23-II-2015, *J.B. Silva* (S3/4-S4/4-A4/4); 20-V-2015, *J.B. Silva* (A4/5-S1/5-S2/5).

Geographical distribution in Brazil: Amazonas (Silva 2002); Minas Gerais (Oliveira 2004); Pernambuco (Cavalcanti 2001); Piauí (Rocha 2002); São Paulo (Beneke & Rogers 1962, Gomes *et al*. 2003, Lyra & Milanez 1974, Milanez 1970, Milanez *et al*. 1994a, Pires-Zottarelli 1990, 1999, Pires-Zottarelli *et al*. 1996b, Rocha & Pires-Zottarelli 2002).

Species characterized by eccentric oospores with pending oogonium stalk (figure 4). The characteristics of the isolates agree with the descriptions of Johnson (1956), Beneke & Rogers (1962), Pires-Zottarelli (1990, 1996, 1999), Rocha (2002) and Nascimento (2010). Gomes & Pires-Zottarelli (2008) reported the presence of larger spherical oogonia, ranging from 35 to 47.5 µm diâm. Rocha (2002) observed for the first time the formation of intercalary and sessile oogonia in this species. Such species is described as a second record for Maranhão State.

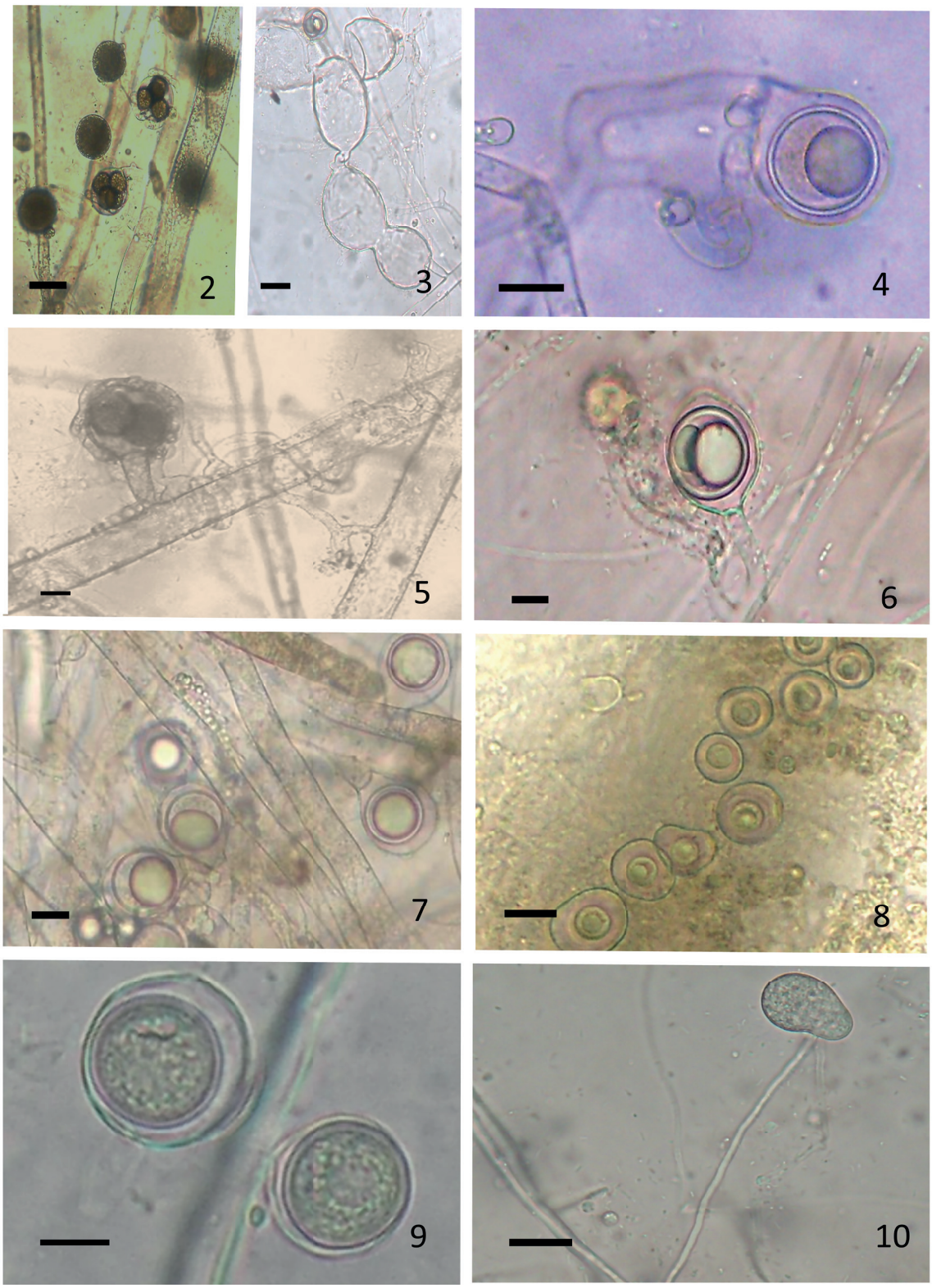
Achlya proliferoides Coker, Saprolegniaceae with notes on other water molds: 115. 1923.
Figure 5

Zoosporangia 259-343 × 19-34 µm, abundant, filiform or fusiform, straight or curved near the apex. Encysted zoospore 10-12 µm diâm. Gemmae abundant. Oogonia 47-55 µm diâm., lateral, spherical, smooth-walled, usually with pores. Peduncle straight smooth, rarely bent. Oosphere often not maturing. Oospore 22-27 µm diâm., spherical, eccentric; 3-8 per oogonium, often not filling the oogonium, abortive. Fertilization tube not observed. Antheridia with diclinous or monoclinous antheridial branches, irregular, branched, usually involving hyphae and oogonial peduncle and/or oogonia. Antheridial cells tubular, irregular, simple or branched, lateral attached or by projections.

Material examined: BRAZIL. Maranhão: Timon, Parque Natural Municipal Lagoa Sambico, soil and water samples at collection spots, 23-II-2015, *J.B. Silva* (S1/4-A4); 20-V-2015, *J.B. Silva* (S1/5-S3/5-S4/5).

Geographical distribution in Brazil: Piauí (Rocha 2002, Pereira 2008, Negreiros 2008, Trindade-Júnior 2013); Amazonas (Silva 2002); Pernambuco (Cavalcanti 2001); Minas Gerais (Beneke & Rogers 1962, Oliveira 2004); São Paulo (Rogers *et al*. 1970, Schoenlein-Crusius *et al*. 1990, Pires-Zottarelli 1999, Gomes *et al*. 2003, Pires-Zottarelli *et al*. 1996b, Rocha & Pires-Zottarelli 2002, Rocha 2004).

The measures of the structures were relatively lower than those reported by the original description by Johnson (1956) and Pires-Zottarelli (1999). However, the isolates agree in general with the data described by Rocha (2002), Nascimento & Pires-Zottarelli (2012) and Sousa (2014). The species has as a differential characteristic, which is the presence of antheridial branches involving hyphae, the stalk and the oogonium (figure 5). This is the first record for Maranhão State.



Figures 2-10. Species of oomycetes (Oomycota) from Parque Natural Municipal Lagoa do Sambico, Timon municipality, Maranhão State, Brazil. 2-3. *Achlya* aff. *diffusa*. 2.Oogonia young. 3. Globose dilatations. 4. *Achlya orion*, oogonium with eccentric oospore, antheridium androgynous, and twisted stalk. 5. *Achlya proliferoides*, oogonium with oospores and antheridial branches involving the main hyphae. 6. *Brevilegnia linearis*, oogonium with oospore eccentric. 7. *Dictyuchus monosporus*, oogonia with parthenogenetic oospores. 8. *Leptolegniella keratinophylla*, resistant spores. 9. *Globisporangium proliferum,* spherical oogonia with oospore hyaline. 10. *Pythiogeton ramosum*, bursiform zoosporangium. Bar: 10 µm.

Brevilegnia linearis Coker, J. Elisha Mitchell Scient. 42:214. 1927.
Figure 6

Zoosporangia 110-505 × 7-15 µm, long, curved or irregular. Zoospores displayed in rows. Gemmae absent. Oogonia spherical, 21-35 µm diâm., lateral or terminal, sometime with papilla. Oospores 1 per oogonia, sphaeric, eccentric, 12-14 µm diâm. Antheridium 1 per oogonia, antheridial branches androgenous.

Material examined: BRASIL. Maranhão: Timon, Parque Natural Municipal Lagoa do Sambico, soil and water samples at collection spots, 20-X-2014, *J.B. Silva* (A1/2- S3/2-S4/2); 23-II-2015, *J.B. Silva* (A1/4-S1/4-S3/4-S4/4).

Geographical distribution in Brazil: Piauí (Trindade-Júnior & Rocha 2013); Amazonas (Johnson *et al*. 2002); Minas Gerais (Oliveira 2004); São Paulo (Pires-Zottarelli & Milanez 1993); Maranhão (Sales 2009).

The isolate presented oospore eccentric (figure 6), and long zoosporangia, in agreement with Trindade-Júnior & Rocha (2013), which were identified as the first occurrence of *B. linearis* in Piauí State. The taxon was found in groundwater wells in Timon municipality by Sales (2009), being the first record for Maranhão State. This is the second record for this State.

Dictyuchus monosporus Leitg. Jb. Wiss. Bot. 7:374. 1870.
Figure 7

Primary zoosporangia produced in the extremity of the main hyphae. Sometimes zoospores germinating of the zoosporangium, leaving the primary cyst wall, forming real network with the wall of zoosporangium. Gemmae absent. Oogonia terminals, 30-45 µm diâm. or formed in single lateral branches, smooth, spherical, thin wall. Oospores 1 per oogonium, 19-35 µm diâm., spherical with a large distinctive lipid droplet, eccentric, wall 2 µm thick. Antheridia diclinous or androgynous or sometimes absent.

Material examined: BRAZIL. Maranhão: Timon, Parque Natural Municipal Lagoa do Sambico, soil and water samples at collection spots, 20-X-2014, *J.B. Silva* (S1/2-S3/2- S4/2-A/2); 18-XII-2014, *J.B. Silva* (S1/4-S3/4).

Geographical distribution in Brazil: Piauí (Pereira 2008, Negreiros 2008); Maranhão (Sales 2009).

It formed oogonia with whole oospores (figure 7). Antheridia diclinous, sometimes, absent. The species is described as a second record for Maranhão State.

Leptolegniellaceae s. lat.

Leptolegniella keratinophila Huneycutt, J. Elisha Mitchell Scient. Soc. 68: 110. (1952).
Figure 8

Micelium very branched, irregular hypha. Zoosporangia branched. Zoospore not observed. Resistance spores 10-16 µm diâm., spherical, internally formed hyphae, sometimes ovoid; with eccentric lipid droplet, thick wall.

Material examined: BRAZIL. Maranhão: Timon, Parque Natural Municipal Lagoa do Sambico, soil and water samples at collection spots, 17-VIII-2014, *J.B. Silva* (A2/1-S1/2-S2/2); 20-X-2014, *J.B. Silva* (S1/3-S2/3-S4/3); 18-XII-2014, *J.B. Silva* (S3/4-S4/4); 23-II-2015, *J.B. Silva* (A2/5-S1/5-S2/5).

Geographical distribution in Brazil: Amazonas (Silva 2002); Piauí (Sousa 2014, Rocha 2002); Pernambuco (Cavalcanti 2001); São Paulo (Milanez 1970, Pires-Zottarelli *et al.* 1996, 1999, Rocha & Pires-Zottarelli 2002); Maranhão (Sales 2009).

The isolate is characterized by resistant spores with thick wall (figure 8). Description of the isolate agrees with original description of the species by Huneycutt (1952) and the data reported by Rocha (2002), Sales (2009), Trindade-Júnior (2013) and Sousa (2014) in Piauí State. It is the second record for Maranhão State.

Pythiaceae s. lat.

Globisporangium proliferum (Cornu) P.P. Kirk, Index Fungorum 191:1. 2014.
Figure 9

Zoosporangia terminal or intercalar, ovoid, elipsoid, globose, with internal proliferation. Oogonia terminal or lateral, spherical, 15-25 µm diâm., oval. Sometimes with papilla. Oospores hyaline, spherical, 13-20 µm diâm., smooth-walled. Antheridia 1-2 per oogonium, monoclinous or hypogynous, sometimes sessile, simple or branched. Antheridial cell simples, clavate, attached apically to oogonium.

Material examined: BRAZIL. Maranhão: Timon, Parque Natural Municipal Lagoa do Sambico, soil sample at collection spots, 20-V-2015, *J.B. Silva* (S4/5).

Geographical distribution in Brazil: Piauí (Rocha *et al*. 2001); São Paulo (Pires-Zottarelli 1999).

Isolates produced typical spherical oogonia with oospore hyaline (figure 9). It agrees with the descriptions of Plaats-Niterink (1981), Pires-Zottarelli (1999) and Rocha (2002) (cited as *Pythium middletonii* Sparrow). Also, according to Plaats-Niterink (1981), its pathogenicity caused disease on seedlings of soybean (*Glycine max*), tomato (*Solanum lycopersicum*), and potato (*Solanum tuberosum*). This is the first record for Maranhão State.

Pythiogeton ramosum Minden, Falck, Mykol. Untersuch. Ber. 1:243. 1910.
Figure 10

Zoosporangia spherical, bursiform, 18-48 × 20-60 µm diâm., terminal. Internal proliferation observed, with a straight or winding release tube forming with the supporting hyphae a right angle. Zoospores encysted 8-11 µm diâm. Sexual reproductive structures not observed.

Material examined: BRASIL. Maranhão: Timon, Parque Natural Municipal Lagoa do Sambico, soil and water samples at collection spots, 23-II-2015, *J.B. Silva* (A4/4-S2/4- S4/4).

Geographical distribution in Brazil: Amazonas (Silva 2002); Pernambuco (Cavalcanti 2001); Piauí (Pereira 2008, Rocha 2002, Trindade-Júnior 2013). Rio de Janeiro (Beneke & Rogers 1970); São Paulo (Rocha & Pires-Zottarelli 2002); Maranhão (Sales 2009).

The characteristics observed corroborate the original description. This sample has a larger spherical zoosporangia (figure 10) than those described by Gomes and Pires-Zottarelli (2008) in which they reported 27-45 µm diâm. Analyses agree with descriptions of Rocha (2002), Negreiros (2008) and Pereira (2008). This is the second record for Maranhão State.

Pythiogeton uniforme A. Lund, Mém. Acad. Roy. Sci. Lett. Danemark, Copenhague, Sect. Sci., ser., 6:54. 1934.
Figure 11

Terminal zoosporangia in lateral long branches, spherical, 29-30 µm diâm., occasionally intercalary. Internal proliferation. Zoospores reniform, biflagellate. Sexual structures not observed.

Material examined: BRAZIL. Maranhão: Timon, Parque Natural Municipal Lagoa do Sambico, soil and water samples at collection spots, 23-II-2015, *J.B. Silva* (A2/4-A4/4-S1/4-S4/4).

Geographical distribution in Brazil: Piauí: (Rocha 2002, Negreiros 2008, Trindade-Júnior 2013).

Isolate produced zoosporangia spherical (figure 11). The data corroborate the original description. It was firstly reported in Brazil by Rocha (2002) in samples of water and soil from the Parque Nacional de Sete Cidades, and later found by Negreiros (2008), in Floriano municipality, and Trindade-Júnior (2013), in Teresina municipality; all in the Piauí State. It is the first record for Maranhão State.

Pythiogeton utriforme Minden. Falck, Mykol. Untersuch. Ber. 1:242. 1916.
Figure 12

Zoosporangia lateral, bursiform with internal proliferation, septum at the base separating it from the supporting hyphae. Thin release tube, long, originated near the base of zoosporangia, forming right angles with the axis of the supportive hyphae. Oogonia terminal, spherical, 33-35 µm diâm. Oospore spherical, 18-19 µm diâm., plerotic or aplerotic, colorless, very thick wall. Antheridia not observed.

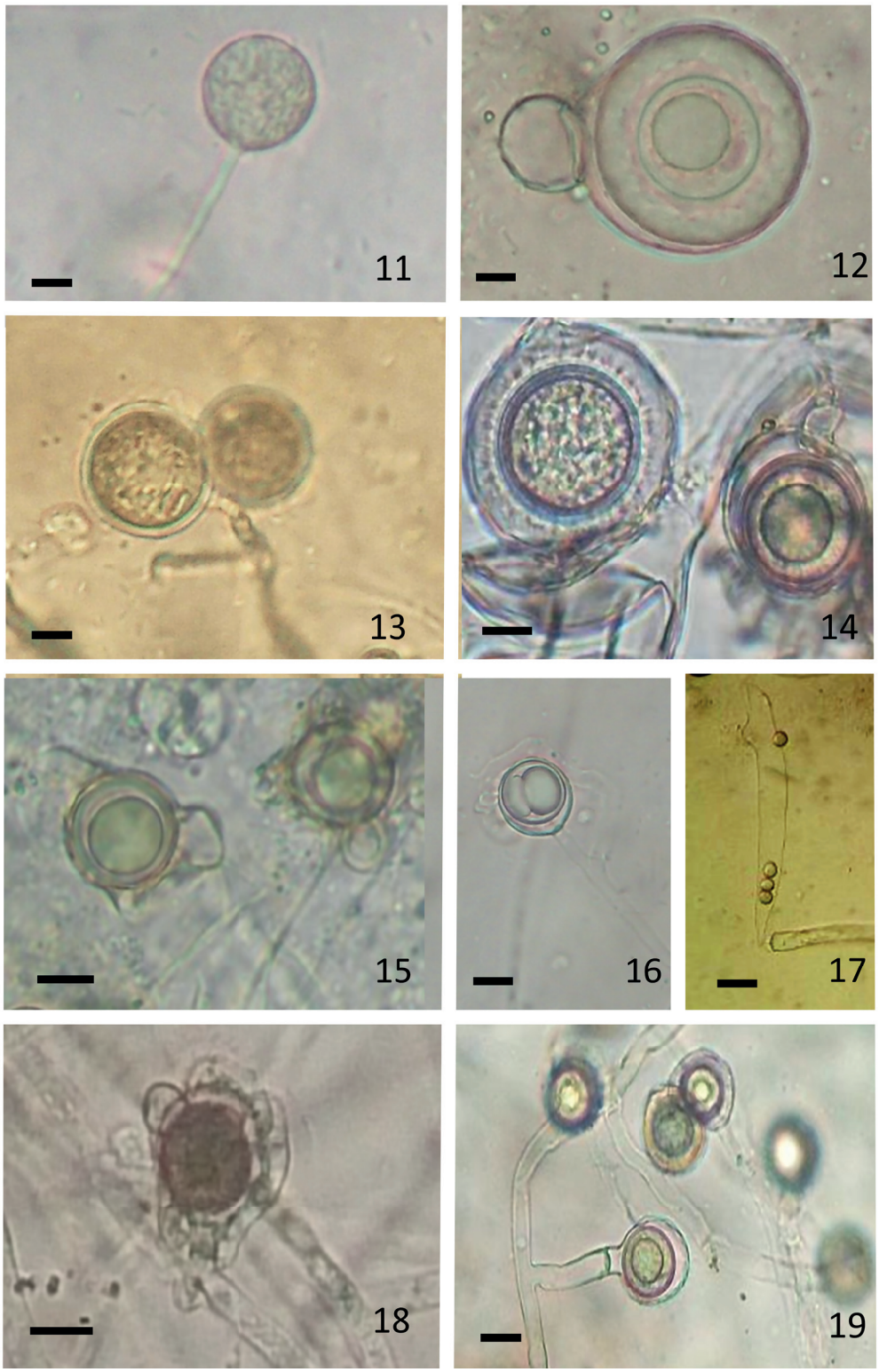
Material examined: BRAZIL. Maranhão: Timon, Parque Natural Municipal Lagoa do Sambico, soil samples at collection spots, 17-VII-2014, *J.B. Silva* (S1/1); 20-X-2014, *J.B. Silva* (S3/2).

Geographical distribution in Brazil: Piauí (Rocha 2002).

The data agree with those presented in the original description. They show a remarkable characteristic: oospores with extremely thick walls with irregular layers (figure 12) and oogonia which can proliferate and originate another oogonium internally with antheridia. This is first record for Maranhão State, and second for Brazil.

Pythium graminicola Subram., Bull. Agric. Res. Inst. Pus. 177:1. 1928.
Figure 13

Zoosporangia terminal or intercalary with filamentous inflated complex. Oogonium 22-26 µm diâm. terminal, globose, smooth. Oospores plerotic, 20-23 µm diâm. Antheridia 1-2 per oogonium, monoclinous, diclinous with apical attachment to oogonium.



Figures 11-19. Species of oomycetes (Oomycota) from Parque Natural Municipal Lagoa do Sambico, Timon municipality, Maranhão State, Brazil. 11. *Pythiogeton uniforme*, spherical zoosporangium. 12. *Pythiogeton utriformis*, spherical oogonium with oospore and very thick wall. 13. *Pythium graminicola*, oogonia with aplerotic oospore. 14. *Olpidiopsis aphanomyces*, oospore covered with conical thorns, parasitizing oogonium of *Aphanomyces keratinophillus* (left). Intact oogonium and oospore of *Aphanomyces keratinophillus* (right). 15. *Aplanopsis terrestris*, oogonia with lipid droplet. 16-17. *Saprolegnia luxurians.* 16. Oogonium with eccentric spores. 17. Zoosporangium with zoospores. 18. *Aphanomyces helicoides*, antheridial cells attached to young oogonium, and antheridial branches coiling about the peduncle of the oogonium. 19. *Aphanomyces keratinophyllus*, oogonia with oospore. Bar: 10 µm.

Material examined: BRAZIL. Maranhão: Timon, Parque Natural Municipal Lagoa Sambico, soil and water samples at collection spots, 20-X-2014, *J.B. Silva* (A2/2-S2/2-S4/2), 23-II-2015, *J.B. Silva* (S3/4-S4/4).

Geographical distribution in Brazil: São Paulo (Ghini *et al.* 2002, Baptista *et al.* 2004); Piauí (Rocha 2002); Maranhão (Sales 2009).

Isolate produced characteristic oogonia and zoosporangia with inflated complex (figure 13). The species is depicted according to the description of Plaats-Niterink (1981). It is highly pathogenic in crop plants such as corn (*Zea mays*), potato (*Solanum tuberosum*), beans (*Phaseolus vulgaris*), pineapple (*Ananas comosus*) and onion (*Allium cepa*). It is second record for Maranhão State.

Olpidiopsidaceae s.lat.

Olpidiopsis aphanomycis Cornu, Annls Sci. Nat., Bot., ser. 5(15): 148. 1872.
Figure 14

Zoosporangia 15-45 µm diâm., solitary or numerous; flat; hyaline or granular gray; oval, spindle-shaped, elongated, but generally spherical; release of one to several tubes that may extend beyond the host cell surface. Zoospore 6-8 µm diâm.; parthenogenetic or sexual; hyaline or brown; oval, spherical. Oospore 2-4 per oogonium, covered with conical thorns; granular content with one or more large refractive droplets. Companion cell or male cell absent.

Material examined: BRAZIL. Maranhão: Timon, Parque Natural Municipal Lagoa Sambico, soil samples at collection spots, 20-X-2014, *J.B. Silva* (S1/2-S2/2); 23-II-2015, *J.B. Silva* (S2/4-S3/4); 20-V-2015, J. B. Silva (S3/5, A2/5).

Geographical distribution in Brazil: Maranhão: this is first record to Brazil.

The examined cultures presented measurements within the variations observed by Cornu (1872), Karling (1944) and Sparrow (1960). *O. aphanomycis* parasite species *Aphanomyces laevis* (Butler & Bysby 1931) and *A. cladogamus* (Whiffen 1942). In *Aphanomyces keratinophillus* the parasite caused severe hypertrophy in the spots of the infected oogonium (figure 14). This record indicates the need for more sampling efforts in order to get a better understanding of diversity and geographical distribution of the species *Olpidiopsis* as well as their role in ecosystems. This new record rises to six species of *Olpidiopsis* recorded in Brazil (Milanez *et al.* 2007). It enlarges the distribution of *O. aphanomycis* in South America, where the nearest record was solely located in Buenos Aires, Argentina (Steciow 2012). This is the first record for Brazil.

Saprolegniaceae s. str.

Aplanopsis terrestris Höhnk, Veröff. Inst. Meeresf, Bremerhaven, Sonderband 1:127. 1952.
Figure 15

Mycelium diffuse, long hyphae. Zoosporangia not observed. Oogonia lateral or terminal, 24-30 µm diâm., with ornamented wall, spherical, globose or ovoid. Oospores subcentric, 18-24 µm diâm., spherical, oval, ellipsoid. Antheridia, when present, androgynous.

Material examined: BRAZIL. Maranhão: Timon, Parque Natural Municipal Lagoa do Sambico, soil and water samples at collection spots, 23-II-2015, *J.B. Silva* (S3/4-S4/4); 20-V-2015, *J.B. Silva* (A2/5-S1/5).

Geographical distribution in Brazil: São Paulo (Rocha *et al.* 2016).

Isolate produced oogonia with lipid droplet (figure 15). First record for Maranhão State and second record for Brazil.

Saprolegnia luxurians (Bhargava & G.C. Srivast) R.L. Seym. Nova Hedwigia 19 (1-2): 55. 1970.
Figures 16-17

Zoosporangia abundant, 150-190 × 19-28 µm, filiform, clavate; straight, rarely bent, renovation by internal proliferation, occasionally basipetalous succession, rarely by simpodial ramification. Zoospore encysted 8-11 µm diâm. Gemmae rare. Oogonia abundant, 32-35 µm diâm., lateral, rarely terminal or intercalary; spherical or subspherical, occasionally pyriform, rarely cylindrical; very rarely catenulate, rarely papillary wall. Oospore eccentric, 22-27 µm diâm., spherical or rarely ellipsoid almost filling the oogonium. Antheridial branch monoclinous or androgynous, often diclinous, rarely branched. Antheridial cell tubular, simple or compound.

Material examined: BRAZIL. Maranhão: Timon, Parque Natural Municipal Lagoa Sambico, soil samples at collection spots, 23-II-2015, *J.B. Silva* (S3/4-S2/5); 20-V-2015, *J.B. Silva* (A/3-A/4).

Geographical distribution in Brazil: Maranhão: This is first record for Brazil.

Isolate produced oogonia with eccentric spores (figure 16), and zoosporangium clavate (figure 17). The morphological data of this species agree with those presented in the original description.

Verrucalvaceae

Aphanomyces helicoides Minden, Krypt.-Fl. Brandenburg (Leipizig) 5:559. 1912.
Figure 18

Zoosporangia with achlyoid release. Oogonia 24-28 µm diâm., lateral or terminal, spherical, smooth-walled, sometimes it becomes wrinkled. Oospore 23-26 µm diâm., light brown, spherical, with thin walls, and a finely granular content with a large central oil globule. Antheridia diclinous or monoclinous. Antheridial cells 1-5 per oogonium.

Material examined: BRAZIL. Maranhão: Timon, Parque Natural Municipal Lagoa do Sambico, soil samples at collection spots, 20-X-2014, *J.B. Silva* (S3/2-S4/2).

Geographical distribution in Brazil: Amazonas (Silva 2002); Minas Gerais (Oliveira 2004); Piauí (Rocha 2002); São Paulo (Pires-Zottarelli 1990, Milanez *et al.* 2007, Miranda & Pires-Zottarelli 2012).

Isolate produced antheridia attached in oogonia (figure 18) and oospore occasionally hyaline but with a predominance of light brown color. Howard *et al.* (1970) assumed that *A. helicoides* and *A. laevis* were synonymous species. Currently, are not synonymous, second Species Fungorum (2016). This is first record for Maranhão State.

Aphanomyces keratinophilus (M. Ôkubo & Kobayasi) R.L. Seym. & T.W. Johnson, Mycology 65(6): 1317. 1974.
Figure 19

Zoosporangia long and filamentous, with achlyoid release of zoospore. Zoospores cylindrical or fusiform. Gemmae absent. Oogonia 16-28 µm diâm., lateral or terminals in variables length branches, spherical or pyriform, sometimes intercalary. Oospores spherical, 13-25 µm diâm., thin-walled, light brown, with the presence of a large lipid droplet in the center. Antheridia branched, androgynous and monoclinous, contorted and irregular.

Material examined: BRAZIL. Maranhão: Timon, Parque Natural Municipal Lagoa do Sambico, soil and water samples at collection spots, 17-VIII-2014, *J.B. Silva* (A1/1-A2/1-S1/1-S3/1); 20-X-2014, *J.B. Silva* (S1/2-S2/2-S3/2); 8-XII-2014, *J.B. Silva* (A2/3-S1/3-S2/3-S3/3); 23-II-2015, *J.B. Silva* (A3/5-A4/5-S1/5-S2/5).

Geographical distribution in Brazil: Piauí (Rocha 2002); Maranhão (Sales 2009).

Isolate produced typical oogonia with oospore (figure 19). The main distinguishing features of *A. keratinophilus* are the affinity for keratin substrates and abundant presence of antheridial branches involving oogonium profusely. Furthermore, the data agree with the descriptions by Rocha (2002), who presented the first description in Brazil, isolating soil samples from Parque Nacional de Sete Cidades, Piauí. This is the second record for Maranhão State.

The current anthropic pressure over ecosystems causes a strong environmental disturbance. Despite global commitments to reduce the impacts of human activities on ecosystems worldwide, loss of biodiversity and ecosystem exploitation continues rising. However, it is little known about the biodiversity of ecosystems in Brazil. Based upon this survey research, new records for Maranhão State and Brazil's Countrywide have been made. Most of the species' records presented herewith were commonly found worldwide (Marano *et al*. 2008, Steciow *et al*. 2012) and have been previously isolated in Brazil (*Gomes et al.* 2003, Gomes & Pires-Zottarelli 2008, Milanez *et al*. 2008, Nascimento & Pires-Zottarelli 2012). Moreover, many other studies were carried out in Piauí State (Negreiros 2008, Pereira 2001, Rocha *et al.* 2001, Sousa 2014, Trindade-Júnior 2013), but a single study in Maranhão State (Sales 2009).

These new records of Oomycetes occurrences enhanced the knowledge about such organisms in Brazil.

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