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Black yeast-like fungi associated with Lethargic Crab Disease (LCD) in the mangrove-land crab, *Ucides cordatus* (*Ocypodidae*)

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ABSTRACT

Lethargic Crab Disease (LCD) caused extensive epizootic mortality of the mangrove land crab *Ucides cordatus* (*Brachyura: Ocypodidae*) along the Brazilian coast, mainly in the Northeastern region. The disease was named after the symptoms of slow movement of infected crabs. Causative agents were suspected to be two black yeast-like fungi of the family *Herpotrichiellaceae* (ascomycete order *Chaetothyriales*), judged by infected tissue biopsies from moribund *U. cordatus*. In the present study, two species were proven to be involved in the disease: the recently described black yeast *Exophiala cancerae*, and a hitherto undescribed fonsecaea-like species, introduced here as *Fonsecaea brasiliense*. Strains were identified by ITS rDNA sequencing, and species borderlines were established by multilocus sequencing and AFLP analysis. The species proved to be closely related to the pathogenic species *Cladophialophora devriesii* which originally was isolated from systemic infection. The virulence of both species was established by artificial inoculation of mangrove crabs.

Keywords:
Ucides cordatus
Mangrove crab
Lethargic Crab Disease (LCD)

Exophiala cancerae

Cladophialophora

Fonsecaea

Virulence

1. INTRODUCTION

Since the year 1997 an extensive epizootic causes high mortality of the mangrove-land crab *Ucides cordatus* (*Brachyura: Ocypodidae*) along the Brazilian coast. The disease was termed Lethargic Crab Disease (LCD) by Boeger et al. (2005) because of clinical signs of ataxia observed in moribund organisms. The histopathology of crabs in diverse stages of development of the disease shows that the most affected tissues are the epidermis, connective tissues, heart, and hepatopancreas, and that the fungus disseminates hematogenously (Boeger et al., 2007). The main signs of disease include increasingly weak motor control, particularly of pereiopods and chelae, causing lethargy and poor balance, followed by the death of the affected crab. Tetany of the claws is also observed in many crabs with other signs of the disease.

Several potential causes and etiological agents of LCD have been informally proposed over the years, including viruses, bacteria, environmental pollution, and substances used in shrimp farming. Despite the importance of the crab species for low-income populations in the coastal regions of Brazil, only few scientific studies addressing LCD are available (e.g. Pie et al., 2011; Orélis-Ribeiro et al., 2011; Boeger et al., 2005). All evidence from a variety of sources (light and electron microscopy, behavioral and experimental tests, and molecular phylogenetics) indicate that LCD signs were associated with the presence of a black yeast-like fungus, *Exophiala cancerae* (de Hoog et al., 2012). However, occasional co-infection may take place by another black yeast-like fungus, an as yet unknown species of *Fonsecaea* or *Cladophialophora*.

Exophiala, Cladophialophora and *Fonsecaea* are members of the ascomycete order *Chaetothyriales*, comprising the black yeasts and allies, which are regularly encountered as causative agents of disorders in humans and animals (Nyaoke et al., 2009; de Hoog et al., 2012;

Kano et al., 2000). In humans, infections by chaetothyrialean fungi range from commensalism or mild cutaneous infection to fatal neurotropism with severe mutilation. All infections are preponderantly found in patients without immune disorder or underlying metabolic disease. Outside humans, particularly cold-blooded waterborne vertebrates are susceptible to a diversity of *Exophiala* species (de Hoog et al., 2012). Some of these are host-specific to particular fish taxa (Nyaoke et al., 2009). As virulence factors the ability to assimilate alkylbenzenes, present in sweat and nervous tissues of mammals and in the toxic skin of amphibians, has been suggested (Prenafeta-Boldú et al., 2006; de Hoog et al., 2012).

Many reports of epizootics from the older literature clearly demonstrate that black yeast infection is a relatively common phenomenon in cold-blood vertebrates (e.g., Fijan, 1969; Richards et al., 1978; Engjom et al., 1983; Reuter et al., 2003). Etiologic agents were often not preserved and were only identified by morphology. However, ITS-sequencing is a prerequisite for correct identification of black yeast species. Recent studies have shown that many different pathogenic species are involved (e.g., Manharth et al., 2005; Nyaoke et al., 2009; de Hoog et al., 2012) which are morphologically very similar. Vakili (1993) was the first to isolate an *Exophiala* species from invertebrate hosts (earthworms); recently Dover et al. (2007) reported on an epizootic in mussels by a *Capronia* species. The mangrove crab epizootic (Boeger et al., 2005, 2007) underlines the ability of black yeasts to infect a larger spectrum of animals.

The present study clarifies the etiology of the disseminated LCD in mangrove-land crabs, describes the epidemiology of the disease and establishes the relative pathogenicity of the agents. The undescribed fonsecaea-like species is introduced here as a novel taxon.

2. MATERIALS AND METHODS

2.1. Fungal strains

A total of 84 strains (Table 1) were analyzed, the set including three isolates of *Exophiala cancerae* and four of an unknown fonsecaea-like species from crab, 24 unidentified environmental isolates, and reference strains from the collection of the Centraalbureau voor

Schimmelcultures (CBS) Fungal Biodiversity Centre, Utrecht, The Netherlands. Stock cultures were maintained on slants of 2% malt extract agar (MEA) and oatmeal agar (OA) at 24°C.

2.2. Microscopy

For morphological studies, MEA slide cultures were prepared and mounted in lactophenol cotton blue. Agar blocks (MEA) of 1 cm^2 were placed on a sterile object glass supported by a V shaped glass bar and inoculated at the four sides. The block was subsequently covered with a sterile cover slip (2 cm^2). Growth was allowed in a closed glass Petri dish; the bottom was covered with sterile filter paper soaked with 5 ml sterile water to ovoid drying of the culture. The chambers were incubated at room temperature for 5, 10 or 14 days. Slides were prepared with lactic acid. Permanent slides were sealed with polyvinyl alcohol. Micrographs were taken using a Nikon Eclipse 80i microscope and DS Camera Head DS-Fi1/DS-5m/DS-2Mv/DS-2MBW using NIS-Element freeware package (Nikon Europe, Badhoevedorp, The Netherlands).

2.3. Physiology

Cardinal growth temperatures were determined on 2% MEA plates incubated in the dark for 3 weeks at temperatures of 12–36°C at intervals of 3°C; growth was also recorded at 37°C and at 40°C.

2.4. DNA extraction

Approximately 1 cm^2 of 14 to 21-day-old cultures was transferred to a 2 ml Eppendorf tube containing 400 µL TEx buffer (pH 9.0) and glass beads (Sigma G9143). The fungal material was homogenized with MoBio vortex for 1 min. Subsequently 120 µL SDS 10% and 10 µL proteinase K were added and incubated for 30 min at 55°C, the mixture was vortexed for 3 min. After addition of 120 µL of 5 M NaCl and 1/10 vol CTAB 10% (cetyltrimethylammonium bromide) buffer, the material was incubated for 60 min at 55°C. Then the mixture was vortexed for 3 min. Subsequently 700 µL SEVAG (chloroform:isoamylalcohol, 24:1) was mixed carefully

by hand and centrifuged for 5 min at 4°C at 20,400 × *g*. The supernatant was transferred to a new Eppendorf tube with 225 µL 5 M NH_4-acetate, mixed carefully by inverting, incubated for 30 min on ice water, and centrifuged again for 5 min at 4°C at 20,400 × *g*. The supernatant was then transferred to another Eppendorf tube with 0.55 vol isopropanol and centrifuged for 5 min at 20,400 *g* force value. Finally, the pellet was washed with 1000 µL ice cold 70% ethanol. After drying at room temperature, it was resuspended in 100 µL TE buffer (Tris 0.12% w/v, Na-EDTA 0.04% w/v).

2.5. Amplification

Fragments of rDNA were amplified using the universal primers V9G and LS266 (Gerrits van den Ende & de Hoog, 1999) for rDNA Internal Transcribed Spacer (ITS) and Large Subunit (LSU) rDNA, NS1 and NS24 for Small Subunit (SSU) rDNA, EF1-728F and EF1-986R for partial Translation Elongation Factor 1-α (*TEF1*), Bt2a and Bt2b for partial β-tubulin (*BT2*) and act-fw and act-bw, eventually combined with EspACTbw for Actin (*ACT1*) in a reaction mixture containing 30 µL sterile water, 5 µL PCR buffer 10×, 10 µL dNTP's (1 mM each), 1 µL of each of the primers (50 pmol / µL, or increased for degenerate primers), 1 µL DNA polymerase 1 U / µL and 1 µL fungal DNA. Thirty five cycles were performed in a GeneAmp, PCR System 9700 (Applied Biosystems), with 5 min delay, and 35 cycles of 94°C 45 s (denaturation), 52°C 30 s (annealing) and 72°C 120 s (extension), with a final delay of 7 min and using the maximum ramp speed for ITS amplification. For SSU the annealing temperature was lowered to 48°C and for *TEF1* amplification raised to 55°C. Five µL of each PCR product, with 2 µL loading buffer, was electrophoresed in 1% agarose gels with $0.5-10^{-5}$ (v/v) ethidium bromide in TAE 1× buffer [200 ml TAE 50× (BioRad: 242 g Tris, 57.1 mL acetic acid, 100 ml, 0.5 M EDTA) mixed with 9800 mL ultrapure water] at 80−100 V for 90 min, and using 5 µL Smart Ladder (Eurogentec, Seraing, Belgium) as marker. Amplicon quality and concentration were estimated on agarose gels (1−1.2 %) and photographed. Amplicons were cleaned using GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences). Sequencing reactions were performed using ITS1 and ITS4 for ITS sequences, EF1-728F, EF1-986R for *TEF1*, Bt2a-Bt2b for *BT2* , ACTfw-ACTbw otherwise combined with EspACTbw for *ACT1* and BF83, Oli1, Oli9, BF951, BF963, BF1438, Oli3 and BF1419 for SSU (de Hoog et al., 2005), following protocols for DYE-ET terminator

cycle sequencing. Reaction mixtures varied with the sample, as follows: 1 µL template DNA (0.1 pmol), 1 µL primer (4 µM), 1 µL sequencing reagent premix, 3 µL dilution buffer completed with 5.5−x µL ultra pure water to 10 µL final volume. Reactions were performed in a GeneAmp in twenty-five cycles of: 95°C for 20 s, 50°C for 15 s, 60°C for 60 s and stopped with cooling to 4°C. Samples were purified with Sephadex G-50 Superfine into a 96 wells of a MultiScreen HV plate and recovered in a standard 96-well microtiter plate. This elution plate was covered with aluminium foil tape (3M Scotch 431, 75 mm) and directly loaded on the ABI 3700 machine for sequence reading or stored at –20 °C.

2.6. Alignment and phylogenetic reconstruction

Sequences were edited using SEQMAN II in the Lasergene software (DNASTAR, Wisconsin, U.S.A.). Iterative alignment was performed automatically and adjusted by hand with BIONUMERICS version 4.61 (Applied Maths, Sint-Martens-Latem, Belgium). The tree was constructed with maximum Likelihood implemented in RaxML version 7.0.4 (Stamatakis, 2008). The model parameters were estimated by RAxML. The GAMMA model of rate heterogeneity up to an accuracy of 0.1000000000 LogLikelihood units with 1000 bootstrap value replicates was used and edited with Mega-4 software (Tamura et al., 2007). The SSU sequences were compared in a large database maintained at CBS for research purposes.

2.7. AFLP analysis

Fifty ng of genomic DNA was adjusted to a combined restriction ligation procedure containing 50 pmol of HpyCH4 IV adapter, 50 pmol *Mse*I adapter, 2 U of HpyCH4 IV (New England Biolabs, Beverly, MA, U.S.A.), 2 U of Mse I (New England Biolabs), and 1 U of T4 DNAligase (Promega, Leiden, The Netherlands) in a total volume of 20 µL of 1× reaction buffer for 1 h at 20°C. Next, the mixture was diluted five times with 10 mM Tris-HCl (pH 8.3) buffer. Adapters were made by mixing equimolar amounts of complementary oligonucleotides (5'-CTCGTAGACTGCGTACC-3' and 5'-CGGGTACGCAGTC-3' for HpyCH4 IV; 5'-GACGATGAGTCCTGAC-3' and 5'-TAGTCAGGACTCAT-3' for *Mse*I) and heating to 95°C, subsequently cooling slowly to ambient temperature. One µL of the diluted restriction-ligation

mixture was used for amplification in a volume of 25 μL under the following conditions: 1 μM HpyCH4 IV primer with one selective residue (underlined) (5'-Flu-GTAGACT GCGTACCCGTC-3'), 1 μM *Mse*I primer with four selective residues (underlined) (5'-GATGAGTCCTGACTAATGAG-3'), 0.2 mM of each deoxynucleoside triphosphate, and 1 U of *Taq* DNA polymerase (Roche Diagnostics, Almere, The Netherlands) in 1× reaction buffer containing 1.5 mM $MgCl_2$. Amplification was done as follows. After an initial denaturation step for 4 min at 94°C in the first 20 cycles, a touchdown procedure was applied: 15 s of denaturation at 94°C, 15 s of annealing at 66°C, with the temperature for each successive cycle lowered by 0.5°C, and 1 min of extension at 72°C. Cycling was then continued for a further 30 cycles with an annealing temperature of 56°C. After completion of the cycles, incubation at 72°C for 10 min was performed before the reaction mixtures were cooled to room temperature. The amplicons were then combined with the ET400-R size standard (GE Healthcare, Diegem, Belgium) and analyzed on a Mega BACE 500 automated DNA platform (GE Healthcare) according to the manufacturer's instructions. Data was inspected visually and was also imported in BIONUMERICS v. 5.1 software (Applied Maths) and analyzed by UPGMA clustering using the Pearson correlation coefficient. The analysis was restricted to DNA fragments in the range from 60−250 bp.

2.8. Experimental infection

Pathogenicity tests were done according to Orélis-Ribeiro et al. (2010). *Ucides cordatus* specimens of comparable age and weight were housed together for one week prior to treatment to ensure acclimation and absence of overt disease. Healthy specimens of the crab *U. cordatus* (5.8−7.8 cm total carapace width) were collected from Ilha das Peças (Antonina Bay, State of Paraná, Brasil), outside the region affected by LCD (Fig. 1). Due to ecdysis period, some months (September till November) were avoided for this procedure. The experimental facility consisted of series of 50 liter plastic aquaria with seawater (15 liter, 25 ppt salinity), permanent aeration (Schulz air compressor), controlled temperature (around 25ºC) and photoperiod (12 h light:12 h dark). Crabs were held individually and fed fish weekly (ad libitum); 50% of the seawater was changed every few days.

Two weeks prior to the inoculation experiment, fresh subcultures of *Exophiala cancerae* CBS 120420 (T) and *Fonsecaea brasiliensis nob*. CBS 119710 (T) were plated on Brain Heart Infusion Agar (BHI) and incubated at 25ºC to obtain active cultures. Conidia and hyphal elements collected from the surface of fresh colonies were resuspended by shaking on a Vortex laboratory shaker in saline solution (2.5%, matching *U. cordatus* hemolymph physiological salinity) (Harris & Santos, 1993) with 1% Tween®20 (Promega, Madison, WI, U.S.A.). Subsequently, the suspension containing 2×10^7 elements per mL, counted in a Neubauer Chamber (EMS, Munich, Germany) with an Olympus BX51 phase contrast microscope (Olympus, Zoeterwoude, The Netherlands) at 400×, was prepared for inoculation (adapted from Iwatsu et al., 1981).

A total of 26 crabs were used for the experiment using non-continuous infections. The crabs were divided in 3 groups per strain of fungus evaluated (1 treatment and 2 controls): (1) 10 specimens were injected with 1 mL inoculum solution; (2) 8 specimens were submitted to injection of 1 mL of saline solution (2.5%) and; (3) remaining 8 crabs were held in the experimental aquaria without manipulations, after having suffered the needle inoculation stress (without any solution). Injections (Injex syringe, 5 mL) were given in the arthrodial membrane at the juncture of the pereiopod basis and carapace, according Boeger et al. (2007). Experiment replicates were accomplished in the same way, just differing in the number of times that inoculum solutions were injected, rose in continuous infections (executed on days 1, 14 and 20). For the mortality rate study, the health status of the infected crabs were monitored twice daily, turning each individual crab upside down and measuring the time (in seconds) until return to the upright position (Boeger et al., 2005, 2007). Gills, heart, hemolymph, hepatopancreas, and thoracic ganglion samples were collected through dissection from dead or sacrificed (euthanized in iced freshwater after 30 days, at experiment conclusion) crabs. Re-isolation of fungus from dead crab tissues during experiments was carried through culturing in Mycosel medium (Pronadisa Conda, Madrid, Spain) for the period of two weeks at 25ºC. Samples of these isolates were still subjected to specific PCR-based markers for confirmation of the identity of the causative agent.

3. RESULTS

3.1. Molecular identification and phylogeny

Agents of Lethargic Crab Disease (LCD) at the mangrove coast of Brazil were isolated from direct culturing of tissues (hepatopancreas and heart) from moribund mangrove crabs (*Ucides cordatus*) showing symptoms of the disease. After isolation and ITS sequencing, two different fungi were encountered and identified. The first was found to be identical to *Exophiala cancerae*. This fungus was isolated from crabs in all stages of the disease in the study area. The isolates from hepatopancreas and heart of moribund crabs are listed in Table 1. In two cases, a co-infection by a second black yeast-like fungus identified as *Fonsecaea* sp. was noticed. Four four available isolates of a *Fonsecaea* species originated from different crab tissues were studied (Table 1).

With SSU/LSU rDNA data and comparison with a reference alignment of *Chaetothyriales* available at CBS, the fonsecaea-like species and *Exophiala cancerae* proved to be located in different clades. The isolates of *E. cancerae* belonged to a clade of waterborne *Exophiala* species which were potentially able to cause infection diseases in cold-blooded animals (Fig. 2). The isolates of the unknown fonsecaea-like species obtained from crab tissues were located in a different clade which also included *C. devriesii* and *C. bantiana*. *Cladophialophora devriesii* was originally described from a fatal disseminated infection in an otherwise healthy Caribbean woman (Gonzalez et al., 1984), while *C. bantiana* is an important agent of human brain infection (de Hoog et al., 2009).

The tree based on rDNA ITS and *BT2* sequencing data (Fig. 3) was built by Maximum Likelihood implemented in RaxML version 7.0.4. In ITS the proportion of gaps and completely undetermined characters in this alignment was 0.099960 with 371 distinct alignment patterns using the substitution model GTR. The Empirical Base Frequencies were pi (A): 0.225551, pi(C): 0.286770, pi(G): 0.239619, pi(T): 0.248060, with 1000 rapid bootstrap inferences. Four environmental isolates of the *Fonsecaea* species were available (Table 1) which had been isolated from the mangrove crab and its direct environment as reported in an earlier study (unpublished data). The strains composed a group separate from *C. devriesii* which was supported by a high bootstrap value (91%; Fig. 3). *Exophiala cancerae* was not isolated from

mangrove environment; it was exclusively found in moribund crabs. A multilocus analysis using sequences of two genes confirmed the results of more widely sampled ITS sequencing, the fonsecaea-like species isolated from crab belonging to a group separate from *C. devriesii* (Fig. 3).

The AFLP profiles of the unknown *Cladophialophora* species were compared to other *Cladophialophora* and *Fonsecaea* species with close phylogenetic affinity, as judged from SSU phylogeny data (de Hoog et al., 2012). Profiles of four strains of the fonsecaea-like species from crab were strictly identical, and clearly different from those of *Cladophialophora* species including *C. devriesii* (ex-type: CBS 147.84), but also from some strains from plant debris in the Brazilian rainforest (Fig. 4). This matched with sequence data (Fig. 3) where heterogeneity within the fonsecaea-like species was noted. Some isolates from mangrove surrounding the crabs in part were identical to crab strains and in part to plant debris strains, and thus are likely to represent the same species. The *C. saturnica* strains (ex-type: CBS 118724) which had been isolated from human infection and from soil polluted hydrocarbon segregated in a separate cluster. The remaining taxa *C. immunda* (ex-type strain: CBS 834.96) originating from clinical sources (CBS 834.96 and CBS 123977) and from environmental samples (CBS 109797 and CBS 110551) clustered together and completely distinct from the fonsecaea-like species.

3.2. Pathogenicity

The maximum growth temperature of three isolates of the fonsecaea-like species from crab tested is 36°C, with a preferred range at 27−30°C. The diameter of colonies observed of environmental isolates after 21 days at 30°C is 30 mm, which is higher than strains of *C. devriesii* (ex-type: CBS 147.84) which under the same conditions is 15 mm (Fig. 5). The maximum growth temperature of *Exophiala cancerae* is 33°C, with absence of growth at 37°C and optimal growth at 24−27°C (de Hoog et al., 2012).

Experimental infection of *Exophiala cancerae* as well as the unknown fonsecaea-like species showed that both species are able to cause infection in healthy crabs. During 29 days following infection, no mortality was observed among crabs artificially infected with the solution containing elements of the fonsecaea-like species. Mortalities were observed on day 29 (20%; n = 2) and on day 33 (30%; n = 3) (Fig. 6). No mortalities were observed in the control group

subjected solely to the stress of needle injection, while just one single death was recorded in the group inoculated with saline solution. Approximately two weeks after plating the collected tissues from crabs that died during the experiment in Mycosel medium, resulting colonies depicted characteristics of the fonsecaea-like species. The identity of the species was verified by morphology and by specific markers of PCR applied to extracts of the recovered colonies (Fig. 7).

4. DISCUSSION

The Lethargic Crab Disease has been reported to cause extensive population depressions in the mangrove-land crab, which represents an important fishery resource for artisanal exploitation by local communities along the Brazilian coast (Alves & Nishida, 2003; Schmidt, 2006). The impact of this infirmity is likely to be severe also due to the ecological importance of the species to West Atlantic mangroves, being responsible for the accelerated decomposition of mangrove litter and thus for nutrient remineralisation and energy transfer into the sediment (Nordhaus, 2003).

Two species are presently associated with the LCD-crab infection, both members of the ascomycete order *Chaetothyriales*. Judging from analysis based on conserved SSU and LSU rDNA genes, *E. cancerea* and an undescribed fonsecaea-like species are located in different clades within the order. *Exophiala cancerea* was recently described by de Hoog et al. (2012) as a member of a clade with prevalently waterborne *Exophiala* species that are potentially able to cause infection in cold-blooded animals. Numerous reports are available describing the occurrence of systemic or superficial infections by meso- to psychrophilic *Exophiala* species in animals such as fish, turtles, seahorses and -dragons, frogs, and mussels (de Hoog et al., 2012; Nyaoke et al, 2009; Dover et al., 2007; Sanchez-Camara et al., 2006). Several *Exophiala* species are relatively host-specific (Nyaoke et al, 2009; de Hoog et al., 2012).

Exophiala cancerae was present in all crabs exhibiting symptoms of Lethargic Crab Disease from all localities where disease outbreaks was investigated. Further, a recent study of artificial infection demonstrated the high pathogenicity of the species and its ability to cause the

disease with comparable clinical signs (Orélis-Ribeiro et al., 2011). Thus, *E. cancerae* is considered to be the main agent of LCD. In a recently completed but unpublished study (R. Schier Guerra, pers. comm.) *E. cancerae* could not be detected from environmental niches of mangrove habitats with a history of LCD outbreaks. This result would suggest that the species is strongly limited to the tissues of the mangrove land crab. De Hoog et al. (2012) reported the same species from water in Germany and in The Netherlands, from a diseased toad in Israel, from several clinical samples in Europe (primarily skin and nail) and from a fruit drink in Australia. A global distribution of the species is, thus, apparent. Despite the large scale of the LCD outbreaks, up to 90% of crabs dying from the disease in certain localities, the world wide occurrence of the species (de Hoog et al., 2012) suggests that it may have been present in Brazil prior to the beginning of the epizootic in 1998. Indeed, reports from elder fishermen of regions distant more that 1500 km from each other along the coast of Brazil indicate the occurrence of extensive mortality events of mangrove-land crabs more than 50 years ago. Assuming that the species has a wide distribution, changes in host or in environmental conditions, rather than emergence of virulent fungal genotypes, are likely causes of the epizootic. The natural niches of the etiologic species of LCD remain unknown.

Recently, Dover et al. (2007) reported an emergent epizootic event occurring in mussels (*Bathymodiolus brevior*) at the Fiji Basin (New Zealand). The causative agent was identified as the black yeast *Capronia* sp. A partial SSU sequence was provided (DQ314803.1), which is 98.8% identical to the type of *Exophiala angulospora*, the purported anamorph of *Capronia coronata*, a waterborne black yeast (de Hoog et al., 2012). Dover et al. (2007) suggested that the outbreak in dense invertebrate communities has the potential to be a major determinant of community change in deep-sea chemosynthetic ecosystems. The possibility that submersible assets may serve as vectors for transport of the fungus warrants further attention. Investigation of the occurrence of *E. cancerae* for example in metazoan organisms may acquire understanding of the dispersive form and probable routes of infection.

The fonsecaea-like species was occasionally encountered co-infecting mangrove crab and contributing to LCD. However, it could not be established whether severity of the disease increased with double infections. A bilocus sequence analysis including ITS and *BT2*, as well as an AFLP comparison with related species indicated that the species of *Fonsecaea* detected in crabs depicting clinical signs of LCD represents a new taxon. The new species is sibling of *C.*

devriesii, which was originally described from a fatal, chronic disseminated case on the Caribbean Grand Cayman Island (Gonzalez et al., 1984; Mitchell et al., 1990). The two species differed phenetically in the ability to grow at 37°C and show slight morphological differences, particularly in the length of the conidial chains.

Several older reports on infections in cold-blooded animals are available which describe melanized fungi with conidia in chains. It is quite possible that among the agents of disease in cold-blooded animals published as '*Cladosporium*' (Reichenbach-Klinke, 1956; Silphaduang et al., 2000; Bube et al., 1992) or '*Fonsecaea*' (Velázquez & Restrepo, 1975) our fonsecaea-like species or a related fungus was reported, but unfortunately no material was available for comparison. Sequencing of voucher specimens is necessary to achieve reliable identification. Cicmanec et al. (1973) and Beneke (1977) described several outbreaks in laboratory-housed toads and frogs (*Bufo marinus* and *Rana pipens*), ascribed to *Fonsecaea pedrosoi* and *F. compacta. Fonsecaea* species were also described by Velázquez & Restrepo (1975) in marine toads. The tissue form of all these cases consisted predominantly of muriform cells. Bube et al. (1992) described recurrent disseminated and neurotropic infections in captive marine toads (*Bufo marinus*), ascribed to *Cladosporium cladosporioides*. Silphaduang et al. (2000) reported on a *Cladosporium*-like infection in a tomato clownfish (*Amphiprion frenatus*) maintained in captivity but originating from a tropical marine farm in Puerto Rico. Elkan & Philpot (1973) described cutaneous and visceral infections in frogs (*Hyla caerulea*) caused by a black fungus reported as '*Phialophora* sp.'; probably an *Exophiala* was concerned.

The experimental model of infection adopted by this study allowed the artificial reproduction of clinical signs of LCD in animals inoculated with the fonsecaea-like species. The lower virulence of the fonsecaea-like species demonstrated in the laboratory assay may explain its behavior in nature as a secondary invader, probably taking advantage of weakened crabs previously affected by *E. cancerae.* From our data it appears that isolates infecting crabs represent a single genotype within the species. The fonsecaea-like species is abundantly present in the crab habitat, whereas *E. cancerae* could never be isolated or detected in the environment (Table 1). The optimum growth temperature of *E. cancerea* is 24−27°C, with a maximum at 33°C, while the maximum of the fonsecaea-like species lies at 37°C, close to that of *C. devriesii*. This suggests that temperature tolerance could be an important factor determining host preference.

The unknown fonsecaea-like species was compared with other *Cladophialophora* and *Fonsecaea* species which are supposedly related judging from SSU phylogeny data (Fig. 2; de Hoog et al., 2012). The dendrogram of AFLP profiles comprising ex-type strains of *Cladophialophora devriesii*, *C. saturnica* and *C. immunda* demonstrated that our fonsecaea-like isolates from crab are closely similar to each other and significantly distinct from all species of *Cladophialophora* sequenced to date (Fig. 4). Bilocus sequence comparison using ITS and *BT2* (Fig. 3) showed that the fonsecaea-like species from mangrove land crab, environmental isolates from the same mangrove area, environmental isolates from dead leaves and bark in Brazil, and a strain from skin composed a clearly delimited group with the ex-type of *Cladophialophora devriesii*, and was confirmed by AFLP profiles (Fig. 4). Hence the strains are judged to represent a novel species. The species proved to be somewhat heterogeneous, but analyzed strains from mangrove crabs were strictly identical with sequence and AFLP data. Several mangrove strains and environmental isolates from land plants had the same genotype (Table 1). Two mangrove strains were deviating and clustered with environmental isolates from Icatu city (Table 1).

The generic attribution of the new taxon is problematic. Morphologically it may be classified in *Fonsecaea*, given the brown and thick-walled hyphae supporting short, branched chains of sympodial conidia, but phylogenetically the taxon clusters amidst species described under the generic name *Cladophialophora* (Fig. 2). The type species of the genus *Fonsecaea*, *F. pedrosoi* is located in the same clade, whereas that of *Cladophialophora*, *C. carrionii*, resides in another clade (de Hoog et al., 2012). If we would adopt a phylogenetic classification system, *Fonsecaea* would be most appropriately applied to the upper clade (Fig. 2). For this reason we classify the new fungus in *Fonsecaea*. Taxonomic concepts in the Chaetothyriales are highly unstable due to the frequent discovery of novel species. For this reason we regard the introduction of further name changes at this stage as premature and clinically counterproductive.

Fonsecaea brasiliensis V.A. Vicente, Najafzadeh, Klaassen & de Hoog, **sp. nov.** – Figs 8, 9. MycoBank MB 561621.

Coloniae fere lente crescentes, ad 30 mm diam post 21 dies 30 °C, velutinae, conidiis matures pulverulentae, olivaceo-nigrae, margine regulariter. Hyphae fertiles suberectae, cylindricae, sursum ramosae, 2.0−2.5 μm latae. Conidia unicellularia, in catenis brevis ramosis connexa persistentibus, ex

hyphis indistinctis orientia, olivacea, levia, tenuitunicata, ellipsoidea vel limonifomes, 6.5−10.0 × 2.0−3.5 μm. Teleomorphosis ignota.
Holotypus CBS- H-20465, exsiccatus in herbariorum CBS praeservatur.

Description based on CBS 119710 at 30°C on MEA and PDA after 3 weeks in darkness. Colonies moderately expanding, 30 mm diam, initially (on day 3) olivaceous black, with olivaceous grey centre and flat margin, later (on day 14) becoming velvety, greyish- black, with grey center. Margin straight, entire. Reverse greyish to olivaceous black. No diffusible pigment produced on any medium. Conida formed in densely branched, acropetal chains of max. 5 conidia, often located on distinct denticles, olivaceous brown, smooth-walled, ellipsoidal to lemon-shaped, narrowed towards both ends, 6.5−10.0 × 2.0−3.5 μm; scars pale pigmented. Teleomorph unknown.
Holotype CBS H-20465, ex-type culture CBS 119710, isolated from mangrove land crab *Ucides cordatus* with lethargic crab disease, mangroves near Brejo Grande city, Sergipe state, Brazil, by W. Boeger.

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Table 1. Strains of analyzed *Cladophialophora*, *Exophiala* and *Fonsecaea* species.

Fig. 1. Map of Brazil showing areas of isolation in Brazil in the state of Paraná and along the Atlantic coasts, where crabs indicate the presence of Lethargic Crab Disease.

Fig. 2. Phylogenetic tree of *Herpotrichiellaceae* based on confidently aligned near-complete SSU and D1/D2 domain of LSU sequences constructed with Maximum likelihood implemented in RAxML. Bootstrap values > 80 from 100 resampled datasets are shown with branches. *Phaeococcomyces catenatus*, CBS 650.76 was taken as outgroup. Rectangles indicate main clades recognized within the by de Hoog *et al.* (2011), and arrows indicate the two agents of LCD, *Exophiala cancaerae* and *Fonsecaea brasiliensis*.

Fig. 3A. Phylogenetic tree of *Fonsecaea / Cladophialophora* based on confidently aligned ITS sequences constructed with Maximum likelihood implemented in RAxML. Bootstrap values > 80 from 100 resampled datasets are shown with branches. *Cladophialophora australiensis*, CBS 122973 was taken as outgroup.

Fig. 3B. Phylogenetic tree of *Fonsecaea / Cladophialophora* based on confidently aligned ITS and partial *BT2* and *TEF1* sequences constructed with Maximum likelihood implemented in RAxML. Bootstrap values > 80 from 100 resampled datasets are shown with branches. *Fonsecaea nubica*, CBS 444.62 was taken as outgroup.

Fig. 4. AFLP fingerprints of *F. brasiliensis* and related species showing DNA bands in the region of 60-250 bp. The dendrogram was constructed by UPGMA clustering using the Pearson correlation coefficient. The scale bar indicates the percentage similarity.

Fig. 5. Temperature relations of *Fonsecaea brasiliensis* (CBS 119710, CBS 119718, CBS 120418) from crabs and *Cladophialophora devriesii* (CBS 147.84) from human patient.

Fig. 6. Mortality curves observed in non-continuous infected crabs with strains of *Exophiala cancereae* and *Fonsecaea brasiliensis*. Symbols: ●, Infected group (with *Exophiala* CBS 120420); ■, Infected group (with *Fonsecaea* CBS 119710); ∆, Control group (needle); ◊, Control group (saline solution).

Fig.7. Amplification of *Fonsecaea brasiliensis* specific fragment supporting the identity of recovered colonies from tissues of artificially infected crabs. Lanes: 1. molecular size markers (1Kb ladder) (L); 2. *Fonsecaea brasiliensis* (positive control) (+C); 3−6. DNA extracts of recovered colonies from thoracic ganglion (3), heart (4) and hemolymph (5 and 6) samples (showing the agar plates with the utilized colonies); 7. Negative control (C).

Fig. 8. *Fonsecaea brasiliensis*, CBS 119710. A, B. Colonies on MEA and PDA, respectively. C. Olivaceous brown hyphae with conidia; D−G. Conidia formed in short acropetal chains; H. Sympodial cluster of conidia; I−Q. Conidia.

Fig. 9. *Fonsecaea brasiliensis*, CBS 119710, line drawing of morphology of the conidial apparatus. Bar represents 10 µm.

Name	Strain number	Cross ref. number	Source	Geography
Exophiala cancerae	CBS 120532	dH 17408 = HFII	Crab	Brazil, Sergipe state, Brejo Grande city, mangrove
Exophiala cancerae	CBS 120420 (T)	dH 17409 = HF	Crab	Brazil, Pernambuco state, Goiana city, mangrove
Exophiala cancerae	CBS 119920	IMI 380731 = Cunningham 179/9	Toad liver	Israel
Fonsecaea brasiliensis	CBS 119710 (T)	dH 16818 = isolate 3 / 2H	Crab	Brazil, Sergipe state, Brejo Grande city, mangrove
Fonsecaea brasiliensis	CBS 119718	dH 16816 = isolate 1 / 2H	Crab	Brazil, Sergipe state, Brejo Grande city, mangrove
Fonsecaea brasiliensis	CBS 120419	dH 17407 = 2E 16/08	Crab	Brazil, Sergipe state, Brejo Grande city, mangrove
Fonsecaea brasiliensis	CBS 120418	dH 17406 = 1G 16/08	Crab	Brazil, Sergipe state, Brejo Grande city, mangrove
Fonsecaea brasiliensis	CBS 127819	dH 21198	Soil in rotten stem of palm	Brazil, Maranhão state, Icatu city
Fonsecaea brasiliensis	CBS 126872	dH 20498	Leaf of Babassu palm tree	Brazil, Maranhão state, Icatu city
Fonsecaea brasiliensis	CBS 126729	dH 20490	Leaf of Babassu palm tree	Brazil, Maranhão state, Pinheiros, village of Fortaleza
Fonsecaea brasiliensis	CBS 126723	dH 20497	Leaf of Babassu palm tree	Brazil, Maranhão state, Icatu city
Fonsecaea brasiliensis	CBS 126732	dH 21169	Leaf of Babassu palm tree	Brazil, Maranhão state, Icatu city
Fonsecaea brasiliensis	CBS 127813	dH 20515 = VM20	Bark of Babassu coconut palm tree	Brazil, Maranhão state, Icatu city
Fonsecaea brasiliensis	CBS 126741	dH 20482 = VM35A5	Leaf of Babassu palm tree	Brazil, Maranhão state, Bacabera city
Fonsecaea brasiliensis	CBS 128148	dH 21295 = VM35A3	Leaf of Babassu palm tree	Brazil, Maranhão state, Bacabera city
Fonsecaea brasiliensis	CBS 126866	dH 21196 = VM53B	Wood of clothesline pole	Brazil, Maranhão state, Pinheiros, village of Fortaleza
Fonsecaea brasiliensis	CBS 127012	dH 20541 = VM54B1	Stem of living Tucum palm tree	Brazil, Maranhão state, Pinheiros, village of Fortaleza
Fonsecaea brasiliensis	CBS 126006	dH 20475 = VM72B1	Rotting wood	Brazil, Maranhão state, Icatu city
Fonsecaea brasiliensis	CBS 126731	dH 20495 = VM72	Rotting wood	Brazil, Maranhão state, Icatu city
Fonsecaea brasiliensis	CBS 126735	dH 20508	Rotting wood	Brazil, Maranhão state, Pinheiros
Fonsecaea brasiliensis	CBS 127816	dH 21167 = VM39B	Decomposing animal tooth	Brazil, Maranhão state, Pinheiros, village of Fortaleza
Fonsecaea brasiliensis	CBS 127010	dH 20494 = VM25A5	Bark of Imbauba palm tree	Brazil, Maranhão state, Icatu city
Fonsecaea brasiliensis	CBS 126721	dH 20486 = VM54A3	Stem of living Tucum palm tree	Brazil, Maranhão state, Pinheiros, village of Fortaleza
Fonsecaea brasiliensis	CBS 127815	dH 20540 = VM52A2	Coconut of Babassu palm tree	Brazil, Maranhão state, Pinheiros, village of Fortaleza
Fonsecaea brasiliensis		dH 12331 = IHM 1712	Lichen	U.S.A., Florida
Fonsecaea brasiliensis	CBS 109629	dH 12334 = IHM 1730	Dead leaf	Uruguay
Fonsecaea brasiliensis		dH 20519 = VM32E1	Soil with horse and cattle manure	Brazil, Maranhão state, Bacabeira city
Fonsecaea brasiliensis	CBS 127898	dH 21422 = R32A1	Mangrove	Brazil, Bahia state, Canavieiras
Fonsecaea brasiliensis	CBS 127905	dH 21423 = R43B1	Branch in mangrove	Brazil, Bahia state, Acupi
Fonsecaea brasiliensis	CBS 127896	dH 21421 = R27A1	Branch in mangrove	Brazil, Bahia state, Canavieiras
Fonsecaea brasiliensis	CBS 127904	dH21431 = R42B1	Branch in mangrove	Brazil, Bahia state, Acupi
Fonsecaea multimorphosa	CBS 102235	dH 11597	Wood	Brazil, Paraná state
Fonsecaea multimorphosa	CBS 102224	dH 11584	wood, *Grevillea*	Brazil, Paraná state
Fonsecaea multimorphosa	CBS 102240	dH 11604	Soil cover	Brazil, Paraná state
Fonsecaea multimorphosa	CBS 102253	dH 11618	Decaying vegetable cover	Brazil, Paraná state
Fonsecaea multimorphosa	CBS 126716	dH 20510	Bark of Babassu coconut tree	Brazil, Maranhão state, Icatu city
Fonsecaea multimorphosa	CBS 102226	dH 11587	Decaying trunk	Brazil, Paraná state
Fonsecaea monophora	CBS 269.37 (T)	dH 12659	Biopsy of chromoblastomycosis lesion	South America
Fonsecaea monophora	CBS 102238	dH 11602	Soil	Brazil, Parana, Ipora
Fonsecaea monophora	CBS 102225	dH 11585	Rotting wood	Brazil, Parana state
Fonsecaea monophora	CBS 102229	dH 11590	Decaying vegetable cover	Brazil, Parana, Ipora

Fonsecaea monophora	CBS 121721	dH 18399 = SUMS0246	Biopsy of chromoblastomycosis lesion	China
Fonsecaea monophora	CBS 126007	dH 20512	Rotting Babassu coconut	Brazil, Maranhão state, Pinheiros, village of Fortaleza
Fonsecaea nubica	CBS 269.64 (T)	dH 15656	Biopsy of chromoblastomycosis lesion	Cameroon
Fonsecaea nubica	CBS 444.62	dH 15586	Biopsy of chromoblastomycosis lesion	Surinam
Fonsecaea nubica	CBS 125191	dH 20423	Biopsy of chromoblastomycosis lesion	China
Fonsecaea pedrosoi	CBS 253 49	dH15620	Biopsy of chromoblastomycosis lesion	Uruguay
Fonsecaea pedrosoi	CBS 271.37 (T)	dH15659	Biopsy of chromoblastomycosis lesion	South America
Fonsecaea pedrosoi	CBS 671 66	dH 16159	Mouse passage	Venezuela
Fonsecaea pedrosoi	CBS 102245	dH 11610	Biopsy of chromoblastomycosis lesion	Brazil
Fonsecaea pedrosoi	CBS 125747	dH 20500	Rotting wood from backyard of patient's	Brazil, Maranhão state, Pinheiros, village of Fortaleza
Fonsecaea pedrosoi	CBS 125749	dH 20488	Rotting wood from backyard of patient's	Brazil, Maranhão state, Pinheiros, village of Fortaleza
Cladophialophora arxii	CBS 102461	dH 11524	Brain	U.S.A., Miami, Florida
Cladophialophora arxii	CBS 306.94 (T)	IFM 52022	Human, tracheal abscess	Germany
Cladophialophora arxii	CBS 409 96	dH 15849	Human, disseminated	Unknown
Cladophialophora australiensis	CBS 112793	CPC1377	Sports drink	Australia
Cladophialophora bantiana	CBS 444.96		Dog, disseminated	South Africa, Pretoria, Onderstepoort
Cladophialophora bantiana	CBS 100429	ATCC 24928	Brain, human	Unkown
Cladophialophora bantiana	CBS 678.79	dH 17412	Skin, cat	U.S.A., Bethesda
Cladophialophora bantiana	CBS 648.96	UAMH 3830	Liver, dog	Barbados
Cladophialophora devriesii	CBS 147.84 (T)	ATCC 56280 = CDC 82-030890	Disseminated infection in human patier	Grand Cayman Islands
Cladophialophora devriesii	CBS 127821	dH 21011	Coconut of Babassu palm tree	Brazil, Maranhão state, Pinheiros, village of Fortaleza
Cladophialophora devriesii	CBS 127019	dH 21170	Wood of clothesline pole	Brazil, Maranhão state, Pinheiros, village of Fortaleza
Cladophialophora emmonsii	CBS 640.96 (T)	dH 17418	Subcutaneous, cat	Unknown
Cladophialophora emmonsii	CBS 979.96	dH 16329 (T)	Forearm human	U.S.A., Virginia
Cladophialophora immunda	CBS 834.96 (T)	dH 21287	Subcutaneous phaeohyphomycosis, ma	U.S.A., Georgia, Atlanta
Cladophialophora immunda	CBS 110551	dH 15250	Soil under gasoline station	The Netherlands
Cladophialophora immunda	CBS 123977	NCPF 4725	Human, subcutaneous	U.K.
Cladophialophora immunda	CBS 102249	dH 11614	Rotten trunk	Brazil, Paraná state, Sarandi
Cladophialophora immunda	CBS 102237	dH 11601	Plant litter	Brazil, Paraná state, Telêmaco Borba
Cladophialophora immunda	CBS 102227	dH 11588	Stem palm	Brazil, Paraná state
Cladophialophora immunda		dH 11601 = F10PLA	Environment	Brazil, Paraná state
Cladophialophora immunda	CBS 109797	dH 11474	Biofilter inoculated with soil	Germany, Kaiserslautern
Cladophialophora minourae	CBS 556.83 (T)	ATCC 52853 = IMI 298056	Decaying wood	Japan
Cladophialophora minuorae	CBS 987.96	IFM 4701 = UAMH 5022	Rotting wood	Japan
Cladophialophora saturnica	CBS 118724 (T)	dH 12939	Interdigital toe lesion, child	Brazil, Paraná state, Curitiba
Cladophialophora saturnica	CBS 109628	dH 12333	Dead tree	Uruguay, Isla Grande del Queguay
Cladophialophora saturnica	CBS 102228	dH 11589	Rotten wood	Brazil, Paraná state, Piraquara
Cladophialophora saturnica	CBS 114326	ATCC 20384	Toluene biofilter	Netherlands, Wageningen
Cladophialophora saturnica	CBS 102230	dH 11591	Vegetable cover and soil	Brazil, Paraná state, Piraquara
Cladophialophora saturnica	CBS 109630	dH 12335	Trunk of cut tree	Uruguay, Isla Grande del Queguay

Table 1. Analyzed strains of *Fonsecaea* and *Exophiala* species involved in Lethargic Crab Disease (LCD), with reference isolates.

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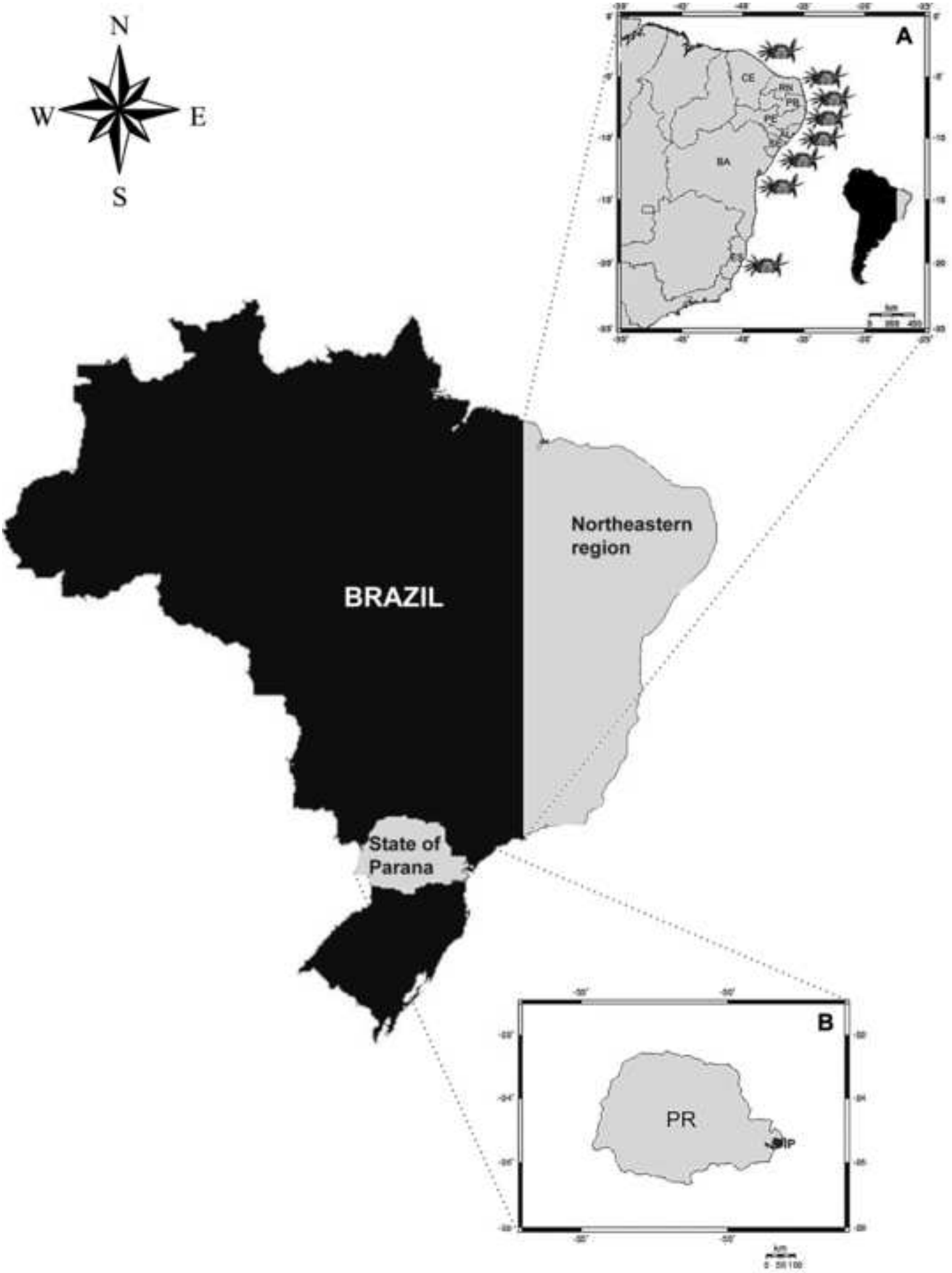


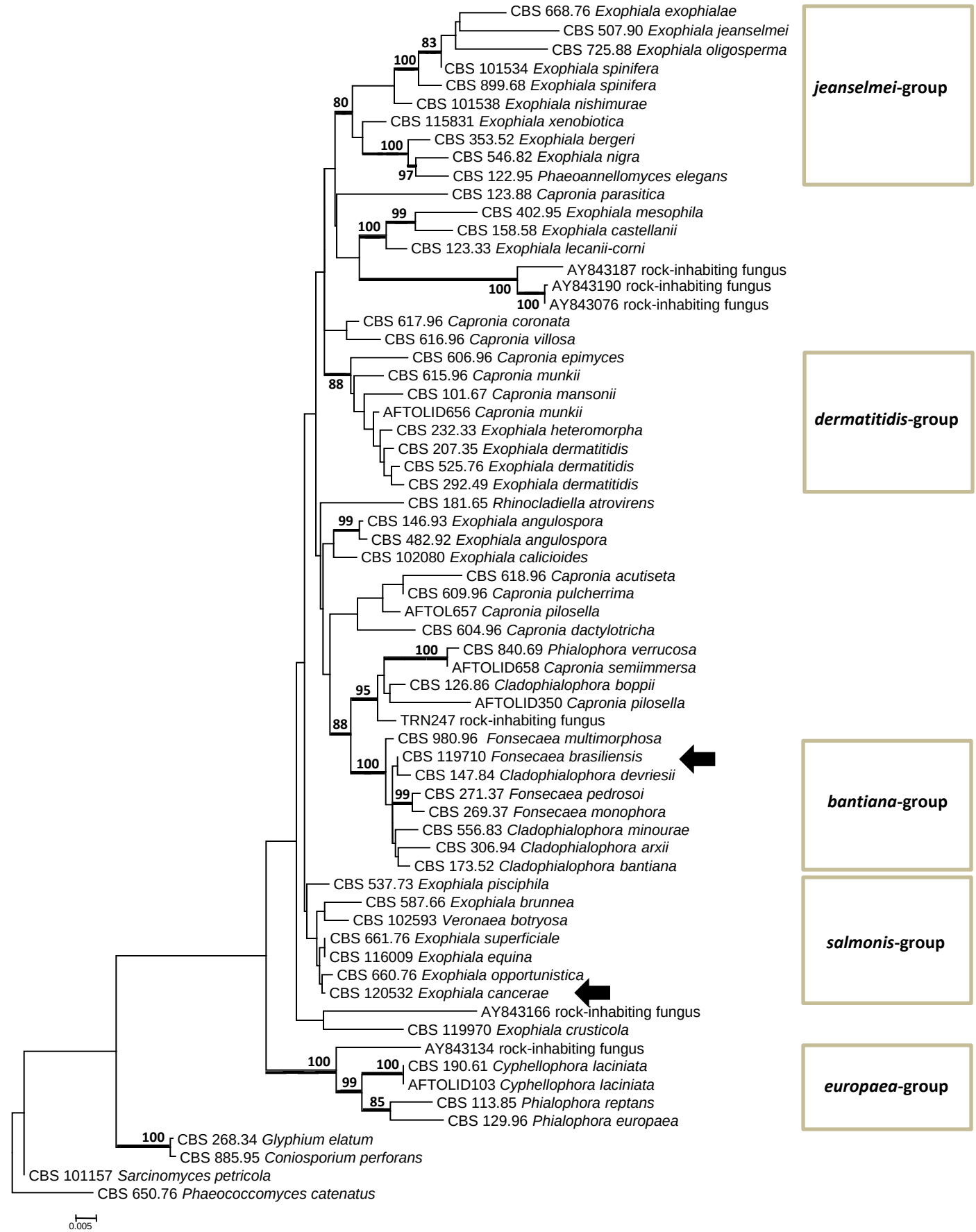
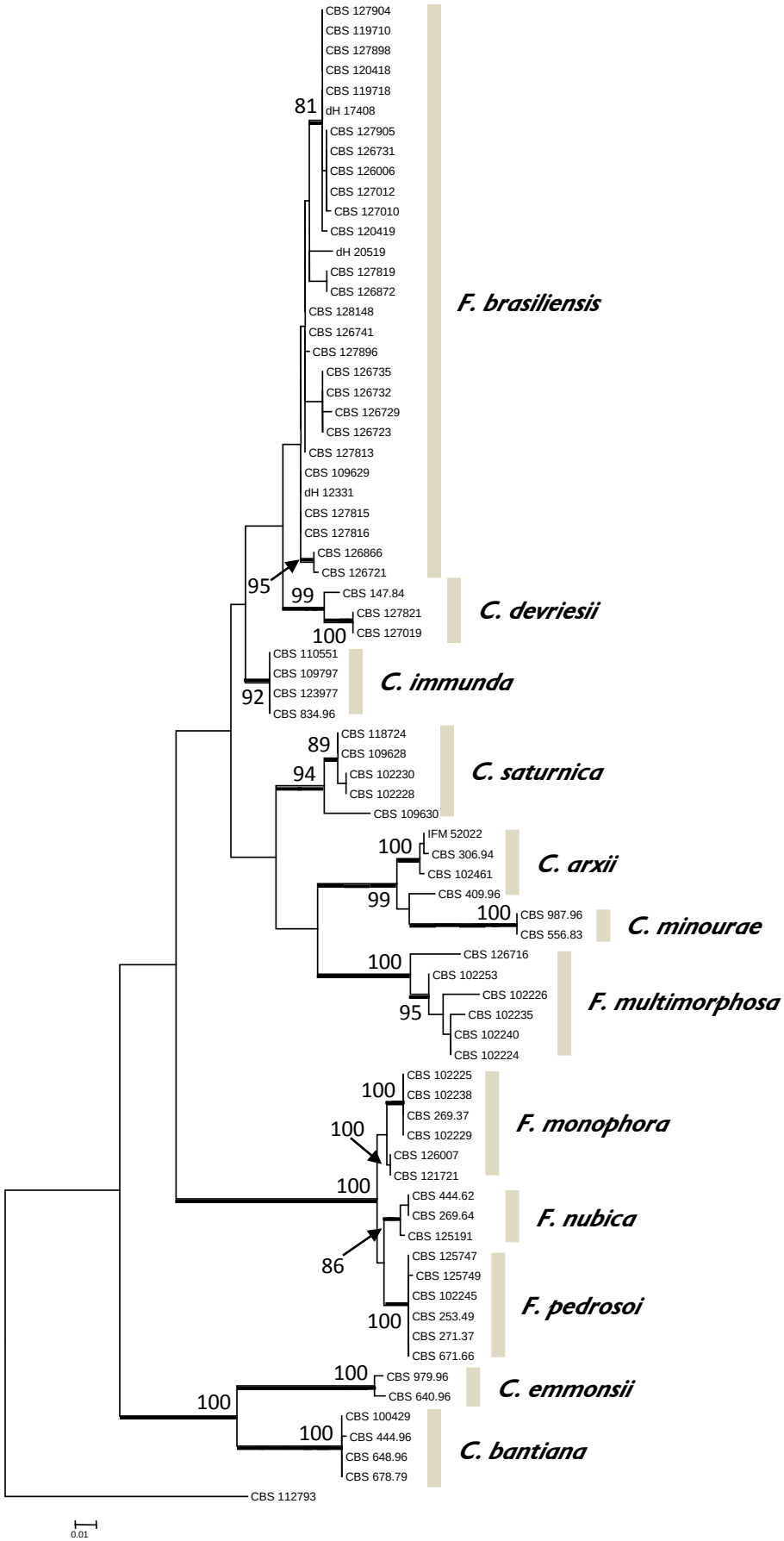

CBS 668.76 Exophiala exophialae
CBS 507.90 Exophiala jeanselmei
CBS 725.88 Exophiala oligosperma
CBS 101534 Exophiala spinifera
CBS 899.68 Exophiala spinifera
CBS 101538 Exophiala nishimurae
CBS 115831 Exophiala xenobiotica
CBS 353.52 Exophiala bergeri
CBS 546.82 Exophiala nigra
CBS 122.95 Phaeoannellomyces elegans
CBS 123.88 Capronia parasitica
CBS 402.95 Exophiala mesophila
CBS 158.58 Exophiala castellanii
CBS 123.33 Exophiala lecanii-corni
AY843187 rock-inhabiting fungus
AY843190 rock-inhabiting fungus
AY843076 rock-inhabiting fungus
CBS 617.96 Capronia coronata
CBS 616.96 Capronia villosa
CBS 606.96 Capronia epimyces
CBS 615.96 Capronia munkii
CBS 101.67 Capronia mansonii
AFTOLID656 Capronia munkii
CBS 232.33 Exophiala heteromorpha
CBS 207.35 Exophiala dermatitidis
CBS 525.76 Exophiala dermatitidis
CBS 292.49 Exophiala dermatitidis
CBS 181.65 Rhinocladiella atrovirens
CBS 146.93 Exophiala angulospora
CBS 482.92 Exophiala angulospora
CBS 102080 Exophiala calicioides
CBS 618.96 Capronia acutiseta
CBS 609.96 Capronia pulcherrima
AFTOL657 Capronia pilosella
CBS 604.96 Capronia dactylotricha
CBS 840.69 Phialophora verrucosa
AFTOLID658 Capronia semiimmersa
CBS 126.86 Cladophialophora boppii
AFTOLID350 Capronia pilosella
TRN247 rock-inhabiting fungus
CBS 980.96 Fonsecaea multimorphosa
CBS 119710 Fonsecaea brasiliensis
CBS 147.84 Cladophialophora devriesii
CBS 271.37 Fonsecaea pedrosoi
CBS 269.37 Fonsecaea monophora
CBS 556.83 Cladophialophora minourae
CBS 306.94 Cladophialophora arxii
CBS 173.52 Cladophialophora bantiana
CBS 537.73 Exophiala pisciphila
CBS 587.66 Exophiala brunnea
CBS 102593 Veronaea botryosa
CBS 661.76 Exophiala superficiale
CBS 116009 Exophiala equina
CBS 660.76 Exophiala opportunistica
CBS 120532 Exophiala cancerae
AY843166 rock-inhabiting fungus
CBS 119970 Exophiala crusticola
AY843134 rock-inhabiting fungus
CBS 190.61 Cyphellophora laciniata
AFTOLID103 Cyphellophora laciniata
CBS 113.85 Phialophora reptans
CBS 129.96 Phialophora europaea
CBS 268.34 Glyphium elatum
CBS 885.95 Coniosporium perforans
CBS 101157 Sarcinomyces petricola
CBS 650.76 Phaeococcomyces catenatus
0.005
jeanselmei-group
dermatitidis-group
bantiana-group
salmonis-group
europaea-group

Fig. 2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dH 20519
CBS 127819
CBS 126872
CBS 128148
CBS 126741
CBS 127896
CBS 126735
CBS 126732
CBS 126729
CBS 126723
CBS 127813
CBS 109629
dH 12331
CBS 127815
CBS 127816
CBS 126866
CBS 126721
F. brasiliensis
95
99
CBS 147.84
CBS 127821
CBS 127019
100
C. devriesii
CBS 110551
CBS 109797
CBS 123977
92
CBS 834.96
C. immunda
89
CBS 118724
CBS 109628
94
CBS 102230
CBS 102228
CBS 109630
C. saturnica
100
IFM 52022
CBS 306.94
CBS 102461
C. arxii
99
CBS 409.96
100
CBS 987.96
CBS 556.83
C. minourae
100
CBS 126716
CBS 102253
CBS 102226
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CBS 102235
CBS 102240
CBS 102224
F. multimorphosa
CBS 102225
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CBS 102238
CBS 269.37
CBS 102229
100
CBS 126007
CBS 121721
F. monophora
100
CBS 444.62
CBS 269.64
CBS 125191
F. nubica
86
CBS 125747
CBS 125749
CBS 102245
CBS 253.49
100
CBS 271.37
CBS 671.66
F. pedrosoi
100
CBS 979.96
CBS 640.96
C. emmonsii
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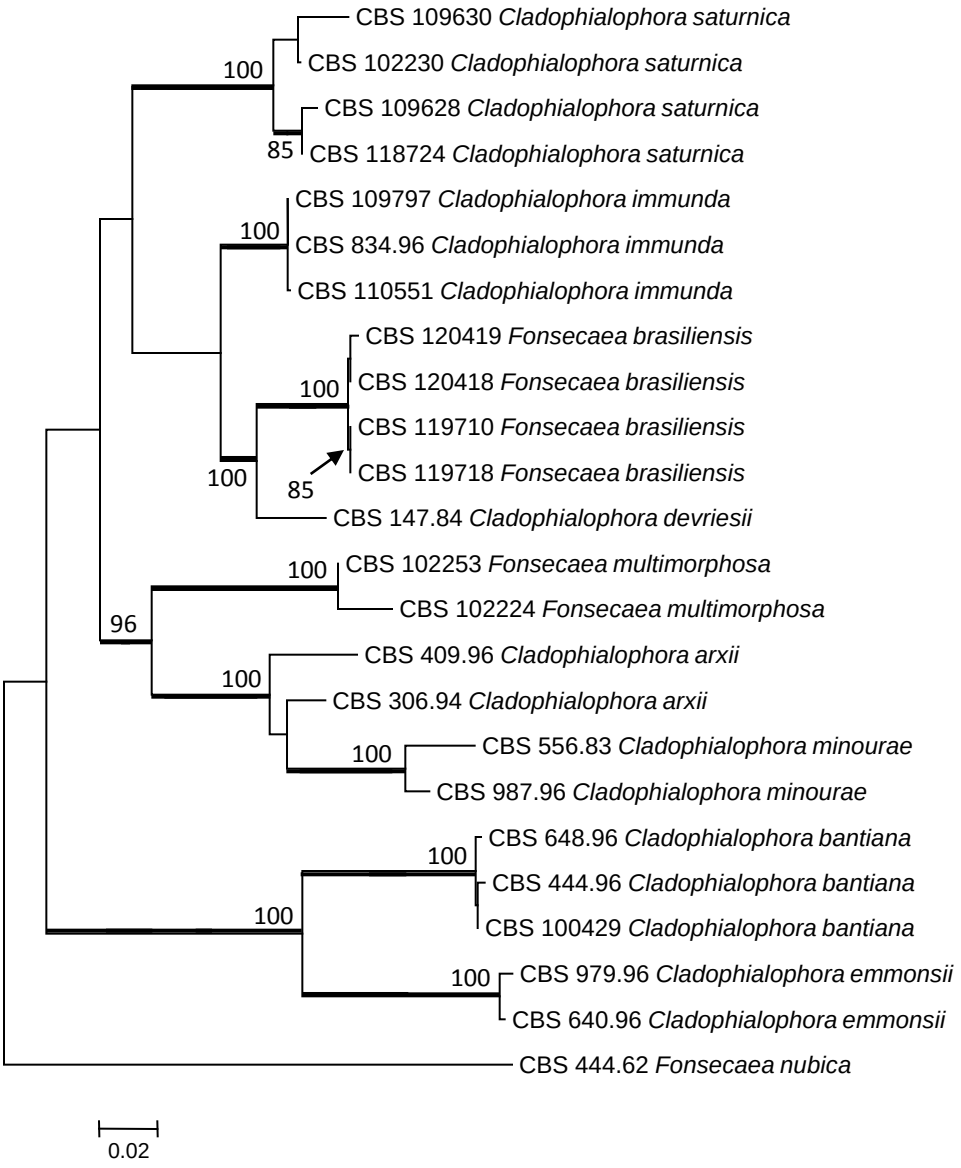
CBS 100429
100
CBS 444.96
CBS 648.96
CBS 678.79
C. bantiana
CBS 112793
0.01

Fig. 3A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CBS 109797 Cladophialophora immunda
CBS 834.96 Cladophialophora immunda
CBS 110551 Cladophialophora immunda
CBS 120419 Fonsecaea brasiliensis
CBS 120418 Fonsecaea brasiliensis
CBS 119710 Fonsecaea brasiliensis
CBS 119718 Fonsecaea brasiliensis
CBS 147.84 Cladophialophora devriesii
CBS 102253 Fonsecaea multimorphosa
CBS 102224 Fonsecaea multimorphosa
CBS 409.96 Cladophialophora arxii
CBS 306.94 Cladophialophora arxii
CBS 556.83 Cladophialophora minourae
CBS 987.96 Cladophialophora minourae
CBS 648.96 Cladophialophora bantiana
CBS 444.96 Cladophialophora bantiana
CBS 100429 Cladophialophora bantiana
CBS 979.96 Cladophialophora emmonsii
CBS 640.96 Cladophialophora emmonsii
CBS 444.62 Fonsecaea nubica
100
85
100
100
100
85
100
96
100
100
100
100
100
0.02

Fig. 3B

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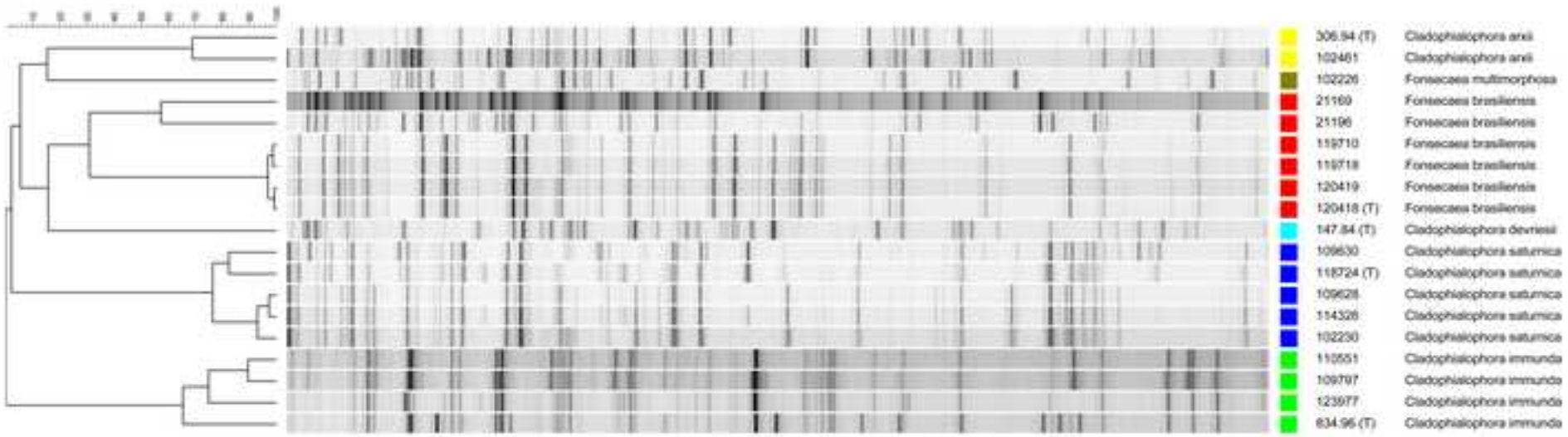

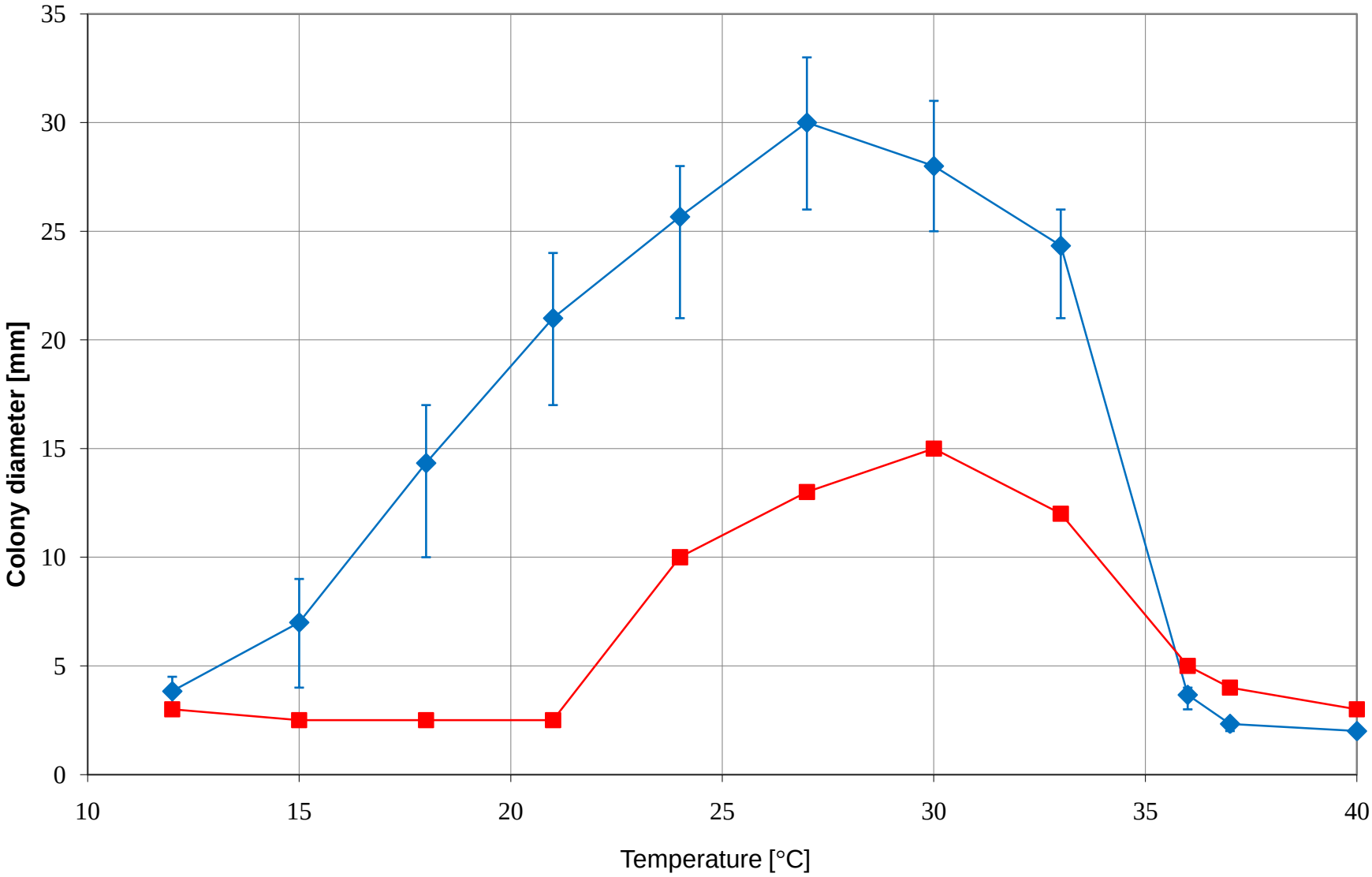
35
30
25
20
15
10
5
0
Colony diameter [mm]
10
15
20
25
30
35
40
Temperature [°C]

Fig. 5

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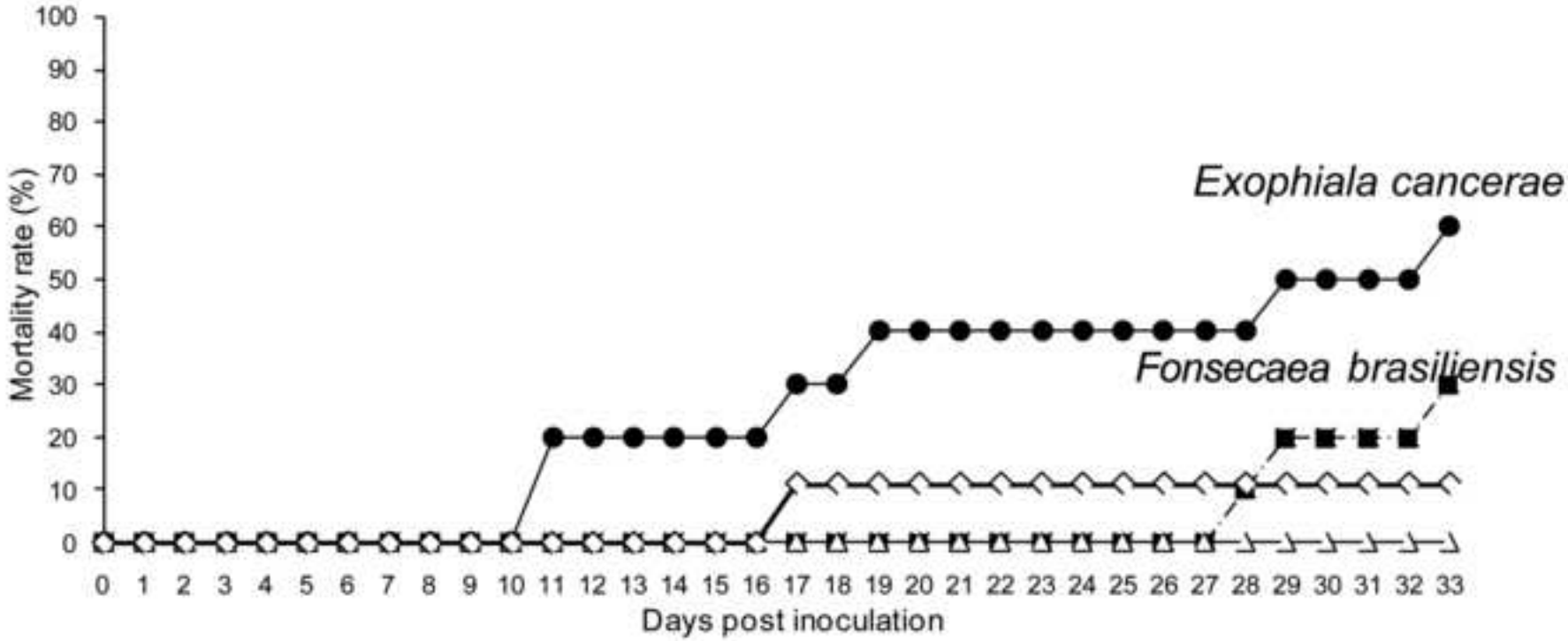
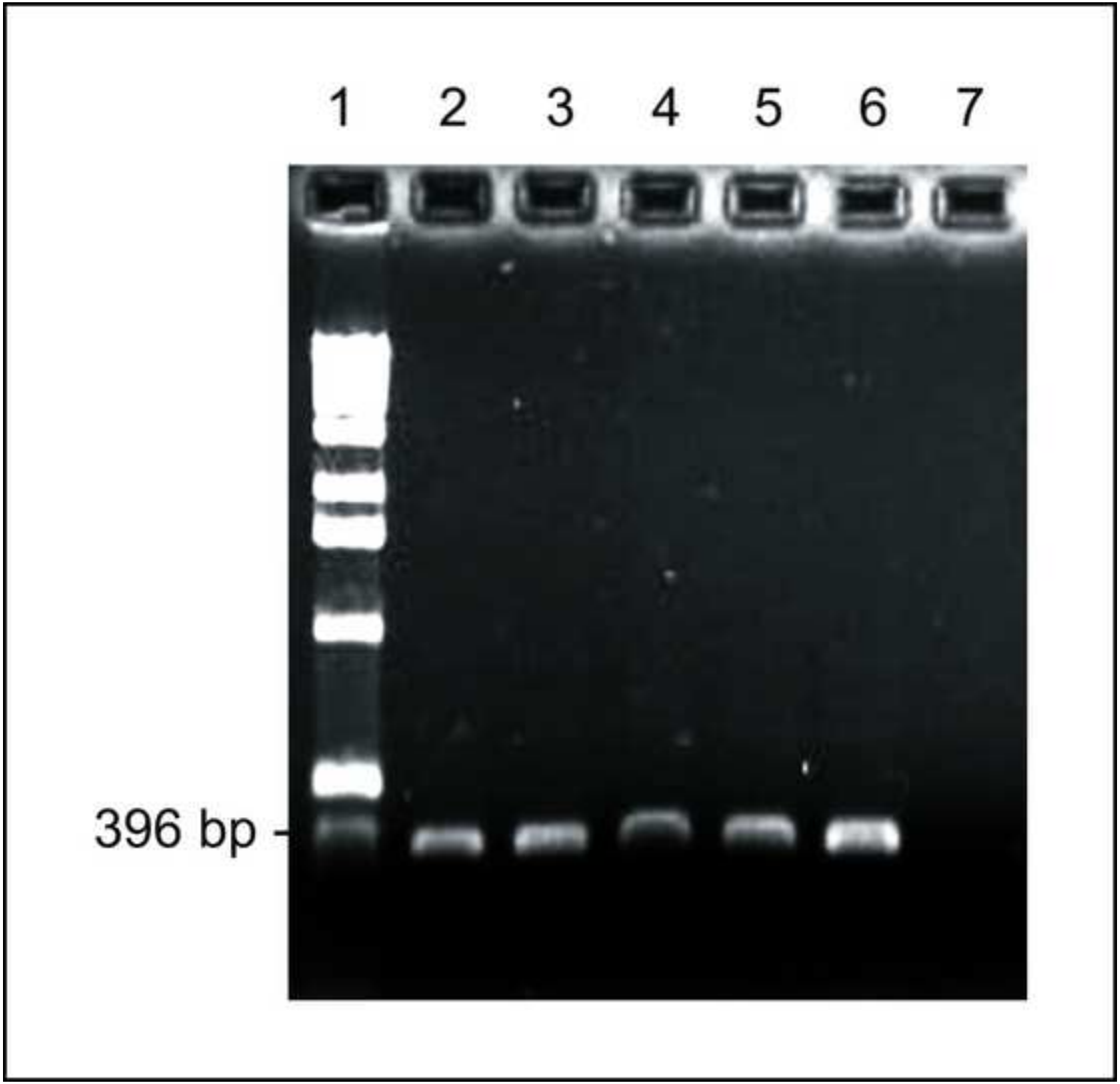


Symbols:

●, Infected group (*Exophiala cancerae)* ; ■, Infected group (*F. brasiliensis*)

◊, Control group (saline solution); △, Control group (needle)



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