TAXONOMIC STUDIES AND THE EVOLUTION OF HABITAT PREFERENCE IN THE CYSTOBASIDIOMYCETES

by

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To my mother for her tenacity and faith.

To my father for teaching me the value of honesty and humbleness

To Juanita, Mariangel and Carolina for being my motivation to never give up

To Rato for loving me and teaching me so much. For making out of me a better man

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ABSTRACT

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Pucciniomycotina is a subphylum with a high diversity in terms of habitat and life history strategies that include plant parasites, animal associates (including opportunistic human pathogens), saprobes and antagonists of other fungi. The class Cystobasidiomycetes within this subphylum is a representative of such diversity and remains understudied. Their role in nature and the associations they establish with their hosts for most of the species is still unknown. In this study we used taxonomic and phylogenetic methods to present an inventory of strains in this class collected for more than 20 years from all over the world and preserved in the Aime Lab Culture Collection. Molecular and morphological data for six new species in the genera *Bannoa, Buckleyzyma, Halobasidium* and *Sakaguchia* were also presented. Additionally, mating experiments were performed by pairing strains of *Bannoa*; this is the second time in the genus that clamp connections and basidia are observed. We also found that newly described species of *Bannoa* can be co-inhabitants of sori of rust fungi. In general, antagonistic interactions can occur through: 1) direct physical contact between two fungi, i.e., mycoparasitism; or, 2) the production of antimicrobial compounds. In the Cystobasidiomycetes, direct physical antagonistic interaction which is associated with sexual states has been reported in species of *Cystobasidium*, *Naohidea, Cyphobasidium* and *Occultifur*. On the other hand, the production of antimicrobial compounds which mainly occurs between the yeast stage of the fungi and other organisms has only been reported in *Cystobasidium pallidum* and *Hasegawazyma lactosa*. We also hypothesize that the common ancestor to Cystobasidiomycetes is a mycoparasite due to the fact that this life strategy is present in most of the lineages in the class. To test this hypothesis, we grouped into five categories the host association or substrate from which strains of extant species in the Cystobasidiomycetes were isolated, i.e., animals, plants, fungi, aquatic or decaying organic matter. We constructed a resolved phylogeny for the class based on seven loci to study the evolutionary origins of mycoparasitism through ancestral character reconstruction with representation of all

described species. Our analysis suggests that the most likely hypothesis is that the most recent common ancestor of the Cystobasidiomycetes was associated to fungi.

CHAPTER 1. A LITERATURE REVIEW OF THE CYSTOBASIDOMYCETES

Pucciniomycotina comprises more than 8400 described species, i.e., more than 8% of described fungi (Kirk et al 2008) and is the sister group to Ustilaginomycotina and Agaricomycotina (Aime et al. 2014). Some species of Pucciniomycotina are known to cause some of the most devastating diseases of crops (e.g., the rust of coffee *Hemileia vastatrix*) as well as causing rare infections in humans (*Cystobasidium minutum* has been reported causing unusual infection in hip-joint prosthesis) (Cutrona et al. 2002), while others have shown potential as biological control organisms of invasive plants and other pathogenic fungi (Evans 1993, Lutz et al. 2004) or can be found asymptomatically in the phylloplane. Circa 90% of the known species in Pucciniomycotina belong to a single order, the Pucciniales or the rust fungi (Kirk et al 2008) in the Pucciniomycetes: the other fungi in the subphylum classified in the eight remaining classes, are very diverse in terms of their ecology, physiology and biology (Aime et al. 2006, Aime et al. 2014). Among the classes in Pucciniomycotina, in which a high diversity in ecology and biology has been documented are the Cystobasidiomycetes.

The Cystobasidiomycetes (Basidiomycota: Pucciniomycotina) proposed by Bauer et al. (2006), is a class in which the majority of species are pink, orange-red or salmon-colored dimorphic or unicellular yeasts. Higher-level classification studies using molecular data of the 18S and 28S ribosomal RNA genes (Aime et al. 2006, Bauer et al. 2006) resolved three orders, Cystobasidiales, Erythrobasidiales and Naohidiales. In 2015, Wang and collaborators, conducted phylogenetic studies using seven genes, including nuclear ribosomal genes and protein-coding genes namely, 18S, ITS, 28S, *rpb1* (RNA polymerase II subunit *rpb1*), *rpb2* (RNA polymerase II subunit *rpb2*), *tef1* (translation elongation factor EF-1 alpha) and the *cytb* (Cytochrome b). Protein-coding gene sequences had not been extensively used in phylogenetic studies on Basidiomycetous yeast (Wang et al. 2015a) and were used to robustly reassess the boundaries of genera in several classes of Pucciniomycotina including the Cystobasidiomycetes.

In that study, it was also possible to accommodate species belonging to the anamorphic genera *Rhodotorula* and *Sporobolomyces* in several genera such as *Bannoa*, *Erythrobasidium* and *Hasegawazyma* in the Erythrobasidiales and in *Cystobasidium* in the Cystobasidiales. In the same study, two genera, *Buckleyzyma* and *Microsporomyces*, were erected to accommodate species

belonging to the former aurantiaca and magnisporus group, respectively (Wang et al. 2015a). In 2016 Spribille and collaborators, identified a new lineage of lichen-inhabitant yeast that were later included within *Cyphobasidium*, erected by Millanes et al. (2016). Subsequently, in 2017 the orders Buckleyzymales and Sakaguchiales were proposed to accommodate species in *Buckleyzyma* and *Sakaguchia*, respectively (Zhao et al. 2017). Currently, the Cystobasidiomycetes consists of 13 genera and over 70 species placed in five orders: Buckleyzymales, Cystobasidiales, Erythrobasidiales, Naohideales and Sakaguchiales (Aime et al. 2006, Bauer et al. 2006, Spribille et al. 2016, Zhao et al. 2017).

Species belonging to the Cystobasidiomycetes have been isolated from different habitats, ranging from aquatic environments, soil, insects, mammals and plants either as endophytes or living on the surface of leaves. Several other species have been found in association or antagonizing other fungi. Mycoparasitism is one of the several antagonistic interactions observed in fungi and consists in the direct physical contact between two fungi and has been reported in some genera within the class namely *Cystobasidium*, *Naohidea*, *Cyphobasidium* and *Occultifur*. On the other hand, antagonism can also occur through the production of toxic compounds and other agents against competing organisms, through the competition for nutrients or by stimulating host defense mechanisms (Karlsson et al. 2015, Narayanasamy 2013). These two phenomena have been reported in the Cystobasidiomycetes only on *Cystobasidium pallidum* and *Hasegawazyma lactosa*. These antagonistic interactions as well as the mechanism that fungi have developed to defend (such as the production of biofilms) from competing organisms has not been compiled for all species in the Cystobasidiomycetes. Considering this, we included in this chapter information collected from the literature and data generated in our lab regarding the formation of a ring when strains are incubated in liquid media (Figure 1.2). This ring can be interpreted as a biofilm (See chapter 5).

1.1.1 *Naohidea*

This monotypic genus is found in an early-diverging lineage within the Cystobasidiomycetes. In nature, hyphae are thin-walled, clamped mycelia are observed and long basidia without a probasidium with four short sterigmata are present (Mcnabb 1965). In the hyphal stage, *Naohidea sebacea* is parasitic on fructifications of other fungi and interacts with its host through intracellular haustoria (Bauer et al. 2006). *Naohidea sebacea* does not produce either orange, red or pink

pigmented colonies, instead, it produces cream-colored or beige to pale yellowish colonies in culture (Sampaio and Chen, 2011). Yeast cells occur singly or in parent-bud pairs, pseudohyphae is absent. Because of the position of *Naohidea* within the Cystobasidiomycetes, it is suggested that the Cystobasidiomycetes may have arisen from nanometer-fusion (i.e., small channel-like structures used to parasitize host cells) mycoparasites (Bauer et al 2004, Bauer et al 2006). *Naohidea sebacea* has been mainly found on old stromata of *Botryosphaeria dothidea* (Piatek 2002), *Botryiodiplodia* sp., *Botryosphaeria quercuum* (Mcnabb 1965), on a sphaeriaceous fungus (Olive 1947) and on pycnidia of a *Phialophorophoma*-like fungus (Bandoni 1973). However, *N. seabacea* also has been isolated from *Fraxinus* sp. and maple (Berk and Broome 1971).

1.1.2 *Cystobasidium*

The genus *Cystobasidium* comprises 20 species, some of them dimorphic. The hyphal septal pore is simple and is occluded by a cystosome, which is a cylindrical structure with a reticulate surface (Sampaio and Franz Oberwinkler 2011). The hyphal stage of *Cystobasidium fimetarium*, the type species of the genus, has been found growing on dung with other ascomycetous fungi such us *Thelebolus crustaceus* and *Lasiobolus equinus* on which it is presumed to be mycoparasitic (Roberts 1999). An asexual stage has not been documented either in nature or laboratory conditions. Other species have been found in association with other fungi during the hyphal stage. For example, *C. sebaceum* was found growing on remnants of an old *Sebacina* sp. on decaying wood (Martin 1939). Also, *C. proliferans* has been found growing on excreta on the inner surface of bark of dead pines (Olive 1952). Out of the three species in which a hyphal stage has been observed, *C. sebaceum* and *C. proliferans* lack molecular data because of it, their phylogenetic affiliation is still unclear. The remaining species have been described from their sexual stage, exhibiting ovoid to elongate cells, occurring singly or in pairs, budding is predominantly polar and colony color ranges from pink to orange or red (Figure 1D, 1E). According to Sampaio and Oberwinkler (2011), species in this genus do not produce ballistoconidia. Species in the genus, may or may not produce a pellicle, ring or sediment when cultured in liquid media. Ring production is observed in *Cystobasidium calyptogenae*, *C. laryngis*, *C. lysinophilum*, *C. minutum*, *C. pinicola*, *C. portillonense*, *C. slooffiae*, and *C. ongulense*. Ring formation has not been observed in *Cystobasidium oligophagum* or *C. benthicum*. Species in *Cystobasidium* have been isolated from

a great variety of habitats and substrates ranging from tropical to Antarctic regions and from marine habitats to plants.

1.1.3 *Occultifur*

Occultifur comprises ten species some, of which are dimorphic, with *O. internus* as the type species for the genus. This fungus is a presumed mycoparasite that interacts with its Dacrymycetales host through haustorial cells (Olive 1954, Bauer et al. 2006). An asexual stage has not been reported either in nature or under laboratory conditions for this species. Other species in *Occultifur* in which the hyphal stage has been observed are *O. corticorum*, isolated from a basidiocarp of *Tylospora fibrillosa* (Roberts 1997), *O. externus* isolated from plant litter and sea water (Sampaio et al. 2013), *O. lumbricifer*; described as an intrahymenial parasite on *Hyphodontia subalutacea* (Roberts 2001), *O. kilbournensis*, isolated in its asexual stage from overwintered maize stalks and for which a hyphal stage was observed under laboratory conditions after being paired with other compatible strains (Kurtzman and Robnett 2015), and *O. rivoirei* isolated from *Hyphodontia arguta* (Trichies 2016). Other species such as *O. brasiliensis*, *O. mephitis, O. tropicalis* and *O. plantarum* are known only from their asexual stage in which true hyphae or pseudo hyphae are not formed (Gomes et al. 2015, Khunnamwong et al. 2015, Kurtzman and Robnett 2015). In the asexual stage, cells occur singly or in parent-bud pairs and budding is mainly polar. *Occultifur brasiliensis* produces white colonies initially (days 1-3) and then colonies turn light pink. Production of a ring or pellicle has not been investigated in the genus.

1.1.4 *Erythrobasidium*

Erythrobasidium, with *E. hasegawianum* as the type species, is a small genus that comprises only three species. The type strain was isolated from an old culture of beer yeast. Under laboratory conditions, *E. hasegawianum*, produces uninucleate mycelia directly from a single cell without mating after 10 days on corn meal agar (CMA, Difco) at 25 C, dikaryotic hyphae were observed when clamp connections were present. After 50-60 days, protrusions emerged laterally from the hyphae to produce a basidium with four terminal basidiospores (Hamamoto et al. 1988). Development of hyphae, clamp connections and sexual structures from a single cell has never been reported in other species in the class. Previous studies such as those conducted on *O. externus* and *O. kilbournensis* in which a hyphal stage was developed, always involved mating between two

compatible strains (Sampaio et al. 2013, Kurtzman and Robnett 2015). However, when we tried to replicate the results obtained by Hamamoto et al. 1988, using the same strain (CBS 8253 = ATCC 9536), media and under the same growing conditions, we were unable to induce the formation of hyphae or any of the structures reported. The genome of *E. hasegawianum* is presumably diploid. If that is the case, it would be the only known member of the Cystobasidiomycetes in which sexual structures are developed from a diploid cell. In other species in the genus, a hyphal stage and sexual structures have not been observed. During the asexual stage, species in *Erythrobasidium* produce orange to red pigmented colonies (Figure 1B, 1C), yeast cells occur in single or in parent-bud pairs. Budding can be lateral or multilateral. Ballistospores have been reported in *E. elongatum* and *E. yunnanense* but not in *E. hasegawianum*. Pseudohyphae are present only in *E. elongatum*. A ring and pellicle are produced in all the species when cultured in liquid media. Species in the genus have been isolated from plants, mangrove sediments, fresh and seawater (Shivas and Rodrigues de Miranda 1983, Bai et al. 2001, Chanprasartsuk et al. 2013, Gomes et al. 2015).

1.1.5 *Bannoa*

The genus *Bannoa* comprises seven species with some being dimorphic such as *B. hahajimensis*, the type species, in which a hyphal stage has been observed (Hamamoto et al. 2002) and *Bannoa tropicalis* sp. nov. Mycelium and clamp connections were observed after pairing of compatible strains. Obovoid basidia-like structures clamped at the base emerging laterally from the hyphae were formed (Hamamoto et al. 2002, Chapter 2). Development of sexual structures has only been observed under laboratory conditions. The asexual stage is characterized by pink to orange or red colonies (Figure 1H). Cells occur singly or in parent-bud pairs and pseudohyphae have not been reported in the genus. Ballistoconidia are produced. (Hamamoto et al. 2002, Chapter 2). When cultured in liquid media a ring is formed in *B. syzygii* and *B. ogasawarensis* but no pellicle. On the other hand, a pellicle is formed in *B. bischofiae* and *B. hahajimensis*. Species in *Bannoa* have been isolated from the phylloplane, dead leaves of various plant species, rust-infected leaves and cloud water (Chiang et al. 2001, Hamamoto et al. 2002, Matheny et al. 2006, Vaïtilingom et al. 2012, James et al. 2016, Raghavendra et al. 2017). *Bannoa* was traditionally known from subtropical regions, however, in recent years it has been found in the tropics, in countries such as Guyana, Mexico, Puerto Rico and Panama (Matheny et al. 2006, James et al. 2016, Chapter 2).

1.1.6 *Cyrenella*

Cyrenella elegans is the only species in this genus. A hyphal stage with clamp connections and spherical teliospores constitutes the sexual stage. Conidia that emerge from dikaryotic hyphae are thin-walled with three to four apical appendages (Gochenaur 1981). They are referred to as tetraradiate conidia and are presumed to be an adaptation to aquatic environments (Gochenaur 1981, Sampaio 2011). In culture, colony color is orange, cells are ovoid to elongate and occur singly or in parent-bud pairs. Budding is predominantly polar, but sometimes multilateral. Ballistoconidia are not formed. Formation of a ring or pellicle in culture conditions have not been observed. *Cyrenella elegans* has been isolated from sand scraped from a submerged stipe of *Laccaria trullisata* (Gochenaur 1981, Sampaio 2011).

1.1.7 *Hasegawazyma*

The only species in the genus is *Hasegawazyma lactosa*. Its sexual stage is unknown. Colonies in media are pink. The asexual stage is characterized by ellipsoid to ovoid yeast cells, occurring singly or in parent-bud pairs, budding is predominantly polar and pseudohyphae are not produced. Ballistospores have not been observed (Hasagawa 1959). After one month in liquid media we were able to see formation of a ring, and a pellicle was formed on the surface when the type strain was cultured in liquid media. *Hasegawazyma lactosa* was isolated from air sampling in cowsheds but also has been isolated from mangrove sediments and sea water (Hasagawa 1959, Sampaio 2011).

1.1.8 *Cyphobasidium*

Until recent years, the phylogenetic position of lichen-inhabiting taxa within Pucciniomycotina such as *Chionosphaera* and *Cystobasidium* (Diederich 2007) was uncertain due to the lack of molecular data. For that reason, lichenicolous fungi in the Cystobasidiomycetes were placed in *Cystobasidium* based merely on morphological characters (Diederich 1996) and only after molecular data became available was possible to establish their real position within the class. *Cyphobasidium* was erected to accommodate *Cyphobasidium usneicola* and *Cyph. hypogymniicola* (Millanes et al. 2016). The genus is characterized by producing galls on the lichen thalli, also species in the genus are the only representatives in the Cystobasidiomycetes in which the hyphal stage (sexual stage) is predominant in nature (Millanes et al. 2016). Contrarily, Spribille et al (2016) suggest that the filamentous stage is rarely observed. The presence of a sexual stage

within the galls suggests that species in the genus complete their life cycle within the lichens (Spribille et al. 2016). On *Hypogymnia physodes*, the species *Cyph. hypogymniicola* induces the formation of galls, the hypha is present but clamp connections and haustorial branches are not observed. Basidia develop from a thick-walled probasidium and basidiospores are ellipsoid to fusiform, more or less symmetrical (Diederich 2007). The asexual stage consists of budding yeasts with round and thin-walled cells embedded in the upper cortex of the lichen (Spribille et al. 2016). *Cyphobasidium hypogymniicola* has been observed on *H. physodes* and *Cavernularia hultenii* while *Cyph. usneicola* was first reported from several species of *Usnea* including *U. subfloridana* (Diederich 1996, 2007, Millanes et al. 2015). Other sequences belonging to *Cyphobaisidium* have been obtained from *Bryoria fremontii* and *B. tortuosa* that are nomenclatural synonyms and are recognized by the production of vulpinic acid. It was hyphotesized that differential gene expresion was responsible for the increased production of *B. tortuosa* in respect to *B. fremontii*. Based on the results obtained by Spribille et al. (2016), both the transcript abundance data and the abundance of cells in thalli, suggested that the higher production of vulpinic acid in *B. tortuosa* implicates *Cyphobasidium* either through the direct production or by inducing the production by the lecanoromycete.

Even though most of the sequences derived from lichens form a monophyletic clade with *Cyphobasidium*, there is a clade that appears closely related to Microsporomyces and *Sakaguchia* (Spribille et al. 2016) meaning, that there is at least another lichen-inhabitant evolutionary lineage within the Cystobasidiomycetes. The finding of related basidiomycetes in 52 lichen genera most of them in the Parmeliaceae from six continents, has changed the traditional definition of the lichen symbiosis and its nature in which only one fungus and a photosynthesizing symbiont is sometimes accompanied by a second photosynthesizing symbiont (Spribille et al. 2016, Hawksworth 1988)

1.1.9 *Symmetrospora*

Symmetrospora consists of seven species with *S. gracilis* as the type species. A sexual state has not been reported in any species of the genus. The asexual stage produces orange to red colonies (Figure 1I); cells occur singly or in parent-bud pairs. Symmetrical ballistospores are produced in all species. When cultured in liquid media, the production of a ring has been reported in *S. coprosmae, S. marina, S oryzicola* and *S. vermiculata*, while a pellicle on the surface of liquid media has been observed in *S. coprosmae, S. folicola* and *S. vermiculata*. Species in the genus have

been isolated from the phylloplane of several plant species, dead leaves, the roots of plants, mangrove sediments, the gut of *Diabrotica virgifera*, aphids (*Aphis spiraecola)*, shrimps (*Peneaus setiferusn*) and the fruiting body of *Pleurotus* sp. (Nakase and Suzuki 1986, Nakase and Takashima 2000, Wang and Bai 2004).

1.1.10 *Buckleyzyma*

Buckleyzyma is a small genus in the Buckleyzymales comprising six species with *B. aurantiaca* as the type species. Rudimentary septate hyphae were observed in *B. kluyveri-nielii* while in *B. phyllomatis* these hyphae were described as adventitiously septate hyphae that delimited terminal lipid-rich cells (Van Der Walt et al 1986, 1988). In both cases, hyphae were produced in single-cell cultures on corn meal agar (CMA, Difco) (Van Der Walt et al. 1986, 1988). The clamp connections or development of sexual structures have not been observed in the genus. The asexual stage in *Buckleyzyma* species consists of pink-salmon to orange colonies (Figure 1A) with ellipsoidal to cylindrical cells that in general occur singly or in parent-bud pairs. In *B. phyllomatis*, cells sometimes appear organized in small clusters. Budding is predominantly polar. Production of ballistoconidia and pseudohyphae has been reported in *B. kluyveri-nielii, B. phyllomatis* and *B. salicina* while it is absent in *B. armeniaca* and *B. aurantiaca*. Formation of a ring when cultured in liquid media has been observed in *B. aurantiaca*, *B. kluyveri-nielii* and *B. phyllomatis*. Formation of a pellicle on the media has not been observed in *Buckleyzyma*. Species in *Buckleyzyma* have been isolated from the phylloplane of several plant species, from air sampling, lake water, mangrove sediments and litter (Shivas and Rodrigues de Miranda 1983, Johri and Bandoni 1984, Van Der Walt et al 1986, 1988).

1.1.11 *Sakaguchia*

Sakaguchia contains six species, in which *S. dacryoidea* is the type. In the type, development of sexual structures and hyphae with clamp connections occur after compatible strains are paired. Two- to four-celled basidia (promycelium) germinate from a thick-walled teliospore. In the basidium, lateral and terminal haploid basidiospores are produced (Fell et al. 1973). For other species in the genus a sexual stage has not been reported. In the asexual stage, species in *Sakaguchia* exhibit pigmented colonies ranging from light pink to orange or red. Cells are arranged in parent-bud pairs (Fell et al. 1973, 2011, Wang et al. 2015b). Cell shape can be ovoidal,

ellipsoidal or cylindrical. Budding is polar or multilateral. Pseudo hyphae are not formed, however cells of *S. cladiensis* and *S. dacryoidea* can be found in short chains (Fell et al. 1973, 2011). Ring formation has only been reported is *S. oryzae*, but not the presence of a pellicle on the liquid media. Species in *Sakaguchia* have been isolated from sea water, fresh water, the gut of fish, tube worm (*Lamellibrachia* sp.), mangrove sediments, and plants such as *Oryza sativa* and *Avicennia germinans* (Fell et al. 1973, Nagahama et al. 2001, Almeida and Sampaio 2003, Libkind et al. 2003, Libkind et al. 2010, Fell et al. 2011, Velmurugan et al. 2013, Raggi et al. 2014, Francis et al. 2016).

1.1.12 *Microsporomyces*

The genus comprises five species for which *Microsporomyces magnisporus* is the type. A sexual stage has not been reported in *Microsporomyces*. The asexual stage generally produces orange or salmon colonies. However, *M. hainanensis* colonies are brownish white. Cells occur singly or in parent-bud pairs, but in *M. magnisporus* cells can be found in small clusters. Budding can be either monopolar or multilateral. Ballistospores are only reported in *M. magnisporus* and pseudohyphae are observed in *M. pini, M. hainanensis* and *M. magnisporus*. Formation of a ring has been reported in *M. hainanensis* and *M. magnisporus* while a pellicle is not formed.

1.1.13 *Halobasidium*

Halobasidium is a recently described monotypic genus in which *Halobasidium xiangyangense* is considered a high-salt tolerant species. A sexual stage is unknown, and its asexual stage exhibits orange to light pink colonies (Figure 1F), cells that are globose or ellipsoidal and occur singly or in parent-bud pairs. Budding is predominantly polar. Sometimes short chains of two or three buds and occasionally Y-shaped groups, but pseudohyphae have not been observed. The type strain was isolated from pickling sauce used to make Datoucai, which is a traditional fermented food made of *Brassica juncea* in China (Guo et al. 2019).

There are over 790 reports in the NCBI, either of strains or sequences of the Cystobasidiomycetes obtained from environmental studies; 378 (47.7%) of these reports have not been affiliated to any of the extant species in the class, while there are other 55 (6.9%) ambiguously classified. This means, that several sequences have been named in a species to which they do not belong. In total, over 54% of sequences included in this dissertation that were retrieved from the

NCBI have some taxonomic uncertainty for which the study of the ecology, host preference and geographic distribution remains obscure (For further detail on species delimitation and classification of sequences in the Cystobasidiomycetes see Chapter 4). However, those are not the only difficulties when studying this class. Reproductive biology of species is also understudied, just a couple of species such as *O. kilbournensis*, *O. externus* and *Bannoa hahajimensis* have been studied under laboratory conditions in this regard. In Chapter 2, we discuss the reproductive biology of a newly described *Bannoa tropicalis* making a contribution to the existing knowledge about the reproductive biology of species in the class. In addition, several species lack molecular data for which it has not been possible to establish their phylogenetic placement, for example, the affiliation of *O. internus* which is the type species for the genus remained unknown until now. We have generated molecular data that would allow to better understand phylogenetic relationships and bring more clarity to the taxonomy of the Cystobasidiomycetes.

In the present chapter, we compile available information of all the 13 genera in the class including features of the most recently described species. We make emphasis on the sexual stage of species in the class, since it is during this stage in which several antagonistic interactions have been documented in nature. Nevertheless, these interactions do not only occur through direct physical contact, i.e., mycoparasitism, but also, they can occur through the production of antimicrobial compounds. This latter phenomenon and how microorganisms defend themselves from these compounds is understudied in the Cystobasidiomycetes

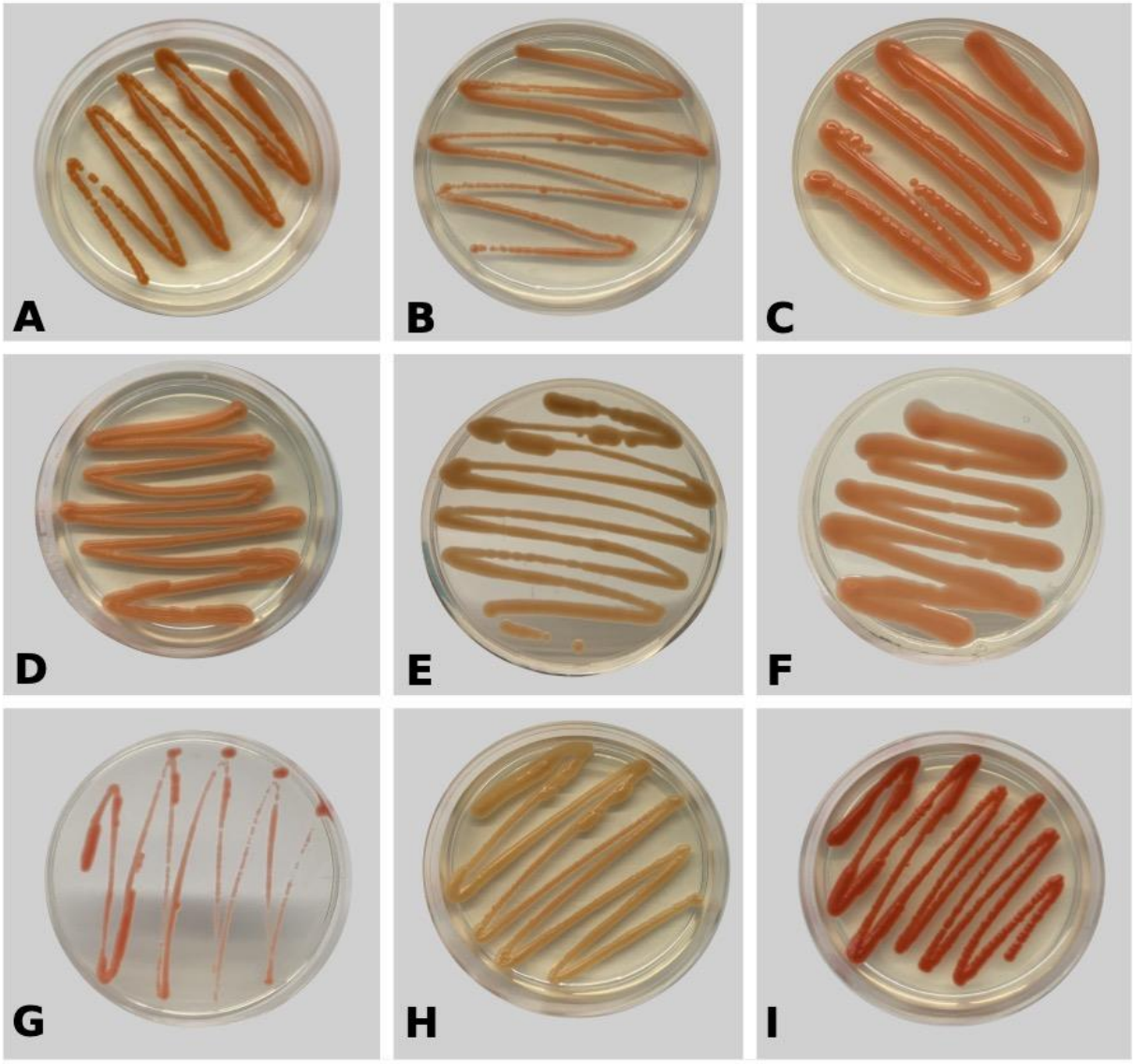

Figure 1.1 Colony pigmentation in some species in the Cystobasidiomycetes. **A.** *Buckleyzyma salicina* **B.** *Erythrobasidium elongatum* **C.** *E. hasegawianum* **D.** *Cystobasidium minutum* **E.** *C. psychroaquaticum* **F.** *Halobasidium xiangyangense* **G.** *Symmetrospora oryzicola* **H.** *Bannoa* sp. **I.** *S. coprosmae.*

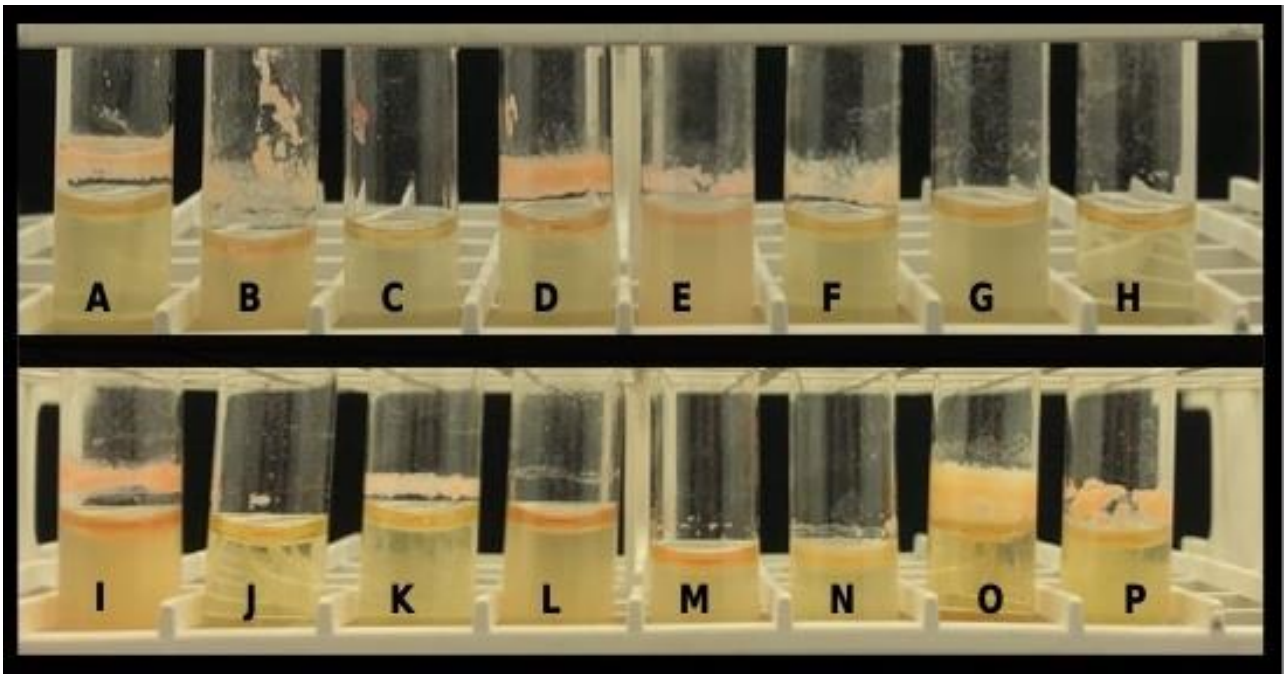

Figure 1.2 Production of a ring in the Cystobasidiomycetes cultured in liquid media containing yeast extract 50 mg/l, Casa amino acid mg/l and 1% glucose. **A.** *Halobasidium umbonatum* (MT254) **B.** *Bannoa tropicalis* (MCA4667) **C.** *Cystobasidium laryngis* (CBS5695) **D.** *Erythrobasidium elongatum* (INDP051) **E.** *Symmetrospora coprosmae* (MT264) **F.** *Cystobasidium* sp. (MCA7633) **G.** *Buckleyzyma butyrosa* (MCA2993) **H.** *Cystobasidium* sp. (MCA7412) **I.** *Symmetrospora* sp. (MCA7676) **J.** *Cystobasidium slooffiae* (SA515) **K.** *Cystobasidium ongulense* (P107) **L.** *Symmetrospora* sp. (P114) **M.** *Symmetrospora* sp. (SA042) **N.** *Cystobasidium minutum* (CBS4407) **O.** *Bannoa* sp. (MCA7674) **P.** *H. xiangyangense* (RK86).

CHAPTER 2. NEW SPECIES OF BANNOA DESCRIBED FROM THE TROPICS AND THE FIRST REPORT OF THE GENUS IN SOUTH AMERICA

2.1 Abstract

The genus *Bannoa* consists of four described species associated with dead leaves in southwestern Japan. In this study we describe three new species, *Bannoa guamensis*, *B. rosea*, and *B. tropicalis*, from the South Pacific island of Guam and Guyana in South America. Isolates were obtained from surfaces of diseased and healthy leaves of plants in the Euphorbiaceae, Asteraceae, and Poaceae. DNA sequences from four gene regions including nuclear rDNA ITS1-5.8S-ITS2 (ITS), D1–D2 domains of nuclear 28S rDNA (28S), nuclear 18S rDNA (18S), and a portion of *tef1*, which encodes translation elongation factor 1-alpha, were used for phylogenetic analysis. Intercompatibility tests were performed and subsequent development of clamp connections and basidia were documented for *B. tropicalis*. Potential life history strategies and association with diseased leaves including rust-infected leaves were evaluated across the genus. This is the first report of a species of *Bannoa* from South America.

2.2 Introduction

Bannoa in the Cystobasidiomycetes was erected as a monotypic genus based on the dimorphic yeast *B. hahajimensis* (Hamamoto et al. 2002) isolated from dead leaves of *Bryophyllum pinnatum* commonly known as cathedral bells is a succulent plant from the Crassulaceae family native to Madagascar, from *Rhaphiolepis wrightiana* known as Japanese-hawthorn that belongs to the Rosaceae family and from *Syzygium buxifolium* know as the boxleaf Eugenia that belongs to the Myrtaceae family , all collected from Haha-jima island in south Japan. Based on analysis of the nuclear 18S rDNA (18S) and nuclear ITS1-5.8S-ITS2 rDNA (ITS) regions of the rDNA repeat, *B. hahajimensis* was placed in the Erythrobasidiales and remained the only recognized species for 15 years (Hamamoto et al. 2002). In 2015, *Sporobolomyces bischofiae*, *S. ogasawarensis*, and *S. syzygii* were transferred to *Bannoa* based on a seven-gene phylogeny (Wang et al. 2015a), raising the total number of known species in *Bannoa* to four. Members of *Bannoa* are rarely isolated in pure culture. Reported *Bannoa* isolates are from dead leaves of various plant species in the

Ogasawara, Iriomote-Jima, and Yakushima islands of subtropical southwestern Japan and from the surface of leaves of *Miscanthus* and other unidentified plants in Taiwan (Chiang et al. 2001). Two other isolates of *Bannoa* have been reported in Panama (Matheny et al. 2006) and from cloud water samplings in France (Vaïtilingom et al. 2012) but have not been named or described. However, environmental sequences indicative of *Bannoa* have been reported from *Fagus sylvatica* (European beech) in Austria (Moll et al. 2011), in Australia from herbaceous plants such as *Mimosa pigra*, *Jatropha gossypiifolia* and *Vachellia nilotica* subsp. *Indica* and trees such as *Parkinsonia aculeata* and *Tamarix aphylla*, (Raghavendra et al. 2017), and from sori of *Hemileia vastatrix* on coffee plant leaves in Mexico and Puerto Rico (James et al. 2016). Taken together this indicates a wider geographic range for the genus. All known isolates of *Bannoa*, except those of Vaïtilingom et al. (2012) from cloud water, have been associated from the phylloplane of dead asymptomatic or infected leaves. The study by James et al. (2016) suggests that some *Bannoa* and other species in the Cystobasidiomycetes such as *Symmetrospora oryzicola* and *S. coprosmae* are capable of inhabiting the sori of rust fungi. However, it is not clear if these species are saprobes, endophytes, or mycoparasites. Examples of mycoparasitic species in the class include *Cystobasidium fimetarium*, *Naohidea sebacea*, and *Occultifur internus*, in which the hyphal stages have been found parasitizing other fungi (Roberts 1999, Piatek 2002, Bauer et al. 2006). The possibility that dimorphic species of *Bannoa* have a mycoparasitic life strategy during the hyphal stage should not be discarded.

During field collecting in Guyana and on Guam, numerous isolates of salmon-pigmented ballistosporic yeasts were isolated from diseased and asymptomatic leaf surfaces. Morphological, physiological, and phylogenetic analyses based on four loci indicate their placement within *Bannoa* in three previously undescribed species, described herein as *B. guamensis, B. rosea,* and *B. tropicalis*. Mating intercompatibility crosses were attempted among isolates, and basidium formation was induced in *B. tropicalis*. *Bannoa tropicalis* represents the first species of *Bannoa* to be recorded from South America.

2.3 Material and Methods

2.3.1 *Sample collection and storage*

Thirteen strains were obtained using a spore-drop method described by Toome et al. (2013) from rust-infected and asymptomatic leaves of live plants in the Asteraceae (*Bidens* sp.), Euphorbiaceae (*Euphorbia cyathophora*) and Poaceae (*Lepturus repens*) collected in Guam in 2012. One additional strain was obtained from an asymptomatic leaf of an unidentified herbaceous plant in Guyana in 2013 (Table 2.1 Collection information and GenBank numbers for material used in this study.). Cultures were maintained on potato dextrose agar (PDA; 39 g/L, Livonia, Michigan) slants at 4 C for short-term storage and in 40% glycerol vials at -80 C for long-term storage. Cultures of isolates MCA4612, MCA4647, and MCA4667 were preserved as dried inert cells and deposited as holotypes in the Kriebel Herbarium (PUL). Live cultures were also deposited at the Westerdijk Fungal Biodiversity Institute (CBS) and the Fungal Biodiversity Center and Agricultural Research Service (NRRL).

2.3.2 *Morphological characterization*

Each strain was cultured on yeast malt agar (YMA, Difco, Franklin Lakes, New Jersey), yeast malt broth (YM broth, Difco), and corn meal agar (CMA, Difco) at 25 C for seven days to study the morphology of colonies. These were characterized by form, elevation, margin, and surface. Colony color was determined from comparisons with the Online Auction Color Chart (oac). Cell morphology was examined using phase-contrast optics under an Olympus BH-2 compound microscope. Twenty individual cells from each strain were measured. Images were captured with an Olympus SC30 camera and cells measured using Olympus cells Sens Entry 1.14 software. The nuclear condition of individual cells and hyphae, where possible, were studied by staining the nucleus with Vectashield mounting medium with DAPI (Burlingame, California) and observed using epifluorescence on a Nikon Eclipse E800 microscope.

2.3.3 *Physiological studies*

Assimilation of carbon and nitrogen compounds in liquid media, fermentation tests, growth on high osmotic media, and growth on vitamin free media were performed according to Kurtzman et al. (2011). The ability of strains to grow on different carbon sources was assessed by using a Yeast

Identification Test Panel (Biolog Inc., Hayward, California) as performed in Kijpornyongpan and Aime (2017).

2.3.4 *DNA extraction, sequencing, and phylogenetic analyses*

DNA extraction was performed from pure cultures grown on YMA using the Promega Wizard genomic DNA purification kit (Promega, Madison, Wisconsin). Phylogenetic placement of newly collected strains was inferred from sequences of ITS, D1–D2 domains of the nuc 28S rDNA (28S), 18S, and from a portion of *tef1*, which encodes translation elongation factor 1-alpha. Amplification and sequencing primers included ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990a), LR0R/LR5 (Vilgalys and Hester 1990), NS1/NS4 and NS3/NS8 (White et al. 1990a), and EF-FC/2218R (Rehner 2005). PCR programs for amplification of the ITS consisted of a denaturation step at 95 C for 5 min, followed by 35 cycles at 94 C for 30 s, annealing T at 45 C for 45 s, 72 C for 45 s, and a final extension step at 72 C for 7 min. For the 18S and 28S regions, the program consisted of a denaturation step at 94 C for 5 min., followed by 35 cycles at 94 C for 30 s, annealing T at 50 C and 58 C for 45 and 30 s for 28S and 18S, respectively, extension at 72 C for 1 min, and a final extension step at 72 C for 7 min. For amplification of *tef1*, the PCR program consisted of a denaturation step at 95 C for 2 min., starting with 9 cycles at 95 C for 1 min, annealing at 66 C for 1 min, and extension at 72 C for 1 min, followed by 35 cycles more at 95 C for 1 min, 56 C for 1 min, then 72 C for 1 min, and a final extension step at 72 C for 7 min. PCR products were separated in a 1% agarose gel and sent to GENEWIZ (South Plainfield, New Jersey) for direct sequencing. Sequences were edited in GENEIOUS 9.1.2 (Biomatters, Auckland, New Zealand).

For phylogenetic reconstructions, taxon selection of related species in the Erythrobasidiales was based on Aime et al. (2006) and Wang et al. (2015b). ITS and 28S regions from our *Bannoa* strains were used to run a BLASTN search against the NCBI GenBank database. Sequences from environmental sampling and previous studies closely related to *Bannoa* were included in our analyses. Accession numbers for sequences of collected strains of *Bannoa* and those from GenBank are provided in Table 2.1. *Microsporomyces magnisporus* and *M. orientalis* were used for outgroup purposes (Wang et al. 2015b). Sequences for each locus were aligned with MAFFT 7.222 (Katoh et al. 2017) using the L-INS-I algorithm. To test the level of congruency of the loci used in this study, a Congruence Among Distance Matrices (CADM) analysis was performed to statistically test the level of congruence among independently built phylogenetic

trees with each gene (Campbell et al. 2011). The APE (Analyses of Phylogenetics and Evolution) package in R contains the CADM module to calculate the Kendall's W statistic that provides an estimate of the degree of congruence of two or more matrices on a scale between 0 (no congruence) and 1 (congruence) (Campbell et al. 2011). In studies performed by Sánchez-García et al. (2016), the value for the W statistic equal to 0.90 showed no significant incongruence among loci. In the present study we used 0.90 as reference value to determine the acceptable level of congruence among the loci used for describing new species in *Bannoa.*

Alignments of the 18S, ITS and 28S in a first dataset and alignments of the 18S, ITS, 28S and *tef1* in a second dataset were concatenated using GENEIOUS 9.1.2 for phylogenetic reconstruction. Ambiguously aligned regions, including *tef1* introns, were removed with GBLOCKS using the less stringent selection option (Talavera and Castresana 2007). The 4-gene concatenated alignment was uploaded to TreeBASE (study no. 23244). The GTR GAMMA-I model was selected as the substitution model for the 3 and 4-gene region alignment according to PARTITIONFINDER2 based on the Akaike Information Criterion (AIC). The protein coding gene (*tef1*) was partitioned by each codon position. Phylogenetic reconstruction was performed using Maximum Likelihood (ML) followed by 1000 bootstrap replicates using GENEIOUS 9.1.2. Bootstrap proportions (BP) are reported below. Bayesian inference (BI) was performed using MRBAYES 3.2.6 (Huelsenbeck and Ronquist 2012) with a GTR model, nts:6, rates=invgamma according and five million generations. The first 25% of trees were discarded as Burn-in.

2.3.5 *Mating intercompatibility*

Single-cell cultures were obtained by serial dilution. Mating experiments were performed according to Yarrow (1998) by pairing ten out of the 13 collected isolates in all possible combinations on CMA . Plates were placed under different growing conditions: Incubation at 25 C under 12:12h light:darkness, incubation at 30 C under 12:12h light:darkness conditions, and incubation at 25 C under complete darkness. Plates were monitored for 12 weeks. Development of mycelium, clamp connections, and basidia were first assessed using an Olympus SZ61 dissecting microscope. If sexual structures were present, then mountings were prepared for further observation under a compound microscope at higher magnification.

2.4 Results

2.4.1 *Phylogenetic analyses*

The 3- and 4-gene combined datasets comprised 2448 and 3222 nucleotide sites. Of these the ITS contributed 504 sites, 28S 557 sites, 18S 1387 sites, and *tef1* 774 sites after exclusion of introns and ambiguously aligned positions. The CADM analysis showed no significant incongruence between the four loci used in the phylogenetic studies ($w = 0.91$; $p < 0.001$). The 3-gene region phylogenetic tree (Figure 2.1 and the 4-gene phylogenetic reconstruction (**Error! Reference source not found.**for *Bannoa* including sequences from tropical isolates formed a monophyletic group with strong support in both Maximum Likelihood (ML) and Bayesian (BI) analysis. The overall topologies of the phylogenetic reconstruction using both 3- and 4-gene regions were similar. At least nine species-level lineages are delineated, three of which are new: *Bannoa guamensis*, *B. rosea* and *B. tropicalis*. *Bannoa rosea* isolated from asymptomatic leaves, is known only from sequences of a single strain collection and appears as an independent evolutionary lineage (BP:68 and PP:1); its sister species *B. ogasawarensis* has only been reported from dead leaves; *B. guamensis* also known from single strain collections was isolated from diseased leaves of *Bidens* sp. and appears phylogenetically related to an unclassified *Bannoa* isolated from rust-infected leaves of coffee (BP:67 and PP:0.75). Lastly, strains associated with asymptomatic leaves reported from Panama (*MP3490*) (Matheny et al. 2006) as *Bannoa* sp. and Thailand (*TY-144*, *TY-177*, *TY-201*, *TY-261*, TY-270 and *TY-288*) previously classified as *Sporobolomyces* sp. (Nakase et al. 2001) are conspecific with *B. tropicalis* (BP:59 and PP:0.80).

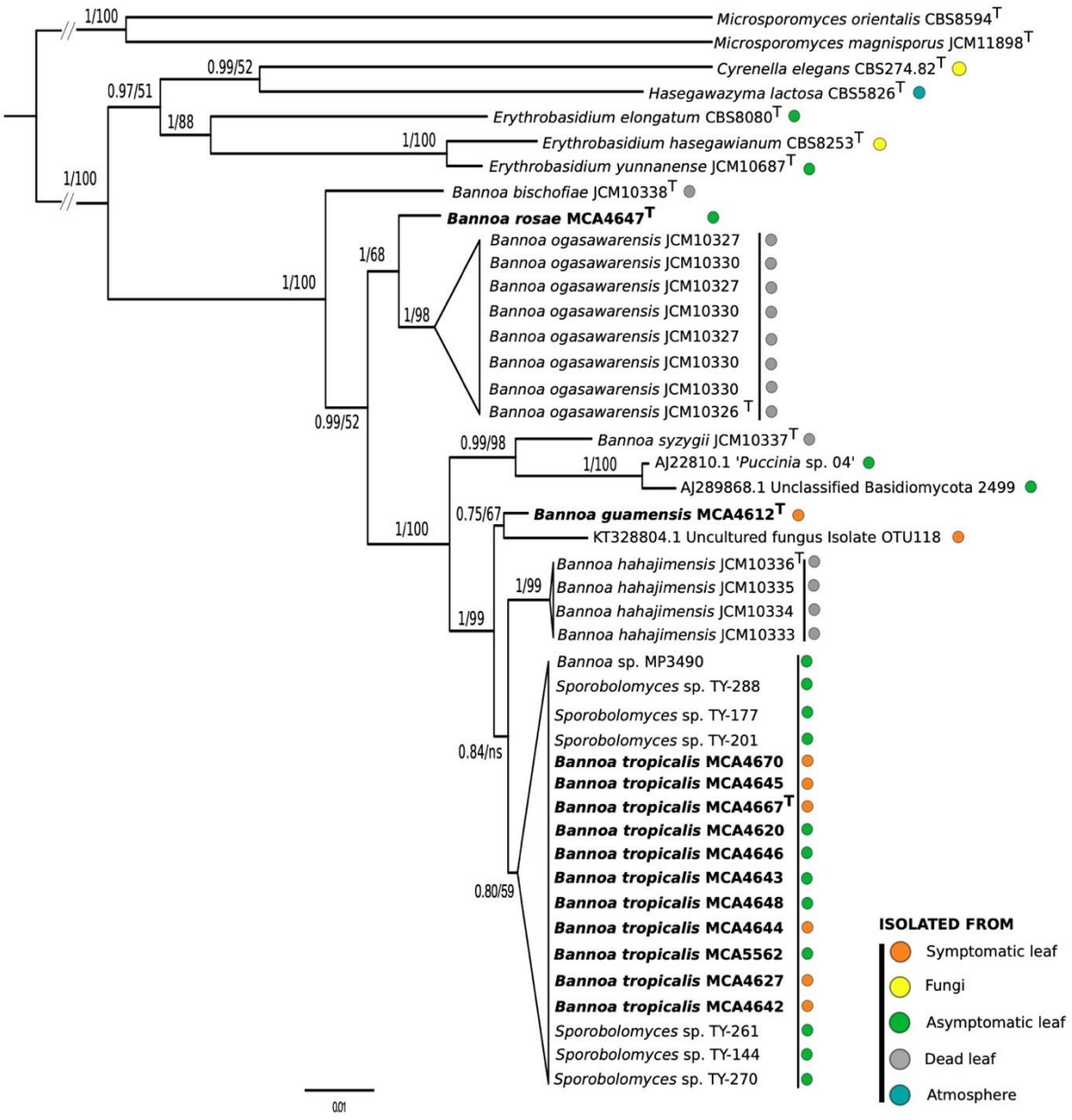

Figure 2.1 Phylogenetic reconstruction for *Bannoa* inferred from combined sequences of ITS, D1–D2 domains of nuclear 28S and nuclear 18S rDNA and *tef1* using ML and BI analysis. New species are indicated in bold. Posterior probability (PP > 0.7) and bootstrap support (BP > 50%) values are shown above nodes. ns: not supported. Tree is midpoint rooted. Scale bar equals expected number of substitutions per site. Strain number is listed in front of the taxon name. ^{T} denotes type species strain. Legend indicates substrate from which each strain was isolated.

Table 2.1 Collection information and GenBank numbers for material used in this study.

SPECIES	STRAIN NUMBER	ISOLATED FROM	LOCATION	COUNTRY	ITS	LSU	SSU	*TEF1α*	Reference
Bannoa guamensis	MCA4612^{T}	Diseased leaf of *Bidens sp.*	University of Guam	Guam	**MK287350**	**MK255006**	**MK254996**	**MK491345**	This study
Bannoa rosea	MCA4647^{T}	Asymptomatic undetermined leaf	Paseo Park	Guam	**MK287351**	**MK255007**	**xxxxxx**	**MK491346**	This study
Bannoa tropicalis	MCA4667^{T}	*Uromyces* diseased leaf of *Bidens* sp.	Andersen Air Force Base	Guam	**MK287360**	**MK255016**	**MK255003**	**MK491353**	This study
Bannoa tropicalis	MCA4620	Asymptomatic undetermined leaf	Paseo Park	Guam	**MK287352**	**MK255008**	**MK254997**	**MK491347**	This study
Bannoa tropicalis	MCA4627	Puccinia-infected leaf of Lepturus sp.	Yigo Experimental Station	Guam	**MK287353**	**MK255009**	--------	--------	This study
Bannoa tropicalis	MCA4642	Diseased leaf of *Euphorbia cyathophora*	Yigo Experimental Station	Guam	**MK287354**	**MK255010**	**MK254998**	--------	This study
Bannoa tropicalis	MCA4643	Palm trash swab	Paseo Park	Guam	**MK287355**	**MK255011**	--------	**MK491348**	This study
Bannoa tropicalis	MCA4644	*Puccinia lepturi* diseased leaf of *Lepturus repens*	Yigo Experimental Station	Guam	**MK287356**	**MK255012**	**MK254999**	**MK491349**	This study
Bannoa tropicalis	MCA4645	*Kweilingia* symptomatic leaf of a Poacea plant	Bernard's Farm	Guam	**MK287357**	**MK255013**	**MK255000**	**MK491350**	This study
Bannoa tropicalis	MCA4646	Asymptomatic undetermined leaf	Paseo Park	Guam	**MK287358**	**MK255014**	**MK255001**	**MK491351**	This study
Bannoa tropicalis	MCA4648	Asymptomatic undetermined leaf	Paseo Park	Guam	**MK287359**	**MK255015**	**MK255002**	**MK491352**	This study
Bannoa tropicalis	MCA4670	*Uromyces* diseased leaf of *Bidens* sp.	Andersen Air Force Base	Guam	**MK287361**	**MK255017**	**MK255004**	--------	This study

Table 2.1 continued

SPECIES	STRAIN NUMBER	ISOLATED FROM	LOCATION	COUNTRY	ITS	LSU	SSU	*TEF1α*	Reference
Bannoa tropicalis	MCA5562	Asymptomatic undetermined leaf	Rockview	Guyana	**MK287362**	**MK255018**	**MK255005**	**MK491354**	This study
Bannoa sp. MP3490	MP3490	*Cintractia axicola* diseased leaf	Unknown	Panama	DQ631900	DQ631898	DQ631899	DQ631902	Matheny et al. (2006),Wang et al. (2015b)
Bannoa sp.	Puccinia sp., isolate fungus 04	*Miscanthus* sp.	Unknown	Taiwan	AJ222810.1	--------	--------	--------	Chiang et al. (2001)
Bannoa sp.	Unclassified basidiomycota 2499	*Miscanthus sinensis*	Unknown	Taiwan	AJ289868.1	--------	--------	--------	Chiang et al. (2001)
Bannoa sp.	Uncultured fungus isolate OTU 118	*Hemileia vastatrix* diseased leaf of coffee	Chiapas	Mexico	KT328804.1	--------	--------	--------	James et al. (2016)
Bannoa sp.	Sporobolomyces sp. TY-144	Undetermined plant	Unknown	Thailand	--------	AY313038.1	--------	--------	Takashima and Nakase (2000)
Bannoa sp.	Sporobolomyces sp. TY-177	Undetermined plant	Unknown	Thailand	--------	AY313042.1	--------	--------	Takashima and Nakase (2000)
Bannoa sp.	Sporobolomyces sp. TY-201	Undetermined plant	Unknown	Thailand	--------	AY313044.1	--------	--------	Takashima and Nakase (2000)
Bannoa sp.	Sporobolomyces sp. TY-261	Undetermined plant	Unknown	Thailand	--------	AY313056.1	--------	--------	Takashima and Nakase (2000)
Bannoa sp.	Sporobolomyces sp. TY-270	Undetermined plant	Unknown	Thailand	--------	AY313058.1	--------	--------	Takashima and Nakase (2000)

Table 2.1 continued

SPECIES	STRAIN NUMBER	ISOLATED FROM	LOCATION	COUNTRY	ITS	LSU	SSU	*TEF1α*	Reference
Bannoa sp.	Sporobolomyces sp. TY-288	Undetermined plant	Unknown	Thailand	--------	AY313060.1	--------	--------	Takashima and Nakase (2000)
Bannoa bischofiae	JCM 10338^{T}	Undetermined plant	Chichi-jima	Japan	AB035721	AB082572	AB035721	KJ707777	Hamamoto et al. (2002), Wang et al. (2015b)
Bannoa hahajimensis	JCM 10336^{T}	Dead leaves of *Bryophyllum pinnatum*	Haha-jima	Japan	AB035897	AB082571	AB035897	KJ707750	Hamamoto et al. (2002), Wang et al. (2015b)
Bannoa hahajimensis	JCM 10335	Dead leaves of *Syzygium buxifolium*	Haha-jima	Japan	AB035894	--------	AB035894	--------	Hamamoto et al. (2002), Wang et al. (2015b)
Bannoa hahajimensis	JCM 10334	Dead leaves of *Rhaphiolepis wrigthiana*	Haha-jima	Japan	AB035895	--------	AB035895	--------	Hamamoto et al. (2002), Wang et al. (2015b)
Bannoa hahajimensis	JCM 10333	Dead leaves of *Bryophyllum pinnatum*	Haha-jima	Japan	AB035896	--------	AB035896	--------	Hamamoto et al. (2002), Wang et al. (2015b)
Bannoa ogasawarensis	JCM 10326^{T}	Dead leaves of *Osmanthus insularis*	Chichi-jima	Japan	AB035713	AB082570	AB035713	KJ707781	Hamamoto et al. (2002), Wang et al. (2015b)
Bannoa syzygii	JCM 10337^{T}	Dead leaves of Syzygium buxifolium	Chichi-jima	Japan	AB035720	AB082573	AB035720	KJ707778	Hamamoto et al. (2002), Wang et al. (2015b)
Erythrobasidium elongatum	CBS8080^{T}	Leaves of *Callistemon viminalis*	Armidale	Australia	AF444561	AF189983	AB021669	KJ707782	Nagahama et al. (2006), Wang et al. (2015b)

Table 2.1 continued

SPECIES	STRAIN NUMBER	ISOLATED FROM	LOCATION	COUNTRY	ITS	LSU	SSU	*TEF1α*	Reference
Erythrobasidium hasegawianum	CBS8253[T]	Culture of beer yeast	Philadelphia	USA	AF444522	AF189899	D12803	KJ707776	Sampaio et al. (1999), Wang et al. (2015b)
Erythrobasidium yunnanense	JCM10687[T]	Leaf of soapberry	Yunnan	China	AB030353	AB127358	AF229176	KJ707779	Bai et al. (2001), Wang et al. (2015b)
Cyrenella elegans	CBS 274.82	Sand scraped from stipe of *Laccaria trullisata*	New York	USA	KJ778626	KJ708454	KJ708360	KJ707830	Wang et al. (2015b)

Note: -------- : No sequence available. Accession numbers in bold were generated in the present study. Other accession numbers were retrieved from the NCBI. **T** denotes type species strain

2.4.2 Physiological tests

The assimilation of carbon compounds such as lactose and methanol is positive (delayed) for *B. guamensis* but negative for *B. tropicalis* and *B hahajimensis*. Assimilation of L-sorbose is negative for *B. guamensis* while positive for *B hahajimensis* but weak in *B. tropicalis*. Assimilation of citrate, salicin, D-arabinose and succinate is negative in *B. rosea* but positive in *B. ogasawarensis*. Nitrogen compounds such as nitrite ($NaNO_2$), cadaverine, and creatinine are weakly assimilated by *B. tropicalis* but not assimilated by *B. guamensis*, *B. rosea*, or any other previously described species in the genus (Table 2.2). In addition, we found that *B. guamensis, B. rosea*, and *B. tropicalis* grew on vitamin-free media whereas previously described species did not (Hamamoto et al. 2002).

Table 2.2 Assimilation of carbon and nitrogen compounds and growth under different temperature and osmotic conditions—comparison of all known *Bannoa* species.

Compound	***B. tropicalis*^{T} MCA4667**	***B. guamensis*^{T} MCA4612**	***B. hahajimensis*^{T}* JCM10336**	***B. syzygii*^{T}* JCM10337**	***B. bischofiae*^{T}* JCM10338**	***B. ogasawarensis*^{T}* JCM10326**	***B. rosea*^{T} MCA4647**
Carbon assimilation							
Glucose	w	+	+	+	+	+	+
Lactose	-	l	-	-	-	v	s
Methanol	-	l	-	-	-	-	-
Ethanol	l	l	v	-	-	-	-
Galactitol	l	-	v	-	-	v	n
DL-Lactate	l	l	v	+	+	v	+
Citrate	-	-	+	+	+	v	-
Propane 1,2 diol	-	l	n	n	n	-	+
Inulin	+	+	-	-	-	-	w
Sucrose	w	w	+	+	+	+	w
Raffinose	w	w	+	+	+	+	w
Melibiose	w	w	+	+	+	v	w
Galactose	w	w	+	+	+	+	w
Trehalose	w	+	+	+	+	+	w
Maltose	w	w	+	+	+	+	w
Melezitose	w	w	+	+	+	+	w
Cellobiose	+	w	+	+	+	v	w
Salicin	w	w	+	+	+	+	-
L-Sorbose	w	-	+	+	+	+	w
L-Rhamnose	w	w	+	+	-	v	w
D-Xylose	w	w	+	+	+	+	w

Table 2.2 continued

Compound	***B. tropicalis*^{T} MCA4667**	***B. guamensis*^{T} MCA4612**	***B. hahajimensis*^{T}* JCM10336**	***B. syzygii*^{T}* JCM10337**	***B. bischofiae*^{T}* JCM10338**	***B. ogasawarensis*^{T}* JCM10326**	***B. rosea*^{T} MCA4647**
L-Arabinose	w	W	+	+	-	+	w
D-Arabinose	w	w	v	-	-	v	-
Glycerol	w	w	+	+	+	+	-
D-Mannitol	w	w	+	+	+	+	w
myo-Inositol	-	+	-	-	-	-	+
Succinate	-	-	+	+	+	+	-
D-Gluconate	w	+	n	n	n	v	w
N-Acetyl-D glucosamine	w	-	n	n	n	+	-
2-Keto-D-gluconate	w	w	+	+	+	+	w
Xylitol	w	w	n	n	n	n	w
Arbutin	-	w	n	n	n	-	-
d-glucuronic acid	l	l	+	n	n	+	+
d-galacturonic acid	-	-	-	n	n	-	-
Aspartic acid	-	-	n	n	n	n	-
L-Glutamic acid	w	w	n	n	n	n	-
Turanose	w	+	n	n	n	n	w
Adonitol	w	-	n	n	n	n	w
Nitrogen assimilation							
D-glucosamine	w	-	n	n	n	+	-
Nitrite	w	-	-	-	-	-	-
Cadaverine	w	-	-	-	-	-	-
Creatinine	w	-	n	n	n	n	-
Growth conditions							

Table 2.2 continued

Compound	***B. tropicalis*^{T} MCA4667**	***B. guamensis*^{T} MCA4612**	***B. hahajimensis*^{T}* JCM10336**	***B. syzygii*^{T}* JCM10337**	***B. bischofiae*^{T}* JCM10338**	***B. ogasawarensis*^{T}* JCM10326**	***B. rosea*^{T} MCA4647**
10% NaCl	-	-	n	n	n	n	-
16% NaCl	-	-	n	n	n	n	-
Growth at 25°C	+	+	n	+	+	+	+
Growth at 30°C	+	+	-	+	+	+	+
Growth at 35°C	-	-	n	-	+	-	-
Vitamin-free	+	+	-	-	-	-	+

+, positive; -, negative; l, delayed positive or latent; S, slow positive; v, variable; w, weak, n, not determined.
*, denotes assimilation data for *B. hahajimensis*, *B. syzygii*, *B. bischofiae* and *B. ogasawarensis* taken from Hamamoto et al. (2002).

2.4.3 Mating intercompatibility experiments

Mycelium and clamp connections were observed in three of the crosses between isolates of *B. tropicalis* (Table 2.3). Basidium-like structures were observed after 42 days of incubation at room T in complete darkness on CMA (Figure 2.3). These structures resemble those described by Hamamoto et al. (2002) as basidia clamped at the base emerging laterally from the hyphae in *B. hahajimensis*. Basidia-like cells were obovoid to obpyriform (6 × 3.5 µm), sometimes with a slender apex. Single cultures of each strain, similarly incubated, did not develop similar structures. In experiments of intercompatibility incubated at 25 C under 12:12h light:darkness and incubated at 30 C under 12:12h light:darkness conditions, development of mycelium and clamp connections was not observed. Mycelium with clamp connections or basidia were not observed when isolates of *B. guamensis* (MCA4612) and *B. rosea* (MCA4647) were crossed with any of the eight isolates of *B. tropicalis*.

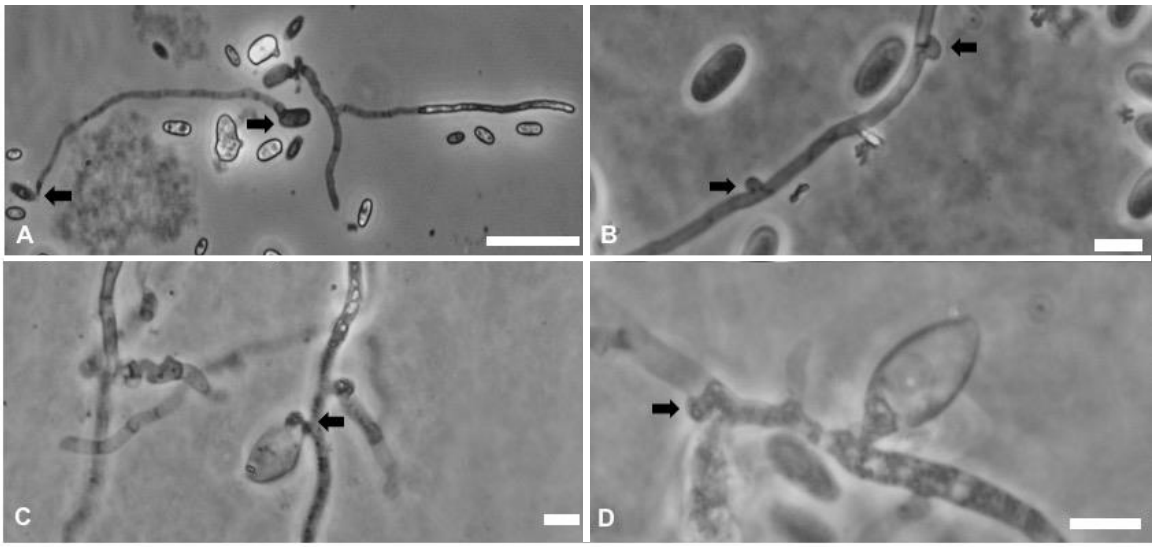

Figure 2.2 Mating in *B. tropicalis*. **A.** Conjugation of cells and germination tube (arrows) in the cross between MCA4667 (holotype) and MCA4648. Bar= 20 µm **B**. Hyphae with clamp connections (arrows) Bar= 2 µm. **C**. Basidium clamped at the base (arrow) Bar= 2 µm, cross between MCA4644 and MCA4667 **D**. Basidium and hyphae with clamp connections (arrows) in cross between MCA4644 and MCA4667 Bar= 5 µm.

Table 2.3 Intercompatibility cross results for ten strains of *Bannoa*

Strain	MCA4612^T	MCA4647^T	MCA4667^T	MCA4620	MCA4643	MCA4644	MCA4645	MCA4646	MCA4648	MCA5562
MCA4612^T	-	-	-	-	-	-	-	-	-	-
MCA4647^T	-	-	-	-	-	-	-	-	-	-
MCA4667^T	-	-	-	-	-	+	-	+	+	-
MCA4620	-	-	-	-	-	-	-	-	-	-
MCA4643	-	-	-	-	-	-	-	-	-	-
MCA4644	-	-	+	-	-	-	-	-	-	-
MCA4645	-	-	-	-	-	-	-	-	-	-
MCA4646	-	-	+	-	-	-	-	-	-	-
MCA4648	-	-	+	-	-	-	-	-	-	-
MCA5562	-	-	-	-	-	-	-	-	-	-

(-): Indicates no compatibility

(+): Indicates the development of hyphae and basidium-like structures.

2.5 Taxonomy

Bannoa guamensis P.P. Parra & Aime, sp. nov. FIGS. 2.4A, 2.4D

MycoBank: MB 823250

Typification: GUAM. MANGILAO: University of Guam campus, from a diseased leaf of *Bidens* sp. (Asteraceae), 31 Jan 2012, *M.C. Aime MCA4612* (**holotype** PUL F21058). Ex-type cultures NRRL Y-63976. GenBank: ITS = MK287350; 28S = MK255006; 18S = MK254996; *tef1* = MK491345.

Etymology: *guamensis* (Latin), referring to the geographical origin of the species.

Description: Asexual stage consisting of uninucleate cells that reproduce by monopolar or bipolar budding. After 7 days of culture in YM broth, cells ellipsoidal 4.5–6.5 × 2.5–4 μm, occurring singly or in parent-bud pairs. Ballistoconidia formed; neither true nor pseudo-hyphae on CMA formed. After 7 days at 25 C on YM agar, colonies orange in color (0ac631), smooth and glistening, butyrous (buttery) in texture. Colony form circular with entire margin and convex elevation. Sexual state unknown.

Fermentation ability negative. Growth was observed at 25 C and 30 C but not at 35 C. When cultured on media containing agar (2%), yeast extract (1%) and supplemented either with 50% glucose, 10% or 16% NaCl, growth was not observed. Assimilation positive for glucose, soluble starch, inulin, D-gluconate, myo-inositol, trehalose, turanose. Weakly assimilated compounds include 2-Keto-D-gluconate, arbutin, cellobiose, D-arabinose, D-mannitol, D-xylose, galactose, glycerol, L-arabinose, L-glutamic acid, L-rhamnose, maltose, melezitose, melibiose, raffinose, salicin and xylitol. Assimilation delayed positive or latent for lactose, methanol, ethanol, DL-Lactate, D-glucuronic acid, propane 1,2 diol. Assimilation of carbon compounds negative for: adonitol, aspartic acid, citrate, d-galacturonic acid, galactitol and succinate. Growth on L-lysine-HCl positive but weak when creatine and D-tryptophan was used as nitrogen sources. Growth negative on cadaverine, creatinine, D-glucosamine, and nitrite. Growth observed on vitamin free media.

Notes: Bannoa guamensis is morphologically indistinguishable from *B. hahajimensis* and *B. tropicalis*, which are the most closely related described species. However, several physiological differences were observed between these three species. Growth of *B. guamensis* in lactose, methanol and myo-inositol is positive unlike for *B. hahajimensis* and *B. tropicalis*. Assimilation of galactitol and L-sorbose is negative in *B. guamensis* but positive for both *B. hahajimensis* and

B. tropicalis. In addition, *B. guamensis* forms a unique phylogenetic position with respect to *B. hahajimensis* and *B. tropicalis*. Considering the most two variable loci, *B. guamensis* differs from *B. hahajimensis* by 2.3% (11/504 bp; ITS) and 2.6% (22/1015 bp; *tef1*). Sequence divergence between *B. guamensis* and *B. tropicalis* is 1.4% (7/504 bp; ITS) and 2.8% (23/1015 bp; *tef1*). *Bannoa guamensis* is the first formally described species to be isolated from rust-diseased leaves and appears as a sister species to the 'Uncultured fungus isolate OTU 118' that was originally reported as *B. hahajimensis* by James et al. (2016) (Figure 1 and Figure 2). It is possible that rust sori may provide a microniche for this lineage, but at this time *B. guamensis* is known only from a single isolate.

Bannoa rosea P.P. Parra & Aime, sp. nov. FIGS. 2.4B, 2.4E

MycoBank: MB 823251

Typification: GUAM. HAGÅTÑA: Paseo Park, from asymptomatic undetermined leaf, Feb 2012, *M.C Aime MCA4647* (**holotype** PUL F21059). Ex-type cultures NRRL Y-63977. GenBank: ITS = MK287351, 28S = MK255007, *tef1* = MK491346.

Etymology: *rosea* (Latin), referring to the dark pink color of the colony.

Description: Asexual stage producing colonies dark pink in color (0ac619), corrugated, and butyrous in texture after 7 days at 25 C on YM agar. Colony form irregular with undulate margin and flat elevation. Uninucleate cells occurring singly or in parent-bud pairs, reproducing by polar budding. In YM broth single cells ellipsoidal, 9–10.5 × 4.5–6 μm after 7 days of incubation. Ballistoconidia formed. On CMA cultures neither pseudo nor true hyphae observed. Sexual stage unknown.

Fermentation of sugars is absent. Growth was observed at 25 C and 30 C but not at 35 C. When cultured on media containing agar (2%), yeast extract (1%) and supplemented either with 50% glucose, 10% or 16% NaCl, growth was not observed. Assimilation positive for glucose, D-glucuronate, myo-inositol, DL-lactate, 1,2-propanediol; slow growth on lactose. Weakly assimilated compounds include 2-keto D-gluconate, adonitol, cellobiose, D-gluconate, D-mannitol, D-melezitose, D-melibiose, D-sorbitol, D-xylose, L-arabinose, L-rhamnose, L-sorbose, maltose, melezitose, melibiose, raffinose, sucrose, trehalose, turanose and xylitol. Assimilation of carbon compounds negative for: citrate, D-arabinose, ethanol, methanol, salicin and succinate. Growth positive when L-lysine-HCl, D-glucosamine and D-tryptophan were used as nitrogen sources.

Growth negative on cadaverine, creatinine, D-glucosamine, and nitrite. Growth observed on vitamin free media.

Notes: Bannoa rosea does not assimilate D-glucosamine, citrate, salicin, glycerol, N-acetyl-D-and glucosamine, while assimilation is positive for *B. ogasawarensis*. *B. rosea* growth in vitamin free media is positive, while *B. ogasawarensis* does not. Sequences of the type strain MCA4647 of *B. rosea* diverge from the type strain JCM10326 of *B. ogasawarensis* by 2.5% (12/504 bp), 0.4% (2/557 bp) and 5.1% (44/1015) for the ITS, nuc 28S and the *tef1* region respectively. *Bannoa rosea* is similar in morphology to *B. ogasawarensis*, but is easily distinguished by its unique assimilation of carbon compounds (Hamamoto et al. 2002, Hamamoto 2011).

Bannoa tropicalis P.P. Parra & Aime, sp. nov. FIGS. 2.4C, 2.4F

MycoBank: MB 823249

Typification: GUAM. YIGO: Andersen Air Force Base, on rust infected leaf of *Bidens* sp. (Asteraceae), 01 Feb 2012, *M.C. Aime MCA4667* (**holotype** PUL F21060). Ex-type cultures: CBS 16087 = NRRL Y-63978. GenBank accession: ITS: MK287360, D1–D2 domains of nuc 28S rDNA: MK255016, nuc 18S rDNA: MK255003, and *tef1*: = MK491353.

Etymology: *tropicalis* (Latin), referring to the pantropical distribution.

Description: Colonies of asexual stage orange (oac631) after 7 days at 25 C on YM agar and CMA; smooth, glistening, and butyrous in texture. Colonies circular with entire margin and convex elevation. In YM broth uninucleate single cells ellipsoidal to narrowly ellipsoidal, 5.5–7 × 2.5–4 μm after 7 days of culture. Ballistoconidia formed; both polar and bipolar budding present. Sexual stage mycelial, hyphae 1.3 to 1.5 μm diam, clamp connections present; basidia 6 × 3.5 μm, obovoid to obpyriform, clamped at the base and sometimes with slender apex, produced after 42 days of incubation on CMA at room T in complete darkness.

Fermentation absent. Growth was positive at 25 C and 30 C and absent at 35 C. When cultured on media containing agar (2%), yeast extract (1%) and supplemented either with 50% glucose, 10% or 16% NaCl, growth was not observed. Assimilation positive for cellobiose, glucose. Assimilation delayed positive or latent for ethanol, galactitol, DL-lactate and D-glucuronic acid. Weakly assimilated compounds include 2-keto D-gluconate, adonitol, D-arabinose, D-gluconate, D-glucosamine, D- mannitol, D-xylose, galactose, glycerol, L-arabinose, L-glutamic acid, L-rhamnose, L-sorbose, maltose, melezitose, melibiose, N-acetyl-D glucosamine, raffinose, salicin,

sucrose, trehalose, turanose and xylitol. Assimilation of carbon compounds negative for 1,2 propane diol, arbutin, aspartic acid, citrate, D- galacturonic acid, lactose, methanol, succinate. Growth on L-lysine-HCl positive, but latent or delayed when D-tryptophan was used as a nitrogen source. Weakly growth on cadaverine, creatine, creatinine and nitrite ($NaNO_2$). Growth is observed on vitamin free media.

Other specimens examined: GUAM. HAGÅTÑA: Paseo Park, asymptomatic undetermined leaf, 01 Feb 2012, *M.C. Aime MCA4620* (PUL F24076); Paseo Park, from Palm trash swap, 01 Feb 2012, *M.C. Aime MCA4643* (PUL F24077); Paseo Park, from *Puccunia lepturi* diseased leaf of *Lepturus repens* (Poaceae), 01 Feb 2012, *M.C. Aime MCA4644* (PUL F24078); Paseo Park, from asymptomatic undetermined leaf, 01 Feb 2012 *M.C. Aime MCA4646* (PUL F24080); Paseo Park, from asymptomatic undetermined leaf, 01 Feb 2012, *M.C. Aime MCA4648* (PUL F24081). YIGO: Bernhard's Farm, *Kweilingia* sp. symptomatic leaf of a plant in Poaceae, 01 Feb 2012, *M.C. Aime MCA4645* (PUL F24079). GUYANA. REGION 9 UPPER TAKUTU-UPPER ESSEQUIBO/ANNAI: Rock View Lodge, from asymptomatic undetermined leaf, 28 Dec 2013, *M.C. Aime MCA5562* (PUL F24082).

Notes: Bannoa tropicalis has been isolated from rust-infected leaves in the Western Pacific and neotropical regions. *Bannoa tropicalis* assimilates inulin and grows on media containing nitrite and cadaverine is positive (weak) as nitrogen source, while growth is negative on *B. hahajimensis* and *B. guamensis*. Growth in vitamin free media is positive; and assimilation is negative for citrate, succinate if compared only to *B. hahajimensis*. Percentages of divergence between *B. tropicalis* and *B. hahajimensis* are 0.15% (2/1387 bp), 1.7% (9/504 bp), 0.6% (3/557 bp) and 3.2% (27/1015 bp) for the 18S, ITS, nuc 28S and the *tef1* respectively.

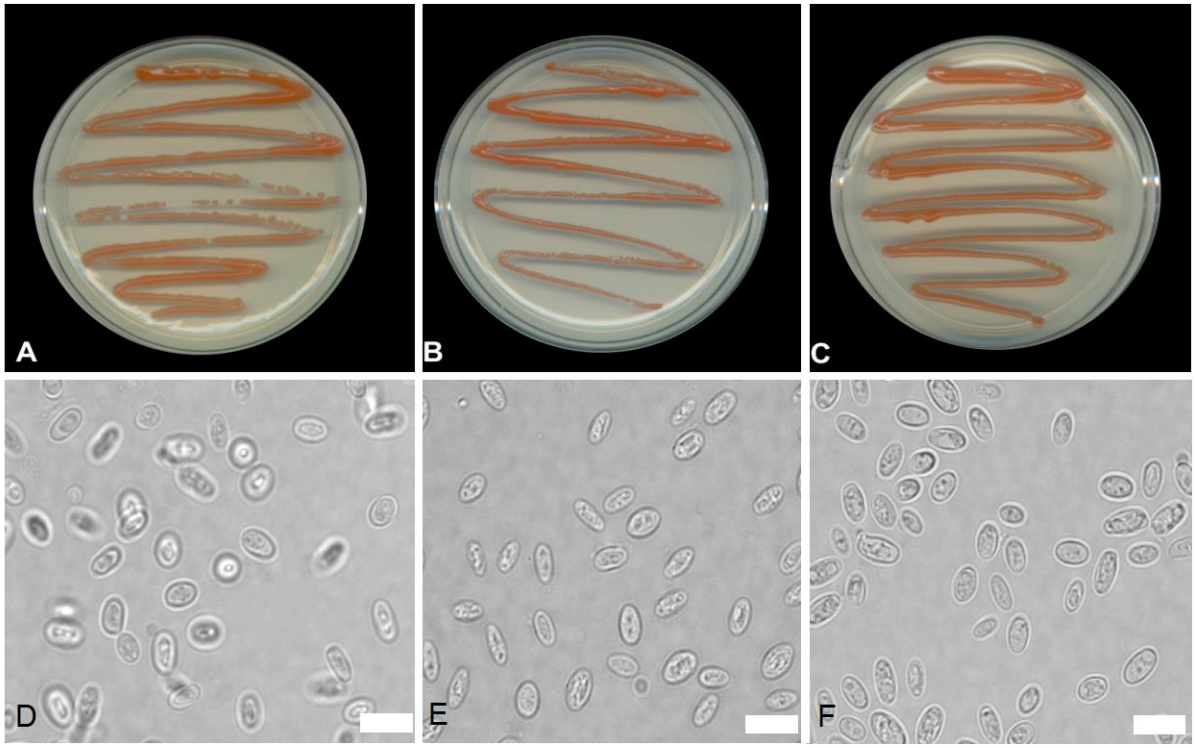

Figure 2.3 Colony and cell morphology of new species of *Bannoa*. **A, D.** *B. guamensis* MCA4612^{T}. **B, E.** *B. rosea* MCA4647^{T}. **C, F.** *B. tropicalis* MCA4667^{T}. Bars: 10 μm. A, B, C and D, E, F were grown on YMA and YMB, respectively, for seven days at room temperature.

2.6 Discussion

In this study, the analysis of three- and four-locus phylogenies allowed to delineate at least nine species-level lineages, three of which we describe as new: *B. guamensis*, *B. rosea* and *B. tropicalis*. Other two species-level lineages from environmental sampling may represent new species; the first lineage appears as closely related to *B. syzygii* and comprises accessions AJ222810.1 and AJ289868.1 isolated from *Miscanthus* sp. and *Miscanthus sinensis*, respectively. The second lineage, comprises the sequence KT328804.1 that was obtained from sori of the coffee rust *Hemileia vastatrix* (James et al. 2016) and appears as sister species to *B. guamensis* that was isolated from a diseased leaf of *Bidens* sp. Furthermore, our data indicate that *B. tropicalis, B. guamensis*, and environmental isolates can be isolated from rust sori and may be co-inhabitants of this microniche during the yeast stage. Of the 12 strains collected in Guam in 2012 and the one collected in Guyana during 2013, seven (or 58%) were associated with diseased leaves (Table 2.1); however, in other studies strains of species in *Bannoa* have been reported from asymptomatic and

dead leaves (Nakase et al. 2001, Hamamoto et al. 2002). Other species such as *Symmetrospora oryzicola* and *Symmetrospora coprosmae*, *Erythrobasidium hasegawianum* and *Hasegawazyma lactosa* are also found in association with other fungi (Hamamoto et al. 2002, Sampaio 2011, James et al. 2016) but the nature of this interaction is unknown. Other species in the Cystobasidiomycetes such as *Naohidea sebacea, Cystobasidium fimetarium* and *Occultifur internus* have been found parasitizing other fungi during their sexual hyphal stage (Roberts 1999, Piatek 2002, Bauer et al. 2006). The results obtained from our intercompatibility studies support the reproductive isolation of the three newly described species *B. tropicalis*, *B. rosea* and *B. guamensis*. Among *B. tropicalis*, the intercompatible crosses indicate that a bifactorial mating system is the most likely system that governs cell identity in this species. We detected at least three mating types; the first mating type comprises strain MCA4667 (A1B1), the second strains MCA4644, MCA4646 and MCA4648 (A2B2) and a third mating type formed by strains MCA4620, MCA4643, MCA4645 and MCA5562 (A1B2/A2B1). According to Coelho et al (2017), in a bifactorial mating system the chances of haploid cells from the same diploid parent are compatible is at most 25%, compared to 50% in an unifactorial mating system. If it were the case of *B. tropicalis*, we could have expected a higher proportion of crosses in which hyphae, clamp connections and basidium were observed. As strain MCA4667 was compatible with other three strains. Similar results were reported by Hamamoto et al (2002), when four strains of *B. hahajimensis* were crossed. A multiallelic bifactorial mating system was suggested to govern sexual identity. Although we only observed a hyphal stage in strains of *B. tropicalis* under laboratory conditions (Figure 2.3), it is uncertain whether the hyphal stage commonly occurs in natural conditions and if this is a mechanism either to reach resources or to interact with other fungi afecting the host plant. As several species in the Cystobasidiomycetes have been shown to be mycoparasites, the association of *Bannoa* with other rust fungi may provide another piece of evidence to hypothesize that dimorphic species in the genus could become parasitic during their dikaryotic hyphal stage.

It has been demonstrated that the description of new species from single strains often leads to a cascade of reported isolations from other locations and substrates (Schelenz et al. 2016, Smith et al 2017, Kijpornyongpan et al. 2018). Here, the description of these three species of *Bannoa* allowed to expand the known range of *Bannoa* westward to South America and southward to Guam. We report new associations and microhabitats of the genus besides *Hemileia vastatrix*-infected leaves of coffee (James et al. 2016), for example *B. tropicalis* can also be found in *Kweilingia* sp.-

infected leaves of an unidentified plant, *Uromyces* sp.-infected leaves of *Bidens* sp. and finally *Puccinia lepturi*-infected leaves of *Lepturus* sp. The description of new species from under sampled geographic regions and microhabitats, is essential to enlarging our knowledge of the scope and distribution of fungal diversity (Graff 1917, Case et al. 2012, Kijpornyongpan and Aime 2016) as is the case for the genus *Bannoa*.

CHAPTER 3. NEW SPECIES IN THE CYSTOBASIDIOMYCETES

3.1 *Buckleyzyma butyrosa* and *Sakaguchia warrensis*: new species in the Buckleyzymales and Sakaguchiales

3.1.1 *Abstract*

Buckleyzyma and *Sakaguchia* are two small genera in the Cystobasidiomycetes with five species each. Both genera contain pink or red pigmented yeast species with different host associations ranging from the gut of beetles and salmonids to marine habitats. New species have not been described in the last 31 and eight years in *Buckleyzyma* and *Sakaguchia*, respectively. In the present study we describe *Buckleyzyma butyrosa* and *Sakaguchia warrensis* isolated from non-volumetric air sampling in Maryland and Indiana, USA. We include morphological, physiological and sequences of the nuclear rDNA ITS1-5.8S-ITS2 (ITS), D1–D2 domains of nuclear 28S rDNA (28S), nuclear 18S rDNA (18S) and the RNA polymerase II subunit *rpb2*. Previous phylogenetic studies focused on generating and including molecular data of several loci for the type strains of extant species, but little has been done to correctly classify and increase the knowledge we have about the biology, distribution and host associations of species in both genera. For that reason we, we compiled available information including sequences from cultures, environmental samplings, geographic distribution and substrate that are available in the NCBI and literature to expand our knowledge of these two understudied genera.

3.1.2 *Introduction*

Rhodotorula and *Sporobolomyces* are artificial genera of mostly pink or red pigmented yeasts, many of which belong in Cystobasidiomycetes (Aime et al. 2014). Within Cystobasidiomycetes these have been placed within several informally named clades: Aurantiaca, Magnisporus, Marina, and Sakaguchia (Aime et al. 2006, Bauer et al. 2006, Wang et al. 2015a). Several studies have focused on the reevaluation of red yeast species, stimulated by the one name one fungus nomenclature in accordance with the International Code of Nomenclature for algae, fungi, and plants (McNeill et al. 2012). It has been shown that *Sporobolomyces* and *Rhodotorula sensu stricto* are allied within Sporidiobolales (Mycobotriomycetes) (Fell et al. 2000, Scorzetti et al. 2002, Boekhout et al. 2011, Sampaio 2011, Wang et al. 2015b), thus, those species of *Sporobolomyces* in the Marina, Magnisporus and Aurantiaca groups have been placed in the new genera

Symmetrospora, *Microsporomyces* and *Buckleyzyma*, respectively, while species of *Rhodotorula* in the *Sakaguchia* group were recombined into *Sakaguchia* (Wang et al. 2015b). While these generic-level relationships have been resolved, the positions of these genera within Cystobasidiomycetes have not been determined (Wang et al. 2015b). The first species to be described in what is now the genus *Buckleyzyma* was originally described in 1922 as *Torula aurantiaca*, isolated from air sampling in Japan. Other strains that appear conspecific with *B. aurantiaca* have been isolated from *Elaeagnus multiflora* and *Pyrus serotina* var. *culta* in South Korea, from lake water in China and from unidentified plants in China, Portugal and Australia (Wuczkowski and Prillinger 2004). Another species, *Buckleyzyma armeniaca*, was isolated from *Banksia colina* in Australia (Shivas and Rodrigues de Miranda 1983) and is the only reported strain for the species. The following year, in 1984, *B. salicina* was described from *Salix* sp. in Canada by Johri and Bandoni, also other strains have been isolated from the phylloplane of *Cotoneaster* sp. in China, from *Vitis vinifera* leaves in USA, from mangrove sediments in India, from forest litter in Austria and from an unidentified plant in Portugal (Wuczkowski and Prillinger 2004, Bourret et al. 2013, Velmurugan et al. 2013). In 1986, *B. kluyveri-nielii* was originally isolated from *Dombeya rotundifolia* in South Africa (Van Der Walt et al 1986), but later, several strains were isolated from mangrove sediments in India (Velmurugan et al. 2013). The last species in the genus to be described is *B. phyllomatis*. This species was originally described as *Sporobolomyces phyllomatis* and isolated from *Dombeya rotundifolia*. *Buckleyzyma* species produce pink-salmon to orange colonies with ellipsoidal to cylindrical cells that in general appear singly or in parent-bud pairs. However, in *B. phyllomatis*, cells sometimes appear organized in small clusters. Production of ballistoconidia and pseudohyphae have been reported in *B. kluyveri-nielii*, *B. phyllomatis* and *B. salicina* but are absent in *B. armeniaca* and *B. aurantiaca*. Rudimentary septate hyphae are observed in *B. kluyveri-nielii* while in *B. phyllomatis* the hyphae were described as adventitiously septate delimited by terminal lipid-rich cells (Van Der Walt et al 1986, 1988). In both cases, hyphae were produced in single-cell cultures on corn meal agar (CMA, Difco) (Van Der Walt et al. 1986, 1988). Clamp connections have not been observed in any of the described species in *Buckleyzyma*.

Like *Buckleyzyma*, *Sakaguchia* is a small genus with only five species. In 1973, *S. dacryoidea* was described as *Rhodosporidium dacryoidum* and was first isolated from sea water in the Antarctic Peninsula (Fell et al. 1973). Subsequently, other strains of *S. dacryoidea* have been

isolated from mangrove sediments, seawater and fresh water in Antarctica, India, New Zealand and Portugal (Almeida and Sampaio 2003, Velmurugan et al. 2013, Francis et al. 2016) and from seawater and the gut of fish from environmental sequencing studies in China and Chile (Raggi et al. 2014, Yu et al. 2017). Several years later in 2001, *S. lameliibrachiae*, first described as *R. lamellibrachii*, was isolated from a tube worm (*Lamellibrachia* sp.) at a depth of 1156 m in Sagami bay, Japan (Nagahama et al. 2001). Other reports of *S. lameliibrachiae* are from environmental sampling from sea water in China (Yu et al. 2017). *Sakaguchia orzyae* was first isolated in Japan from paddy rice and was originally described as *R. oryzae* in 2003. In more recent years, *S. meli* was described from the Argentinean Patagonia (Libkind et al. 2010), while other strains have been isolated from fresh water, from soil in USA and from a glacial lagoon in Argentina. This latest strain has been classified as *Rhodotorula* sp. (Libkind et al. 2003). Lastly, *S. cladiensis* was isolated from fresh water and mangrove habitat in Florida bay (Fell et al. 2011). This species is only known from this location.

Species in *Sakaguchia* share several macro- and micromorphological characteristics with species in *Buckleyzyma* and other genera in the Cystobasidiomycetes such as colored colonies that range from pink, orange to red and in some species single cells are arranged in parent-bud pairs. Cell shape can be ovoidal, ellipsoidal or cylindrical. Hyphae or pseudohyphae are not formed, however cells of *S. cladiensis* and *S. dacryoidea* can be found in short chains (Fell et al. 1973, 2011). Development of sexual structures and hyphae with clamp connections has only been observed in *S. dacryoidea* (Fell et al. 1973). New species have not been described during the last 31 and eight years in *Buckleyzyma* and *Sakaguchia*, respectively, nonetheless, there are several sequences from next-generation sequencing studies and strains isolated from fungal community studies that show new host associations with insects like *Diabrotica virgidera* and *Atta texana* (Molnár et al. 2008, Rodrigues et al. 2009, Poli et al. 2018) in species of *Buckleyzyma*. Additionally next-generation sequencing studies also show several sequences in species of *Sakaguchia* associated with fish like *Cillus gilberti* (Raggi et al. 2014).

Buckleyzyma and *Sakaguchia* remain understudied, as is true for other genera in the Cystobasidiomycetes, and their role in nature remains unknown. Phylogenetic studies have focused on generating and including molecular data of several loci for the type strains of extant species, but little has been done to increase the knowledge we have about their biology, host associations and habitat preference. We compiled the available information that has been generated

in recent years from different studies, including several unclassified accessions in the NCBI database that are key to understanding geographical distribution and new host associations of species in these two genera. Lastly, during field collections performed in Maryland and Indiana, United States, several strains of pink and orange pigmented yeast were isolated by using the non-volumetric air sampling methodology. In this paper, we present morphological, physiological and phylogenetic analysis based on four loci of the known species and describe two new species, *B. butyrosa* and *S. warrensis*. Also, we report the occurrence of *B. salicina* in the United States, that has only been reported before from *Vitis vinifera* inflorescences in Washington state (Bourret et al. 2013). these findings do not only indicate a wider distribution but also new host associations of this species.

3.1.3 *Material and Methods*

3.1.3.1 Sample collection and storage

Three strains were obtained by using the non-volumetric sampling method (that consists in leaving a petri plate with culture media and without a lid for a determined period of time) in Maryland in 2003 and Indiana in 2013 and 2015. For JDSF10, we used 50% glucose medium. Cultures were maintained on potato dextrose agar (PDA; 39g/L, Livonia, Michigan) slants at 4 C for short-term storage and in 40% glycerol vials at -80 C for long-term storage. Cultures of the isolates MCA2993, P117 and JDSF10 were dried and deposited as holotypes in the Kriebel Herbarium (PUL). Living cultures also were deposited in the Westerdijk Fungal Biodiversity Institute (CBS) and the Fungal Biodiversity Center and Agricultural Research Service (NRRL).

3.1.3.2 Morphological and physiological studies

To study macro- and micromorphological features of the collected strains, they were cultured on yeast malt agar (YMA, Difco, Franklin Lakes, New Jersey), yeast malt broth (YM broth, Difco) and corn meal agar (CMA, Difco) at 25 C for seven days. Features such as color of the colonies, cell shape and cell measurement, was performed as in section 2.3.2. Equipment used for cell morphology studies consisted of a compound microscope, OLYMPUS BH-2 (Olympus, Japan) under phase-contrast microscopy. Assimilation of carbon and nitrogen compounds as well as fermentation tests were performed in liquid media, while growth of strains on high osmotic media and growth on vitamin-free media were performed in solid media according to Kurtzman et al.

(2011). Likewise, the ability of strains to grow on other carbon sources was assessed by using a Yeast Identification Test Panel (Biolog Inc., Hayward, California) as performed in Kijpornyongpan and Aime (2017).

3.1.3.3 DNA extraction and phylogenetic studies

DNA extraction was performed from pure cultures grown on YMA using the Promega Wizard genomic DNA purification kit (Promega, Madison, WI). Phylogenetic placement of newly collected strains was inferred from sequences of the internal transcribed spacer (ITS) bar code region (nuc rDNA ITS1-5.8S-ITS2), D1–D2 domains of the nuc 28S rDNA (28S), the nuc 18S rDNA (18S) and the RNA polymerase II subunit *rpb2*, with primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990b), LR0R/LR5 (Vilgalys and Hester 1990), NS1/NS4 and NS3/NS8 (White et al. 1990b) and 5F/6R1 and 6F/7CR (Liu et al. 1999), respectively. PCR programs for amplification were followed according to section 2.3.4. PCR products were separated in a 1% agarose gel and sent to GENEWIZ (South Plainfield, New Jersey) for sequencing. Sequences were edited using GENEIOUS 9.1.2 (Biomatters, Auckland, New Zealand). All available sequences of the 18S, ITS and 28S from environmental sequencing and collections from previous studies for species in *Buckleyzyma* and *Sakaguchia* were aligned in two different datasets. Ambiguously aligned regions for each locus were removed with Gblocks using the less stringent selection option (Talavera and Castresana 2007). Two datasets consisting of four loci were generated: The first dataset was generated for the phylogenetic analysis of species in *Buckleyzyma*, while another dataset was used for the phylogenetic reconstruction of *Sakaguchia*. Taxon sampling consisted of sequences of the ITS, 28S and 18S of type strains of extant species in *Buckleyzyma* and *Sakaguchia*; were used to run a BLASTN search against the NCBI GenBank database, sequences from culture collections and environmental samplings were included in the analysis. *Naohidea sebacea* was used as outgroup (Aime et al. 2006) in both analyses. Sequences for each locus were first aligned with MAFFT 7.222 (Katoh et al. 2002) using the L-INS-I algorithm. Subsequently, alignments of the four loci used were concatenated using GENEIOUS 9.1.2 (Biomatters, New Zealand) to perform the multi-locus phylogenetic reconstruction.

The General time reversible model with substitution-rate among sites of gamma distribution (GTR+G) was selected as the substitution model of evolution for both datasets according to

PARTITIONFINDER2 based on the Akaike Information Criterion (AIC) (Stamatakis 2014, Lanfear et al. 2012, 2016). Phylogenetic reconstruction was performed using Maximum Likelihood (ML) followed by 1000 bootstrap replicates using GENEIOUS 9.1.2. Bootstrap proportions (BP) are reported below. Bayesian inference (BI) was performed using MRBAYES 3.2.6 (Huelsenbeck and Ronquist 2012) with a GTR model, nts:6, rates=invgamma according and five million generations. The first 25% of trees were discarded as Burn-in.

3.1.4 *Results*

3.1.4.1 Phylogenetic placement of collected strains

The first dataset for *Buckleyzyma* was composed of 1668, 580, 596 and 658 bp while the second dataset for *Sakaguchia* was composed of 1024, 540, 486 and 674 bp for the nuc 18S, ITS, nuc LSU and the *rpb2* sequences, respectively. The four concatenated loci consisted of 3502 bp for *Buckleyzyma* and 2724 bp for *Sakaguchia* phylogenetic reconstruction. The multi-locus phylogenetic reconstruction along with morphological and physiological data support two new species: *Buckleyzyma butyrosa* and *Sakaguchia warrensis*.

Buckleyzyma butyrosa appears as phylogenetically related to *B. armeniaca* and *B. phyllomatis* (Figure 3.1). Neither molecular data from culture collections nor environmental sequences appear clustered together with *B. butyrosa*. Our phylogenetic analysis shows several unclassified sequences that belong to already described species in the genus such as *B. aurantiaca, B. salicina* and *B. kluyveri-nielli*. Likewise, a cluster of sequences that appear as sister species of *B. aurantiaca* may represent a new species. *S*train P117 isolated from air sampling belongs to *B. salicina* and appears as the second known report of this species in the USA.

For *S. warrensis*, only one sequence from environmental sampling (Uncultured fungus clone CMH332) in the United States from indoor environments appears conspecific (Figure 3.2). In our phylogenetic analysis, several independent evolutionary lineages that may represent new species are identified. Additionally, several accessions retrieved from the NCBI had incorrect names.

Figure 3.1 Phylogenetic reconstruction of *Buckleyzyma* inferred from concatenated sequences of ITS, D1–D2 domains of nuclear 28S, nuclear 18S rDNA, and *rpb2* using maximum likelihood. New species indicated in bold. Posterior probability (PP > 0.7) and bootstrap support (BP > 50%) values are shown above nodes. ns: not supported. Tree is midpoint rooted. Scale bar equals expected number of substitutions per site. Strain number is listed in front of the taxon name. ^{T} denotes type species strain.

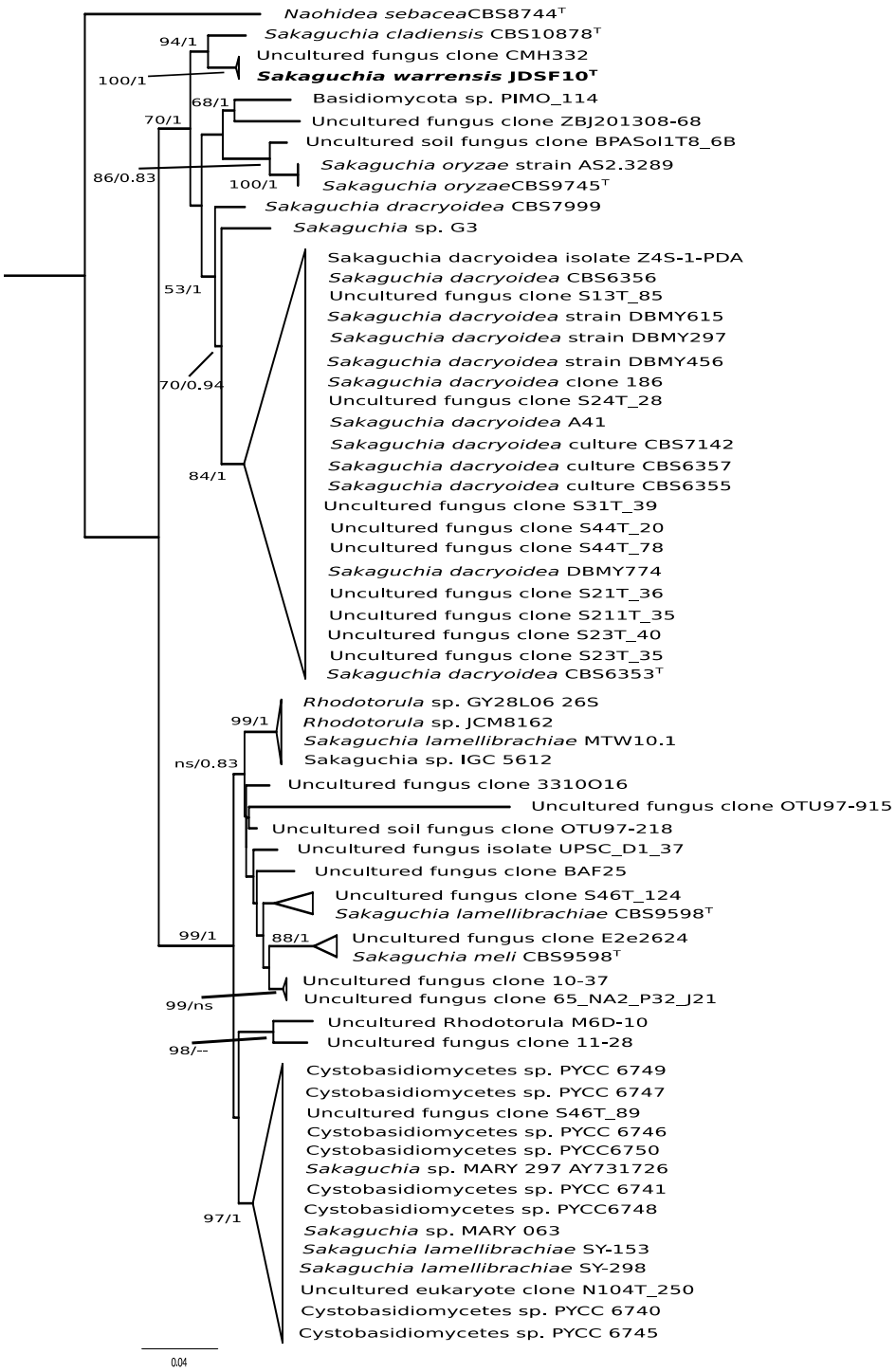

Figure 3.2 Phylogenetic reconstruction of *Sakaguchia* inferred from concatenated sequences of ITS, D1–D2 domains of nuclear 28S, nuclear 18S rDNA, and *rpb2* using maximum likelihood. New species indicated in bold. Posterior probability (PP > 0.7) and bootstrap support (BP > 50%) values are shown above nodes. ns: not supported. Tree is midpoint rooted. Scale bar equals expected number of substitutions per site. Strain number is listed in front of the taxon name. ^{T} denotes type species strain.

3.1.5 *Taxonomy*

Buckleyzyma butyrosa P.P. Parra & Aime, sp. nov. FIGS. 3.3A, 3.3C

MycoBank: MB 831219

Typification: UNITED STATES. MARYLAND: Prince George County, aerial sampling, 16 September, 2005. *M.C. Aime MCA2993*. (**holotype** PUL: F24083). Ex-type cultures CBS 16088 (NRRL Y-63999). GenBank: ITS = MK990657, D1–D2 domains of nuc 28S rDNA = MK990684, nuc 18S rDNA = MK990638.

Etymology: Referring to the buttery texture of the colony.

Description: After 7 days at 25 C on YM agar colonies orange colored (0ac651), dull surface and butyrous in texture. Colony form is irregular with entire margin and crateriform elevation. Cells occur singly or in parent-bud pairs and reproduce asexually by multilateral budding. In YM broth (YM broth, Difco) after 7 days, cells are ellipsoidal to narrowly ellipsoidal 3–4.7 × 6.8–9.5 μm with some pyriform cells. On CMA agar, colonies are orange in color (0ac652), neither true nor pseudohyphae are formed. Ballistoconidia were not observed.

Fermentation is absent. Growth is observed at 25 C and absent at 30 C and 35 C. When cultured on media containing agar (2%), yeast extract (1%) and supplemented either with 50% glucose, 10% or 16% NaCl colonies were not formed. Growth is observed on vitamin free media. The following carbon compound are assimilated: 1,2-propanediol, dextrin, dextrin + xylose, glucose, D-glucuronate, DL-lactate (slow), lactose (slow) and methanol. Weakly assimilated compounds are:, 1,2-propanediol + xylose, a-D-glucose, a-D-lactose + xylose, arbutin, b-Me-D-glucoside, cellobiose, D-gluconic acid, inulin, D-galactose, D-melezitose, D-psicose, D-mannitol, D-sorbitol, D-arabitol, L-aspartic acid, D-arabitol, D-galactose, D-galactose + xylose, D-gluconic acid, D-glucuronic acid + xylose, D-mannitol, D-melibiose + xylose, D-sorbitol, D-xylose, gentiobiose, inulin, L-sorbose, m-inositol + xylose, glycerol, maltotriose, L-arabinose, L-glutamic acid, L-sorbose, NAc-L-glutamic acid, L-proline, maltose, Me-succinate + xylose, mentiobiose, salicin, succinic acid, sucrose, palatinose, propionic acid and xylitol. Nitrogen assimilation on D-Glucosamine, D-Tryptophan and L-lysine-HCl (slow) are positive (Table 3.1).

Notes: Assimilation of carbon compounds of *Buckleyzyma butyrosa* is positive in methanol, weak for cellobiose, D-gluconic acid, inulin, salicin and sucrose and slow for lactose if compared with its sister species *Buckleyzyma armeniaca* for which growth is negative. When sequences of *B. butyrosa* are compared to *B. armeniaca*, sequences divergence is 0.24% (4/1668 bp, no gaps), 2.7%

(16/580 bp, two gaps), 0.2 % (1/596bp, no gaps) and 11% (73/658 bp, two gaps) for the nuc 18S, ITS, nuc 28S and the *rpb2* respectively.

Sakaguchia warrensis P.P. Parra & Aime, sp. nov. FIGS. 3.3B, 3.3D

MycoBank: MB 831220

Typification: UNITED STATES. INDIANA: Warren county, isolated from non-volumetric sampling in media containing 50% glucose in the Weiler-Leopold Nature reserve. Collected during the A. H. Smith Great Lakes States Foray. September 27, 2015. *Jorge Diaz-Valderrama, JDSF10*. (**holotype** PUL: F24088). Ex-type cultures CBS 16089 = NRRL Y-64000. GenBank: ITS = MK990682, D1–D2 domains of nuc 28S rDNA = MK990698, nuc 18S rDNA = MK990648.

Etymology: Referring to the Warren County located in western Indiana, United States between the Illinois State line and the Wabash River where the strain was collected.

Description: After 7 days at 25 C on YM agar colonies salmon colored (0ac617), glistering surface and butyrous in texture. Colony form circular with curled margin and umbonate elevation. Cells occur singly or in clusters and reproduce asexually by multilateral budding. In YM broth (YM broth, Difco) after 7 days, cells are oblong, 2.3–3.4 × 4.2–8.6 μm. On CMA agar, colonies are orange in color (0ac618).

Growth was observed at 25 C and 30 C but not at 35 C. When cultured on media containing agar (2%), yeast extract (1%) and supplemented either with 50% glucose, 10% or 16% NaCl growth was not observed. Growth on vitamin free media is positive. Assimilates 1,2-propanediol, dextrin, D-glucuronate, DL-lactate, glucose, and soluble starch. Assimilation of carbon is weak for the following compounds: 2-keto D-gluconate, a-D-glucose, a-Me-D-glucoside, adonitol, amygdalin, b-Me-D-glucoside, bromosuccinic acid, cellobiose, D-psicose, D-arabinose, dextrin + xylose, gamma-aminobutyric acid, D-arabitol, D-galactose + xylose, D-gluconic acid, inulin, D-glucuronic acid + xylose, D-mannitol, D-melezitose, D-sorbitol, D-xylose, dextrin, fumaric acid, gentiobiose, glycerol, guinic acid + xylose, inulin, Me succinate, Me-succinate + xylose, L-glutamic acid, L-sorbose, maltitol, maltose, maltotriose, mentiobiose, palatinose, ribose, sucrose, trehalose, turanose, sucrose, tween 80, trehalose, xylitol. Assimilation of nitrogen on ethylamine-HCl, L-lysine-HCl, D-glucosamine and D-tryptophan is positive, weak growth in cadaverine-HCl and slow on D-galacturonate and methanol (Table 3.1).

Notes: *Sakaguchia warrensis* growth is positive in DL-lactate and soluble starch; weak in 2-keto D-gluconate, D-ribose, D-xylose, inulin, L-sorbose, slow in D-galacturonate and methanol while is negative for *Sakaguchia cladiensis* which is the sister species. Comparison of *S. warrensis* and *S. cladiensis* sequences show a divergence of 0% (0/1024 bp no gaps), 1.6% (9/540 bp, no gaps), 2.9% (14/486 bp, 5 gaps) and 9.19% (62/674 bp, no gaps) for the 18S, ITS, nuc 28S and the *rpb2* respectively.

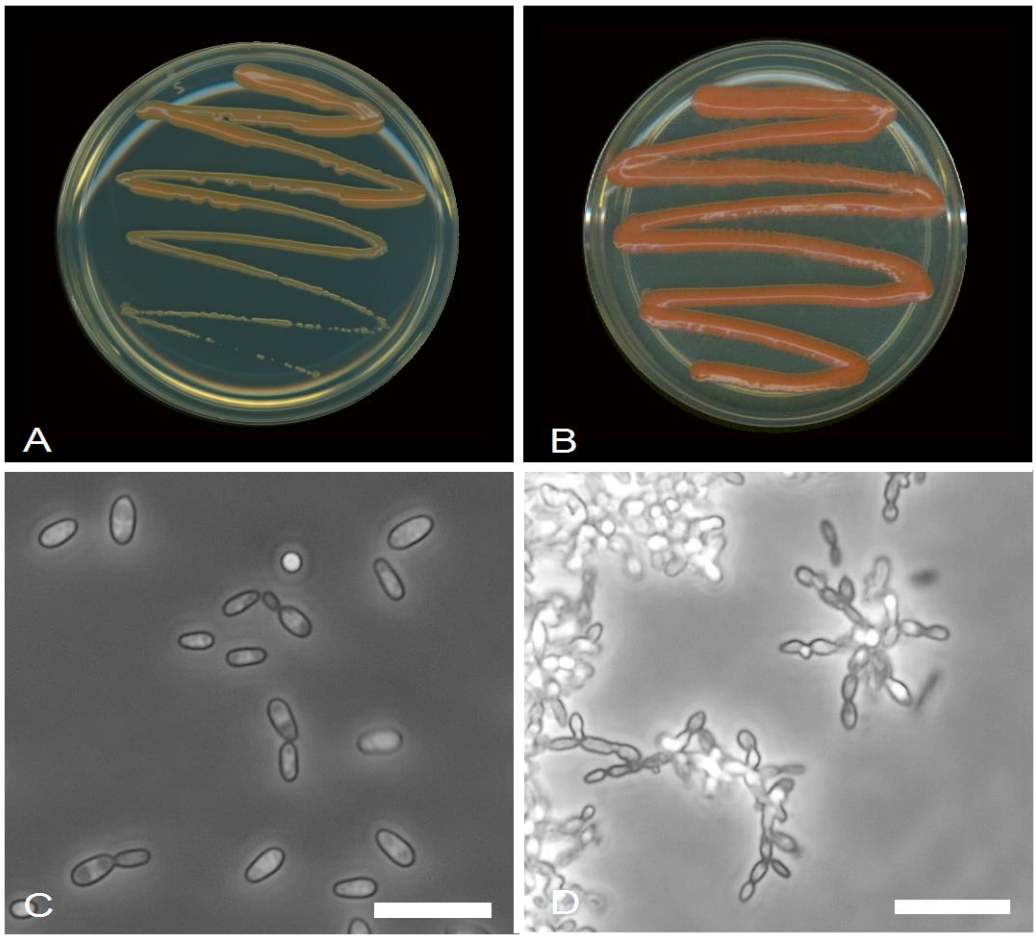

Figure 3.3 Colony and cell morphology. **A. C.** *Buckleyzyma butyrosa* MT254^{T}. **B. D.** *Sakaguchia warrensis* SFJD10^{T}. Bars: 20 μm. A, B, and C, D, were grown on YMA and YMB, respectively, for seven days at room temperature.

Table 3.1 Assimilation of carbon and nitrogen compounds and growth under different temperature and osmotic conditions of new species of *Buckleyzyma* and *Sakaguchia* and sister species.

Compound	***Buckleyzyma butyrosa* MCA2993^T**	***Buckleyzyma armeniaca** CBS8076^T**	***Buckleyzyma salicina** JCM2959^T**	***Sakaguchia warrensis* JDSF10^T**	***Sakaguchia cladiensis** CBS10878^T**
Carbon assimilation	-	n	-	-	n
Acetic acid	-	n	n	-	n
Formic acid	-	n	n	-	n
Propionic acid	w	n	n	-	n
Succinic acid	w	+	+	w	-
Me succinate	-	n	n	w	n
L-Aspartic acid	w	n	n	-	n
L-Glutamic acid	w	n	n	w	n
L-Proline	w	n	n	-	n
D-gluconic acid	w	-	-	-	+
Dextrin	+	n	n	+	n
Cellobiose	w	-	+	w	v
Mentiobiose	w	n	n	w	n
Maltose	w	+	-	w	+
Maltotriose	w	n	n	w	n
Palatinose	w	n	n	w	n
Sucrose	w	-	+	w	+
Turanose	-	n	n	w	n
D-Galactose	w	+	+	-	+
D-Psicose	w	n	n	w	n
Salicin	w	-	+	-	S
D-Arabitol	w	n	n	-	n
Tween 80	w	n	n	w	n
Fumaric acid	-	n	n	w	n
L-Malic acid	-	n	n	-	n
Bromosuccinic acid	-	n	n	w	n
Gammna-aminobutyric acid	-	n	n	w	n
a-ketoglutarate	-	n	n	-	n
2-keto D-gluconate	-	n	-	w	-
Inulin	w	-	-	w	-
Gentiobiose	w	n	n	w	n
D-Melezitose	w	-	-	w	+
D-Melibiose	-	-	-	-	-
D-Raffinose	-	-	+	-	v

Table 3.1 continued

Compound	***Buckleyzyma butyrosa* MCA2993^T**	***Buckleyzyma armeniaca** CBS8076^T**	***Buckleyzyma salicina** JCM2959^T**	***Sakaguchia warrensis* JDSF10^T**	***Sakaguchia cladiensis** CBS10878^T**
Table continued					
Trehalose	-	v	+	w	+
Nac-D-glucosamine	-	n	n	-	-
a-D-Glucose	w	n	n	w	n
L-Rhamnose	-	+	-	-	s
L-Sorbose	w	+	-	w	-
a-Me-D-glucoside	-	-	-	w	v
b-Me-D-glucoside	w	n	n	w	n
Amygdalin	-	n	n	w	n
Arbutin	w	n	n	-	n
Maltitol	-	n	n	w	n
D-Mannitol	w	+	+	w	S
D-Sorbitol	w	n	n	w	n
Adonitol	-	n	n	w	n
Xylitol	w	+	n	w	n
i-Erythritol	-	-	-	-	-
Glycerol	w	+	+	w	+
L-Arabinose	w	+	+	-	-
D-Arabinose	-	-	+	w	v
D-Ribose	-	+	-	w	-
D-Xylose	w	+	+	w	-
Me-succinate+xylose	w	n	n	w	n
NAc-L-glutamic acid	w	n	n	-	n
Guinic acid+xylose	-	n	n	w	n
D-Glucuronic acid+xylose	w	n	-	w	n
Dextrin + xylose	+	n	n	w	n
a-D-Lactose + xylose	w	n	n	-	n
D-Melibiose+xylose	w	n	n	-	n
D-galactose + xylose	w	n	n	w	n
m-Inositol + xylose	w	n	n	-	n
1,2-propanediol + xylose	w	n	n	-	n
Acetonin + xylose	-	n	n	-	n
Citrate	-	-	+	-	-

Table 3.1 continued

Compound	***Buckleyzyma butyrosa* MCA2993^T**	***Buckleyzyma armeniaca** CBS8076^T**	***Buckleyzyma salicina** JCM2959^T**	***Sakaguchia warrensis* JDSF10^T**	***Sakaguchia cladiensis** CBS10878^T**
Ethanol	-	-	-	-	-
D-Galacturonate	-	n	-	s	n
Glucose (+ ctrl)	+	+	+	+	+
D-Glucuronate	+	n	-	+	+
Inositol	-	-	-	-	-
DL-Lactate	s	+	-	+	-
Lactose	s	-	-	-	-
Methanol	+	-	-	s	-
Table continued					
1,2-propanediol	+	n	n	+	n
Soluble starch	-	-	-	+	-
Nitrogen assimilation	-	n	n	-	n
KNO3	-	-	+	-	-
NaNO2	-	-	-	-	n
Ethylamine-HCl	-	n	-	+	n
L-lysine-HCl	s	n	-	+	n
Cadaverine-HCl	-	n	w	w	n
Creatine	-	n	n	-	s
D-Glucosamine	+	-	n	+	n
Imidazole	-	n	n	-	n
D-Tryptophan	+	n	n	+	n
(NH4)2SO4	+	n	n	+	n
50% Glucose	-	n	-	-	-
10% NaCl	-	n	n	-	n
16% NaCl	-	n	n	-	n
Vitamin free medium	+	-	-	+	+
25 C (C5 tube)	+	+	n	+	n
30 C	-	-	+	+	+

+, positive; -, negative; l, delayed positive or latent; s, slow positive; v, variable; w, weak; n, not determined.

*, denotes assimilation data for *B. armeniaca*, *B. salicina* and *S. cladiensis* taken from Shivas and Rodrigues de Miranda (1983) and Nakase and Mutsumi Itoh (1988).

Table 3.2 Collection information and GenBank accession numbers for material used in the phylogenetic reconstruction of *Buckleyzyma*.

SPECIES/ACCESSION	HOST/SUBSTRATE	COUNTRY	ITS	LSU	SSU	*rpb2*
Buckleyzyma armeniaca /CBS 8076^{T}	Healthy leaves of *Callistemon viminalis*	Australia	AF444523	AF189920	AB126644	KJ708211
Buckleyzyma sp. CBS10208	Plants	Portugal	--------	EU002829	--------	--------
Buckleyzyma aurantiaca HB12-1	Flower of *Pyrus serotina* var. culta	South Korea	--------	KJ507255	--------	--------
Buckleyzyma aurantiaca HB31-4	*Elaeagnus multiflora*	South Korea	--------	KJ507280	--------	--------
Buckleyzyma aurantiaca PDD-26b-27	Cloud water	Unknown	--------	JF706572	--------	--------
Buckleyzyma aurantiaca YM25880	Lake water	China	--------	KC494744	--------	--------
Buckleyzyma aurantiaca CBS317^{T}	Air sampling	Japan	AF444538	AF189921	KJ708436	KJ708212
Buckleyzyma aurantiaca culture CBS8074	Plants	Australia	KY101785	KY106235	--------	--------
Buckleyzyma aurantiaca isolate RP432_13	Endophyte on plant	Germany	KX096691	--------	--------	--------
Buckleyzyma aurantiaca JCM 11358	Apple leaf and fruits	Germany	AB093528	--------	--------	--------
Buckleyzyma aurantiaca JCM8163	Unknown	Japan	AB038113	--------	--------	--------
Buckleyzyma aurantiaca MUT5471	*Padina pavonica*	Italy	--------	KU314991	--------	--------

Table 3.2 continued

SPECIES/ACCESSION	HOST/SUBSTRATE	COUNTRY	ITS	LSU	SSU	*rpb2*
Buckleyzyma cf. *aurantiaca* CBS10211	Plants	Portugal	--------	EU002832	--------	--------
Buckleyzyma cf. *aurantiaca* DBVPG 5753	Air sampling	Italy	--------	KC433806	--------	--------
Buckleyzyma sp. ATT147	*Atta texana*	USA	--------	FJ743613	--------	--------
Buckleyzyma sp. CBS10207	Plants	Portugal	--------	EU002828	--------	--------
Buckleyzyma sp. CBS10209	Plants	Portugal	--------	EU002830	--------	--------
Buckleyzyma sp. CBS10210	Plants	Portugal	--------	EU002831	--------	--------
Fungal endophyte isolate 31	*Populus* sp./Roots/Endophyte	Canada	KU179266	--------	--------	--------
Rhodotorula sp. HB 1211	Beetle gut	Austria	AM160641	--------	--------	--------
Rhodotorula sp. P44D004	*Vitis vinifera* leaf	USA	JX188233	--------	--------	--------
Uncultured Basidiomycota Cluster13783	Alder leaves from boreal streams	Finland	KT160889	--------	--------	--------
Uncultured fungus clone OTU13	Gut of Staphylinidae	Canada	KT965045	--------	--------	--------
uncultured fungus_Fungal endophyte	Field retted *Cannabis sativa*	Denmark	LT622084	--------	--------	--------

Table 3.2 continued

SPECIES/ACCESSION	HOST/SUBSTRATE	COUNTRY	ITS	LSU	SSU	*rpb2*
Uncultured *Rhodotorula* clone KL_2d_2F03	Leaf Litter: F*agus sylvatica*	Austria	JF495232	--------	--------	--------
Uncultured *Rhodotorula* clone KL_2d_2G04	Leaf Litter: F*agus sylvatica*	Austria	JF495243	--------	--------	--------
Uncultured *Rhodotorula* clone KL_2w_A02	Leaf Litter: F*agus sylvatica*	Austria	JF449588	--------	--------	--------
Uncultured *Rhodotorula* clone SW_2d_G11	Leaf Litter: F*agus sylvatica*	Austria	JF449839	--------	--------	--------
Uncultured *Rhodotorula* clone SW_2w_B07	Leaf Litter: F*agus sylvatica*	Austria	JF449860	--------	--------	--------
Buckleyzyma butyrosa MCA2993^{T}	Air sampling	USA	**MK990657**	**MK990684**	**MK990638**	XXXXX
Buckleyzyma kluyveri-nielii DBMY626	Mangrove sediments	India	KJ706843	--------	--------	--------
Buckleyzyma kluyveri-nielii DBMY785	Mangrove sediments	India	KJ707002	--------	--------	--------
Buckleyzyma kluyveri-nielii CBS 7168^{T}	*Dombeya rotundifolia*	South Africa	AF444544	AF189988	AB021674	KJ708310
Buckleyzyma kluyveri-nielii DBMY308	Mangrove sediments	India	KJ706525	--------	--------	--------
Buckleyzyma kluyveri-nielii DBMY467	Mangrove sediments	India	KJ706684	--------	--------	--------
Buckleyzyma phyllomatis JCM7549^{T}	*Dombeya rotundifolia*	South Africa	AF444515	AF189991	AB021685	KJ708328
Buckleyzyma salicina CBS10203	Plants	Portugal	--------	EU002824	--------	--------

Table 3.2 continued

SPECIES/ACCESSION	HOST/SUBSTRATE	COUNTRY	ITS	LSU	SSU	*rpb2*
Buckleyzyma salicina DBMY470	Mangrove sediments	India	KJ706687	--------	--------	--------
Buckleyzyma salicina DBMY629	Mangrove sediments	India	KJ706846	--------	--------	--------
Buckleyzyma salicina DBMY788	Mangrove sediments	India	KJ707005	--------	--------	--------
Buckleyzyma salicina CBS10204	Plants	Portugal	--------	EU002825	--------	--------
Buckleyzyma salicina DBMY311	mangrove sediments	India	KJ706528	--------	--------	--------
Buckleyzyma salicina HB11400	Forest litter	Austria	--------	AM039681	--------	--------
Buckleyzyma salicina JCM2959^{T}	*Salix* sp.	Canada	AF444511	AF189995	AB021687	--------
Buckleyzyma salicina P117	Air sampling	USA	--------	--------	--------	--------
Rhodotorula sp. P34D004	*Vitis vinifera* leaf	USA	JX188231	--------	--------	--------
Buckleyzyma salicina XJ10B5	Plant Leaf of *Cotoneaster* sp.	China	HE650887	HE650896	--------	--------
Naohidea sebacea^{T}	Perithecia of *Botryosphaeria. quercuum*, *Botryodiplodia* sp.	Taiwan	DQ911616	DQ831020	KP216515	KF706535

-------- : No sequence available. Accession numbers in bold were generated in the present study.

T denotes type species strain.

Table 3.3 Host/substrate and GenBank accession numbers for the material in the phylogenetic reconstruction of *Sakaguchia*.

SPECIES /ACCESSION	HOST/ SUBSTRATE	LOCATION	ITS	LSU	SSU	*rpb2*
Naohidea sebacea^{T} CBS8477^{T}	*Botryosphaeria. quercuum, Botryodiplodia* sp.	Taiwan	DQ911616	DQ831020	KP216515	KF706535
Sakaguchia warrensis JDSF10^{T}	Air sampling 50% glucose	USA	**MK990682**	**MK990698**	**MK990648**	XXXXX
Sakaguchia dacryoidea culture CBS7999	Brewery	France	--------	KY109529	--------	--------
Sakaguchia lameliibrachiae MTW10.1	Water	Thailand	--------	LC435582	--------	--------
Sakaguchia cladiensis CBS10878^{T}	Sawgrass (*Cladium jamaicense*)	USA	FJ008055	FJ008049	KJ708354	KJ708219
Sakaguchia dacryoidea A41	Sea water	Portugal	--------	AF485998	--------	--------
Sakaguchia dacryoidea clone 186	Fish gut microbiota	Chile	--------	KF488791	--------	--------
Sakaguchia dacryoidea culture CBS6356	Seawater	Antarctica			--------	--------
Sakaguchia dacryoidea culture CBS6357	Seawater	Antarctica	KY105303	KY109532	--------	--------
Sakaguchia dacryoidea culture CBS6355	Seawater	Antarctica	KY105300	KY109528	--------	--------
Sakaguchia dacryoidea culture CBS7142	Seawater		KY105305	KY109527	--------	--------

Table 3.3 continued

SPECIES /ACCESSION	HOST/ SUBSTRATE	LOCATION	ITS	LSU	SSU	*rpb2*
Sakaguchia dacryoidea isolate Z4S-1-PDA	Seaweed	New Zealand	KT819324	--------	--------	--------
Sakaguchia dacryoidea strain DBMY297	Mangrove sediments	India	KJ706514	--------	--------	--------
Sakaguchia dacryoidea strain DBMY456	Mangrove sediments	India	KJ706673	--------	--------	--------
Sakaguchia dacryoidea strain DBMY615	Mangrove sediments	India	KJ706832	--------	--------	--------
Sakaguchia dacryoidea strain DBMY774	Mangrove sediments	India	KJ706991	--------	--------	--------
Uncultured fungus clone S13T_85	Seawater	China	KU163855	--------	--------	--------
Uncultured fungus clone S211T_35	Seawater	China	KU164039	--------	--------	--------
Uncultured fungus clone S21T_36	Seawater	China	KU164098	--------	--------	--------
Uncultured fungus clone S23T_35	Seawater	China	KU164241	--------	--------	--------
Uncultured fungus clone S23T_40	Seawater	China	KU164246	--------	--------	--------
Uncultured fungus clone S24T_28	Seawater	China	KU164298	--------	--------	--------
Uncultured fungus clone S31T_39	Seawater	China	KU164446	--------	--------	--------
Uncultured fungus clone S44T_20	Seawater	China	KU164584	--------	--------	--------

Table 3.3 continued

SPECIES /ACCESSION	HOST/ SUBSTRATE	LOCATION	ITS	LSU	SSU	*rpb2*
Uncultured fungus clone S44T_78	Seawater	China	KU164608	--------	--------	
Sakaguchia dacryoidea CBS6353^{T}	Seawater	Antarctica	AF444597	AF189972	D13459	KJ708348
Uncultured fungus clone S46T_124	Seawater	China	KU164637	--------	--------	--------
Sakaguchia lamellibrachiae CBS9598^{T}	*Lamellibrachia* sp./Seawater	Japan	AB025999	AB025999	AB126646	KJ708314
Uncultured fungus clone E2e2624	Soil	USA	--------	KF750497	--------	--------
Sakaguchia meli CBS10797^{T}	Glacier meltwater	Argentina	FJ807683	KJ708452	KJ708355	KJ708245
Sakaguchia oryzae AS2.3289	Unknown	Unknown	KJ778624	--------	KJ708353	--------
Sakaguchia oryzae CBS9745^{T}	Paddy rice	Japan	AY335160	AY335161	KJ708352	KJ708250
Basidiomycota sp. PIMO_114	*Pinus monticola*	USA	JF705938	--------	--------	--------
Cystobasidiomycetes sp. PYCC6740	Seawater	Portugal	KU171059	KU171058	--------	--------
Cystobasidiomycetes sp. PYCC6741	Seawater	Portugal	KU171063	KU171062	--------	--------
Cystobasidiomycetes sp. PYCC6745	Seawater	Portugal	KU171061	KU171060	--------	--------
Cystobasidiomycetes sp. PYCC6746	Seawater	Portugal	KU171065	KU171064	--------	--------
Cystobasidiomycetes sp. PYCC6747	Seawater	Portugal	KU171067	KU171066	--------	--------

Table 3.3 continued

SPECIES /ACCESSION	HOST/ SUBSTRATE	LOCATION	ITS	LSU	SSU	*rpb2*
Cystobasidiomycetes sp. PYCC6748	Seawater	Portugal	KU171069	KU171068	--------	--------
Cystobasidiomycetes sp. PYCC6749	Seawater	Portugal	KU171071	KU171070	--------	--------
Cystobasidiomycetes sp. PYCC6750	Seawater	Portugal	KU171073	KU171072	--------	--------
Rhodotorula sp. GY28L06 26S	Plants	Taiwan	--------	FJ527100	--------	--------
Rhodotorula sp. JCM8162	Unknown	Unknown	KJ778625	--------	--------	--------
Sakaguchia lamellibrachiae SY-153	Sediment/ hydrothermal vents	Unknown	AB263119	--------	--------	--------
Sakaguchia lamellibrachiae SY-298	*Calyptogena* sp.	Unknown	AB263120	--------	AB263120	
Sakaguchia sp. G3	Strawberry leaf, Bronchial secretion	Netherlands	JN636811		--------	--------
Sakaguchia sp. IGC 5612			--------	AF387145	--------	--------
Sakaguchia sp. strain JCM8162	Unknown		--------		KJ708356	
Sakaguchia sp. MARY 063	Seawater	Portugal	--------	AY731725	--------	--------
Sakaguchia sp. MARY 297	Seawater	Portugal	--------	AY731726	--------	--------
Uncultured eukaryote clone N104T_250	Seawater	China	GU941203	--------	--------	--------
Uncultured fungus clone 10-37	Soil	UK	KM374342	--------	--------	--------

Table 3.3 continued

SPECIES /ACCESSION	HOST/ SUBSTRATE	LOCATION	ITS	LSU	SSU	*rpb2*
Uncultured fungus clone 11-28	Soil	UK	KM374368	--------	--------	--------
Uncultured fungus clone 3310O16	Soil	USA	KF617787	--------	--------	--------
Uncultured fungus clone 65_NA2_P32_J21	Soil	Canada	KC965397	--------	--------	--------
Uncultured fungus clone BAF25	Surface of a beetle	Canada	AY761181	--------	--------	--------
Uncultured fungus clone S46T_89	Seawater	China	KU164673	--------	--------	--------
Uncultured fungus isolate UPSC_D1_37	*Populus tremula*	Sweden	GU564992	--------	--------	--------
Uncultured soil fungus clone OTU97-218	Soil	USA	JQ310961	--------	--------	--------
Uncultured soil fungus clone OTU97-915	Soil	USA	JQ311732	--------	--------	--------
Uncultured fungus clone CMH332	Indoor environments	USA	KF800423	--------	--------	--------
Uncultured fungus clone ZBJ201308-68	Rainwater	China	KX515421	--------	--------	--------
Uncultured marine eukaryote clone B29	Gut content of *Calanus sinicus*	China	--------	--------	KT825609	--------
Uncultured *Rhodotorula*	Cave sediment	France	HE605258	--------	--------	--------
Uncultured soil fungus clone BPASol1T8_6B	*Picea glauca* rhizosphere	Canada	--------	EU691645	--------	--------

Note: -------- : No sequence available. Accession numbers in bold were generated in the present study. Other accession numbers were retrieved from the NCBI. **T** denotes type species strain

3.1.6 *Discussion*

The number of sequences available in the NCBI for genera like *Buckleyzyma* and *Sakaguchia* is significantly smaller if compared with those of *Cryptococcus* or *Bullera* that are commonly isolated from the phylloplane (Fonseca and Inácio 2006). However, several strains and sequences from environmental studies in *Buckleyzyma* and *Sakaguchia* have been reported in recent years that allow the inference of new habitats for species in both genera (Table 3.2, Table 3.3). *Buckleyzyma aurantiaca* was originally obtained from air sampling for which a substrate or host was not possible to establish at that time; in recent days several other strains and sequences of *B. aurantiaca* have been reported from roots of *Populus* sp. (Bourdel et al. 2016), the gut of insects and marine algae (Stefani et al. 2016). Species in *Buckleyzyma* have been traditionally found associated with the phylloplane of different families such as Elaeagnaceae, Malvaceae, Proteaceae, Salicaceae and Vitaceae, however, the host associations observed in the genus are not exclusive with plants but also have been isolated from mangroves or soils and other substrates.

Among the four loci, the 28S region presented the least divergence (0.2%) when *B. butyrosa* was compared with its sister species *B. armeniaca*, nonetheless there was a higher divergence for the ITS (2.7%) and the *rpb2* (11%) locus that provided more resolution in differentiating *B. butyrosa* from its closest relative. The use of *rpb2* in addition to the 18S, ITS and 28S region, was more useful in providing more phylogenetic resolution in the case of *Buckleyzyma*, when the 28S did not allow to separate *B. butyrosa* from *B. armeniaca*. Assimilation of nitrogen and carbon compounds of *B. butyrosa* shows several differences to *B. armeniaca*, its closest related species. Also, we provide additional physiological data by testing other compounds that were not assessed before in other species of the genus.

Our second described species is *S. warrensis* isolated from 50% glucose medium during non-volumetric air sampling. The fact that this strain was isolated from a high-osmotic pressure medium may indicate that this is a xerotolerant species, however, in subsequent attempts to culture the strain in media containing 10% NaCl and 50% glucose it failed to grow. The sequence divergence of the 28S region and the *rpb2* was 2.9% and 9.2% when *S. warrensis* was compared with *S. cladiensis*. The sequence divergence of 28S was higher (2.9%) in *S. warrensis* when compared with *S. cladiensis* than it was between *B. butyrosa* and *B. cladiensis* (0.2%), however, the divergence of the *rpb2* sequences in both cases was similar (11% and 9.2% in *Buckleyzyma* and *Sakaguchia* respectively). Both species, *B. butyrosa* and *S. warrensis*, were formally described

from single strain collections and since strains MCA2993 and JDSF10 of *B. butyrosa* and *S. warrensis* respectively, were obtained from non-volumetric air sampling a host is not recognized but this will serve as a reference for future strains or sequences that may be conspecific and help to better understand the host associations of these two species. Our analyses did not identify any other sequences that appear to be conspecific to *B. butyrosa*. However, there is one ITS sequence that was isolated from house dust in the United States that appears to represent *S. warrensis* (Accession: KF800423) (Rittenour et al. 2014). This study contributes not only with the description of new species in both genera, but also provides a more complete physiological profile and features that may have not been documented in species already described in both genera. Lastly, we document in this paper the second report of *B. salicina* (P117) from *Pinus nigra* in the United States enlarging the known geographic distribution of this species; the other reported strain in the country was isolated from *Vitis vinifera* inflorescences (Bourret et al 2013). *Buckleyzyma salicina* also has been isolated from *Salix* sp. from Canada and from the phylloplane in China and Portugal (Inácio et al 2002). Apart from plants this species has been isolated from mangrove sediments in India (Velmurugan 2013). The description of new species from a single strain has been controversial, but several authors have provided arguments in favor to circumvent the major concerns of this practice (Graff 1917, Kurtzman and Robnett 1998, Kurtzman 2010, Case et al. 2012, Kijpornyongpan and Aime 2016). For example, the use of multigene comparison for the description *B. butyrosa* and *S. warrensis* has been a useful tool to get more phylogenetic resolution when single-gene phylogenies do not allow it.

3.2 *Halobasidium umbonatum* sp. nov. and the first report of *H. xiangyangense* in North America

3.2.1 *Abstract*

The Cystobasidiales is one of the five orders in the Cystobasidiomycetes with a single family. This order includes *Cystobasidium, Occultifur* and the recently described *Halobasidium*. The genus *Halobasidium* with one species *H. xiangyangense*, was described from a traditional high-salt fermented food in China. In this study we used morphological, physiological and DNA sequences of the nuc rDNA ITS1-5.8S-ITS2 (ITS), D1–D2 domains of nuc 28S rDNA (28S), nuc 18S rDNA (18S) and the protein coding genes RNA polymerase II subunit *rpb2* and the cytochrome b *cytb* to propose *Halobasidium umbonatum*. A single strain MT254 of this species was isolated from

Gymnosporangium sp. infected leaves of *Crataegus* sp. Previous reports of sequences conspecific with *H. xiangyangense* are from China, Korea and Brazil, for which this is the first report in North America. Isolates RK86 and SA444 were obtained from *Dryopteris erythrosora* and watermelon skin.

3.2.2 *Introduction*

The Cystobasidiales includes anamorphic and dimorphic yeasts in a single family, the Cystobasidiaceae, comprises three genera: *Cystobasidium, Halobasidium* and *Occultifur* (Roberts 1997, Bauer et al. 2006, Guo 2019). The sexual stage of species of *Cystobasidium* and *Occultifur* are morphologically similar in terms of the formation of mycelium with clamp connections and their septal pore structure, which is occluded by a peculiar organelle called the cystosome (Weiss 2004, Sampaio et al. 1999). A sexual stage is not known in *Halobasidium*. Among the species in the Cystobasidiales in which a hyphal stage has been described are *C. fimetarium, C. sebaceum, C. proliferans, O. corticorum, O. externus, O. internus* and *O. kilbournensis* (Martin 1939, Olive 1952, Olive 1954, Roberts 1997, Roberts 1999, Sampaio et al 1999, Kurtzman and Robnett 2015). For the remaining species in *Cystobasidium, Halobasidium* and *Occultifur* only the asexual yeast stage has been reported, mostly exhibiting orange to pink colonies when cultured on artificial media.

Species in the Cystobasidiales have been isolated from habitats ranging from tropical regions to Antarctic environments, from marine to fresh water and terrestrial ecosystems, living presumably as saprobes on the surface of leaves, or as endophytes in the xylem of pine trees (Zhao et al. 2002). Also they have been isolated from plant litter, the rhizosphere or found associated with animals and parasitizing other fungi (Reiersøl 1954, Roberts 1999, Zhao et al. 2002, Nagahama et al. 2003, Satoh et al. 2013, Khunnamwong et al. 2015, 2017) or from fermented food as in *Halobasidium,* in which *H. xiangyangense* is considered a high-salt tolerant species. (Guo et al. 2019). During the last 10 years with the help of recent advances in molecular systematics it has been possible to phylogenetically place and describe species new in *Cystobasidium* and *Occultifur* and also to identify several sequences of isolates in independent evolutionary lineages within the Cystobasidiales. Most of these unclassified sequences correspond to the nuclear rDNA ITS1-5.8S-ITS2 (ITS barcode) and the D1–D2 domains of nuclear 28S rDNA (28S) and were generated from environmental samplings but many others correspond to isolates kept in private culture collections.

In the present study, based on morphological, physiological and molecular data we propose a new species namely, *Halobasidium umbonatum* sp. nov., isolated from *Gymnosporangium* sp. infected leaves of *Crataegus* sp. and also, we report for the first time in the western hemisphere *H. xiangyangense* isolated from *Dryopteris erythrosora* and watermelon skin.

3.2.3 *Materials and Methods*

3.2.3.1 Sample collection and storage

The spore-drop method described in Toome et al. (2013) was used to obtain isolates from *Crataegus* sp. leaves infected with *Gymnosporangium* sp. (MT254) and *Dryopteris erythrosora* (SA444) while strain RK86 was isolated from watermelon skin. Cultures were maintained on potato dextrose agar (PDA; 39 g/L, Livonia, Michigan) slants at 4 C for short-term storage and in 40% glycerol vials at -80 C for long-term storage. Cultures of strains of the new species were dried and deposited in the Kriebel Herbarium (PUL) at Purdue University. Live cultures were also deposited at the Westerdijk Fungal Biodiversity Institute (CBS) and the Fungal Biodiversity Center and Agricultural Research Service (NRRL).

3.2.3.2 Morphological and physiological studies

Colony and cell morphology were analyzed by culturing strains on yeast malt agar (YMA, Difco, Franklin Lakes, New Jersey), YM broth (YM broth, Difco) and Corn Meal Agar (CMA, Difco) for seven days at 25 C. Among the macro-morphological characteristics recorded were surface, form, texture, elevation and margin. Cell morphology was studied using a compound microscope OLYMPUS BH-2 (Olympus, Japan) under phase-contrast microscopy. Pictures of cells were taken by using OLYMPUS SC30 camera and the images were analyzed with the Olympus cells Sens Entry 1.14 software. A total of 20 cells was measured in width and length determine average cell dimensions. Assimilation of carbon compounds, growth in vitamin free-media and fermentation tests were performed in liquid media while assimilation of nitrogen compounds and growth on high osmotic conditions were performed in solid media according to Kurtzman et al. (2011). The ability of strains to grow on different carbon sources was assessed by using a Yeast Identification Test Panel (Biolog Inc., Hayward, California) as performed in Kijpornyongpan and Aime (2017).

3.2.3.3 DNA extraction, sequencing and phylogenetic studies

DNA extraction was made from fresh cultures using the Promega Wizard genomic DNA purification kit (Promega, Madison, WI). Sequences of the internal transcribed spacer (ITS) bar code region (nuclear rDNA ITS1-5.8S-ITS2) were amplified with primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990a); the D1–D2 domains of the nuc 28S rDNA (28S) with the primers LR0R/LR5 (Vilgalys and Hester 1990); the nuc 18S rDNA (18S) with primers NS1/NS4 and NS3/NS8 (White et al. 1990a) for all strains stored in the Aime collection. Finally, RNA polymerase II subunit 2, *rpb2* and cytochrome b (*cytb*) were amplified with primers 5F/6R1 and 6F/7CR (Liu et al. 1999) and E1M4/E2Mr3 (Biswas et al. 2001), respectively for strains of the new species. PCR programs for amplification of ribosomal genes are described in section 2.3.4. Protein-coding genes were amplified by using the following programs. For the *rpb2* we used the same PCR programs used in section 3.3.3. For the *cytb*, an initial denaturation step at 94 C during 2 min, followed by 35 cycles at 94 C for 30 s, annealing temperature 50 C for 30 s, 72 C for 1 min and a final extension step at 72 C for 10 min. PCR products were separated in a 1% agarose gel and sent for sequencing at GENEWIZ (South Plainfield, New Jersey). Sequences were edited, aligned and concatenated using GENEIOUS 9.1.2 (Biomatters, Auckland, New Zealand); GenBank accession numbers of sequences used in this study are listed in Table 3.4. The dataset consisted of the nuc 18S, ITS, nuclear 28S region and the protein-coding genes for the *rpb2* and the *cytb* from type strains of species in *Occultifur*, *Halobasidium* and *Cystobasidium* from previous studies. The ITS and 28S sequences of MT254, SA444 and RK86 were used to run a BLASTN search against the NCBI GenBank database, sequences of closely related accessions were included. The genus *Symmetrospora* was used as outgroup (Wang et al. 2015a). Sequences were aligned with the MAFFT V 7.222 program (Katoh et al. 2002) using the L-INS-I algorithm. For aligned sequences of each locus, ambiguously aligned regions were removed with Gblocks using the less stringent selection option (Talavera and Castresana 2007). Lastly, alignments were concatenated using GENEIOUS 9.1.2. (Biomatters, Auckland, New Zealand). The GTR GAMMA-I model was selected as the substitution model for the 5-gene region alignment according to PARTITIONFINDER2 based on the Akaike Information Criterion (AIC). Phylogenetic reconstruction was performed using Maximum Likelihood (ML) followed by 1000 bootstrap replicates using GENEIOUS 9.1.2. Bootstrap proportions (BP) are reported below. Bayesian inference (BI) was performed using

MRBAYES 3.2.6 (Huelsenbeck and Ronquist 2012) with a GTR model, nts:6, rates=invgamma according and five million generations. The first 25% of trees were discarded as Burn-in.

3.2.4 *Results*

3.2.4.1 Phylogenetic studies

The multi-locus phylogeny was reconstructed using a dataset consisting of 4031 nucleotide positions. One species was delimited: *Halobasidium umbonatum* sp. nov. (MT254) (Figure 3.4). Among the sequences that are closely related to *H. umbonatum* are KC966072 from an environmental study in artic soil, LM644065 isolated from *Arrhenatherum elatius* in Germany, JQ320370 from an unidentified plant in China, JF706656 isolated from flowers of an unidentified plant in Korea, U65613 isolated from *Chimonobambusa marmorea* in China and LT602889 isolated from *Arabidopsis* phylloplane in Finland (Yan et al 2013, Timling et al. 2014, Yurkov et al. 2015, Wang et al. 2016). Accessions that appear closely related to *H. xiangyangense* are KY986543 and EU678949 isolated from skin scales and a unidentified source in Brazil, respectively, LC272869 from seawater in Korea, and KT819328 isolated from seaweed in New Zealand, while FJ238089, FJ238090, FJ238091and FJ865356 were all isolated from a solar saltern in Korea (Table 3.4) (Spader et al. 2017, Chin et al. 2017, Francis et al. 2016).

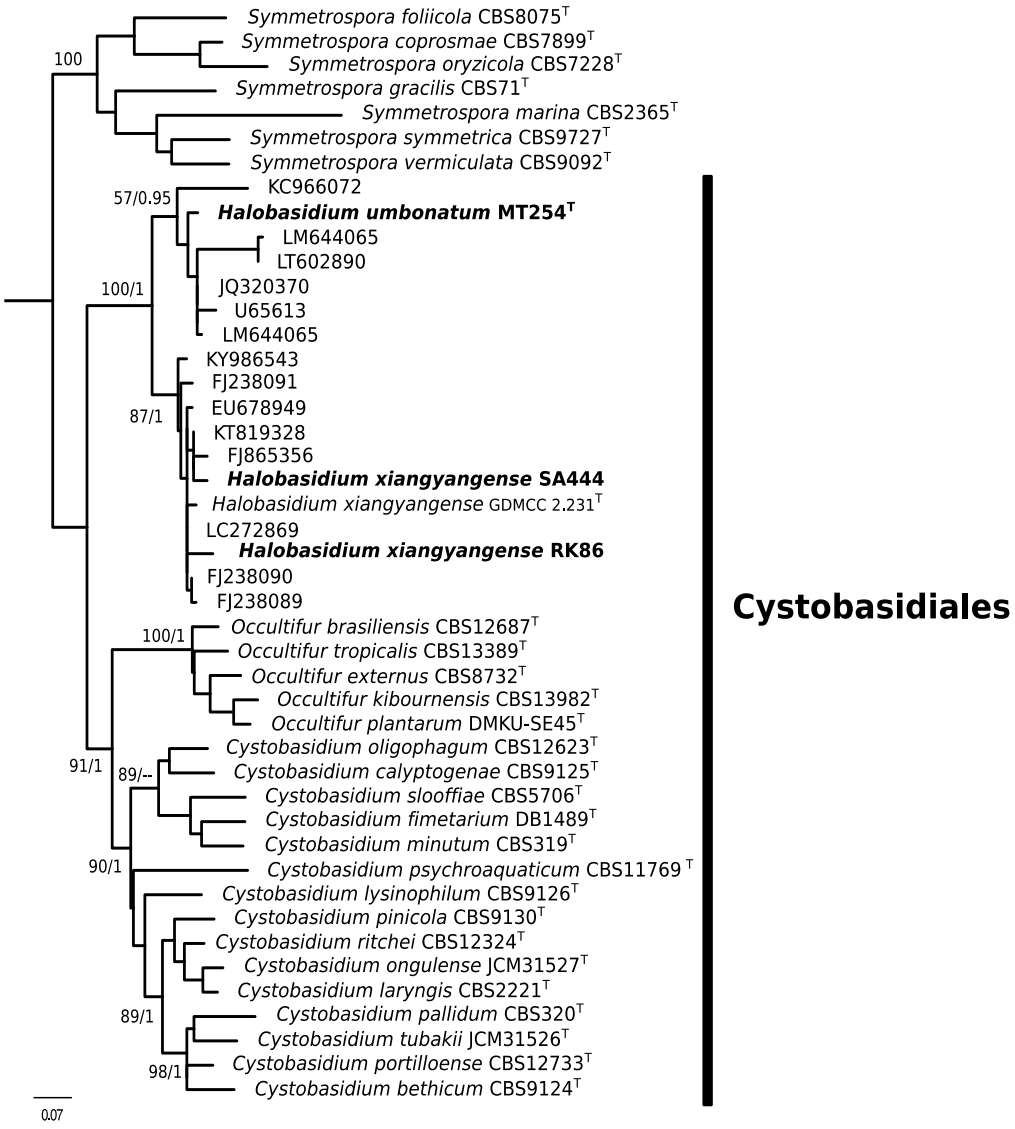

Figure 3.4 Phylogenetic reconstruction of the Cystobasidiales inferred from concatenated sequences of ITS, D1–D2 domains of nuclear 28S, nuclear 18S rDNA, *rpb2* and *cytb* using maximum likelihood. New species and strains that are new reports for western hemisphere are indicated in bold. Posterior probability (PP > 0.7) and bootstrap support (BP > 50%) values are shown above nodes. ns: not supported. Tree is midpoint rooted. Scale bar equals expected number of substitutions per site. Strain number is listed in front of the taxon name. ^{T} denotes type species strain.

3.2.5 *Taxonomy*

Halobasidium umbonatum P. P. Parra & Aime, sp. nov. FIGS. 3.5A, 3.5C

Mycobank: MB 831221

Typification: UNITED STATES. INDIANA: Tippecanoe County, West Lafayette, Purdue University, West Lafayette Campus, front side of Whistler Agricultural Research Building. Collected from *Crataegus* sp. leaves infected with *Gymnosporangium* sp. 29 of August 2013. *M. Toome MT254.* (Holotype preserved as dried inert cells at PUL: F24085). Ex-type cultures CBS 16090 = NRRL- 63998. GenBank: ITS = MK990658, D1–D2 domains of nuc 28S rDNA = MK990685, nuc 18S rDNA = MK990639.

Etymology: *umbonatum* = Referring to the umbonate elevation of colony when cultured on YMA.

Description: After 7 days at 25 C on YM agar colonies are pink/light coral (0ac619), glistering and butyrous in texture. Colony form is circular with entire margin and umbonate elevation. Cells occur singly or in parent-bud pairs and reproduce asexually by monopolar budding. In YM broth (YM broth, Difco) after 7 days, cells are subglobose to ellipsoidal (2.2–3.3 × 3.5–4.6). On CMA agar, colonies are dark pink in color (0ac611), neither true nor pseudohyphae are formed. Ballistoconidia are not produced on CMA.

Fermentation is absent. Growth is observed at 25 C and 30 C. When cultured on media containing agar (2%), yeast extract (1%) and supplemented either with 50% glucose, 10% NaCl colonies were observed but no colonies were formed when media was supplemented with 16% NaCl. Growth on vitamin free media is positive. Assimilation of a-D-glucose, D-gluconic acid, , mentiobiose, D-glucuronate, inositol, DL-lactate, lactose, 1,2-propanediol is positive. Assimilation is slow on D-galacturonate, methanol and weak on 1,2-propanediol + xylose, 2-keto D-gluconate, a-D-lactose + xylose, inulin, cellobiose, a-D-glucose, b-Me-D-glucoside, adonitol, arbutin, bromosuccinic acid, D-melezitose, D-mannitol, D-arabinose, dextrin, dextrin + xylose, D-arabitol, L-aspartic acid, L-malic acid, D-galactose, D-galactose + xylose, D-glucuronic acid + xylose, D-melibiose + xylose, D-sorbitol, D-xylose, fumaric acid, gentiobiose, turanose, L-sorbose, m-inositol + xylose, glycerol, L-arabinose, Me-succinate + xylose, L-glutamic acid, Nac-D-glucosamine, L-sorbose, maltitol, NAc-L-glutamic acid, maltose, palatinose, sucrose and trehalose. Nitrogen assimilation is positive on L-lysine-HCl, D-glucosamine, D-tryptophan, KNO3 (w), cadaverine-HCl (w) and imidazole (w) (Table 3.5).

Notes: Halobasidium umbonatum MT254^{T} is morphologically undistinguishable from its sister species *H. xiangyangense* strain GDMCC2.2.31^{T}, but there are several differences in their assimilation profiles. Assimilation is positive for DL-lactate, methanol (slow), lactose, D-glucosamine (weak), inositol while is negative in *H. xiangyangense* strain GDMCC2.2.31^{T}. Sequence divergence with strain GDMCC2.2.31^{T} is 1.6% (2.2/506 bp) and 1.2% (7/575). When *H. umbonatum* sequences are compared with *H. xiangyangense* for the other strains of *H. xiangyangense* strain SA444 the difference is 1.6% (8/506 bp), 1.5% (9/575), 15.1% (96/962) and 8.8% (35/429), for the ITS, 28S, *rpb2* and *cytb* respectively.

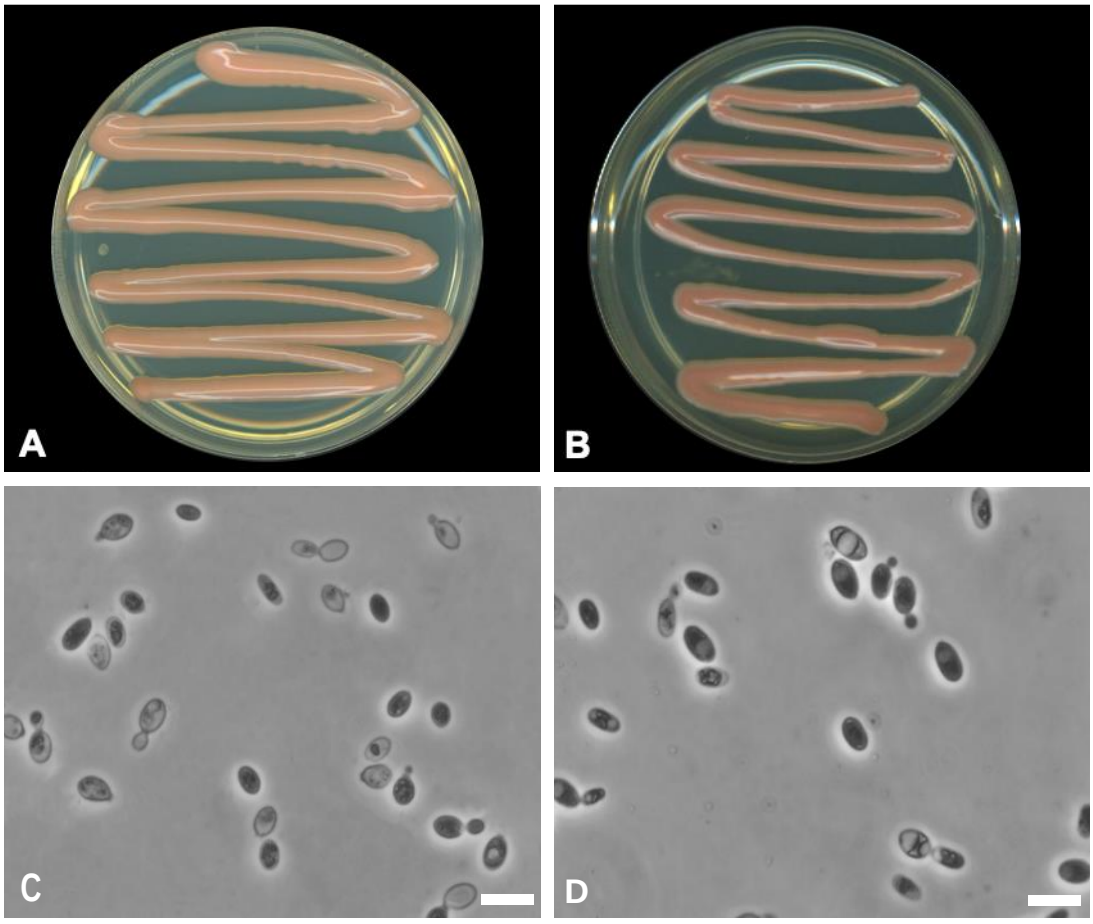

Figure 3.5 Colony and cell morphology. **A. C.** *Halobasidium umbonatum* MT254^{T}. **B. D.** *H. xiangyangense* SA444. Bars: 5 μm. A, B, and C, D, were grown on YMA and YMB, respectively, for seven days at room temperature.

Table 3.4 List of taxa and accession numbers used in this study

SPECIES/ACCESSION	HOST/SUBSTRATE	LOCATION	ITS	LSU	SSU	*rpb2*	*cytb*
Cystobasidium lysinophilum CBS9126^{T}	unknown	Japan	AB078501	AB078501	AB126650	KJ708243	KJ707721
Cystobasidium benthicum CBS9124^{T}	*Lamellibrachia* sp.	Japan	AB026001	AB026001	AB126647	KJ708214	KJ707691
Cystobasidium calyptogenae CBS9125^{T}	*Calyptogena* sp.	Japan	AB025996	AB025996	AB126648	KJ708218	KJ707690
Cystobasidium fimetarium DB1489^{T}	*Thelebolus crustaceus* on grouse dung	Denmark	KP053250	AY512843	AY124479	--------	--------
Cystobasidium laryngis CBS2221^{T}	Laryngeal swabs	Norway	AF190014	AF189937	--------	KJ708240	KJ707619
Cystobasidium minutum CBS319^{T}	Air sampling	Japan	AF190011	AF189945	D45367	KJ708246	KJ707562
Cystobasidium oligophagum CBS12623^{T}	Koala nasal smears	Australia	AB702968	AB702967	LM644061	--------	--------
Cystobasidium ongulense JCM31527^{T}	Soil	Antarctica	LC155915	LC155915	LC158351	--------	LC158355
Cystobasidium pallidum CBS320^{T}	mycotic nodule in white rat.	unknown	AB078492	AF189962	AB126651	KJ708253	KJ707621
Cystobasidium pinicola CBS9130^{T}	*Pinus tabulaeformis*	China	AF444292	AF444293	AB126652	KJ708257	KJ707579
Cystobasidium portillonense CBS12733^{T}	Rock surface	Chile	JQ769323	JQ769312	--------	--------	--------
Cystobasidium psychroaquaticum CBS11769^{T}	leatherleaf *Chamaedaphne calyculata*	Russia	FN868153	KY107444	LM644062	--------	--------
Cystobasidium ritchiei CBS12324^{T}	leatherleaf *Chamaedaphne calyculata*	Russia	LM644066	LM644066	LM644063	--------	--------

Table 3.4 continued

SPECIES/ACCESSION	HOST/SUBSTRATE	LOCATION	ITS	LSU	SSU	*rpb2*	*Cytb*
Cystobasidium slooffiae CBS5706^T	laryngeal swab	Hungary	AF444627	AF189965	AB126653	KJ708266	KJ707629
Cystobasidium tubakii JCM31527^T	Soil	Antarctica	LC155913	LC155913	LC158350	--------	LC158354
Halobasidium xiangyangense GDMCC2.231^T	high-salt fermented food	China	MH209248	MH212153	--------	--------	--------
Halobasidium umbonatum MT254^T	Crataegus sp. with *Gymnosporangium* sp.	USA	**MK990685**	**MK990658**	**MK990639**	--------	--------
Halobasidium xiangyangense RK086	watermelon skin	USA	**MK990659**	**MK990686**	**MK990640**	--------	--------
Halobasidium xiangyangense SA444	*Dryopteris erythrosora* leaves	USA	**MK990660**	**MK990687**	**MK990641**	--------	--------
Cystobasidiomycetes sp. BI218	Unknown	R. of Korea	FJ865356	EU678949	--------	--------	--------
Cystobasidiomycetes sp. KSS-2008 strain 3-23T	Solar saltern	R. of Korea	FJ238089	FJ238091	--------	--------	--------
Cystobasidiomycetes sp. KSS-2008 strain 5-19	Solar saltern	R. of Korea	FJ238090	--------	--------	--------	--------
Cystobasidiomycetes sp. MJL-2011 strain JS-40	flowers	R. of Korea	JF706656	--------	--------	--------	--------
Rhodotorula sp. YM24636	Unknown	China	JQ320370	--------	--------	--------	--------
Uncultured fungus clone 112_NA3_P31_E20	Soil	Artic	KC966072	--------	--------	--------	--------
Rhodotorula sp. P2S-PDA	Seaweeds	New Zealand	KT819328	--------	--------	--------	--------
Cystobasidiomycetes sp. isolate 62134	skin scales	Brazil	KY986543	--------	--------	--------	--------

Table 3.4 continued

SPECIES/ACCESSION	HOST/SUBSTRATE	LOCATION	ITS	LSU	SSU	*rpb2*	*cytb*
Cystobasidiomycetes sp. DSM 28479	False oat-grass *Arrhenatherum elatius*	Germany	LM644065	--------	--------	--------	--------
Cystobasidiomycetes sp. M17	Wild Arabidopsis leaf	Finland	LT602890	--------	--------	--------	--------
Basidiomycete from a bamboo	*Chimonobambusa marmorea*	China	U65613	--------	--------	--------	--------
Occultifur brasiliensis CBS12687 T	Water tanks of *Vriesea minarum*	Brazil	KC698874	KC698874	--------	--------	--------
Occultifur externus CBS8732 T	Plant litter	Portugal	AF444567	AF131062	AB055193	KJ708199	KJ707689
Occultifur kilbournensis CBS13982 T	Soil	USA	KP413162	KP413160	--------	--------	--------
Occultifur plantarum DMKU-SE45 T	Sugarcane leaf	Thailand	--------	--------	--------	--------	--------
Occultifur tropicalis CBS13389 T	Sugarcane leaf	Thailand	AB921282	AB921280	--------	--------	--------
Symmetrospora coprosmae CBS7899 T	*Coprosma tenuifolia*	New Zealand	AF444577	AF189980	D66880	KJ708296	KJ707742
Symmetrospora foliicola CBS8075 T	Leaves of *Banksia collina*	Australia	AF444521	AF189984	AB021671	KJ708302	KJ707589
Symmetrospora gracilis CBS71T	*Phertinia glabra* leaves	Australia	AF444578	AF189985	KJ708433	KJ708304	KJ707705
Symmetrospora marina CBS2365 T	*Peneaus setiferusn* (Shrimp)	USA	AF444504	AF189944	AB126645	KJ708244	AB040635
Symmetrospora oryzicola CBS7228 T	Dead leaf of *Oryza sativa*	Japan	AF444546	AF189990	AB021677	KJ708324	KJ707712
Symmetrospora symmetrica CBS9727 T	Wilting leaves of *Betula platyphylla*	China	AY364836	AY364836	KJ708350	KJ708337	KJ707582

Table 3.4 continued

SPECIES/ACCESSION	HOST/SUBSTRATE	LOCATION	ITS	LSU	SSU	*rpb2*	*cytb*
Cystobasidium sp.	Seawater	R. of Korea	--------	LC272869	--------	--------	--------
Symmetrospora vermiculata CBS9092T	Dead leaf of *Pennisetum pediocellatum*	Thailand	AB030335	AF460176	--------	KJ708342	KJ707675

Note: -------- : No sequence available. Accession numbers in bold were generated in the present study. Other sequences were retrieved from the NCBI.

T denotes type species strain

Table 3.5 Assimilation of carbon and nitrogen compounds and growth under different temperature and osmotic conditions of new species of *Halobasidium umbonatum* and strains of its sister species *H. xiangyangense*

Compound	***Halobasidium umbonatum* MT254^{T}**	***Halobasidium xianyangense* RK86**	***Halobasidium xianyangense* SA444**	***Halobasidium xianyangense* GDMCC2.2.31^{T*}**
Carbon assimilation				
D-gluconic acid	+	w	w	+
Dextrin	w	-	w	n
Acetic acid	-	-	w	n
Formic acid	-	-	-	n
Propionic acid	-	-	-	n
Succinic acid	-	-	-	+
L-Aspartic acid	w	-	w	n
L-Proline	-	-	-	n
Cellobiose	w	-	-	n
Trehalose	w	w	w	+
Turanose	+	-	w	n
Mentiobiose	+	w	w	n
D-Melezitose	w	w	+	+
D-Raffinose	-	-	-	-
a-D-Glucose	+	w	w	n
D-Psicose	-	-	-	n
Salicin	-	-	w	+
1,2-propanediol	+	+	+	n
Citrate	-	-	-	+
D-Galacturonate	s	-	+	
D-Glucuronate	+	+	+	n
DL-Lactate	+	+	+	-
Ethanol	-	-	-	+
Glucose (+ ctrl)	+	+	+	n
Inositol	+	+	-	-
Lactose	+	+	s	-
Methanol	s	-	-	-
Soluble starch	-	-	-	-
Dextrin	-	w	-	n
Inulin	w	+	+	+
Fumaric acid	w	w	-	n
L-Malic acid	w	w	w	n
Me succinate	-	-	-	n
Bromosuccinic acid	w	w	w	n

Table 3.5 continued

Compound	***Halobasidium umbonatum* MT254^{T}**	***Halobasidium xianyangense* RK86**	***Halobasidium xianyangense* SA444**	***Halobasidium xianyangense* GDMCC2.2.31^{T*}**
Gammna-aminobutyric acid	-	w	-	n
a-ketoglutarate	-	w	-	n
2-keto D-gluconate	w	w	-	n
Sucrose	w	+	w	+
Trehalose	w	+	w	+
Gentiobiose	w	+	w	n
Maltose	w	w	w	+
Maltotriose	w	+	w	n
D-Melibiose	-	-	-	w
Palatinose	w	w	w	n
Nac-D-glucosamine	w	-	w	-
Amygdalin	w	w	-	n
Arbutin	w	w	-	n
D-Galactose	w	w	w	+
L-Rhamnose	-	-	-	n
L-Sorbose	w	w	w	+
a-Me-D-glucoside	-	w	-	w
b-Me-D-glucoside	w	+	w	n
a-methyl-D-glucoside	-	-	-	n
D-Glucose	-	-	-	+
Melezitose	-	-	-	n
Melibiose	-	-	-	n
Raffinose	-	-	-	n
Starch	-	-	-	n
Maltitol	w	-	w	n
L-Arabinose	w	w	w	+
D-Arabinose	w	+	w	w
D-Ribose	-	-	w	+
D-Mannitol	w	w	w	+
D-Sorbitol	w	+	w	n
Adonitol	w	w	w	n
D-Arabitol	w	w	w	n
i-Erythritol	-	-	-	-
Glycerol	w	w	w	n
Tween 80	-	w	-	n

Table 3.5 continued

Compound	***Halobasidium umbonatum* MT254^{T}**	***Halobasidium xianyangense* RK86**	***Halobasidium xianyangense* SA444**	***Halobasidium xianyangense* GDMCC2.2.31^{T*}**
Gelatin liquefaction				n
D-Xylose	w	-	w	+
m-Inositol + xylose	w	-	-	n
1,2-propanediol + xylose	w	-	-	n
Acetonin + xylose	-	-	-	n
Me-succinate+xylose	w	-	-	n
NAc-L-glutamic acid	w	-	-	n
Table 3.5 continued				
Guinic acid+xylose	w	-	w	n
D-Glucuronic acid+xylose	w	w	w	n
Dextrin + xylose	w	-	-	n
a-D-Lactose + xylose	w	-	-	n
D-Melibiose+xylose	w	-	-	n
D-galactose + xylose	w	-	w	n
Nitrogen assimilation				n
(NH4)2SO4	+	+	+	w
Cadaverine-HCl	w	w	w	-
Creatine	-	-	w	w
D-Glucosamine	+	+	+	w
D-Tryptophan	+	+	+	n
Ethylamine-HCl	-	+	-	w
Imidazole	w	-	-	w
KNO3	w	-	w	w
L-lysine-HCl	+	+	-	n
NaNO2	-	-	-	-
10% NaCl	+	+	+	n
16% NaCl	-	-	-	n
50% Glucose	+	+	+	n
25 C (C5 tube)	+	+	+	n
30 C	+	+	+	+
Vitamin free medium	+	+	+	w

+, positive; -, negative; v, variable; w, weak; n, not determined. *, denotes assimilation data for *Halobasidium xiangyangense* from Guo et al. (2019)

3.2.6 *Discussion*

In this study, we describe a new species in the genus *Halobasidium.* The asexual stage of *Halobasidium* species share several characteristics with other species in the Cystobasidiales such as pink to orange colonies and ellipsoidal cells that occur singly or in parent-bud pairs (Figure 3.5A, 3.5C). The absence of pseudohyphae formation is another shared characteristic with species in most of the class. It is not possible to differentiate species in *Halobasidium* from species in *Cystobasidium* or *Occultifur* morphologically, but it is possible to identify strains based on the 28S region. When a five-gene phylogeny using the 18S, ITS, 28S, *rpb2* and *cytb* was built, these genera are separated into three highly supported monophyletic genera (Figure 3.4). According to Hibbett et al. (2011) an average of 1200 species were identified per year from 2001 to 2011. Although the number of described species is rising with the help of molecular techniques, there are several taxa of fungi that receive less attention due to their cryptic nature in ecosystems or just because their role in nature is still unknown.

During the last ten years, only ten species in the Cystobasidiales have been formally described; nonetheless, there are still several lineages represented by strains that have been reported in research papers but that remain undescribed. One example is evidenced by at least 12 accessions deposited in the NCBI that appear closely related to *Halobasidium* but remained undescribed until recently (Guo et al. 2019). From these reports, the first collection was made in 1997 from bamboo (Zhang et al. 1997), meaning that strains belonging to the now described *Halobasidium* were found 21 years ago but did not elicit a formal description and proposal for a long time. These records of sequences related to *Halobasidium* indicate that its geographic distribution is not limited to China or the United States, but it also can be found in Finland, Germany and Korea. The habitats in which sister taxa have been isolated range from plants to extreme noches such as artic soil or salterns which are characterized by hypersaline waters (>3.5% NaCl). This coincides with the fact that both species of *Halobasidium* can grow on media containing 10% NaCl, as observed in other species of the Cystobasidiales such as *Occultifur externus, C. calyptogenae, C. minutum, C. lysinophillum, C. oligophagum* and *C. tubakii* (Nagahama et al. 2003, Tsuji et al. 2016). With the discovery of the ability of species in *Halobasidium* to grow under high osmotic conditions (media containing 50% glucose) we identified other habitats in which species in the Cystobasidiales can be found. The only xerotolerant species in the Cystobasidiomycetes previously reported is *Cystobasidium pallidum*.

CHAPTER 4. TAXONOMIC REVISION AND THE EVOLUTION OF HABITAT PREFERENCE IN THE CYSTOBASIDIOMYCETES

4.1 Introduction

The Cystobasidiomycetes (Basidiomycota: Pucciniomycotina) consists of 13 genera and over 70 species, currently placed in five orders: Buckleyzymales, Cystobasidiales, Erythrobasidiales, Naohideales and Sakaguchiales (Aime et al. 2006, 2014, Bauer et al. 2006, Zhao et al. 2017). Species belonging to the Cystobasidiomycetes have been isolated from different habitats, ranging from aquatic environments, soil, insects, mammals and plants. Several species have been found in association with or parasitizing other fungi, also known as mycoparasitism. Different interactions that occur in nature between organisms are not well documented or studied; instead the habitat preference that refers to where the fungus was found, could then reflect a preference towards a certain life strategy or substrate (Chaverri and Samuels 2011) either parasitic, saprotrophic, endophytic, etc. The early-diverging position of *Naohidea sebaceae* (a mycoparasite) within the class and the occurrence of mycoparasitism and host associations found in several lineages of the Cystobasidiomycetes has led to the hypothesis that the Cystobasidiomycetes may have arisen from nanometer-fusion mycoparasites (Bauer 2004). Hence the possibility of mycoparasitism or close association with other fungi being an ancestral character should not be neglected.

Mycoparasitism, which consists in the direct physical contact between two fungi is one of the several antagonistic interactions observed in fungi. Antagonism can also occur through the production of toxic compounds and other agents against competing organisms, through the competition for nutrients or by stimulating host defense mechanisms (Karlsson et al. 2015, Narayanasamy 2013). Mycoparasitism and the production of toxic compounds have been reported in the Cystobasidiomycetes; out of these two phenomena, the less studied is the production of toxic compounds. This type of antagonism that mainly occurs between the yeast stage of the fungi and other organisms was first described in *Saccharomyces cerevisiae* (Bevan and Makeower 1963) and has been more extensively studied in ascomycetous yeasts. In Basidiomycota only 50 yeast species have been reported as producers of these compounds, in the Cystobasidiomycetes only two species, *Cystobasidium pallidum* and *Hasegawazyma lactosa,* have been reported to produce toxic compounds (Klassen et al. 2017).

Another type of antagonism; mycoparasitism, involves direct physical interaction. Mycoparasitism, is associated with sexual states and has been reported in species of *Cystobasidium*, *Naohidea, Cyphobasidium* and *Occultifur*. In the Naohideales, the species *Naohidea sebacea* has been found in association with plants such as *Fraxinus* sp. and maple (Berk and Broome 1971), but also has been reported as a parasite of old stromata of *Botryosphaeria dothidea* (Piatek 2002), *Botryiodiplodia* sp., *Botryosphaeria quercuum* (Mcnabb 1965), on a sphaeriaceous fungus (Olive, 1947) and also observed on pycnidia of a *Phialophorophoma*-like fungus (Bandoni 1973). In the Cystobasidiales, the hyphal stage of *Cystobasidium fimetarium* has been found growing on dung with other ascomycetous fungi like *Thelebolus crustaceus* and *Lasiobolus equinus*, on which it is presumed to be mycoparasitic (Robets 1999). Similarly, *C. sebaceum* was found growing on remnants of an old *Sebacina* sp. on decaying wood (Martin 1939). In the genus *Occultifur, O. corticiorum* was isolated from a basidiocarp of *Tylospora fibrillosa* (Roberts 1997), *O. internus* was isolated from *Dacrymyces stillatus* (Olive 1954), *O. rivoirei* was found parasitizing *Hyphodontia arguta* (Trichies 2016) and *O. lumbricifer* has been described as an intrahymenial parasite on *Hyphodontia subalutacea* (Roberts 2001). Lastly, *Cyphobasidium hypogymniicola* and *Cyphobasidium usneicola* have been found in association with lichens on several species of *Usnea, Bryoria* and another 50 genera (Diederich 1996, 2007, Spribille et al. 2016, Millanes et al. 2015).

Fungal association of species in the Cystobasidiomycetes have not only been reported during the hyphal stage; other species such as *Erythrobasidium hasegawianum* have been isolated from an old culture of beer yeast, *Cystobasidium psychroaquaticum* has been found in association with the lichen *Cladonia pocillum* (Zhang et al. 2015) and *Hasegawazyma lactosa* has been isolated from fungus gardens of *Atta texana* presumably antagonizing other microbes (Rodrigues et al. 2009). Additionally, sequences from environmental samplings have evidenced the presence of Cystobasidiomycetes in *Hemileia vastatrix* pustules in coffee leaves (James et al. 2016).

It should be considered that several species in the Cystobasidiales found in association with other fungi have been described only from morphological characters. Among these species are *C. sebaceum* (Martin 1939) while in *Occultifur* there are five species: *O. corticorum* (Roberts 1997); *O. rivoirei* (Trichies 2016); *O. internus* (Olive 1954); and *O. lumbricifer* (Roberts 2001). As stated by Crous et al. (2014), it is of vital importance for fungal taxonomy the application of generic names based on DNA sequence data when possible and not only on morphology and ecology, although it is not always possible. Such importance is demonstrated in *Occultifur,* for which the

taxonomic affiliation of the type species *O. internus* is unknown leading to a taxonomic uncertainty for the genus (for more details about the genus see Chapter 1).

Due to the lack of molecular data for the type species *O. internus*, the species *O. externus* for which molecular data are available is not only the most studied species in the genus, but also has been used as the reference species for the genus in most of the phylogenetic studies (Sampaio et al. 1999, Wang et al. 2015b). In this chapter, for the first time, we use molecular data to establish the phylogenetic affiliation of *O. internus* and we present evidence of mycoparasitism as a life strategy in another lineage in the Cystobasidiomycetes that remained unknown until now. Additionally, we made phylogenetic reconstructions using the ITS and 28S gene regions to classify sequences from strains and environmental sampling available in the NCBI that are conspecific to extant species of the Cystobasidiomycetes and were not classified before or were not classified correctly. In this way, we can identify host associations that remained unknown for these species and include them in further analyses. Lastly, a seven-locus phylogenetic reconstruction of the class and ancestral state reconstruction analyses were performed to study the evolution of habitat preference of species in the class and to test whether the Cystobasidiomycetes could have arisen from a mycoparasitic ancestor.

4.2 Materials and Methods

4.2.1 *Specimens and strains*

Herbarium specimens KM068754 and KM094773, were acquired from the Royal Botanical Gardens, KEW, and brought to the Aime lab for DNA extraction. Also, cultures of strains belonging to eight genera of the Cystobasidiomycetes collected in different countries were included in the Aime Lab Collection (See section 2.2.1) for further analyses.

4.2.2 *DNA extraction, PCR and sequencing*

DNA extraction from specimens KM68754 and KM94773 along with strains of Cystobasidiomycetes in the Aime Lab collection was performed using the Promega Wizard genomic DNA purification kit (Promega, Madison, Wisconsin). The ITS, D1–D2 domains of the nuclear 28S rDNA (28S) and *tef1* (for *O. internus* specimens) were amplified by PCR following the conditions used in section 2.2.4. PCR products were separated in a 1% agarose gel and sent to GENEWIZ (South Plainfield, New Jersey) for direct sequencing. Sequences were edited in

GENEIOUS 9.1.2 (Biomatters, Auckland, New Zealand). Sequences of newly described species in previous studies (Chapter 2 and Chapter 3) and sequences from strains of the Cystobasidiomycetes in the Aime Lab collection were included in the analyses.

4.2.3 *Phylogenetic analyses*

Sequences for the ITS1-5.8S-ITS2 (ITS), the D1–D2 domains of nuclear 28S rDNA (28S), nuclear 18S rDNA (18S), and portions of the *tef1*, *rpb1*, *rpb2* and *cytb* genes were retrieved from the NCBI repository for the type strain of all extant species. Also, sequences of newly described species in the previous chapters were included. The ITS, nuclear 28S and nuclear 18S sequences of type strains of extant species in the class were searched against the NCBI GenBank database using BLASTn; the most closely related accessions and sequences of strains of the Cystobasidiomycetes in the Aime Lab Collection were used to build single-gene phylogenies for species delimitation. Duplicates of sequences in each dataset were removed using the remove duplicates built-in function in GENEIOUS 9.1.2 (Biomatters, Auckland, New Zealand). Phylogenetic reconstructions were made using: 1) Seven concatenated gene regions for extant species in the class. *Sterigmatomyces hyphaenes* was used for outgroup purposes (Wang et al. 2015b); 2) Three gene regions including the ITS, 28S and 18S to resolve the phylogenetic position of *Occultifur internus*; and 3) Single-gene region phylogenies for species in each genus of the Cystobasidiomycetes using ITS, 28S and 18S sequences. GenBank accession numbers for sequences used to build a resolved phylogeny of the Cystobasidiomycetes are provided in Table 4.1, while accession numbers for single-gene phylogenies used in the species delimitation are provided in Table A1. Sequences used in all phylogenies were aligned with MAFFT 7.222 (Katoh et al. 2017) using the L-INS-I algorithm. Alignments of the seven- and four-gene regions were concatenated in GENEIOUS 9.1.2 (Biomatters, Auckland, New Zealand). The GTR GAMMA-I model was selected as the substitution model for the seven- and four-gene phylogenies according to PARTITIONFINDER2 based on the Akaike Information Criterion (AIC). Phylogenetic reconstruction using seven- and four- gene regions were performed using Maximum Likelihood (ML) followed by 1000 bootstrap replicates, and Bayesian inference (BI) was performed using MRBAYES 3.2.6 (Huelsenbeck and Ronquist 2012) with a GTR, an INVGAMMA model and five million generations. For species delimitation using single-gene regions only ML analysis was performed. Both, ML and BI analyses were run using the packages available in the Purdue University super computer cluster.

4.2.4 *Species delimitation and host association*

We used a phylogenetic species concept (PSC) to delimit species based either on the ITS, 28S or 18S region. When sequences of multiple loci were available for one strain, their phylogenetic placement was determined according to genealogical concordance for phylogenetic species recognition based on individual gene phylogenies (GCSR). The purpose of this strategy was to identify more accurately the phylogenetic placement of sequences reported in the NCBI, strains in the Aime Lab Collection and to include in our datasets all the sequences available for the species in the Cystobasidiomycetes. In summary, we built phylogenies of the ITS, 28S and 18S sequences for every genus in the class. Once each accession from the NCBI was assigned to a species, information about location or country, isolation source (substrate) and whether the sequence corresponds to a culture collection or environmental sampling was recorded.

4.2.5 *Ancestral character reconstruction and host preference reconstruction*

According to Chaverri and Samuels (2013), the habitat preference specifically refers to where the fungus was isolated and this host association could reflect a preference for a particular life strategy. Based on this concept we used the substrate of isolation as a proxy to investigate the trophic strategy of species in the class. We grouped these associations into five categories: Animals (A), aquatic (B), fungi (C), plants (D) or decaying organic matter (E). The ancestral character reconstruction to study the evolution of the host preference of species in the Cystobasidiomycetes was made using BayesTraits v3.0.1 with two approaches: 1) Maximum Likelihood (ML) and 2) Markov Chain Monte Carlo (MCMC). As one species can have more than one host association, we used a multistate approach. The ML ancestral character reconstruction was run using a multistate approach and default parameters. For the MCMC approach we used a reversible-jump MCMC, burn-in of 10 000, sample frequency: 1 000, and 1 000 000 iterations. Posterior probability for each possible ancestral state was calculated for both ML and MCMC by averaging across post-burn-in estimates of 1000 trees of each node.

4.3 Results

4.3.1 *Phylogenetic studies: A resolved phylogeny of the Cystobasidiomycetes*

Individual alignments for type strains of species in the Cystobasidiomycetes consisted of 593 (ITS), 606 (28S), 1 690 (18S), 1003 (*tef1*), 787 (*rpb1*), 974 (*rpb2*) and 432 (*cytb*) nucleotide positions after exclusion of ambiguously aligned positions. We obtained sequences for the ITS, 28S and the *tef1* from specimens KM68754 and KM94773 of *Occultifur internus* and included them in our seven-gene region phylogeny. To determine the phylogenetic placement of *O. internus*, datasets consisting of three (18S, ITS and 28S) and seven (18S, ITS, 28S and *tef1*, *rpb1*, *rpb2* and *cytb*) combined gene regions comprising 2 889 and 6 085 nucleotide sites, respectively, were used. Based on the phylogenetic reconstructions using these datasets, we established the non-monophyletic nature of *Occultifur*. In our analysis, *O. internus* appears as congeneric to species in *Microsporomyces* using the two datasets and appears as sister species of *Microsporomyces bloemfonteinensis* (Figure 4.1). Based on the results from our phylogenetic reconstructions and aiming to clarify the phylogenetic affiliations of species such as *O. internus* for which molecular data is presented for the first time, we propose several taxonomic changes in the Cystobasidiomycetes, in particular in *Occultifur* and *Microsporomyces* (See section 4.4). As *O. internus* is the type species for the genus, species in *Microsporomyces* should be transferred to *Occultifur*. Consequently, the former *Occultifur* should be renamed.

Once the phylogenetic relationships in the Cystobasidiomycetes were resolved (Figure 4.2), we proceeded to perform species delimitation based on the PSC and GCSR. We assigned sequences available in the NCBI as well as sequences generated in this study to extant species in the class. In this chapter we do not only classify sequences (listed as uncultured fungus or that were not classified correctly), but also, we identified new associations, substrates, habitats and locations in which members of the Cystobasidiomycetes were not reported in the past.

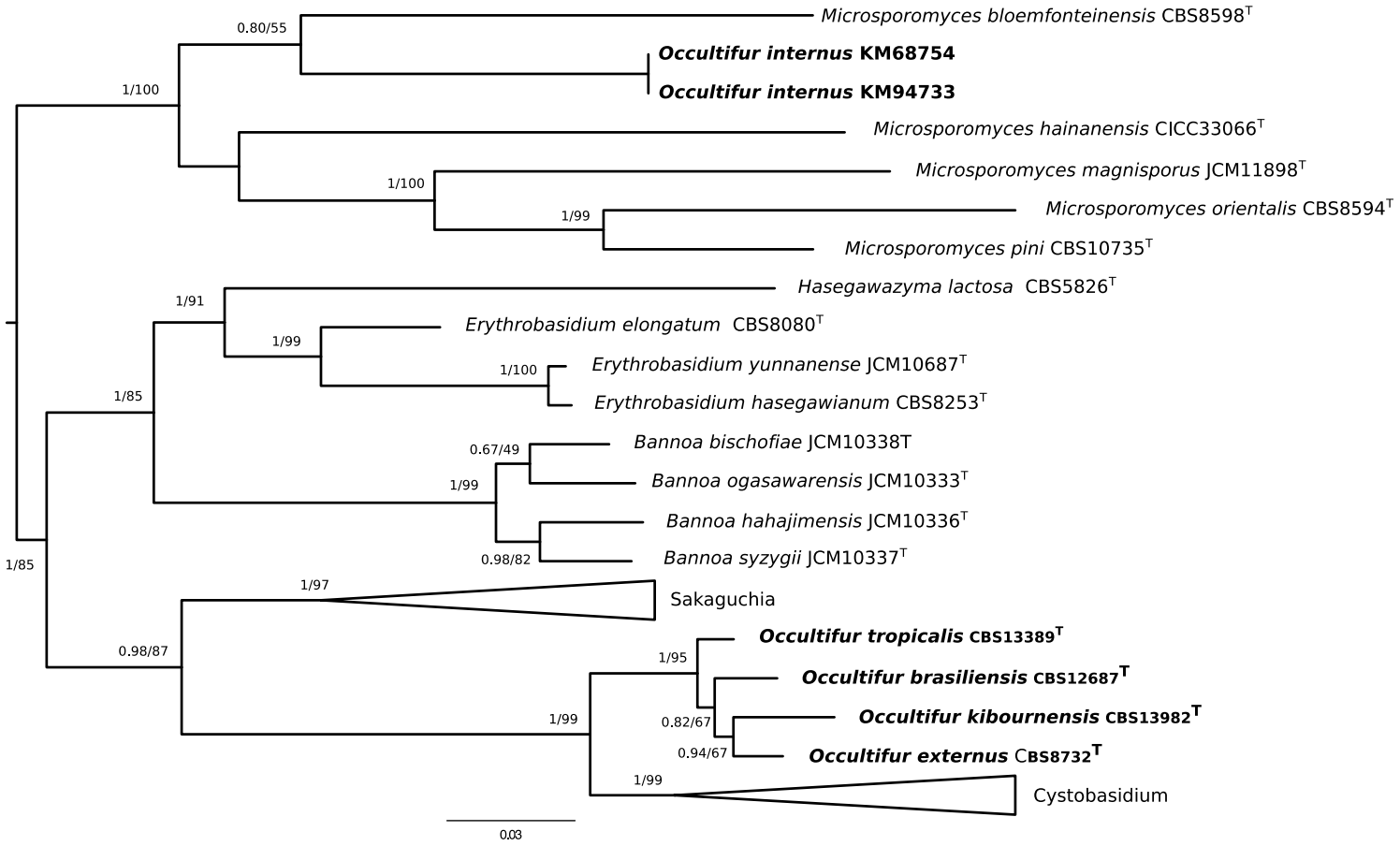

Figure 4.1 Phylogenetic placement of *Occultifur internus* inferred from combined sequences of ITS, D1–D2 domains of nuc 28S and nuc 18S rDNA using maximum likelihood and Bayesian analysis. Species in *Occultifur* are indicated in bold. Posterior Probability (PP>0.7) and Bootstrap support (BP>50%) values are shown above nodes. Bar 0.03 substitutions per nucleotide position. ^{T} denotes type species strain.

Figure 4.2 Phylogenetic reconstruction of the Cystobasidiomycetes inferred from combined sequences of ITS, D1–D2 domains of nuc 28S, nuc 18S rDNA, *tef1*, *rpb1*, *rpb2* and *cytb* using maximum likelihood and Bayesian analysis. Posterior Probability (PP>0.7) and Bootstrap support (BP>50%) values are shown above nodes. ns: not supported. Bar 0.04 substitutions per nucleotide position. ^{T} denotes type species strain.

Table 4.1 List of taxa and accession number of type species in the Cystobasidiomycetes used in this study

SPECIES	**STRAIN**	**SSU**	**ITS**	**LSU**	***tef1***	***rpb1***	***rpb2***	***cytb***
Bannoa bischofiae	JCM10338^{T}	AB035721	AB035721	AB082572	KJ707777	KJ708018	KJ708292	KJ707684
Bannoa guamensis	MCA4612^{T}	**MK254996**	**MK287350**	**MK255006**	**MK491345**	--------	--------	--------
Bannoa hahajimensis	JCM 10336^{T}	AB035897	AB035897	AB082571	KJ707750	KJ708014	KJ708146	KJ707682
Bannoa ogasawarensis	JCM 10326^{T}	AB035713	AB035713	AB082570	KJ707781	KJ708017	KJ708323	KJ707681
Bannoa rosea	MCA4647^{T}	--------	**MK287351**	**MK255007**	**MK491353**	--------	--------	--------
Bannoa syzygii	JCM 10337^{T}	AB035720	AB035720	AB082573	KJ707778	KJ708011	KJ708338	KJ707683
Bannoa tropicalis	MCA4667^{T}	**MK255003**	**MK287360**	**MK255016**	**MK491346**	--------	--------	--------
Buckleyzyma armeniaca	JCM 8977^{T}	AB126644	AF444523	AF189920	KJ707762	KP216521	KJ708211	AB040615
Buckleyzyma aurantiaca	JCM 3771^{T}	KJ708436	AF444538	AF189921	KJ707757	KJ707970	KJ708212	AB040616
Buckleyzyma butyrosa	MCA2993^{T}	**MK990638**	**MK990657**	**MK990684**	--------	--------	--------	--------
Buckleyzyma kluyveri-nielii	JCM 6356^{T}	AB021674	AF444544	AF189988	KJ707760	KJ707977	KJ708310	KJ707638
Buckleyzyma phyllomatis	JCM 7549^{T}	AB021685	AF444515	AF189991	KJ707761	KJ707976	KJ708328	KJ707728
Buckleyzyma salicina	JCM 2959^{T}	AB021687	AF444511	AF189995	KJ707758	--------	--------	KJ707703
Cyphobasidium hypogymniicola	S-F264671^{T}	KU587705	KU587700	KU587694	--------	--------	--------	--------
Cyphobasidium usneicola	S-F264672^{T}	KU587706	KU587703	KU587698	--------	--------	--------	--------
Cyrenella elegans	CBS274.82^{T}	KJ708360	KJ778626	KJ708454	KJ707830	KJ708080	KJ708168	KJ707620
Cystibasidium lysinophilum	CBS 9126^{T}	AB126650	AB078501	AB078501	KJ707845	KJ708074	KJ708243	KJ707721
Cystobasidium alpinum	CBS 14809^{T}	--------	KC455920	KC433879	--------	--------	--------	--------
Cystobasidium benthicum	CBS9124T	AB126647	AB026001	AB026001	KJ707842	KJ708081	KJ708214	KJ707691
Cystobasidium calyptogenae	CBS9125^{T}	AB126648	AB025996	AB025996	KJ707840	KJ708075	KJ708218	KJ707690

Table 4.1 continued

SPECIES	STRAIN	SSU	ITS	LSU	*tef1*	*rpb1*	*rpb2*	*cytb*
Cystobasidium fimetarium	DB1489	AY124479	KP053250	AY512843	LM644071	--------	--------	--------
Cystobasidium halotolerans	CBS5706^{T}	--------	MH828324	MH828330	--------	--------	--------	--------
Cystobasidium iriomotense	JCM24594^{T}	--------	AB726571	AB726571	--------	--------	--------	--------
Cystobasidium laryngis	CBS2221^{T}	--------	AF190014	AF189937	KJ707824	KJ708055	KJ708240	KJ707619
Cystobasidium minutum	CBS319^{T}	D45367	AF190011	AF189945	KJ707825	KJ708059	KJ708246	KJ707562
Cystobasidium oligophagum	CBS12623^{T}	LM644061	AB702968	AB702967	--------	--------	--------	--------
Cystobasidium ongulense	JCM31527^{T}	LC158351	LC155915	LC155915	LC158353	--------	--------	LC158355
Cystobasidium pallidum	CBS320^{T}	AB126651	AB078492	AF189962	KJ707826	KJ708056	KJ708253	KJ707621
Cystobasidium pinicola	CBS9130^{T}	AB126652	AF444292	AF444293	KJ707827	KJ708057	KJ708257	KJ707579
Cystobasidium portillonense	CBS12733^{T}	--------	JQ769323	JQ769312	--------	--------	--------	--------
Cystobasidium proliferans	unknown	--------	--------	--------	--------	--------	--------	--------
Cystobasidium psychroaquaticum	CBS11769^{T}	LM644062	FN868153	KY107444	LM644068	--------	--------	--------
Cystobasidium ritchiei	CBS12324^{T}	LM644063	LM644066	LM644066	LM644069	--------	--------	--------
Cystobasidium sebaceum	GWM3686^{T}	--------	--------	--------	--------	--------	--------	--------
Cystobasidium slooffiae	CBS5706^{T}	AB126653	AF444627	AF189965	KJ707828	KJ708058	KJ708266	KJ707629
Cystobasidium tubakii	JCM31526^{T}	LC158350	LC155913	LC155913	LC158352	/	/	LC158354
Erythrobasidium elongatum	CBS8080^{T}	AB021669	AF444561	AF189983	KJ707782	KJ708012	KJ708300	KJ707570
Erythrobasidium hasegawianum	CBS8253^{T}	D12803	AF444522	AF189899	KJ707776	KF706506	KF706534	KJ707563
Erythrobasidium yunnanense	JCM10687^{T}	AF229176	AB030353	AB127358	KJ707779	KJ708015	KJ708344	KJ707576
Halobasidium xiangyangense	GDMCC 2.231^{T}	--------	MH209248	MH212153	--------	--------	--------	--------
Halobasidium umbonatum	MT254^{T}	**MK990639**	**MK990658**	**MK990685**	--------	--------	--------	--------

Table 4.1 continued

SPECIES	STRAIN	SSU	ITS	LSU	*tef1*	*rpb1*	*rpb2*	*cytb*
Hasegawazyma lactosa	CBS5826^{T}	D45366	AF444540	AF189936	AB127098	KJ708016	KJ708239	AB040633
Occultifur internus	KM068754	--------	**MK990680**	**MK990697**	--------	--------	--------	--------
Occultifur internus	KM094773	--------	**MK990681**	--------	--------	--------	--------	--------
Occultifur bloemfonteinensis	CBS8598^{T}	KJ708359	EU075189	EU075187	--------	KJ708082	KJ708215	KJ707657
Occultifur hainanensis	CBS14092^{T}	--------	KU296948	KU296947	--------	--------	--------	--------
Occultifur magnisporus	JCM11898^{T}	KJ708428	AB112077	AB111955	KJ707780	KJ708013	KJ708317	KJ707695
Occultifur orientalis	CBS8594^{T}	KJ708358	HM559719	HM559718	KJ707843	KJ708078	KJ708249	KJ707656
Occultifur pini	CBS10735^{T}	KJ708357	NR_120007	EU075188	KJ707832	KJ708084	KJ708258	KJ707601
Naohidea sebacea	CBS8477^{T}	KP216515	DQ911616	DQ831020	KF706487	KF706508	KF706535	KJ707654
Pseudoccultifur brasiliensis	CBS12687^{T}	--------	KC698874	KC698874	--------	--------	--------	--------
Pseudoccultifur corticiorum	KM33238^{T}	--------	--------	--------	--------	--------	--------	--------
Pseudoccultifur externus	CBS8732^{T}	AB055193	AF444567	AF131062	KJ707829	KJ708060	KJ708199	KJ707689
Pseudoccultifur kilbournensis	CBS13982^{T}	--------	KP413162	KP413160	--------	--------	--------	--------
Pseudoccultifur lumbricifer	KM33234^{T}	--------	--------	--------	--------	--------	--------	--------
Pseudoccultifur mephitis	CBS14611^{T}	--------	KX929055	LT594852	--------	--------	--------	--------
Pseudoccultifur plantarum	CBS14554^{T}	--------	LC158346	LC158346	--------	--------	--------	--------
Pseudoccultifur rivoirei	LY BR-6532^{T}	--------	--------	--------	--------	--------	--------	--------
Pseudoccultifur tropicalis	CBS13389T	--------	AB921282	AB921280	--------	--------	--------	--------
Sakaguchia cladiensis	CBS10878^{T}	KJ708354	FJ008055	FJ008049	KJ707847	--------	KJ708219	KJ707603
Sakaguchia dacryoidea	JCM3795^{T}	D13459	AF444597	AF189972	KP216514	KJ708102	KJ708348	KJ707709
Sakaguchia lamellibrachiae	CBS9598^{T}	AB126646	AB025999	AB025999	KJ707876	KJ708098	KJ708314	KJ707667

Table 4.1 continued

SPECIES	**STRAIN**	**SSU**	**ITS**	**LSU**	***tef1***	***rpb1***	***rpb2***	***cytb***
Sakaguchia meli	CBS10797^{T}	KJ708355	FJ807683	KJ708452	KJ707855	KJ708085	KJ708245	KJ707602
Sakaguchia warrensis	SFJD10^{T}	**MK990648**	**MK990682**	**MK990698**	--------	--------	--------	--------
Sakaguchia oryzae	CBS9745^{T}	KJ708352	AY335160	AY335161	KJ707853	KJ708100	KJ708250	KJ707587
Sterigmatomyces hyphaenes	CBS7811^{T}	AY665775	AF444553	AF177406	KJ707749	KJ707965	KJ708145	KJ707645
Symmetrospora coprosmae	CBS7899^{T}	D66880	AF444577	AF189980	KJ707798	KJ707966	KJ708296	KJ707742
Symmetrospora foliicola	CBS8075^{T}	AB021671	AF444521	AF189984	KJ707797	KJ707969	KJ708302	KJ707589
Symmetrospora gracilis	CBS71^{T}	KJ708433	AF444578	AF189985	KJ707799	KJ707968	KJ708304	KJ707705
Symmetrospora marina	CBS2365^{T}	AB126645	AF444504	AF189944	KJ707795	KJ707973	KJ708244	AB040635
Symmetrospora oryzicola	CBS7228^{T}	AB021677	AF444546	AF189990	KJ707955	KJ707974	KJ708324	KJ707712
Symmetrospora symmetrica	CBS9727^{T}	KJ708350	AY364836	AY364836	KJ707800	KJ707975	KJ708337	KJ707582
Symmetrospora vermiculata	CBS9092^{T}	--------	AB030335	AF460176	KJ707801	KJ707967	KJ708342	KJ707675

Note: -------- : No sequence available. Accession numbers in bold were generated in the present study. Other accession numbers were retrieved from the NCBI. **T** denotes type species strain. Type strain species for each genus appears in bold.

4.3.2 *Species delimitation, host associations and geographic distribution*

A total of 794 sequences were available from both cultures and environmental samplings in the Cystobasidiomycetes retrieved from the NCBI. The database also included sequences generated from strains in the Aime Lab. Out of the 794 sequences, 608 (76.6%) were assigned to one of the extant species in the class, while 186 (23.4%) sequences remained unclassified because they appeared as independent evolutionary lineages and may represent new species. Sequences of the Cystobasidiomycetes have been isolated from all over the world, being Asia where more occurrences have been reported (394), while North America and Europe showed similar numbers, 121 and 141 respectively. In Oceania and South America, the number of occurrences is lower with 40 and 52 respectively; followed by Africa and Antarctica with 24 and 27 occurrences. Lastly, the lowest number of occurrences are for Central America and the Caribbean with 1 and 3 respectively.

The country with the higher number of occurrences is China with 156 in 26 species belonging to the genera *Buckleyzyma*, *Cystobasidium*, *Erythrobasidium*, *Halobasidium*, *Hasegawazyma*, *Occultifur*, *Pseudoccultifur*, Sakaguchia and *Symmetrospora*; i.e., in nine out of the 13 genera of the Cystobasidiomycetes. The second country with the highest number is the USA with 98 occurrences in 30 species belonging to the 9 genera listed above and *Cyrenella*. In Europe, Portugal is the country with the highest number of occurrences (37) of the Cystobasidiomycetes in 13 species of eight genera.

At the genus-level, the highest number of occurrences are in *Symmetrospora* (190), *Cystobasidium* (149) and *Erythrobasidium* (87), with species isolated from the all over the world. Contrarily, the monotypic genera *Cyrenella* has only one occurrence in Brazil, Germany, Portugal and the USA respectively. The genus *Hasegawazyma* has been mostly reported from Asia and only one occurrence in the USA.

From the phylogenies built with the 18S, ITS and 28S gene regions it was possible to delimit species and identify new host associations that were not reported until today. For example, new species of *Bannoa* that have been documented in Chapter 2 not only represent an increase in the number of known species for the genus but also, a larger geographic distribution. Additionally, other strains (MCA7643, MCA7637, MCA7670, MCA7672, MCA7674) that are congeneric to *Bannoa* have been isolated from Vanuatu and may represent new species. In *Buckleyzyma*, for the species *B. aurantiaca* our analyses indicate an association with the gut of insects in the family

Chrysomelidae and Staphylinidae. This species has also been isolated from *Atta texana* in which it is presumed to play an antagonistic role against other microorganisms. Both associations had never been reported. In terms of geographic distribution, *B. aurantiaca* in the present study is reported for the first time in Austria, Canada, Portugal and Finland (Prillinger et al. 1996, Rodrigues et al. 2009, Stefani et al. 2016). New associations of *Buckleyzyma salicina* also have been identified from an unclassified sequence on *Vitis vinifera*, while strain P117 from the Aime lab has been isolated from *Pinus nigra*. Both reports are the two only known of this species for the United States.

Cystobasidium calyptogenae has been reported from Japan, India and Taiwan, however, several sequences from an uncultured fungus isolated from seawater have been reported from China which is a new report for this species. Also, sequences classified as *Cystobasidium* sp. "classicola" that appeared conspecific to *C. calyptogenae* have been isolated from Thailand and Canada, that along with the strain SA628 from the Aime Lab Collection are the first reports of the species in the western hemisphere. For *Cystobasidium laryngis* we have identified uncultured and unclassified sequences that were isolated from a coprophilous fungus in Norway, this is not only the first time that this species is reported to be associated with fungi but also is the first report of the species from Norway. Also, in the current study we show for the first time that *Cystobasidium minutum* strain MCA4210, reported from the United States isolated from the mycangia of Sirex wasp. Other strains such as MCA7620 and MCA7407 were isolated from *Lygodium* sp.; MCA7423 and MCA7611 from Vanuatu are the first report for this location. Despite *C. minutum* has been already reported in China from *Camellia sinensis*, in this study we identified environmental sequences that were isolated from seawater, a habitat from which this species had not been reported.

Cystobasidium ongulense, previously only known from Antarctica, based on our analyses this species has a wider geographic distribution. Collection P107 from the Aime Lab isolated from *Rubus* sp., TAR415 from *Dryopteris erythrosora* and MCA715 from an unknown plant were obtained from the USA, Iceland and Canada, respectively. Also accessions KU145512 and MF062208 isolated from Chile and China that were classified as *C. laryngis*, are actually conspecific to *C. ongulense.* Other sequences from environmental sampling, KP314689 and KR004864, that appear as *C. ongulense* were isolated from a coprophilous fungus (Zhang et al. 2015) and lichen, respectively, in Norway. *Cystobasidium psychroaquaticum* comprised only two classified sequences, the type strain from Russia from *Chamaedaphne calyculata* and one

additional strain from Bulgaria from a Springtail Sminthuridae feeding on fungus. Other unclassified sequences, that are conspecific to C. *psychroaquaticum,* were isolated from the lichen *Cladonia pocillum* and from a coprophilous fungus in Norway. Association with fungi was never reported previously. Also, we report the strain *MT096* isolated from *Dryopteris erythrosora* in the USA, the first report of this species in the American continent. For *C. slooffiae* new geographic distributions include, the Republic of Korea from seawater culture (LC272874), also seawater in China (KU163884), while in Finland *C. slooffiae* has been isolated from indoor environments (AB726571), in Japan from soil samples (AB726571) and in the USA, from leaves, stem and kernels of *Zea mays*. Lastly, strain SA515, stored in the Aime Lab collection, along with accessions EF505775 and KF800332, are the only reports for this species in North America (Table A1).

In *Erythrobasidium*, we identified sequences of from environmental sampling labeled as "Uncultured fungus" isolated from *Hemileia vastatrix* pustules in Coffee leaves in Mexico (James et al. 2016). Accession KT328766 was identified in the study conducted by James et al (2016) as *Symmetrospora oryzicola,* however, our analyses placed this sequence as conspecific to *Erythrobasidium yunnanense.* Similarly, we identified a sequence from an uncultured fungus (KT328645) conspecific to *E. elongatum* cohabiting *Hemileia vastatrix* pustules, this time in Puerto Rico (James et al. 2016). Lastly, despite the type species of *Erythrobasidium hasegawianum* being isolated from an old culture of yeast beer, it has been traditionally found in association with plants in Portugal (e.g., EU002836, EU002836, EU002836) and lake water in China (KT239097, KT345331, KT345330). We identified from the Aime Lab Collection, the strain MCA6865 as *E. hasegawianum*; this strain was isolated from an unidentified leaf infected by the rust fungus *Puccinia coronata* in Russia. Also, we identified two sequences (AM901803, KF815655) of uncultured fungus conspecific to *E. hasegawianum* that were isolated from house dust and air sampling in Finland and Slovakia, respectively. Both are new reports for *E. hasegawianum* in these countries.

Occultifur internus has been reported previously from the fungi *Peniophora* sp., *Hyphoderma argillaceum* and Dacrymycetales hosts; however, because of the new sequences of *O. internus* generated in this study, it was possible to identify environmental sequences that remained unclassified that are conspecific to *O. internus*. Sequences KU948766 and KU948779 were isolated from the lichens *Cladonia multiformis* and *Cetraria ericetorum,* respectively, in the United States. These findings suggest that this mycoparasitic species is not only found on fungi

but also that it may be a parasite of lichens. Also, accession KF274414 was isolated from *Picea abies* represents the first non-fungal host association and the first report of *O. internus* in Finland. Lastly, other unclassified *Symmetrospora* sequences from environmental sampling and cultures were assigned to *Symmetrospora coprosmae*, these sequences represent the first report of this species in countries such as in Belgium where accession KP100170 was obtained from *Aphis spiraecola*, while in Slovakia, France, Thailand and Czech Republic, *S. coprosmae* has been reported from overwintering grape (KT933355), air sampling (HG008765), corn phylloplane (HG008765), and roots of *Neottia ovata*. One additional sequence was obtained in Mexico from *Hemileia vastatrix* pustules by James et al. (2016 (See Chapter 1). A compilation of host and substrates in which extant species in the Cystobasidiomycetes have been isolated is shown in Table 4.2. Detailed data of the accession number, substrate, host associations and geographic distribution for every sequence used the species delimitation for host association studies in this chapter are listed in Table A1.

Table 4.2 Ecological characteristics, geographic distribution for extant of the Cystobasidiomycetes from sequences available in the NCBI. Formation or ring and pellicle is also listed.

SPECIES	COUNTRY/ LOCATION	HOST/ SUBSTRATE	RING/ PELLICLE	REFERENCE
Bannoa bischofiae	Japan	Dead leaves of *Bischofia javanica*	+/-	Hamamoto et al. (2002)
Bannoa guamensis	Guam	Diseased leaf of *Bidens* sp.	+/+ **(MCA4612)**	Chapter 2
Bannoa hahajimensis	Japan	Dead leaves of *Bryophyllum pinnatum*	-/+	Hamamoto et al. (2002)
Bannoa ogasawarensis	Japan	Dead leaves of *Schima mertensiana*, *Osmanthus insularis*, *Syzygium buxifolium*	+/-	Hamamoto et al. (2002)
Bannoa rosea	Guam	Asymptomatic undetermined leaf	+/+ **(MCA4647)**	Chapter 2
Bannoa syzygii	Japan	Dead leaves of *Syzygium buxifolium*	Present	Hamamoto et al. (2002)
Bannoa tropicalis	Guam, France, Panama, Taiwan	Cloud water, altitude 1465m, healthy leaves, diseased leaf of *Euphorbia cyathophora*, *Kweilingia* symptomatic leaf, *Puccinia lepturi* diseased leaf of *Lepturus repens*, *Uromyces* diseased leaf of *Bidens* sp.	+/+ **(MCA4667)**	Chapter 2, Matheny et al. (2006), Nakase el al. (2001),
Buckleyzyma armeniaca	Australia	Healthy leaves of *Callistemon viminalis*	+/-	Shivas and Rodrigues de Miranda (1983)
Buckleyzyma butyrosa	USA	Media containing 50% Glucose - Air sampling	-/-	Chapter 3

Table 4.2 continued

SPECIES	COUNTRY/ LOCATION	HOST/ SUBSTRATE	RING/ PELLICLE	REFERENCE
Buckleyzyma aurantiaca	Japan, Canada, China, Finland, Germany, Italy, Portugal, Rep. of Korea, USA	*Cistus albidus*, *Callistemon viminalis*, roots of Populus sp., seawater, beech litter, gut of *Diabrotica virgifera* Posterior gut of Staphylinidae, Lake water, leaves from boreal streams, apple fruit surface, endophyte on plant, air sampling *Padina pavonica* (Algae), flower of *Elaeagnus multiflora*, flower of *Pyrus serotina* var. culta, *Atta texana* (Ant) nest, *Canabis sativa*, *Vitis vinifera*	--/--	Rodrigues et al. (2009), Wuczkowski and Prillinger (2004) Loder (1934)
Buckleyzyma kluyveri-nielii	South Africa, India	*Dombeyarotundifolia*, mangrove sediments	+/-	Van der Walt et al. (1986)
Buckleyzyma phyllomatis	South Africa	Leaves of *Dombeya rotundifolia*	+/-	Van der Walt et al. (1988)
Buckleyzyma salicina	Canada, France, India, Portugal, USA,	Healthy leaves of *Salix* sp., mangrove sediments, *Vitis vinifera*, Forest litter	+/--	Bourret et al. (2013), Wuczkowski and Prillinger (2004), Nakase and Itoh (1988)
Cyphobasidium hypogymniicola	Canada, Finland, Norway, Russia, and the USA, etc.	*Hypogymnia physodes*, more rarely on *H. hultenii*, *H. imshaugii*, *H. incurvoides*, *H. krogiae*, *H. vittata*	--/--	Millanes et al. (2016), Spribille et al. (2016) Diederich (2007), Diederich (1996),
Cyphobasidium usneicola	Canada, Colombia Costa Rica, Mexico, and the USA, Peru, Ecuador, Macronesia, etc.	*Usnea* spp., *Usnea subfloridan*	--/--	Millanes (2015), Diederich (2007)

Table 4.2 continued

SPECIES	COUNTRY/ LOCATION	HOST/ SUBSTRATE	RING/ PELLICLE	REFERENCE
Cyrenella elegans	USA, Brazil, Germany, Portugal, USA	Isolated from sand scraped from the submerged stipe of a mushroom, *Laccaria trullisata*	--/--	Gochenaur 1981
Cystobasidium alpinum	Arctic	Glacial sediments	+/--	Turchetti et al. (2018)
Cystobasidium benthicum	Japan, China, Canada	Tube worm *Lamellibrachia* sp., Seawater	+/-	Nagahama et al. 2003
Cystobasidium calyptogenae	Japan, Canada, China, India, Taiwan, Thailand, USA	*Calyptogena* sp., a genus of giant white clam, phylloplane, soil	+/-	Nagahama et al. 2003
Cystobasidium fimetarium	Denmark	*Thelebolus crustaceus* on grouse dung or cow dung and *Lasiobolus equinus*	--/--	Roberts 1999
Cystobasidium halotolerans	Qatar, Arabian Gulf	Seawater		
Cystobasidium iriomotense	China, Japan	Dead branch of an unidentified tree, sandy soil	+/--	Tanimura et al. (2018)
Cystobasidium laryngis	Norway, Antarctica, Canada, Chile, China, Finland, Germany, Hungary, Sweded, USA	Seawater and ice core, water treatment plant, soil, barley, indoor dust, child feces, wood stump, *Zea mays* field bulk, laryngeal, reindeer dung coprophilous fungus	+/- **(CBS5695^{T})**	Reiersøl 1954

Table 4.2 continued

SPECIES	COUNTRY/ LOCATION	HOST/ SUBSTRATE	RING/ PELLICLE	REFERENCE
Cystobasidium lysinophilum	Japan, Antarctica, Canada, Japan	Goat dung, from butter, from glacier ice, soil and animals collected from the deep-sea	+/-	Nagahama et al 2013
Cystobasidium minutum	Japan, China, Egypt, Taiwan, USA, Vanuatu	Shrimp, seawater, freshwater, reed (plant), humans, foliar lesions of *Camellia sinensis*, air samplings, fresh orange juice, *Lygodium* sp., mycangia of Sirex wasp.	+/- **(MCA4210)**	Saito (1922)
Cystobasidium oligophagum	Australia	Koala nasal smears,	Absent	Satoh et al. (2013)
Cystobasidium ongulense	Antarctica, Canada, Chile, China, Iceland, Norway, USA	Soil, glacier fragment, rocks, wood, Glacie. soil *Dryopteris erythrosora*, lichens, pond, reindeer dung coprophilous fungus *Rubus* sp.	-/- **(+ for TAR415)**	Tsuji et al 2016
Cystobasidium pallidum	Japan	Isolated from a mycotic nodule in white rat, was isolated from plant bark in India	--/--	Loder (1934)
Cystobasidium pinicola	China, France, Germany	*Pinus tabulaeformis*, freshwater, ice core, *Rubus* sp. and nematode	+/-	Zao et al (2002)
Cystobasidium portillonense	Chile, Antarctica	isolated from a rock surface collected from shallow-water	+/-	Laich et al (2013)
Cystobasidium proliferans	USA	Insect excreta	--/--	Olive 1952

Table 4.2 continued

SPECIES	COUNTRY/ LOCATION	HOST/ SUBSTRATE	RING/ PELLICLE	REFERENCE
Cystobasidium psychroaquaticum	Russia, Antarctica, Bulgaria, China, Norway, Russia, USA	Leatherleaf (*Chamaedaphne calyculata,* reindeer dung, glacier surface, estuary, phylloplane, lichen: *Cladonia pocillum*, springtail Sminthuridae (insects) feeding on fungus	-/- **(+ for MT09)**	Yurkov et al. 2017
Cystobasidium ritchiei	Russia	Leatherleaf: *Chamaedaphne calyculata*	--/--	Yurkov et al (2015)
Cystobasidium sebaceum	Colombia	Decaying wood remnants of an old *Sebacina*	--/--	Martin 1939
Cystobasidium slooffiae	Hungary, Brazil, China, Finland, Hungary, India, Japan, New Zealand, West Pacific Ocean, Portugal, South Africa, Republic of Korea, Spain, Sweden, Taiwan, USA	Human infections, seawater, *Vitis vinifera*, seaweeds, house dust, *Holcus lanatus* (poaceae) and *Melocactus zehntneri*	-/- **(+ for SA515)**	Novak and Felkai, (1962)
Cystobasidium tubakii	Antarctica	Soil	-/-	Tsuji et al 2016
Erythrobasidium elongatum	Australia, Brazil, India, Portugal, Puerto Rico, USA, Australia	Healthy leaf of *Callistemon viminalis*, mangrove sediments, bromeliad water thank, coffee leaf infected by *Hemileia vastatrix*, *Bidens* sp., stem tissue of *Smilax rotundifolia*	+/- **(INDP051)**	Nagahama et al. (2006)

Table 4.2 continued

SPECIES	COUNTRY/ LOCATION	HOST/ SUBSTRATE	RING/ PELLICLE	REFERENCE
Erythrobasidium hasegawianum	USA, China, Finland, Portugal, Russia, Slovakia, Taiwan, Brazil	from an oldculture of beer yeast, phylloplane, Coffee plants, aromatic plant tissue, lake water, stem of Poplar house dust, *Spartina maritima, Puccinia coronata infected leave,* air sampling, seawater	+/- **(P36^{T})**	Sampaio et al. (1999)
Erythrobasidium yunnanense	China, Brazil, Italy, Mexico, Portugal, South Africa, Republic of Korea, Taiwan, United Kingdom, USA	Dried leaf of *Sapindus delavayi*, phylloplane, oribatid mite, sugar cane leaf, freshwater sediment, seawater, Oil-contaminated mangrove soil, apple fruit surface, phylloplane, rainwater, roots of *Rhododendron argyrophyllum*, seawater, Coffee leaf infected by *Hemileia vastatrix*, flower of *Silene* sp., human Skull and leaf of *Platycladus orientalis*	+/-	Bai et al. (2001)
Halobasidium umbonatum	Canada, China, Finland, Germany, Republic of Korea, USA	Soil, *Chimonobambusa marmorea*, seaweeds, wild Arabidopsis leaf, *Arrhenatherum elatius*, flowers/plants and *Crataegus* sp.	+/+ **(MT254^{T})**	Chapter 3,
Halobasidium xiangyangense	Brazil, China, Rep. of Korea, New Zealand, USA	Datoucai, skin scales (Human skin), Solar saltern, seaweeds and *Dryopteris erythrosora*	+/- **(RK86)**	Chapter 3, Guo et al 2019
Hasegawazyma lactosa	India, Taiwan	air of dairies and cowsheds, mangrove sediments and seawater, *Atta texana*	+/- **(CBS5826)**	
Naohidea sebacea	England, Poland, Taiwan	Isolated *Fraxinus* sp, perithecia of *Botryosphaeria* sp. and *Botryodiplodia* sp.	-/-	Piatek 2002, Bandoni (1973), Mcnabb 1965, Olive 1947

Table 4.2 continued

SPECIES	COUNTRY/ LOCATION	HOST/ SUBSTRATE	RING/ PELLICLE	REFERENCE
Occultifur bloemfonteinensis	South Africa, China Portugal	Pine trees, radiation-polluted soil	-/-	Pohl (2011)
Occultifur hainanensis	China	*Oryza sativa*	+/-	Bai et al. (2016)
Occultifur internus	England, Finland, USA	*Peniophora* sp., *Hyphoderma argillaceum*, *Picea abies*, Dacrymycetales,*Cetraria ericetorum* (Lichen)	-/-	Khunnamwong et al. (2015)
Occultifur magnisporus	Japan, Taiwan, Thailand	*Miscanthus floridulus* and *Saurauia oldhamii*, marine sediments/Plants Rotting leaves of *Pisonia umbellifera*	+/-	Nakase et al. (2003)
Occultifur orientalis	Malaysia	Soil	--/--	Pohl (2011)
Occultifur pini	India, South Africa	Air sampling, mangrove sediments and phylloplane	--/--	Pohl (2011)
Pseudoccultifur brasiliensis	Brazil	Water tanks of *Vriesea minarum*	--/--	Gomes et al (2015)
Pseudoccultifur corticiorum	United Kingdom	*Hyphoderma praetermissum*, *Peniophorella praetermissa*	--/--	Roberts (1997)
Pseudoccultifur externus	Portugal, Brazil, Canada, China, Taiwan, Thailand	Plant litter, Mangrove, seawater collected, Coffee plants, exudate from Maple tree *Acer* sp., Coffee plants Stems and flowers of *Baccharis dracunculifolia*,fresh water, plant litter, *Forcipomia taiwana* (Fly), leaves of Sugarcane (*Saccharum officinarum*)	--/--	Sampaio (1999)

Table 4.2 continued

SPECIES	COUNTRY/ LOCATION	HOST/ SUBSTRATE	RING/ PELLICLE	REFERENCE
Pseudoccultifur kilbournensis	Brazil, China, Germany, Hungary, USA, Republic of Korea	*Zea mays* field bulk soil, Leaves, stems, kernels of *Zea mays*, seawater, rhizosphere *Picea glauca* (white spruce), fruit surface, cow rumen	--/--	Kurtzman and Robnett (2015)
Pseudoccultifur lumbricifer	Scotland	*Hyphodonlia subalutacea* (fungi)	--/--	Robert (2000)
Pseudoccultifur mephitis	Slovenia	Soil, Leaf of *Cistus albidus*	--/--	Sibanac et al. (2018)
Pseudoccultifur plantarum	Brazil, India, Japan, Thailand	sugarcane leaf tissue and corn leaf tissue, bromeliad *Neoreglia cruenta* and magrove soil	--/--	Khunnamwong et al. (2017)
Pseudoccultifur rivoirei	France	*Hyphodontia arguta*	--/--	Trichies (2016)
Pseudoccultifur tropicalis	Brazil, Thailand and Republic of Korea	Water tanks of *Vriesea minarum*, indoor environment, leaves of Sugarcane (*Saccharum officinarum*)	--/--	Khunnamwong et al. (2015)
Sakaguchia cladiensis	USA	Sawgrass (*Cladium jamaicense*), fresh water, grasses, (*Rhizophora mangle*) and black (*Avicennia germinanus*)	--/--	Fell et al (2011)
Sakaguchia dacryoidea	USA, Antarctica, Chile, China, India, Portugal	Seawater (1738 depth), wild *Cilus gilberti* (Fish)/Salmonids, mangrove sediments	+/-	Francis et al. (2016), Velmurugan (2013), Almeida and Sampaio (2003), Fell et al (1973)
Sakaguchia lamellibrachiae	Japan, China	Tubeworm (*Lamellibrachia* sp.)	--/--	Yu et al. (2017), Nagaham et al. (2011)

Table 4.2 continued

SPECIES	COUNTRY/ LOCATION	HOST/ SUBSTRATE	RING/ PELLICLE	REFERENCE
Sakaguchia meli	Argentina, USA	Fresh water and soil	--/--	Libkind (2010)
Sakaguchia oryzae	Japan, Canada	*Oryzae sativa* and rhizosphere of *Picea glauca* (white spruce)	+/-	Bai et al. (2004)
Sakaguchia warrensis	USA	Air sampling, 50% Glucose House dust	--/--	Chapter 2, Rittenour et al. (2014)
Symmetrospora coprosmae	New Zealand, Austria, Belgium, Brazil, China, Czech Republic, France, Germany, India, Mexico, Portugal, Slovakia, Spain, Switzerland, Thailand, Taiwan, USA	*Coprosma tenuifolia*, beetle gut of *Diabrotica virgifera*, beech litter, *Aphis spiraecola* (aphid), Leaves soybean, Leaves of *Gossypium hirsutum*, mycorrhizal root *Neottia ovata*, air sampling, mangrove sediments, Coffee leaf infected by *Hemileia vastatrix, Xylem sap from Vitis vinifera, corn leaf (Zea mays), commercial romaine lettuce, Vigna sp., Pinus nigra* and *Puccinia smilax* infected leaf	-/+ **+ for MT264**	James et al. (2016), Hamamoto and Nakase (1995)
Symmetrospora foliicola	Australia, China, India, South Africa, USA	Leaves of *Banksia collina*, gut of larva, mangrove sediments, seawater, indoor air, apple fruit surface	-/+	Velmurugan et al. (2013), Shivas and Rodrigues (1983)
Symmetrospora gracilis	Australia, Czech Republic, Germany, India, New zealand, Portugal	*Phertinia glabra*, roots of *Populus* sp., *Phragmites australis*, mangrove sediments, seawater, dead leaf of *Coprosma tenuifolia*	--/--	Hamamoto and Nakase (1995)
Symmetrospora marina	Brazil, Israel, Japan, Qatar, Taiwan, USA	*Peneaus setiferusn* (Shrimp), seawater, coffee plants, sugar cane leaf and floral nectar	+/-	Wang et al. 2003, Scorzetti, 2002, Derx (1930)

Table 4.2 continued

SPECIES	COUNTRY/ LOCATION	HOST/ SUBSTRATE	RING/ PELLICLE	REFERENCE
Symmetrospora oryzicola	Japan, Republic of Korea, Thailand, Taiwan	Dead leaf of *Oryza sativa*, *Aphis spiraecola* (insect), Willow like tree and *Vigna* sp.	+/-	Chapter 4, Nakase and Suzuki (1986)
Symmetrospora symmetrica	China, Netherlands, Portugal, USA	Fruiting body *Pleurotus eryngii* Rainwater, Seawater, Talli of *Fucus* sp. (seaweed), P*inus nigra*, from wilting leaves of *Betula platyphylla*	-/-	Chapter 4 ,Wang and Bai (2004)
Symmetrospora vermiculata	Thailand, China	Dead leaf of *Pennisetum pedicellatum*, leaves of corn leaf (*Zea mays*) and sugar cane leaf	+/+	Takashima and Nakase ()

Note: RING/PELLICLE. +: Indicates formation of a ring or pellicle. -: no formation or ring or pellicle. --: No information or unknown

In bold and parenthesis: Strains in which the formation of ring and pellicle were tested in our Lab.

4.3.3 *Host preference evolution in the Cystobasidiomycetes*

In the present study, we show a seven-gene phylogeny including 72 taxa in the Cystobasidiomycetes, of which 66 are described species and four taxa that are in process of formal description in the Aime Lab. Also, we present information regarding the habitat preference of species in one or several categories defined: Animals, fungi, plants, aquatic habitat or decaying organic matter (Figure 4.3). Based in our analyses, there is not an evident pattern of specialization towards a particular substrate or habitat. For example, a species can be found in association with animals or plants, or even isolated from aquatic environments, fungi, or decaying organic matter. Also, single species have been found in association with plants of different families or insects that are phylogenetically distant (e.g., *Erythrobasidium yunnanense*, *Buckleyzyma aurantiaca,* see Table 4.2).

By using the seven-gene region phylogeny and the known host association for species in the class according to five categories (Animals, aquatic, fungi, plants or decaying organic matter), we reconstructed 19 nodes for the habitat preference of the most recent common ancestor (MRCA) in the Cystobasidiomycetes. Our ancestral character reconstruction using the MCMC (Marco Chain Monte Carlo) shows that the host association of the MRCA of the Cystobasidiomycetes is uncertain, however it is likely that the MRCA was associated with either plants (Figure 4.3, Node 1, PP: 0.43), fungi (Node 1, PP: 0.31) or decaying organic matter (Node 1, PP: 0.19). Preference for a habitat was gained several times in the class, for example: The MRCA of the Erythrobasidiales appears to have a habitat preference for decaying organic matter (Node 8, PP: 0.82) but there was a gain in habitat preference towards plants for the MRCA of the *Erythrobasidium* and *Bannoa* (Node 11, PP:0.78). Also, a gain in habitat preference toward plants is observed in the MRCA of *Buckleyzyma* (Node 4, PP: 0.93) and *Symmetrospora* (Node 7, PP: 0.97). However, species in these genera have also been found in association with decaying organic matter, animals and fungi. In the Cystobasidiales, habitat preferences were gained in the evolution of the order, from plants (Node 14, PP: 0.55) to animals in the MRCA of *Cystobasidium* (Node 18, PP: 0.67) even when several species in the genus also have been found in association with plants and fungi. Lastly, the MRCA of *Halobasidium* gained habitat preference towards plants and aquatic environments (Node 19, PP: 0.45). Multiple changes in habitat preference have been reported in the evolution of the Cystobasidiomycetes, it seems like the preference for habitat or host association with fungi is apparently lost. Our analyses also show that habitat preference

towards fungi in some MRCAs has been lost in recent lineages (e.g. Node 2, 3, 5, 8). We obtained similar reconstructions for the ancestral character when the analysis was performed using the ML approach.

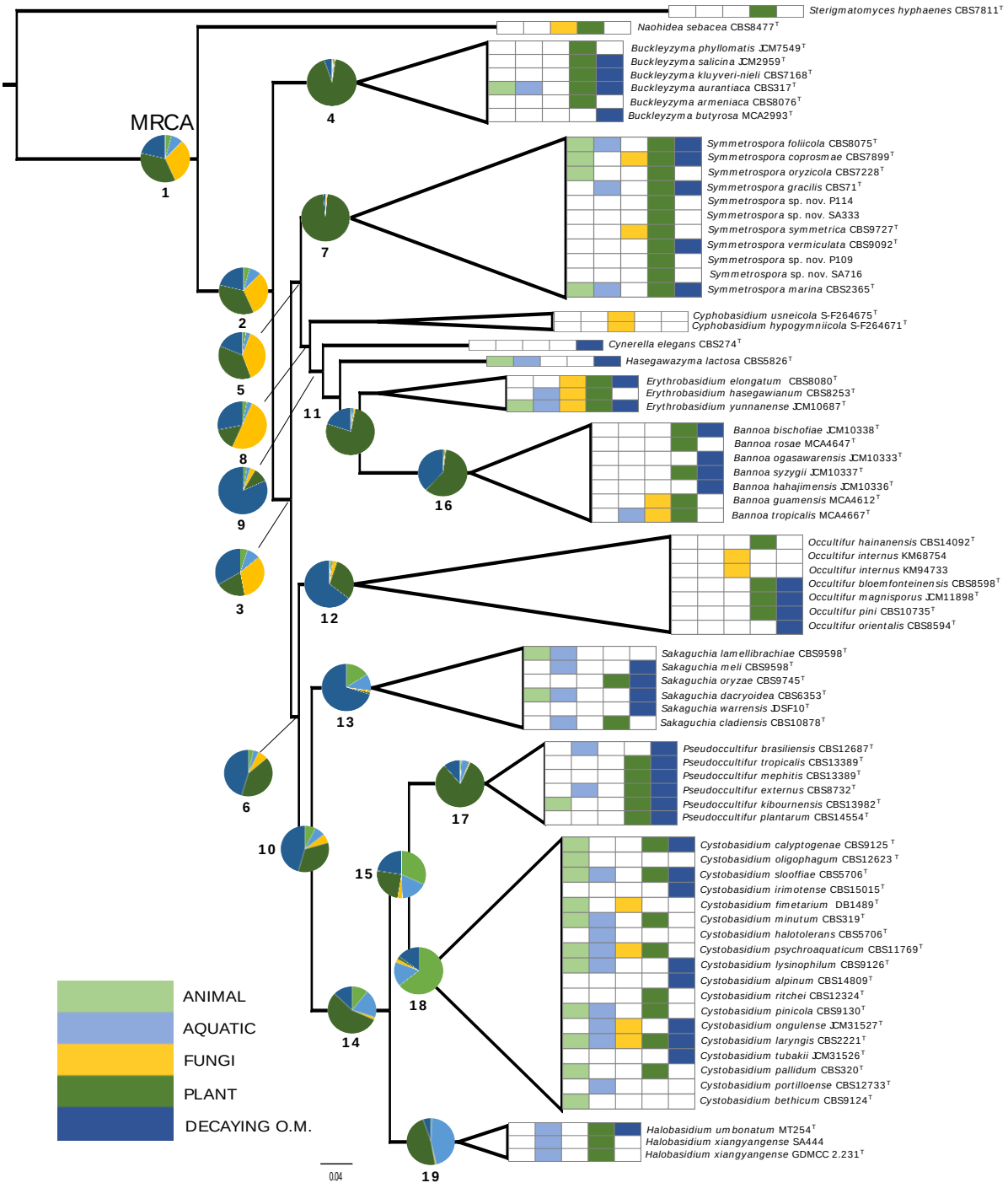

Figure 4.3 Ancestral character reconstruction using MCMC (Markov Chain Monte Carlo). Colors represent the different categories of habitat preference of species in the Cystobasidiomycetes. Backbone was built based on the ITS, D1–D2 domains of nuc 28S, nuc 18S rDNA, *tef1*, *rpb1*, *rpb2* and *cytb* using ML.

4.4 Taxonomy

Occultifur Oberw. 1990. Reports of the Tottori Mycological Institute 28: 119 (1990).
MB#25570

Type species: *Occultifur internus* (L.S. Olive) Oberw. 1990.

Basionym: *Platygloea peniophorae* var. interna L.S. Olive, Bulletin of the Torrey Botanical Club 81: 331 (1954)
The description is that of *Platygloea peniophorae* var. interna L.S. Olive 1954.

Occultifur bloemfonteinensis (C.H. Pohl, M.S. Smit & Albertyn) P.P. Parra & Aime, comb. nov.
MycoBank: 814789

Synonym: *Microsporomyces bloemfonteinensis* (C.H. Pohl, M.S. Smit & Albertyn) Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout, Studies in Mycology 81: 177 (2015).
≡Rhodotorula bloemfonteinensis C.H. Pohl, M.S. Smit & Albertyn, International Journal of Systematic and Evolutionary Microbiology 61: 2324 (2011).

Occultifur hainanensis (F. Bai & Yang Liu) P.P. Parra & Aime, comb. nov.
MycoBank: 815471

Synonym: *Microsporomyces hainanensis* F. Bai & Yang Liu, Current Microbiology 73 (4): 572 (2016).

Occultifur magnisporus (Nakase, Tsuzuki, F.L. Lee, Sugita, Jindam. & M. Takash) P.P. Parra & Aime, comb. nov.
MycoBank: 813137

Synonym: *Microsporomyces magnisporus* (Nakase, Tsuzuki, F.L. Lee, Sugita, Jindam. & M. Takash.) Q.M. Wang, F.Y. Bai, M. Groenew & Boekhout, Studies in Mycology 81: 177 (2015).
≡*Sporobolomyces magnisporus* Nakase, Tsuzuki, F.L. Lee, Sugita, Jindam. & M. Takash., Journal of General and Applied Microbiology Tokyo 49 (6): 341 (2003).

Occultifur orientalis (C.H. Pohl, M.S. Smit & Albertyn) P.P. Parra & Aime, comb. nov.
MycoBank: 813327

Synonym: *Microsporomyces orientalis* (C.H. Pohl, M.S. Smit & Albertyn) Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout, Studies in Mycology 81: 177 (2015).
≡*Rhodotorula orientis* C.H. Pohl, M.S. Smit & Albertyn, International Journal of Systematic and Evolutionary Microbiology 61: 2325 (2011).

Occultifur pini (C.H. Pohl, M.S. Smit & Albertyn) P.P. Parra & Aime, comb. nov.
MycoBank: 813138

Basionym: *Microsporomyces pini* (C.H. Pohl, M.S. Smit & Albertyn) Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout, Studies in Mycology 81: 177 (2015).
≡Rhodotorula pini C.H. Pohl, M.S. Smit & Albertyn, International Journal of Systematic and Evolutionary Microbiology 61: 2323 (2011).

Pseudoccultifur P.P. Parra & Aime, gen. nov.
MycoBank: MB#813135

Etymology: *Pseudo*, false. False *Occultifur*.

Neotype: *Pseudoccultifur externus* (J.P. Samp., R. Bauer & Oberw.) P.P. Parra & Aime, comb. nov.
The description is that of *Occultifur externus* J.P. Samp., R. Bauer & Oberw., Mycologia 91 (6): 1095 (1999).
Holotype: CBS8732, permanently preserved in a metabolically inactive state at Westerdijk Fungal Biodiversity Institute (CBS). Ex-type culture: CBS8732 = IGC 4817 = JCM 10725.

Pseudoccultifur externus (J.P. Samp., R. Bauer & Oberw.) P.P. Parra & Aime, comb. nov.
MycoBank: 460058

Basionym: *Occultifur externus* J.P. Samp., R. Bauer & Oberw., Mycologia 91 (6): 1095 (1999).

Pseudoccultifur brasiliensis (F.C.O. Gomes, Safar, A. Marques, A.O. Medeiros, A.R.O. Santos, C. Carvalho, Lachance, J.P. Samp. & C.A. Rosa) P.P. Parra & Aime, comb. nov.
MycoBank: 809816

Basionym: *Occultifur brasiliensis* F.C.O. Gomes, Safar, A. Marques, A.O. Medeiros, A.R.O. Santos, C. Carvalho, Lachance, J.P. Samp. & C.A. Rosa, Antonie van Leeuwenhoek 107 (2): 608 (2015).

Pseudoccultifur corticiorum **(**P. Roberts) P.P. Parra & Aime, comb. nov.
MycoBank: 437200

Basionym: *Occultifur corticiorum* P. Roberts, Mycotaxon 63: 202 (1997)

Pseudoccultifur kilbournensis **(**Kurtzman & Robnett) P.P. Parra & Aime, comb. nov.
MycoBank: 811259

Basionym: *Occultifur kilbournensis* Kurtzman & Robnett, Antonie van Leeuwenhoek 107 (5): 1325 (2015) [MB#811259]

Pseudoccultifur lumbricifer **(**P. Roberts) P.P. Parra & Aime, comb. nov.
MycoBank: 371011

Basionym: *Occultifur lumbricifer* (P. Roberts) Trichies, Bulletin de la Société Mycologique de France 119: 325 (2004).
≡Achroomyces lumbricifer P. Roberts, Sydowia 53: 152 (2001).

Pseudoccultifur mephitis **(**Zalar, Šibanc & J.P. Samp.) P.P. Parra & Aime, comb. nov.
MycoBank: 818568

Basionym: *Occultifur mephitis* Zalar, Šibanc & J.P. Samp., International Journal of Systematic and Evolutionary Microbiology: [8] (2018).

***Pseudoccultifur plantarum* (**P. Khunnamw., J.R.A. Ribeiro, Hagler, M. Takash., Sugita, Jindam. & Limtong) P.P. Parra & Aime, comb. nov.

MycoBank: 819736

Basionym: *Occultifur plantarum* P. Khunnamw., J.R.A. Ribeiro, Hagler, M. Takash., Sugita, Jindam. & Limtong, International Journal of Systematic and Evolutionary Microbiology 67 (8): 2630 (2017).

***Pseudoccultifur rivoirei* (**G. Trichies**)** P.P. Parra & Aime, comb. nov.

MycoBank: 818646

Basionym: *Occultifur rivoirei* Trichies, Bulletin Mensuel de la Société Linnéenne de Lyon 86 (1-2): 20 (2016).

4.5 Discussion

The genus *Occultifur* was erected by Oberwinkler (1990) to accommodate *O. internus*, which is the type species for the genus. This species is presumed to be a mycoparasite during its hyphal stage on fungi in the Dacrymycetales (Bauer et al. 2006). The hyphal stage has never been reported in species of the former genus *Microsporomyces* and has never been found in association with other fungi. Instead, species in the genus have been mostly isolated from plants such as *Oryza sativa, Miscanthus floridulus* and pines trees (Pohl et al. 2011, Bai et al. 2016). The presence of a hyphal stage of *O. internus* not only suggests that other species in this lineage may be dimorphic but also indicates the occurrence of mycoparasitism in another evolutionary lineage within the Cystobasidiomycetes. Mycoparasitism as life strategy in *Pseudoccultifur* requires further investigation. Despite species such as *Pseudoccultifur lumbricifer* being found as an intrahymenial parasite on *Hyphodontia subalutacea* (Roberts 2001), *P. corticorum* has been isolated from *Hyphoderma praetermissum* (Roberts 1997) and *P. rivoirei* was recently described growing parasitically on *Hyphodontia arguta* (Trichies 2016), molecular data is still not available for these species. The lack of molecular data as in *O. internus* makes more difficult the task of establishing their phylogenetic affiliation and to better understand the evolution of host preferences and life strategy in this genus. The case of *O. internus* is the best example of the relevance of molecular data in fungal taxonomy, especially when the type species of a genus lacks it.

In general, the life strategy for most of the species in the class is still unknown, however, as more strains or reports from environmental samplings are available from different habitats and

substrates, it may be possible to identify the habitat preferences of species in the class and their most likely life strategy if additional efforts are invested to study these species in natural ecosystems. Additional efforts should be made to correctly classify sequences in public repositories since they are the source to identify geographic distribution and host preferences of species in the class. Despite species of the Cystobasidiomycetes have a global distribution, the number of occurrences is not uniform, for example, in Central America there is only one report, which does not mean that Cystobasidiomycetes are not distributed in this region, but it is due likely to under sampling or sampling bias. We have found that over 40% (Chapter 1) of the sequences available for the Cystobasidiomycetes were not assigned to one of the extant species in the class, making the task of studying habitat preference more demanding and more difficult to establish their possible role in nature.

The hypothesis is that closely or phylogenetically related taxa may have the same role or trait in nature because of the shared ancestor (Powell et al. 2009, Parrent et al. 2010). Nevertheless, there are some challenges in defining the life strategy of dimorphic species, since the kind of interactions a particular species may have during the yeast stage can greatly change during the hyphal stage. It is then possible that the yeast stage of a species exhibits preferences towards a habitat while these preferences switch during the hyphal stage. In our case, we based our ancestral character reconstruction mostly on host preference of the asexual stage, for this reason it is of vital importance to know the interactions that species of this class have in nature during the hyphal stage. After more information is gathered, studies like this can be revisited to draw definitive conclusions about the life strategy and host preference. According to Chaverri and Samuels (2013), most of the shifts in habitat preference are observed among organisms that are more closely related phylogenetically while interkingdom shifts are less frequent, however, in the Cystobasidiomycetes we observed interkingdom shifts in most of the lineages during the asexual stage, which means that a single species can be found either on fungi, animals or plants.

CHAPTER 5. FINAL REMARKS AND FUTURE WORK

For over 20 years, the Aime Lab has put a huge amount of effort to understand the phylogenetic relationships among species in Basidiomycota, the phylum to which the Cystobasidiomycetes belong. Over 7800 fungal specimens collected from all over the world are stored in the Aime Lab collection and represent an invaluable resource for research purposes. Among the 80 strains of Cystobasidiomycetes in the collection, there is representation of 13 already described species and at least 10 potential new species of which six have been described in this dissertation.

We have observed from the host association studies in the fourth chapter of this dissertation, that the Cystobasidiomycetes is a very diverse class of fungi in terms of host associations with species of different kingdoms and habitats. However, for most species in the class we still do not know what kind of interactions they have and their role in nature. Additional questions regarding their reproductive biology are still unresolved. The main motivation for conducting studies on this class consisted in the potential of describing a big number of new species to science that deposited in the Aime Lab culture collection. By formally describing these species and investigating their habitat preference, their phylogenetic relationships and possible roles in nature, we could contribute to the understanding we have of the class and the possible applications they may have in agriculture or biotechnology.

In the first chapter we collected available information from the literature to make a general review of the 13 genera in the Class. We emphasized on the sexual stage of species when present, and also some of the host associations and known geographic distribution. One of the main objectives of our studies was to describe new species to science that were stored in the Aime Lab Culture collection. In Chapter 2, we described three new species, *B. guamensis*, *B. rosea* and *B. tropicalis*, raising the number of species in this genus to seven. Previously described species were all obtained from the south of Japan and just a small number of sequences had been reported from other locations. Two species-level lineages from environmental sampling may represent new species in the genus. We also reported for the first time a member of *Bannoa, B. tropicalis* from South America, indicating a broader geographic distribution of the genus. We also performed intercompatibility studies on ten strains of *Bannoa*. We were able to report the development of a hyphal stage when compatible strains of *B. tropicalis* were crossed and incubated in the dark in

comparison with those incubated at higher temperatures and other light:darkness regimes. Despite it is uncertain whether the hyphal stage commonly occurs in natural conditions, it is possible that this is a mechanism either to reach resources or to interact with other fungi afecting the host plant. Our data indicate that *B. tropicalis, B. guamensis*, and environmental isolates can be isolated from rust sori and may be co-inhabitants of this microniche during the yeast stage. Further studies should be focused to study the interactions between species of *Bannoa* and its host, in this way it would be possible to determine if there is either a parasitic or epiphytic relationship.

We also described new species in three genera of the Cystobasidiomycetes in Chapter 3 namely: *Buckleyzyma butyrosa*, *Halobasidium umbonatum* and *Sakaguchia warrensis* (Figures 3.3 and 3.5). In our phylogenetic analyses, we included available sequences in the NCBI and information from the literature to expand the range of distribution and substrates in which species of these three genera can be isolated. Also, we identified several lineages in *Sakaguchia* that may represent new species (Figure 3.2). Based on the phylogenies built in Chapter 2 and 3, it is possible to conclude, that there is still a lot of diversity to be discovered in these four genera.

The basis for understanding the evolution of traits and relationships among species consists in having the best possible taxon sampling to build the phylogenies and to include information about the ecological features of each taxon in the analysis. In the Cystobasidiomycetes, several species in *Cystobasidium* and *Occultifur* lack molecular data for which it is not possible to know with certainty their phylogenetic affiliation. This is especially important if the species for which molecular data is missing is the type species of the genus, this is the case of *Occultifur internus*. We established that *O. internus* was not conspecific to other described species in the genus. Instead, it was phylogenetically related to *Microsporomyces* (Figure 4.1). This finding led us to propose new combinations and the proposal of a new name for those species in the former *Occultifur* (See section 4.4). In this dissertation we have mentioned several times that mycoparasitism has been reported in several generic lineages in the class, but for most of the species their role in nature remains unknown. Considering this, we performed an ancestral character reconstruction to test whether mycoparasitism is ancestral. After gathering information from over 790 available reports in the NCBI and assigning them to extant species based on PSC and GCSR we identified host associations and geographic distribution that were unknown for many species in the class (Section 4.3.2). Patterns of specialization were not identified, in fact, species can have associations with species in different kingdoms during the asexual stage. As they

have not been regularly studied in nature, the occurrence of the hyphal stage and the level of specialization during this stage remains understudied.

Over 50% of the sequences available in the NCBI for species in the Cystobasidiomycetes are not or are ambiguously classified, the value behind the effort of conducting species delimitation of these sequences is to enlarge not only the host range but also their geographic distribution. In addition, our Chapter 4 highlight the importance of having a criterion for the assignation of a species (or at least genus) to a strain or sequence obtained from environmental samplings. From the ancestral character reconstruction study, we observed several changes in host preference in lineages such as *Buckleyzyma* and *Symmetrospora* and to aquatic environments in *Sakaguchia*. However, these results are not conclusive due to the bias that could exist due to the fact that plants are more commonly sampled that any other substrate, for example other fungi.

Future research in the Cystobasidiomycetes also should be focused on studying the interaction of these species with their host in natural conditions, especially, because specific growing conditions are not always replicable under laboratory conditions. In this dissertation we reported that Cystobasidiomycetes can be co-inhabitants of rust sori, lichens, and fruiting bodies of other fungi. These niches should be considered for future samplings and studies to better understand the interactions that may occur. For example, in case there is mycoparasitism of species of Cystobasidiomycetous yeast that are co-inhabiting pustules of *Hemileia vastatrix*, they could be used as a biological control alternative to fight this devastating disease.

Mycoparasitism is not the only antagonistic interactions the Cystobasidiomycetes may have in natural ecosystems, also the production of antimicrobial compounds to outcompete other microorganisms has been reported during the asexual stage. Taking advantage of the considerable amount of strains in the Aime Lab collection, we evaluated the presence of antimicrobial compounds of 54 strains. We used the following methodology: A sensitive strain was allowed to grow for 24h on media containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1.0% glucose, and 2.0% agar, supplemented with 0.003% methylene blue and pH 4.2. Each strain in the Cystobasidiomycetes tested for killer activity was also incubated for 24h on Yeast Malt agar and inoculated by making a single streak on the plate containing the sensitive strain. Cultures were evaluated every 24h for 5 days for the presence of an inhibition zone with no growth. Over 900 dual cultures were made using the previously described system and we found that *Kluveromyces lactis* strain NRRL Y-6628 is weakly sensitive to substances produced by *Bannoa guamensis* strain

MCA4612. Similarly, we found that *Papiliotrema laurentii* strain MCA7145 is sensitive to *Symmetrospora coprosmae* strain MT264 (data not shown).

Lastly, the production of biofilms, that is defined as the association of yeast cells or other microorganisms to improve their performance (Mittelbach and Vannette 2017) (e.g., as a defense mechanism against toxic compounds), has not been studied in a wider context in the Cystobasidiomycetes. However, we have taken the first step and we collected available information from the literature; also we performed experiments in our laboratory to test which strains from our collection produce a ring (that can be interpreted as a biofilm) when cultured in liquid media (yeast extract 50 mg/l, casa amino acid mg/l and 1% glucose) (Table 4.2). Despite we did not have the opportunity to study the biofilms and how relevant they are in the defense mechanism of the asexual stage in nature we understand the importance of documenting these preliminary results and the need for further research in this topic in yeast fungi, especially in the Cystobasidiomycetes.

APPENDIX

Table A.1 Accession number and host association for sequences of the Cystobasidiomycetes used in species delimitation.

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Bannoa bischofiae	JCM10338^{T}	C	AB035721	Dead leaves of *Bischofia javanica*	Japan
Bannoa guamensis	**MCA4612^{T}**	C	**MK287350**	Diseased leaf of *Bidens* sp.	Guam
Bannoa hahajimensis	JCM10336T	C	AB035897	Dead leaves of *Bryophyllum pinnatum*	Japan
Bannoa ogasawarensis	JCM10326^{T}	C	AB035713	Dead leaves of *Schima mertensiana*, *Osmanthus insularis*, and *Syzygium buxifolium*	Japan
Bannoa rosea	MCA4647^{T}	C	**MK287351**	Asymptomatic undetermined leaf	Guam
Bannoa sp.	KL_2d_2H09	UC	JF495257	Beech litter	Austria
Bannoa sp.	**MCA7799**	C	**MK990655**	Fern	Cameroon
Bannoa sp.	IP055	C	AB726312	Plant/Soil	Japan
Bannoa sp.	OTU118	UC	KT328804	Coffee leaf infected by rust fungus (*Hemileia vastatrix*)	Mexico
Bannoa sp.	Chiang2499	C	AJ289868	*Miscanthus sinensis* var. transmorrisonensis	Taiwan
Bannoa sp.	**MCA7643**	C	**MK990651**	Unknown	Vanuatu

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Bannoa sp.	**MCA7637**	C	**MK990650**	Unknown	Vanuatu
Bannoa sp.	**MCA7670**	C	**MK990652**	Unknown	Vanuatu
Bannoa sp.	**MCA7672**	C	**MK990653**	Unknown	Vanuatu
Bannoa sp.	**MCA7674**	C	**MK990654**	Unknown	Vanuatu
Bannoa syzygii	JCM10337^{T}	C	AB035720	Dead leaves of *Syzygium buxifolium*	Japan
Bannoa tropicalis	PDD-28b-2	C	JN176592	Cloud water, altitude 1465m	France
Bannoa tropicalis	**MCA4620**	C	**MK287352**	Asymptomatic undetermined leaf	Guam
Bannoa tropicalis	**MCA4646**	C	**MK287358**	Asymptomatic undetermined leaf	Guam
Bannoa tropicalis	**MCA4648**	C	**MK287359**	Asymptomatic undetermined leaf	Guam
Bannoa tropicalis	**MCA5562**	C	**MK287362**	Asymptomatic undetermined leaf	Guam
Bannoa tropicalis	**MCA4642**	C	**MK287354**	Diseased leaf of *Euphorbia cyathophora*	Guam
Bannoa tropicalis	**MCA4645**	C	**MK287357**	*Kweilingia* symptomatic leaf of a Poacea	Guam
Bannoa tropicalis	**MCA4643**	C	**MK287355**	Palm trash swab	Guam

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Bannoa tropicalis	**MCA4644**	C	**MK287356**	*Puccinia lepturi* diseased leaf of *Lepturus repens*	Guam
Bannoa tropicalis	**MCA4627**	C	**MK287353**	Puccinia-infected leaf of *Lepturus sp.*	Guam
Bannoa tropicalis	**MCA4667^{T}**	C	**MK287360**	*Uromyces* diseased leaf of *Bidens* sp.	Guam
Bannoa tropicalis	**MCA4670**	C	**MK287361**	*Uromyces* diseased leaf of *Bidens* sp.	Guam
Bannoa tropicalis	NIP056	C	AB726636	Unknown	Japan
Bannoa tropicalis	MP3490	C	--------	Water cloud sampling	Panama
Bannoa tropicalis	TY-144	C	AY313038	Phylloplane	Taiwan
Bannoa tropicalis	TY-177	C	AY313042	Phylloplane	Taiwan
Bannoa tropicalis	TY-201	C	AY313044	Phylloplane	Taiwan
Bannoa tropicalis	TY-261	C	AY313056	Phylloplane	Taiwan
Bannoa tropicalis	TY-270	C	AY313058	Phylloplane	Taiwan
Bannoa tropicalis	TY-288	C	AY313060	Phylloplane	Taiwan
Buckleyzyma armeniaca	JCM8977^{T}	C	AF444523	Undamaged leaves of *Callistemon viminalis*	Australia
Buckleyzyma aurantiaca	YM24635	C	HQ637571	Phylloplane	--------

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Buckleyzyma aurantiaca	Clone31	C	KU179266	Roots of *Populus* sp.	--------
Buckleyzyma aurantiaca	JCM8163	C	AB038113	Sea surface microlayer and underlying water	--------
Buckleyzyma aurantiaca	CBS8074	C	KY101785	Phylloplane	Australia
Buckleyzyma aurantiaca	MUCL30645	C	KY106235	Phylloplane	Australia
Buckleyzyma aurantiaca	KL_2w_A02	UC	JF449588	Beech litter	Austria
Buckleyzyma aurantiaca	SW_2d_G11	UC	JF449839	Beech litter	Austria
Buckleyzyma aurantiaca	SW_2w_B07	UC	JF449860	Beech litter	Austria
Buckleyzyma aurantiaca	KL_2d_2F03	UC	JF495232	Beech litter	Austria
Buckleyzyma aurantiaca	KL_2d_2G04	UC	JF495243	Beech litter	Austria
Buckleyzyma aurantiaca	HB1211	C	AM160641	beetle gut of *Diabrotica virgifera*	Austria
Buckleyzyma aurantiaca	OTU13	UC	KT965045	Posterior gut of Staphylinidae	Canada
Buckleyzyma aurantiaca	YM25880	C	KC494744	Lake water	China
Buckleyzyma aurantiaca	Cluster13783	UC	KT160889	Leaves from boreal streams	Finland
Buckleyzyma aurantiaca	JCM11358	C	AB093528	Apple fruit surface	Germany

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Buckleyzyma aurantiaca	RP432_13	C	KX096691	Endophyte on plant	Germany
Buckleyzyma aurantiaca	DBVPG5753	C	KC433806	Air sampling	Italy
Buckleyzyma aurantiaca	MUT5471	C	KU314991	Mediterranean Sea *Padina pavonica* (Algae)	Italy
Buckleyzyma aurantiaca	CBS10207	C	EU002828	Phylloplane	Portugal
Buckleyzyma aurantiaca	CBS10208	C	EU002829	Phylloplane	Portugal
Buckleyzyma aurantiaca	CBS10209	C	EU002830	Phylloplane	Portugal
Buckleyzyma aurantiaca	CBS10210	C	EU002831	Phylloplane	Portugal
Buckleyzyma aurantiaca	CBS10211	C	EU002832	Phylloplane	Portugal
Buckleyzyma aurantiaca	HB31-4	C	KJ507280	Flower of *Elaeagnus multiflora*	Republic of Korea
Buckleyzyma aurantiaca	HB12-1	C	KJ507255	Flower of *Pyrus serotina* var. culta	Republic of Korea
Buckleyzyma aurantiaca	ATT147	C	FJ743613	*Atta texana* nest	USA
Buckleyzyma aurantiaca	Unkown	UC	LT622084	*Canabis sativa*, Hemp fibres	USA
Buckleyzyma aurantiaca	P44D004	C	JX188233	*Vitis vinifera*	USA
Buckleyzyma aurantiaca	JCM3771^{T}	C	AF444538	Air sampling	Japan

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Buckleyzyma butyrosa	**MCA2993^{T}**	C	**MK990657**	Air sampling	USA
Buckleyzyma kluyveri-nielii	DBMY308	C	KJ706525	Mangrove sediments	India
Buckleyzyma kluyveri-nielii	DBMY467	C	KJ706684	Mangrove sediments	India
Buckleyzyma kluyveri-nielii	DBMY626	C	KJ706843	Mangrove sediments	India
Buckleyzyma kluyveri-nielii	DBMY785	C	KJ707002	Mangrove sediments	India
Buckleyzyma kluyveri-nielii	JCM6356^{T}	C	AF444544	*Dombeya rotundifolia*	South Africa
Buckleyzyma phyllomatis	JCM7549^{T}	C	AF444515	*Dombeya rotundifolia*	South Africa
Buckleyzyma salicina	HB1140	C	AM039681	Forest litter	Austria
Buckleyzyma salicina	XJ10B5	C	HE650887	Phylloplane	China
Buckleyzyma salicina	DBMY311	C	KJ706528	Mangrove sediments	India
Buckleyzyma salicina	DBMY470	C	KJ706687	Mangrove sediments	India
Buckleyzyma salicina	DBMY629	C	KJ706846	Mangrove sediments	India
Buckleyzyma salicina	DBMY788	C	KJ707005	Mangrove sediments	India
Buckleyzyma salicina	CBS10203	C	EU002824	Phylloplane	Portugal

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Buckleyzyma salicina	CBS10204	C	EU002825	Phylloplane	Portugal
Buckleyzyma salicina	**P117**	C	**MK990656**	*Pinus nigra*	USA
Buckleyzyma salicina	JCM2959^{T}	C	AF444511	Healthy leaves of *Salix* sp.	Canada
Cyphobasidium hypogymniicola	S-F264671^{T}	C	KU587700	*Usnea* sp.	--------
Cyphobasidium sp.	T1650	UC	KU948918	*Usnea antarctica*	Antarctica
Cyphobasidium sp.	T1651	UC	KU948928	*Usnea antarctica*	Antarctica
Cyphobasidium sp.	T1594	UC	KU948893	*Bryoria capillaris*	Austria
Cyphobasidium sp.	T1632	UC	KU948914	*Flavopunctelia flaventior*	Austria
Cyphobasidium sp.	T1664	UC	KU948905	*Hypotrachyna laevigata*	Costa Rica
Cyphobasidium sp.	T1705	UC	KU948925	*Oropogon colibor*	Costa Rica
Cyphobasidium sp.	T1630	UC	KU948924	*Bulbothrix sensibilis*	Ethiopia
Cyphobasidium sp.	T1629	UC	KU948911	*Parmelinopsis swinscowii*	Ethiopia
Cyphobasidium sp.	T1631	UC	KU948913	*Parmotrema* cf. *durumae*	Ethiopia
Cyphobasidium sp.	T1633	UC	KU948915	*Parmotrema lobulascens*	Ethiopia

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Cyphobasidium sp.	T1637	UC	KU948907	*Parmotrema sancti-angeli*	Ethiopia
Cyphobasidium sp.	T1635	UC	KU948908	*Pseudoparmelia simplicior*	Ethiopia
Cyphobasidium sp.	T1638	UC	KU948909	*Pseudoparmelia simplicior*	Ethiopia
Cyphobasidium sp.	T1642	UC	KU948910	*Xanthoparmelia kiboensis*	Ethiopia
Cyphobasidium sp.	T1655	UC	KU948923	*Menegazzia subsimilis*	Japan
Cyphobasidium sp.	T1599	UC	KU948895	*Bryoria capillaris*	Sweden
Cyphobasidium sp.	T1435	UC	KU948881	*Bryoria tortuosa*	USA
Cyphobasidium sp.	T1557	UC	KU948904	*Esslingeriana idahoensis*	USA
Cyphobasidium sp.	T1397	UC	KU948912	*Hypogymnia apinnata*	USA
Cyphobasidium sp.	T1460	UC	KU948883	*Hypogymnia physodes*	USA
Cyphobasidium sp.	T1644	UC	KU948896	*Hypogymnia wilfiana*	USA
Cyphobasidium sp.	T1560	UC	KU948906	*Imshaugia aleurites*	USA
Cyphobasidium sp.	T1645	UC	KU948917	*Kaernefeltia merrillii*	USA
Cyphobasidium sp.	T1670	UC	KU948920	*Melanelia hepatizon*	USA

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Cyphobasidium sp.	T1711	UC	KU948926	*Physcia biziana*	USA
Cyphobasidium sp.	VT005	UC	KU948899	*Tuckermannopsis platyphylla*	USA
Cyphobasidium sp.	VT006	UC	KU948900	*Vulpicida canadensis*	USA
Cyphobasidium sp.	VT008	UC	KU948901	*Vulpicida canadensis*	USA
Cyphobasidium sp.	VT009	UC	KU948902	*Vulpicida canadensis*	USA
Cyphobasidium usneicola	S-F264672^{T}	C	KU587703	*Usnea* sp.	--------
Cyrenella elegans	BSS353	C	MH909047	Unknown	Brazil
Cyrenella elegans	TUBF181	C	AY512842	Unknown	Germany
Cyrenella elegans	CCMF791	C	EF450544	Unknown	Portugal
Cyrenella elegans	CBS274.82^{T}	C	KJ778626	Sand scraped from the submerged stipe of a mushroom, Laccaria trullisata	USA
Cystobasidium alpinum	CBS14809^{T}	C	KC455920	Supraglacial sediments	Arctic
Cystobasidium benthicum	CBS12080	C	KY107428	Unknown	--------
Cystobasidium benthicum	SJ249	C	AY953950	Unknown	Canada

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Cystobasidium benthicum	N702T_13	UC	GU942279	Seawater	China
Cystobasidium benthicum	CBS9124^{T}	C	AB026001	Tube worm *Lamellibrachia* sp.	Japan
Cystobasidium calyptogenae	CBS11058	C	KY103126	Unknown	--------
Cystobasidium calyptogenae	CBS11318	C	KY103127	Unknown	--------
Cystobasidium calyptogenae	CBS11134	C	KY103129	Unknown	--------
Cystobasidium calyptogenae	SJ007	C	AY953945	Unknown	Canada
Cystobasidium calyptogenae	N203T_256	UC	GU941333	Seawater	China
Cystobasidium calyptogenae	N205T_374	UC	GU941374	Seawater	China
Cystobasidium calyptogenae	NS7T_223	UC	KJ180533	Seawater	China
Cystobasidium calyptogenae	NS11T_219	UC	KJ180902	Seawater	China
Cystobasidium calyptogenae	-------	UC	KP099877	Seawater	China
Cystobasidium calyptogenae	S11T_55	UC	KU163766	Seawater	China
Cystobasidium calyptogenae	S14T_95	UC	KU163903	Seawater	China
Cystobasidium calyptogenae	S24T_07	UC	KU164288	Seawater	China

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Cystobasidium calyptogenae	S31T_109	UC	KU164421	Seawater	China
Cystobasidium calyptogenae	S46T_49	UC	KU164655	Seawater	China
Cystobasidium calyptogenae	RY47	C	KM014601	continental shelf sediments	India
Cystobasidium calyptogenae	CBS9125^{T}	C	AB025996	*Calyptogena*, giant white clam	Japan
Cystobasidium calyptogenae	UA78	C	FJ515209	sea surface microlayer and underlying water	Taiwan
Cystobasidium calyptogenae	4107	C	EU669877	Seawater	Taiwan
Cystobasidium calyptogenae	SN59	C	FJ515245	Seawater	Taiwan
Cystobasidium calyptogenae	36-2A(IAM14905)	C	AB055196	Unknown	Thailand
Cystobasidium calyptogenae	**SA628**	C	**MK990672**	Unknown	USA
Cystobasidium fimetarium	DB1489	C	KP053250	*Thelebolus crustaceus*, *Lasiobolus equinus*	Denmark
Cystobasidium halotolerans	IPM32-16	C	AB726384	Seawater	Qatar
Cystobasidium halotolerans	CGMCC 2.5570^{T}	C	MH828324	Seawater	Arabian Gulf
Cystobasidium iriomotense	JCM24594^{T}	C	AB726571	Dead branch of an unidentified tree	China
Cystobasidium iriomotense	MH828324	C	MH828324	Sandy soil, Dead branch of an unidentified tree	Japan

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Cystobasidium laryngis	21B-1-9	C	LC203673	Soil	Antarctica
Cystobasidium laryngis	21B-1-10	C	LC203674	Soil	Antarctica
Cystobasidium laryngis	21B-1-13	C	LC203675	Soil	Antarctica
Cystobasidium laryngis	21B-2-2	C	LC203677	Soil	Antarctica
Cystobasidium laryngis	21B-2-12	C	LC203680	Soil	Antarctica
Cystobasidium laryngis	JCM10953	C	AB078500	Animals collected from the deep-sea	Canada
Cystobasidium laryngis	M22-22C-8	C	KU145526	Soil	Chile
Cystobasidium laryngis	7_337	UC	KC884334	Soil	China
Cystobasidium laryngis	11-143	UC	KC884414	Soil	China
Cystobasidium laryngis	R106	C	HG532076	Barley	Finland
Cystobasidium laryngis	BF92	C	AM901696	Indoor dust	Finland
Cystobasidium laryngis	2_69	UC	KF274247	Wood stump	Finland
Cystobasidium laryngis	CBML151a	C	MH047192	Aquatic	Germany
Cystobasidium laryngis	09S70C31	UC	HG937096	*Zea mays* field bulk soil	Germany

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Cystobasidium laryngis	CBS5695	C	KY103131	Human	Hungary
Cystobasidium laryngis	CBS2221^{T}	C	AF190014	Laryngeal swabs	Norway
Cystobasidium laryngis	I14F-1273	C	KR698869	Pond	Norway
Cystobasidium laryngis	SH04-01	C	KP314712	Reindeer dung coprophilous fungus	Norway
Cystobasidium laryngis	SH04-02	C	KP314713	Reindeer dung coprophilous fungus	Norway
Cystobasidium laryngis	SH05-01	C	KP314718	Reindeer dung coprophilous fungus	Norway
Cystobasidium laryngis	SH07-04	C	KP314734	Reindeer dung coprophilous fungus	Norway
Cystobasidium laryngis	CBS8020	C	KY103130	Seawater	Sweden
Cystobasidium laryngis	CMH285	UC	KF800376	House dust	USA
Cystobasidium laryngis	Y17494	C	AF444607	Unknown	USA
Cystobasidium lysinophilum	21A-H1	C	LC202040	Soil	Antarctica
Cystobasidium lysinophilum	21A-H2	C	LC203672	Soil	Antarctica
Cystobasidium lysinophilum	SJ036	C	AY953946	Unknown	Canada
Cystobasidium lysinophilum	JCM8105	C	AB038117	Animals collected from the deep-sea	Japan

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Cystobasidium lysinophilum	CBS9126^{T}	C	AB078501	Unknown	Japan
Cystobasidium minutum	CBS2177	C	AF190010	Unknown	--------
Cystobasidium minutum	CBS5475	C	KY107435	Water	--------
Cystobasidium minutum	CBS4408	C	KY107433	Unknown	--------
Cystobasidium minutum	LH227	C	HQ832824	Foliar lesions of *Camellia sinensis*	China
Cystobasidium minutum	F4-3-10	C	KX349460	Reed field	China
Cystobasidium minutum	F5-3-20	C	KX349469	Reed field	China
Cystobasidium minutum	N704T_295	UC	GU942321	Seawater	China
Cystobasidium minutum	NS8T_230	UC	KJ180628	Seawater	China
Cystobasidium minutum	S11T_35	UC	KU163756	Seawater	China
Cystobasidium minutum	S13T_19	UC	KU163827	Seawater	China
Cystobasidium minutum	S13T_28	UC	KU163830	Seawater	China
Cystobasidium minutum	S14T_39	UC	KU163873	Seawater	China
Cystobasidium minutum	S14T_41	UC	KU163875	Seawater	China

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Cystobasidium minutum	S15T_06	UC	KU163905	Seawater	China
Cystobasidium minutum	S21T_69	UC	KU164121	Seawater	China
Cystobasidium minutum	S21T_87	UC	KU164136	Seawater	China
Cystobasidium minutum	S21T_91	UC	KU164139	Seawater	China
Cystobasidium minutum	S23T_10	UC	KU164226	Seawater	China
Cystobasidium minutum	S43T_58	UC	KU164547	Seawater	China
Cystobasidium minutum	S43T_60	UC	KU164548	Seawater	China
Cystobasidium minutum	S43T_76	UC	KU164558	Seawater	China
Cystobasidium minutum	S44T_100	UC	KU164573	Seawater	China
Cystobasidium minutum	S44T_56	UC	KU164602	Seawater	China
Cystobasidium minutum	S46T_13	UC	KU164639	Seawater	China
Cystobasidium minutum	S46T_22	UC	KU164649	Seawater	China
Cystobasidium minutum	S46T_74	UC	KU164665	Seawater	China
Cystobasidium minutum	S47T_128	UC	KU164684	Seawater	China

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Cystobasidium minutum	S47T_78	UC	KU164700	Seawater	China
Cystobasidium minutum	AUMC10791	C	KY495759	Fresh orange juice	Egypt
Cystobasidium minutum	CBS319^{T}	C	AF190011	Air sampling	Japan
Cystobasidium minutum	CBS4407	C	KY107437	Air sampling	Japan
Cystobasidium minutum	SN35	C	FJ515189	Sea surface microlayer and underlying water	Taiwan
Cystobasidium minutum	UN7	C	FJ515211	Sea surface microlayer and underlying water	Taiwan
Cystobasidium minutum	**MCA4210**	C	**MK990665**	Mycangia of Sirex wasp	USA
Cystobasidium minutum	**MCA7620**	C	**MK990669**	*Lygodium* sp.	Vanuatu
Cystobasidium minutum	**MCA7407**	C	**MK990666**	*Lygodium* sp.	Vanuatu
Cystobasidium minutum	**MCA7423**	C	**MK990667**	Unknown	Vanuatu
Cystobasidium minutum	**MCA7611**	C	**MK990668**	Unknown	Vanuatu
Cystobasidium oligophagum	CBS12623^{T}	C	AB702968	Koala nasal smears	Australia
Cystobasidium ongulense	CLQCA-ANT-047	C	HF545224	Glacier	Antarctica
Cystobasidium ongulense	CCFEE5186	C	JX124722	Rocks	Antarctica

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Cystobasidium ongulense	BB13	C	FJ236004	Soil	Antarctica
Cystobasidium ongulense	A5	C	HM589289	Soil	Antarctica
Cystobasidium ongulense	B4	C	HM589371	Soil	Antarctica
Cystobasidium ongulense	21B-2-1	C	LC203676	Soil	Antarctica
Cystobasidium ongulense	21B-2-3	C	LC203678	Soil	Antarctica
Cystobasidium ongulense	21B-2-4	C	LC203679	Soil	Antarctica
Cystobasidium ongulense	CBS8923	C	AY033642	Unknown	Antarctica
Cystobasidium ongulense	BC22	C	DQ317357	Wood	Antarctica
Cystobasidium ongulense	87_NA5_P34_I3	UC	KF296995	Soil	Canada
Cystobasidium ongulense	MCA7215	C	MK990663	Unknown	Canada
Cystobasidium ongulense	D27-10C-2	C	KU145512	Soil	Chile
Cystobasidium ongulense	MX-29	C	MF062208	Glacier	China
Cystobasidium ongulense	JCM31527^{T}	C	LC155915	Soil	Antarctica
Cystobasidium ongulense	**TAR415**	C	MK990674	*Dryopteris erythrosora*	Iceland

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Cystobasidium ongulense	L116-09	C	KR004864	Lichen	Norway
Cystobasidium ongulense	I14F-1275	C	KR698871	Pond	Norway
Cystobasidium ongulense	SH01-02	C	KP314689	Reindeer dung coprophilous fungus	Norway
Cystobasidium ongulense	**P107**	C	MK990661	*Rubus* sp.	USA
Cystobasidium pallidum	CBS320^{T}	C	AB078492	isolated from a mycotic nodule in white rat	Japan
Cystobasidium pinicola	CBS9130^{T}	C	AF444292	*Pinus tabulaeformis*	China
Cystobasidium pinicola	**MCA5101**	C	**MK990662**	*Rubus* sp.	France
Cystobasidium pinicola	MYf129	C	KX079906	Nematode	Germany
Cystobasidium portillonense	CBS12733^{T}	C	JQ769323	Rock surface/marine sediment	Antarctica
Cystobasidium proliferans	unknown	C	--------	Insect excreta	USA
Cystobasidium psychroaquaticum	CBS8913	C	AY040648	Unknown	Antarctica
Cystobasidium psychroaquaticum	BB2	C	FJ235993	Wood	Antarctica
Cystobasidium psychroaquaticum	D88R	C	JN572894	Springtail Sminthuridae feeding on fungus	Bulgaria

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Cystobasidium psychroaquaticum	TP-Snow-Y142	C	JQ768925	Glacier	China
Cystobasidium psychroaquaticum	I14F-1325	C	KR698921	Estuary	Norway
Cystobasidium psychroaquaticum	I14F-1313	C	KR698909	Ice	Norway
Cystobasidium psychroaquaticum	L003-01	C	KR004827	Lichen *Cladonia pocillum*	Norway
Cystobasidium psychroaquaticum	SH04-04	C	KP314715	Reindeer dung coprophilous fungus	Norway
Cystobasidium psychroaquaticum	SH06-03	C	KP314726	Reindeer dung coprophilous fungus	Norway
Cystobasidium psychroaquaticum	SH07-01	C	KP314731	Reindeer dung coprophilous fungus	Norway
Cystobasidium psychroaquaticum	CBS11769^{T}	C	FN868153	Leatherleaf: *Chamaedaphne calyculata*	Russia
Cystobasidium psychroaquaticum	**MT096**	C	**MK990670**	Unknown	USA
Cystobasidium ritchiei	CBS12324^{T}	C	LM644066	Leatherleaf: *Chamaedaphne calyculata*	Russia
Cystobasidium sebaceum	GWM3686^{T}	C	--------	Decaying wood remnants of an old *Sebacina*	Colombia
Cystobasidium slooffiae	CBS2622	C	KY107439	Humans	--------
Cystobasidium slooffiae	JCM10954	C	AB052212	Seawater	--------

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Cystobasidium slooffiae	CBS7094	C	AF444552	Unknown	--------
Cystobasidium slooffiae	CBS7093	C	AF444554	Unknown	--------
Cystobasidium slooffiae	PYCC4689	C	AF444588	Unknown	--------
Cystobasidium slooffiae	PYCC4887	C	AF444589	Unknown	--------
Cystobasidium slooffiae	CBS7295	C	AF444619	Unknown	--------
Cystobasidium slooffiae	URM7234	C	KX811210	*Melocactus zehntneri*	Brazil
Cystobasidium slooffiae	N418T_41	UC	GU942002	Seawater	China
Cystobasidium slooffiae	NS8T_210	UC	KJ180611	Seawater	China
Cystobasidium slooffiae	S13T_66	UC	KU163848	Seawater	China
Cystobasidium slooffiae	S14T_64	UC	KU163884	Seawater	China
Cystobasidium slooffiae	S15T_18	UC	KU163908	Seawater	China
Cystobasidium slooffiae	S15T_75	UC	KU163921	Seawater	China
Cystobasidium slooffiae	S43T_31	UC	KU164529	Seawater	China
Cystobasidium slooffiae	S43T_73	UC	KU164557	Seawater	China

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Cystobasidium slooffiae	S43T_86	UC	KU164564	Seawater	China
Cystobasidium slooffiae	S44T_25	UC	KU164588	Seawater	China
Cystobasidium slooffiae	S46T_122	UC	KU164636	Seawater	China
Cystobasidium slooffiae	S46T_76	UC	KU164667	Seawater	China
Cystobasidium slooffiae	S46T_91	UC	KU164674	Seawater	China
Cystobasidium slooffiae	DN18	C	KY781369	Sediments	China
Cystobasidium slooffiae	BF-OTU160	UC	AM901759	House dust	Finland
Cystobasidium slooffiae	BF-OTU645	UC	FR682388	House dust	Finland
Cystobasidium slooffiae	CBS5706^{T}	C	AF444627	Humans	Hungary
Cystobasidium slooffiae	ABP_28	UC	JF497131	Marine sediment	India
Cystobasidium slooffiae	R28Pi	C	JQ665423	Marine sediment	India
Cystobasidium slooffiae	ISM28-8s	C	AB726571	Soil/Plants	Japan
Cystobasidium slooffiae	J3S-STD	C	KT819329	Seaweeds	New Zealand
Cystobasidium slooffiae	Y-1002	C	AM117832	Sediment	West Pacific Ocean

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Cystobasidium slooffiae	WP02-1-2-B	C	AM117834	Sediment	West Pacific Ocean
Cystobasidium slooffiae	PYCC4761	C	FJ807685	Paper mill effluent	Portugal
Cystobasidium slooffiae	IWBT-Y820	C	JQ993376	*Vitis vinifera*	South Africa
Cystobasidium slooffiae	GY11314PS	C	LC272867	Seawater	Republic of Korea
Cystobasidium slooffiae	GY113164PS	C	LC272868	Seawater	Republic of Korea
Cystobasidium slooffiae	GY113157PS	C	LC272870	Seawater	Republic of Korea
Cystobasidium slooffiae	GY11318PS	C	LC272871	Seawater	Republic of Korea
Cystobasidium slooffiae	GY113366PS	C	LC272872	Seawater	Republic of Korea
Cystobasidium slooffiae	GY113273PS	C	LC272873	Seawater	Republic of Korea
Cystobasidium slooffiae	GY113276PS	C	LC272874	Seawater	Republic of Korea
Cystobasidium slooffiae	GY113367PS	C	LC272875	Seawater	Republic of Korea
Cystobasidium slooffiae	3209	C	FN386295	*Holcus lanatus*	Spain
Cystobasidium slooffiae	CBS8019	C	KY107440	Seawater	Sweden
Cystobasidium slooffiae	KB02_HCn_B4	C	AB081118	Fungus garden of *Odontotermes* sp.	Taiwan

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Cystobasidium slooffiae	SN2	C	FJ515193	sea surface microlayer and underlying water	Taiwan
Cystobasidium slooffiae	UN64	C	FJ515213	sea surface microlayer and underlying water	Taiwan
Cystobasidium slooffiae	UN71	C	FJ515215	sea surface microlayer and underlying water	Taiwan
Cystobasidium slooffiae	CMH241	UC	KF800332	House dust	USA
Cystobasidium slooffiae	15-17-28	UC	EF505775	Leaves, stems, kernels of *Zea mays*	USA
Cystobasidium slooffiae	**SA515**	C	**MK990671**	Unknown	USA
Cystobasidium sp.	MX-6	C	MF062211	Glacier	China
Cystobasidium sp.	S15T_02	UC	KU163904	Seawater	China
Cystobasidium sp.	S43T_05	UC	KU164512	Seawater	China
Cystobasidium sp.	S47T_38	UC	KU164691	Seawater	China
Cystobasidium sp.	22214	C	HQ670684	Shrimp and shrimp culture pond water	China
Cystobasidium sp.	CI9	UC	KC922111	Stool cattle	China
Cystobasidium sp.	AY952	C	MG250437	Inner surface of termite's tapetum	Namibia
Cystobasidium sp.	**MCA7633**	C	**MK990664**	*Lygodium* sp.	Vanuatu

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Cystobasidium sp.	**MCA7412**	C	**MK990673**	Phylloplane	Vanuatu
Cystobasidium tubakii	JCM31526^{T}	C	LC155913	Soil	Antarctica
Erythrobasidium elongatum	UFMG-ABT330	C	KM527115	Bromeliad water phytotelm	Brazil
Erythrobasidium elongatum	UFMG-ABT330	C	KM527116	Bromeliad water phytotelm	Brazil
Erythrobasidium elongatum	BI113	C	EU678952	Unknown	Brazil
Erythrobasidium elongatum	BRT170	C	MH885312	Unknown	Brazil
Erythrobasidium elongatum	DBMY304	C	KJ706521	Mangrove sediments	India
Erythrobasidium elongatum	DBMY463	C	KJ706680	Mangrove sediments	India
Erythrobasidium elongatum	DBMY622	C	KJ706839	Mangrove sediments	India
Erythrobasidium elongatum	DBMY781	C	KJ706998	Mangrove sediments	India
Erythrobasidium elongatum	CBS10205	C	EU002826	Phylloplane	Portugal
Erythrobasidium elongatum	CBS10206	C	EU002827	Phylloplane	Portugal
Erythrobasidium elongatum	OTU294	UC	KT328645	Coffee leaf infected by rust fungus (*Hemileia vastatrix*)	Puerto Rico
Erythrobasidium elongatum	**INDP051**	C	**MK990675**	*Bidens* sp.	USA

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Erythrobasidium elongatum	**P127**	C	**MK990676**	*Bidens* sp.	USA
Erythrobasidium elongatum	10B-T-2t-1	C	KP122300	Stem tissue of *Smilax rotundifolia*	USA
Erythrobasidium elongatum	CBS8080^{T}	C	AF444561	Undamaged leaf of *Callistemon viminalis*	Australia
Erythrobasidium hasegawianum	IA28	C	KM246204	Coffee plants	Brazil
Erythrobasidium hasegawianum	YDMJ72	C	KC160570	Aromatic plant tissue	China
Erythrobasidium hasegawianum	NDZYD14	C	KC160629	Aromatic plant tissue	China
Erythrobasidium hasegawianum	NDZYP32	C	KC160630	Aromatic plant tissue	China
Erythrobasidium hasegawianum	YM24993	C	KT239097	Lake water	China
Erythrobasidium hasegawianum	YM25281	C	KT345330	Lake water	China
Erythrobasidium hasegawianum	YM25219	C	KT345331	Lake water	China
Erythrobasidium hasegawianum	CBS10213	C	EU002833	Phylloplane	China
Erythrobasidium hasegawianum	CBS10212	C	EU002880	Phylloplane	China
Erythrobasidium hasegawianum	M13F_D10_13J	C	KF293978	Stem of Poplar	China
Erythrobasidium hasegawianum	BF-OTU159	UC	AM901803	House dust	Finland

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Erythrobasidium hasegawianum	CBS10215	C	EU002835	Phylloplane	Portugal
Erythrobasidium hasegawianum	CBS10216	C	EU002836	Phylloplane	Portugal
Erythrobasidium hasegawianum	CBS10218	C	EU002837	Phylloplane	Portugal
Erythrobasidium hasegawianum	CBS10214	C	EU002882	Phylloplane	Portugal
Erythrobasidium hasegawianum	AV3BVC9	C	KX263815	*Spartina maritima*	Portugal
Erythrobasidium hasegawianum	**MCA6865**	C	**MK990677**	*Puccinia coronata* infected leave	Russia
Erythrobasidium hasegawianum	K-215-12-5	UC	KF815655	Air sampling	Slovakia
Erythrobasidium hasegawianum	UN85	C	FJ515203	Sea surface microlayer and underlying water	Taiwan
Erythrobasidium hasegawianum	CBS8253^{T}	C	AF444522	Old culture of beer yeast	USA
Erythrobasidium sp.	MBP33-29	UC	FJ237198	Soil	Austria
Erythrobasidium sp.	IA28	C	KM246121	Coffee plants	Brazil
Erythrobasidium sp.	IMUFRJ52011	C	FN428879	Sugar cane leaf	Brazil
Erythrobasidium sp.	clone6.1	UC	AJ876495	*Paradamaeus clavipes*	Germany
Erythrobasidium sp.	Ms7Mb20	UC	AJ875349	*Phragmites australis*	Germany

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Erythrobasidium sp.	OTUL71	UC	KR906007	Pacific Ocean deep-sea sediment	India
Erythrobasidium sp.	DBVPG5083	C	GQ911513	Glacier	Italy
Erythrobasidium sp.	LA	C	MG515899	Macabeo grape must	Mexico
Erythrobasidium sp.	M8	C	MH382784	Macabeo grape must	Mexico
Erythrobasidium sp.	CBS10217	C	EU002885	Phylloplane	Portugal
Erythrobasidium sp.	JCM1545	C	AF131058	Unknown	Portugal
Erythrobasidium sp.	ZIM664	C	AM748534	Grape berries	Slovenia
Erythrobasidium sp.	HB34-1	C	KJ507252	Flower of *Vitis vinifera* L.	Republic of Korea
Erythrobasidium sp.	MOM_882	C	HG421447	Floral nectar	Spain
Erythrobasidium sp.	OH120523.2	C	MH931270	Phylloplane	Switzerland
Erythrobasidium sp.	F28	C	AY560616	Phylloplane	Taiwan
Erythrobasidium sp.	UN85	C	FJ515258	sea surface microlayer and underlying water	Taiwan
Erythrobasidium yunnanense	TSBF1_53	UC	HQ257440	Oil-contaminated mangrove soil (10cm depth)	Brazil
Erythrobasidium yunnanense	TA203	C	JQ219309	Apple surface	China

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Erythrobasidium yunnanense	YG20	C	MF045464	Apple fruit surface	China
Erythrobasidium yunnanense	BJ7	C	JQ219308	Apple surface	China
Erythrobasidium yunnanense	CBS8906	C	KY107682	Phylloplane	China
Erythrobasidium yunnanense	ZBJ201207-47	UC	KX514957	Rainwater	China
Erythrobasidium yunnanense	ZBJ201207-49	UC	KX514959	Rainwater	China
Erythrobasidium yunnanense	ZBJ201208-44	UC	KX515007	Rainwater	China
Erythrobasidium yunnanense	ZBJ201208-47	UC	KX515010	Rainwater	China
Erythrobasidium yunnanense	ZBJ201208-49	UC	KX515012	Rainwater	China
Erythrobasidium yunnanense	ZSH201205-19	UC	KX515476	Rainwater	China
Erythrobasidium yunnanense	ZSH201206-14	UC	KX515509	Rainwater	China
Erythrobasidium yunnanense	R061230	UC	GU256960	Roots of *Rhododendron argyrophyllum*	China
Erythrobasidium yunnanense	N104T_255	UC	GU941204	Seawater	China
Erythrobasidium yunnanense	N104T_259	UC	GU941206	Seawater	China
Erythrobasidium yunnanense	NS4T_214	UC	KJ180251	Seawater	China

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Erythrobasidium yunnanense	NS4T_218	UC	KJ180254	Seawater	China
Erythrobasidium yunnanense	NS4T_238	UC	KJ180273	Seawater	China
Erythrobasidium yunnanense	NS4T_266	UC	KJ180296	Seawater	China
Erythrobasidium yunnanense	NS4T_284	UC	KJ180312	Seawater	China
Erythrobasidium yunnanense	NS4T_287	UC	KJ180314	Seawater	China
Erythrobasidium yunnanense	NS4T_293	UC	KJ180320	Seawater	China
Erythrobasidium yunnanense	DBP-2011	C	JF702915	Marble statue	Italy
Erythrobasidium yunnanense	RS	C	JF758860	Marble statue	Italy
Erythrobasidium yunnanense	OTU158	UC	KT328766	Coffee leaf infected by rust fungus (*Hemileia vastatrix*)	Mexico
Erythrobasidium yunnanense	CBS10213	C	EU002881	Phylloplane	Portugal
Erythrobasidium yunnanense	CBS11710	C	KY103393	Flower of *Silene* sp.	South Africa
Erythrobasidium yunnanense	GY1131127PS	C	LC272890	Seawater	Republic of Korea
Erythrobasidium yunnanense	GY113362PS	C	LC272891	Seawater	Republic of Korea
Erythrobasidium yunnanense	UN68	C	FJ515217	Sea surface microlayer and underlying water	Taiwan

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Erythrobasidium yunnanense	UN68	C	FJ515272	Seawater	Taiwan
Erythrobasidium yunnanense	Clone8	UC	MG976255	Human Skull	United Kingdom
Erythrobasidium yunnanense	Clone59	UC	MG976305	Human Skull	United Kingdom
Erythrobasidium yunnanense	9279	C	EF419971	Leaf of *Platycladus orientalis*	USA
Erythrobasidium yunnanense	JCM10687^{T}	C	AB030353	Semi dried leaf *Sapindus delavayi*	China
Halobasidium sp.	M22	C	LT602889	Wild *Arabidopsis* leaf	Finland
Halobasidium umbonatum	NA3_P31_E20	UC	KC966072	Soil	Canada
Halobasidium umbonatum	Clone5	C	BSU65613	*Chimonobambusa marmorea*	China
Halobasidium umbonatum	YM24636	C	JQ320370	Seaweeds	China
Halobasidium umbonatum	M17	C	LT602890	Wild *Arabidopsis* leaf	Finland
Halobasidium umbonatum	DSM28479	C	LM644065	*Arrhenatherum elatius*	Germany
Halobasidium umbonatum	JS-40	C	JF706656	Flowers/plants	Korea
Halobasidium umbonatum	**MT254^{T}**	C	**MK990658**	*Crataegus* sp. with *Gymnosporangium* sp.	USA
Halobasidium xiangyangense	62134	C	KY986543	Skin scales	Brazil

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Halobasidium xiangyangense	HBUAS51001	C	MH209248	Datoucai	China
Halobasidium xiangyangense	KSS-2008	C	FJ238090	Solar saltern	Korea
Halobasidium xiangyangense	BI218	C	FJ865356	Unknown	Korea
Halobasidium xiangyangense	P2S-PDA	C	KT819328	Seaweeds	New Zealand
Halobasidium xiangyangense	GY1131128PS	C	LC272869	Seawater	Republic of Korea
Halobasidium xiangyangense	**SA444**	C	**MK990660**	*Dryopteris erythrosora*	USA
Halobasidium xiangyangense	**RK086**	C	**MK990659**	Watermelon Skin	USA
Halobasidium xiangyangense	GDMCC2.231^{T}	C	MH209248	Pickling sauce	China
Hasegawazyma lactosa	DBMY268	C	KJ706485	Mangrove sediments	India
Hasegawazyma lactosa	DBMY427	C	KJ706644	Mangrove sediments	India
Hasegawazyma lactosa	DBMY586	C	KJ706803	Mangrove sediments	India
Hasegawazyma lactosa	DBMY745	C	KJ706962	Mangrove sediments	India
Hasegawazyma lactosa	SN34	C	FJ515187	Seawater	Taiwan
Hasegawazyma lactosa	UN1	C	FJ515208	Seawater	Taiwan

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Hasegawazyma lactosa	UN1	C	FJ515263	Seawater	Taiwan
Hasegawazyma lactosa	ATT258	C	FJ743626	*Atta texana* fungus garden in nest	USA
Hasegawazyma lactosa	CBS5826^{T}	C	AF444540	Air of dairies and cowsheds	Japan
Hasegawazyma sp.	EJ	C	GQ372843	Endophyte: Seeds and roots of *Dendrobium candidum*	China
Naohidea sebacea	CBS8477^{T}	C	DQ911616	*Botryosphaeria. quercuum*, *Botryodiplodia* sp.	USA
Occultifur bloemfonteinensis	CHAC201	C	KY931516	Polluted soil	China
Occultifur bloemfonteinensis	CBS10219	C	EU002838	Phylloplane	Portugal
Occultifur bloemfonteinensis	CBS8598^{T}	C	EU075189	Pine tree	South Africa
Occultifur hainanensis	CICC33066	C	KU296947	*Oryza sativa*	China
Occultifur hainanensis	CBS14092^{T}	C	KU296948	Oryza sativa	China
Occultifur internus	**KM068754**	S	**MK990680**	*Hyphoderma argillaceum*, Dacrymycetales	England

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Occultifur internus	**KM094773**	S	**MK99068**1	*Hyphoderma argillaceum*, Dacrymycetales	England
Occultifur internus	3_81	UC	KF274414	*Picea abies* stumps	Finland
Occultifur internus	T1646	UC	KU948779	*Cetraria ericetorum*	USA
Occultifur internus	T1615	UC	KU948766	*Cladonia multiformis*	USA
Occultifur magnisporus	IP029	C	AB726290	Marine sediments/Plants	Japan
Occultifur magnisporus	NIP008	C	AB726599	Unknown	Japan
Occultifur magnisporus	NIP020	C	AB726606	Unknown	Japan
Occultifur magnisporus	FK93	C	AB111954	Phylloplane	Taiwan
Occultifur magnisporus	TY-212	C	AY313068	Phylloplane	Thailand
Occultifur magnisporus	PisoRtLf1761	UC	JX242401	Rotting leaves of *Pisonia umbellifera*	--------
Occultifur magnisporus	JCM11898^{T}	C	AB112077	*Miscanthus floridulus* and *Saurauia oldhamii*	Taiwan
Occultifur mephitis	RS106	C	AB727268	Marine sediments/Plants	Japan
Occultifur orientalis	ATCCMYA-4818	C	JN874514	Unknown	USA

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Occultifur orientalis	CBS8594^{T}	C	HM559719	Soil of Eucapyptus plantation	Malaysia
Occultifur pini	APBSWTPF132	C	MG569687	Cultivable from aerosol samples	India
Occultifur pini	DBMY287	C	KJ706504	Mangrove sediments	India
Occultifur pini	DBMY446	C	KJ706663	Mangrove sediments	India
Occultifur pini	DBMY605	C	KJ706822	Mangrove sediments	India
Occultifur pini	DBMY764	C	KJ706981	Mangrove sediments	India
Occultifur pini	CBS10735^{T}	C	NR_120007	Pine leaf	South Africa
Occultifur sp.	10.10.L31	C	KU057818	Lichen	Antarctica
Occultifur sp.	SW005_H11	UC	KP889342	Soil	Canada
Occultifur sp.	SG042_F09	UC	KP889526	Soil	Canada
Occultifur sp.	NIP038	C	AB726620	Plant/Soil	Japan
Occultifur sp.	OTU461	UC	KF617486	*Picea mariana* forest soil organic horizon	USA
Occultifur sp.	OTU341	UC	KF617929	*Picea mariana* forest soil organic horizon	USA
Occultifur sp.	C31_A02	UC	EU490032	savanna soil (0 to 10 cm) under C3 perennial grasses	USA

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Occultifur sp.	OTU97-86	UC	JQ311670	Soil	USA
Pseudoccultifur brasiliensis	CBS12687^{T}	C	KC698874	water tanks of Vriesea minarum	Brazil
Pseudoccultifur brasiliensis	UFMG-ABT396	C	KM235221	Bromeliad water phytotelm	Brazil
Pseudoccultifur brasiliensis	UFMG-BRO-170	C	KC698874	*Vriesea minarum* (Bromeliaceae)	Brazil
Pseudoccultifur corticiorum	KM33238^{T}	C	--------	*Hyphoderma praetermissum, Peniophorella praetermissa*	United Kingdom
Pseudoccultifur externus	A19	C	KM246006	Coffee plants	Brazil
Pseudoccultifur externus	AM02	C	KM246014	Coffee plants	Brazil
Pseudoccultifur externus	AM08	C	KM246018	Coffee plants	Brazil
Pseudoccultifur externus	AM09	C	KM246019	Coffee plants	Brazil
Pseudoccultifur externus	AM14	C	KM246025	Coffee plants	Brazil
Pseudoccultifur externus	M02	C	KM246123	Coffee plants	Brazil
Pseudoccultifur externus	MG14	C	KM246130	Coffee plants	Brazil
Pseudoccultifur externus	MG17	C	KM246132	Coffee plants	Brazil

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Pseudoccultifur externus	MG18	C	KM246133	Coffee plants	Brazil
Pseudoccultifur externus	Bd25	C	KP731462	Stems and flowers of *Baccharis dracunculifolia*	Brazil
Pseudoccultifur externus	UCDFST68-934.2	C	KU609481	Exudate from Maple tree *Acer* sp.	Canada
Pseudoccultifur externus	YM25246	C	KT345338	Lake water	China
Pseudoccultifur externus	CBS8732^{T}	C	AF444567	Plant litter	Portugal
Pseudoccultifur externus	JYC1082	C	KM555203	*Forcipomia taiwana*	Taiwan
Pseudoccultifur externus	DMKU-SP214	C	LC053873	Leave of Sugarcane (Saccharum officinarum)	Thailand
Pseudoccultifur kilbournensis	BEL22	C	KX356666	Macrophyte	Brazil
Pseudoccultifur kilbournensis	TAYL-4	UC	KC442922	Fruit surface	China
Pseudoccultifur kilbournensis	10S10C35	UC	HG937091	*Zea mays* field bulk soil	Germany
Pseudoccultifur kilbournensis	NCAIMY.02205	C	MG250367	*Zea mays* field bulk soil	Hungary
Pseudoccultifur kilbournensis	FF11	UC	AY464846	Cow rumen	Korea
Pseudoccultifur kilbournensis	MWGM3T0_4H	UC	EU692384	Rhizosphere of *Picea glauca* (white spruce)	USA

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Pseudoccultifur kilbournensis	GY113279PS	C	LC272898	Seawater	Republic of Korea
Pseudoccultifur kilbournensis	29-16-13	UC	EF505781	Leaves, stems, kernels of *Zea mays*	USA
Pseudoccultifur kilbournensis	37-18-67	UC	EF505783	Leaves, stems, kernels of *Zea mays*	USA
Pseudoccultifur kilbournensis	NRRLY-12747	C	KP413161	Macrophyte	USA
Pseudoccultifur kilbournensis	JJP-2009a	C	FJ210641	*Zea mays*	USA
Pseudoccultifur kilbournensis	NRRLY-63695	C	KP413162	*Zea mays*	USA
Pseudoccultifur kilbournensis	CBS13982^{T}	C	KP413162	*Zea mays*	USA
Pseudoccultifur lumbricifer	KM33234^{T}	C	--------	*Hyphodonlia subalutacea*	Scotland
Pseudoccultifur mephitis	PYCC6067	C	KY308183	High CO2 meadow mofette soil	Slovenia
Pseudoccultifur mephitis	EXF-6436	C	LT594852	High CO2 meadow mofette soil	Slovenia
Pseudoccultifur mephitis	EXF-6437	C	LT594853	High CO2 meadow mofette soil	Slovenia
Pseudoccultifur mephitis	EXF-6473	C	LT594863	High CO2 meadow mofette soil	Slovenia
Pseudoccultifur mephitis	CBS10223	C	EU002842	Leaf of *Cistus albidus*	Slovenia
Pseudoccultifur mephitis	CBS14611^{T}	C	KX929055	High CO2 meadow mofette soil	Slovenia

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Pseudoccultifur plantarum	CBS14576	C	KY305118	Bromeliad	Brazil
Pseudoccultifur plantarum	IMUFRJ51954	C	FN424101	*Neoreglia cruenta*	Brazil
Pseudoccultifur plantarum	IMUFRJ52018	C	FN428895	Sugar cane leaf	Brazil
Pseudoccultifur plantarum	IMUFRJ52019	C	FN428901	Sugar cane leaf	Brazil
Pseudoccultifur plantarum	DBTIOC-ML3	C	KJ528561	Magrove soil	India
Pseudoccultifur plantarum	IPM31-24	C	AB726366	Unknown	Japan
Pseudoccultifur plantarum	DMKU-CE36	C	LC158344	Corn leaf (Zea mays L.)	Thailand
Pseudoccultifur plantarum	DMKU-SE24	C	LC158345	Sugar cane leaf	Thailand
Pseudoccultifur plantarum	DMKU-SE45	C	LC158346	Sugar cane leaf	Thailand
Pseudoccultifur plantarum	DMKU-SE129	C	LC158347	Sugar cane leaf	Thailand
Pseudoccultifur plantarum	DMKU-SE134	C	LC158348	Sugar cane leaf	Thailand
Pseudoccultifur plantarum	CBS14554^{T}	C	LC158346	Sugarcane leaf tissue and corn leaf tissue	Thailand
Pseudoccultifur plantarum	FLITS10F09	UC	KF675690	Soil	USA
Pseudoccultifur rivoirei	LYBR-6532T	C	--------	*Hyphodontia arguta*	France

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Pseudoccultifur sp.	PYCC4823	C	AF444642	Unknown	--------
Pseudoccultifur sp.	UFMG-ABT396	C	KM527117	Bromeliad water	Brazil
Pseudoccultifur sp.	BEL107	C	KU854927	Macrophyte	Brazil
Pseudoccultifur sp.	BEL99	C	KX356664	Macrophyte	Brazil
Pseudoccultifur sp.	BEL41	C	KX356665	Macrophyte	Brazil
Pseudoccultifur sp.	UFMG-CM-Y376	C	KM248526	*Vriesea minarum* (Bromeliaceae)	Brazil
Pseudoccultifur sp.	UCDFST68-934.2	C	KU609539	Exudate from Maple tree Acer sp.	Canada
Pseudoccultifur sp.	BF-OTU490	UC	FR682233	House dust	Finland
Pseudoccultifur sp.	10S10C37	UC	HG937089	*Zea mays* field bulk soil	Germany
Pseudoccultifur sp.	EXF-6482	C	LT594867	High CO2 meadow mofette soil	Slovenia
Pseudoccultifur tropicalis	SC17d100p18-12	C	HQ631017	*Saccharum officinarum*	--------
Pseudoccultifur tropicalis	UFMG-CM-Y6349	C	MG832887	Bromeliad tank of Vriesea minarum	Brazil
Pseudoccultifur tropicalis	IMUFRJ52020	C	FN428928	Sugar cane leaf	Brazil
Pseudoccultifur tropicalis	CBS13389^{T}	C	AB921282	Sugar cane	Brazil and Thailand

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Pseudoccultifur tropicalis	16l2n11	UC	KJ461429	Indoor environment air	Republic of Korea
Pseudoccultifur tropicalis	DMKU-SE59	C	AB921280	Leave of Sugarcane (Saccharum officinarum)	Thailand
Pseudoccultifur tropicalis	DMKU-SE38	C	AB921283	Leave of Sugarcane (Saccharum officinarum)	Thailand
Pseudoccultifur tropicalis	DMKU-SP385	C	AB924026	Leave of Sugarcane (Saccharum officinarum)	Thailand
Sakaguchia cladiensis	CBS10878^{T}	C	FJ008055	sawgrass (*Cladium jamaicense*)	USA
Sakaguchia dacryoidea	CBS6357	C	KY105303	Unknown	--------
Sakaguchia dacryoidea	CBS7142	C	KY109527	Water	--------
Sakaguchia dacryoidea	CBS6355	C	KY105300	Seawater	Antarctica
Sakaguchia dacryoidea	CBS6356	C	KY105302	Seawater	Antarctica
Sakaguchia dacryoidea	Clone186	C	KF488791	wild *Cilus gilberti* (Fish)/Salmonids	Chile
Sakaguchia dacryoidea	S13T_85	UC	KU163855	Seawater	China
Sakaguchia dacryoidea	S211T_35	UC	KU164039	Seawater	China
Sakaguchia dacryoidea	S21T_36	UC	KU164098	Seawater	China

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Sakaguchia dacryoidea	S23T_35	UC	KU164241	Seawater	China
Sakaguchia dacryoidea	S23T_40	UC	KU164246	Seawater	China
Sakaguchia dacryoidea	S24T_28	UC	KU164298	Seawater	China
Sakaguchia dacryoidea	S31T_39	UC	KU164446	Seawater	China
Sakaguchia dacryoidea	S44T_20	UC	KU164584	Seawater	China
Sakaguchia dacryoidea	S44T_78	UC	KU164608	Seawater	China
Sakaguchia dacryoidea	DBMY297	C	KJ706514	Mangrove sediments	India
Sakaguchia dacryoidea	DBMY456	C	KJ706673	Mangrove sediments	India
Sakaguchia dacryoidea	DBMY615	C	KJ706832	Mangrove sediments	India
Sakaguchia dacryoidea	DBMY774	C	KJ706991	Mangrove sediments	India
Sakaguchia dacryoidea	A41	C	AF485998	Seawater	Portugal
Sakaguchia dacryoidea	JCM3795^{T}	C	AF444597	Sea water, at 1738 depth	USA
Sakaguchia lamellibrachiae	S46T_124	UC	KU164637	Seawater	China
Sakaguchia lamellibrachiae	CBS9598^{T}	C	AB025999	Tube worm, *Lamellibrachia* sp.	Japan

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Sakaguchia meli	CRUB1032	C	AY158654	Glacial Lagoon,	Argentina
Sakaguchia meli	E2e2624	UC	KF750497	Soil	USA
Sakaguchia meli	CBS10797^{T}	C	FJ807683	Fresh water	Argentina
Sakaguchia oryzae	AS2.3289	C	KJ708353	Unknown	--------
Sakaguchia oryzae	BPASol1T8_6B	UC	EU691645	Rhizosphere of *Picea glauca* (white spruce)	Canada
Sakaguchia oryzae	CBS9745^{T}	C	AY335160	paddy rice	Japan
Sakaguchia sp.	JCM8162	C	KJ778625	Unknown	--------
Sakaguchia sp.	MARY063	C	AY731725	Seafloor and sub-Seafloor	Artic
Sakaguchia sp.	MARY297	C	AY731726	Seafloor and sub-Seafloor	Artic
Sakaguchia sp.	G3	C	JN636811	epiphytic yeasts associated with strawberry	Belgium
Sakaguchia sp.	NA2_P32_J21	UC	KC965397	Soil	Canada
Sakaguchia sp.	ZBJ201308-68	UC	KX515421	Rainwater	China
Sakaguchia sp.	N104T_250	UC	GU941203	Seawater	China
Sakaguchia sp.	S46T_89	UC	KU164673	Seawater	China

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Sakaguchia sp.	BAF25	UC	AY761181	Surface of a beetle	China
Sakaguchia sp.	M6D-10	UC	HE605258	black stain on cave sediment	France
Sakaguchia sp.	CBS7999	C	KY109529	Brewery	France
Sakaguchia sp.	SY-298	C	AB263120	*Calyptogena* sp.	Japan
Sakaguchia sp.	CBS5143	C	AF387145	Bronchial secretion	Netherlands
Sakaguchia sp.	Z4S-1-PDA	C	KT819324	Unknown	New Zealand
Sakaguchia sp.	SY-153	C	AB263119	Sediment near hydrothermal vents	Papua New Guinea
Sakaguchia sp.	PYCC6740	C	KU171058	Seawater	Portugal
Sakaguchia sp.	PYCC6745	C	KU171060	Seawater	Portugal
Sakaguchia sp.	PYCC6741	C	KU171062	Seawater	Portugal
Sakaguchia sp.	PYCC6746	C	KU171064	Seawater	Portugal
Sakaguchia sp.	PYCC6747	C	KU171066	Seawater	Portugal
Sakaguchia sp.	PYCC6748	C	KU171068	Seawater	Portugal
Sakaguchia sp.	PYCC6749	C	KU171070	Seawater	Portugal

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Sakaguchia sp.	PYCC6750	C	KU171072	Seawater	Portugal
Sakaguchia sp.	**MCA7921**	C	**MK990679**	Unknown	Puerto Rico
Sakaguchia sp.	UPSC_D1_37	UC	GU564992	*Populus tremula* fungal endophyte	Sweden
Sakaguchia sp.	GY28L06	C	FJ527100	Phylloplane	Taiwan
Sakaguchia sp.	MTW10.1	C	LC435582	water	Thailand
Sakaguchia sp.	11_37	UC	KM374342	Soil	United Kingdom
Sakaguchia sp.	11_28	UC	KM374368	Soil	United Kingdom
Sakaguchia sp.	**MCA7812A**	C	**MK990678**	Crabapple unsymptomatic leaf	USA
Sakaguchia sp.	OTU340	UC	KF617436	*Picea mariana* forest soil organic horizon	USA
Sakaguchia sp.	PIMO_114	C	JF705938	*Pinus monticola*	USA
Sakaguchia warrensis	**SFJD10^{T}**	C	**MK990682**	Air sampling 50% Glucose	USA
Sakaguchia warrensis	CMH332	UC	KF800423	House dust	USA
Sakkaguchia sp.	OTU749	UC	KF617787	*Picea mariana* forest soil organic horizon	USA
Sakkaguchia sp.	OTU97-218	UC	JQ310961	Soil	USA

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Sakaguchia sp.	OTU97-915	UC	JQ311732	Soil	USA
Sterigmatomyces hyphaenes	CBS7811^{T}	C	AF444553	*Trichopteryx nigritiana*	Congo
Symmetrospora sp.	**SA308**	C	**KJ701225**	*Dryopteris erythrosora*	USA
Symmetrospora sp.	**SA333**	C	**KJ701228**	*Lygodium japonicum*	USA
Symmetrospora sp.	**WRP07**	C	**KJ701231**	*Quercus virginiana*	USA
Symmetrospora sp.	**WRP08**	C	**KJ701234**	*Salix* sp.	USA
Symmetrospora sp.	**INDP057**	C	--------	*Pinus nigra*	USA
Symmetrospora sp.	**INDP060**	C	--------	*Pinus nigra*	USA
Symmetrospora sp.	**SA337**	C	--------	Unknown	USA
Symmetrospora aff. Oryzicola	**MCA6805**	C	--------	FABACEAE - *Vigna* sp.	Russia
Symmetrospora aff. Symmetrica	**RAK95**	C	--------	Unknown	--------
Symmetrospora aff. Symmetrica	**RAKYE1**	C	--------	Unknown	--------
Symmetrospora aff. Symmetrica	**S/N039**	C	--------	Unknown	USA
Symmetrospora aff. Symmetrica	**INDP053**	C	--------	*Bidens* sp.	USA

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Symmetrospora aff. Symmetrica	**INDP53W**	C	--------	*Bidens* sp.	USA
Symmetrospora aff. Symmetrica	**INDP53Y**	C	--------	*Bidens* sp.	USA
Symmetrospora coprosmae	SW_2d_A09	UC	JF449781	Beech litter	Austria
Symmetrospora coprosmae	HB1219	C	AM160645	beetle gut of *Diabrotica virgifera*	Austria
Symmetrospora coprosmae	HB1056	C	AJ510199	sea surface microlayer and underlying water	Austria
Symmetrospora coprosmae	AsA5.1	C	KP100170	*Aphis spiraecola*	Belgium
Symmetrospora coprosmae	C18	C	JQ936284	Leaves soybean	Brazil
Symmetrospora coprosmae	C67	C	JQ936285	Leaves soybean	Brazil
Symmetrospora coprosmae	C21	C	JQ936286	Leaves soybean	Brazil
Symmetrospora coprosmae	C91.2	C	JQ936288	Leaves soybean	Brazil
Symmetrospora coprosmae	CAP11A	C	JQ936332	Leaves soybean	Brazil
Symmetrospora coprosmae	YM25172	C	KT345332	Lake water	China
Symmetrospora coprosmae	DQ447767	C	DQ447767	Leaves of *Gossypium hirsutum*	China
Symmetrospora coprosmae	XJ13E2	C	HE650893	Phylloplane	China

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Symmetrospora coprosmae	OUTPUC1	UC	KJ188588	mycorrhizal root *Neottia ovata*	Czech Republic
Symmetrospora coprosmae	F0210-53Y4	C	HG008765	Air sampling	France
Symmetrospora coprosmae	LX042767-122-058-A06	UC	GU054299	Air filter sample	Germany
Symmetrospora coprosmae	LX042767-122-058-A11	UC	GU054304	Air filter sample	Germany
Symmetrospora coprosmae	LX042767-122-058-B11	UC	GU054315	Air filter sample	Germany
Symmetrospora coprosmae	LX042767-122-058-F11	UC	GU054323	Air filter sample	Germany
Symmetrospora coprosmae	LX042767-122-058-G06	UC	GU054330	Air filter sample	Germany
Symmetrospora coprosmae	LX042767-122-058-G07	UC	GU054331	Air filter sample	Germany
Symmetrospora coprosmae	L046973-122-077-B07-unis	UC	JF289161	Air filter sample	Germany
Symmetrospora coprosmae	S210	UC	FJ820698	Air sampling	Germany
Symmetrospora coprosmae	DSM101926	C	KX067833	*Phaseolus vulgaris* and *Vicia faba*	Germany
Symmetrospora coprosmae	DBMY302	C	KJ706519	Mangrove sediments	India
Symmetrospora coprosmae	DBMY314	C	KJ706531	Mangrove sediments	India
Symmetrospora coprosmae	DBMY461	C	KJ706678	Mangrove sediments	India

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Symmetrospora coprosmae	DBMY473	C	KJ706690	Mangrove sediments	India
Symmetrospora coprosmae	DBMY620	C	KJ706837	Mangrove sediments	India
Symmetrospora coprosmae	DBMY632	C	KJ706849	Mangrove sediments	India
Symmetrospora coprosmae	DBMY779	C	KJ706996	Mangrove sediments	India
Symmetrospora coprosmae	DBMY791	C	KJ707008	Mangrove sediments	India
Symmetrospora coprosmae	OTU317	UC	KT328631	Coffee leaf infected by rust fungus (*Hemileia vastatrix*)	Mexico
Symmetrospora coprosmae	MSRY_15	C	KY952845	Rhizosphere	Mexico
Symmetrospora coprosmae	MSRY_45	C	KY952851	Rhizosphere	Mexico
Symmetrospora coprosmae	CBS7899^{T}	C	AF444577	Dead leaf of *Coprosma tenuifolia*	New Zealand
Symmetrospora coprosmae	Cohort_18-1	C	JN377457	*Vitis vinifera*	New Zealand
Symmetrospora coprosmae	CBS10201	C	EU002822	Phylloplane	Portugal
Symmetrospora coprosmae	CBS10202	C	EU002823	Phylloplane	Portugal
Symmetrospora coprosmae	38140	C	KT933355	Overwintering grape	Slovakia
Symmetrospora coprosmae	0210LASC53Y-4	C	FR799501	Air from a cave	Spain

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Symmetrospora coprosmae	I-Y365b	C	GU585171	Xylem sap from *Vitis vinifera*	Switzerland
Symmetrospora coprosmae	JCM8772	C	AB030343	Phylloplane	Thailand
Symmetrospora coprosmae	121	C	JN544044	Phylloplane	Taiwan
Symmetrospora coprosmae	C105NR30	UC	KT388147	Corn leaf (Zea mays L.)	Thailand
Symmetrospora coprosmae	R65NP-19	UC	KJ937770	*Oryza sativa*	Thailand
Symmetrospora coprosmae	HU-9256	C	MG589167	Commercial Romaine lettuce	USA
Symmetrospora coprosmae	HU-9059	C	MG589205	Commercial Romaine lettuce	USA
Symmetrospora coprosmae	**MT264**	C	**KJ701205**	FABACEAE - *Vigna* sp.	USA
Symmetrospora coprosmae	**P116**	C	**KJ701203**	Leaf of *Pinus nigra*	USA
Symmetrospora coprosmae	**MT262**	C	**KJ701199**	Melampsora sp.	USA
Symmetrospora coprosmae	**P116**	C	**KJ701202**	*Pinus nigra*	USA
Symmetrospora coprosmae	**MT236**	C	--------	*Puccinia smilax* leaf	USA
Symmetrospora coprosmae	P34C004	C	JX188240	*Vitis vinifera*	USA
Symmetrospora foliicola	CBS8075^{T}	C	AF444521	Leaf of *Banksia collina*	Australia

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Symmetrospora foliicola	TA205	C	JQ219310	Apple fruit surface	China
Symmetrospora foliicola	YG19	C	MF045463	Apple fruit surface	China
Symmetrospora foliicola	DBMY469	C	KJ706686	Mangrove sediments	India
Symmetrospora foliicola	DBMY628	C	KJ706845	Mangrove sediments	India
Symmetrospora foliicola	DBMY787	C	KJ707004	Mangrove sediments	India
Symmetrospora foliicola	DBMY310	C	--------	Mangrove sediments	India
Symmetrospora foliicola	CAB564	C	KR995805	Seawater	South Africa
Symmetrospora foliicola	CAB472	C	KR995806	Seawater	South Africa
Symmetrospora foliicola	CAB1355	C	KR995807	Seawater	South Africa
Symmetrospora foliicola	CAB1529	C	KR995808	Seawater	South Africa
Symmetrospora foliicola	CAB1837	C	KR995809	Seawater	South Africa
Symmetrospora foliicola	CMH434	UC	KF800525	Indoor air	USA
Symmetrospora gracilis	CBS71^{T}	C	AF444578	*Phertinia glabra*	Australia
Symmetrospora gracilis	PCR79	C	KY436124	Roots of *Populus* sp.	Czech Republic

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Symmetrospora gracilis	Ms28Mb91	UC	AJ875378	*Phragmites australis*	Germany
Symmetrospora gracilis	DBMY301	C	KJ706518	Mangrove sediments	India
Symmetrospora gracilis	DBMY460	C	KJ706677	Mangrove sediments	India
Symmetrospora gracilis	DBMY619	C	KJ706836	Mangrove sediments	India
Symmetrospora gracilis	DBMY778	C	KJ706995	Mangrove sediments	India
Symmetrospora gracilis	JCM8771	C	AB178481	Dead leaf of *Coprosma tenuifolia*	New zealand
Symmetrospora gracilis	CBS10197	C	EU002819	Phylloplane	Portugal
Symmetrospora gracilis	CBS10198	C	EU002820	Phylloplane	Portugal
Symmetrospora marina	A31	C	KM246010	Coffee plants	Brazil
Symmetrospora marina	IMUFRJ52027	C	FN428896	Sugar cane leaf	Brazil
Symmetrospora marina	HAI-Y-011	C	JX901092	Unknown	Israel
Symmetrospora marina	fn_58	C	LC333515	Floral nectar	Japan
Symmetrospora marina	2Y186	C	KY744115	Seawater	Qatar
Symmetrospora marina	2Y190A	C	KY744116	Seawater	Qatar

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Symmetrospora marina	QCC/Y22/17	C	KY744133	Seawater	Qatar
Symmetrospora marina	QCC/Y23/17	C	KY744134	Seawater	Qatar
Symmetrospora marina	36955	C	AF515477	Seawater	Taiwan
Symmetrospora marina	183	C	JN544039	Unknown	Taiwan
Symmetrospora marina	CBS2365	C	KY105572	Crusta	USA
Symmetrospora marina	CBS2365	C	KY109809	Crusta	USA
Symmetrospora marina	CBS2365^{T}	C	AF444504	*Peneaus setiferusn* (Shrimp)	USA
Symmetrospora oryzicola	CBS7228^{T}	C	AF444546	Dead leaf of *Oryza sativa*	Japan
Symmetrospora oryzicola	F2-O6	UC	JX984698	Urban air sampling	Republic of Korea
Symmetrospora oryzicola	JCM5399	C	AB030349	Phylloplane	Thailand
Symmetrospora oryzicola	**MCA4505**	C	--------	4443, unk path on willow like tree	Taiwan
Symmetrospora oryzicola	**MCA4496**	C	**KJ701193**	FABACEAE - *Vigna* sp.	Taiwan
Symmetrospora oryzicola	**MCA4497**	C	**KJ701196**	FABACEAE - *Vigna* sp.	Taiwan
Symmetrospora sp.	LB68_1	C	KJ159055	*Atta sexdens* nest	Brazil

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Symmetrospora sp.	BPT209	C	KY305116	Bromeliad	Brazil
Symmetrospora sp.	UFMG-ABT670	C	KM527125	Bromeliad water phytotelm	Brazil
Symmetrospora sp.	IMUFRJ52025	C	FN428894	Sugar cane leaf	Brazil
Symmetrospora sp.	IMUFRJ52026	C	FN428925	Sugar cane leaf	Brazil
Symmetrospora sp.	BI217	C	EU678948	Unknown	Brazil
Symmetrospora sp.	------	C	BSU65599	*Bashania fargesii*	China
Symmetrospora sp.	------	C	BSU65598	*Sasa variegata*	China
Symmetrospora sp.	S13T_49	UC	KU163841	Seawater	China
Symmetrospora sp.	S13T_69	UC	KU163849	Seawater	China
Symmetrospora sp.	S13T_93	UC	KU163857	Seawater	China
Symmetrospora sp.	S14T_75	UC	KU163891	Seawater	China
Symmetrospora sp.	S16T_94	UC	KU163938	Seawater	China
Symmetrospora sp.	S23T_33	UC	KU164240	Seawater	China
Symmetrospora sp.	S23T_77	UC	KU164270	Seawater	China

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Symmetrospora sp.	S24T_06	UC	KU164287	Seawater	China
Symmetrospora sp.	S24T_53	UC	KU164312	Seawater	China
Symmetrospora sp.	S32T_62	UC	KU164379	Seawater	China
Symmetrospora sp.	S34T_03	UC	KU164402	Seawater	China
Symmetrospora sp.	S43T_09	UC	KU164515	Seawater	China
Symmetrospora sp.	S43T_79	UC	KU164559	Seawater	China
Symmetrospora sp.	S44T_103	UC	KU164574	Seawater	China
Symmetrospora sp.	S44T_69	UC	KU164605	Seawater	China
Symmetrospora sp.	S44T_92	UC	KU164613	Seawater	China
Symmetrospora sp.	S52T_22	UC	KU164703	Seawater	China
Symmetrospora sp.	rs-jx40	UC	AB711991	Soil	China
Symmetrospora sp.	rs-jx51	UC	AB712002	Soil	China
Symmetrospora sp.	**MCA6148**	C	--------	*Uredo cajani* infected leaf	Jamaica
Symmetrospora sp.	JS-14	C	JF706653	flower of *Forsythia koreana*	Republic of Korea

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Symmetrospora sp.	OTU126	UC	KT328795	Coffee leaf infected by rust fungus (*Hemileia vastatrix*)	Mexico
Symmetrospora sp.	CBS10199	C	EU002878	Phylloplane	Portugal
Symmetrospora sp.	2Y207	C	KY744117	Seawater	Qatar
Symmetrospora sp.	QCC/Y24/17	C	KY744135	Seawater	Qatar
Symmetrospora sp.	YY49	C	KR912282	Pear surface	South Africa
Symmetrospora sp.	SM10	C	FJ515188	sea surface microlayer and underlying water	Taiwan
Symmetrospora sp.	TY-246	C	AY313075	Phylloplane	Thailand
Symmetrospora sp.	DMKU5-4	C	LC216897	Sea sponge	Thailand
Symmetrospora sp.	DMKU-SP200	C	AB826440	Sugar cane leaf	Thailand
Symmetrospora sp.	DMKU-SP213	C	AB826441	Sugar cane leaf	Thailand
Symmetrospora sp.	DMKU-SE44	C	LC176964	Sugar cane leaf	Thailand
Symmetrospora sp.	DMKU-SE130	C	LC177043	Sugar cane leaf	Thailand
Symmetrospora sp.	**MASI_BG9_1**	C	--------	Unknown	USA
Symmetrospora sp.	**MASI_BG9_4_2**	C	--------	Unknown	USA

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Symmetrospora sp.	**SA716**	C	**KJ701216**	*Dryopteris erythrosora*	USA
Symmetrospora sp.	CMH175	UC	KF800266	House dust	USA
Symmetrospora sp.	**P109**	C	--------	*Pinus nigra*	USA
Symmetrospora sp.	**P114**	C	**KJ701219**	*Taxus cuspidata*	USA
Symmetrospora sp.	**P115**	C	**KJ701222**	*Taxus cuspidata*	USA
Symmetrospora sp.	**SA042**	C	**KJ701213**	Unknown	USA
Symmetrospora sp.	BG02-5-27-3-2-2	C	AY520389	Gut of staphylinid beetle	USA
Symmetrospora sp.	**MCA7675**	C	--------	Unknown	Vanuatu
Symmetrospora sp.	**MCA7676**	C	--------	Unknown	Vanuatu
Symmetrospora symmetrica	JZB2120003	C	KF314801	Fruiting body *Pleurotus eryngii*	China
Symmetrospora symmetrica	CB64	C	AY364836	Phylloplane	China
Symmetrospora symmetrica	ZBJ201207-44	UC	KX514954	Rainwater	China
Symmetrospora symmetrica	ZSH201206-45	UC	KX515540	Rainwater	China
Symmetrospora symmetrica	ZSH201207-16	UC	KX515562	Rainwater	China

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Symmetrospora symmetrica	S12T_03	UC	KU163787	Seawater	China
Symmetrospora symmetrica	S12T_46	UC	KU163803	Seawater	China
Symmetrospora symmetrica	S21T_19	UC	KU164087	Seawater	China
Symmetrospora symmetrica	S46T_11	UC	KU164633	Seawater	China
Symmetrospora symmetrica	S54T_96	UC	KU164724	Seawater	China
Symmetrospora symmetrica	FP-027-B6	C	MH102094	Talli of *Fucus* sp. (seaweed)	Netherlands
Symmetrospora symmetrica	CBS10192	C	EU002816	Phylloplane	Portugal
Symmetrospora symmetrica	CBS10196	C	EU002818	Phylloplane	Portugal
Symmetrospora symmetrica	**P118**	C	**KJ701210**	*Pinus nigra*	USA
Symmetrospora symmetrica	**SA107**	C	**KJ701207**	Unknown	USA
Symmetrospora symmetrica	CBS9727^{T}	C	AY364836	From wilting leaves of *Betula platyphylla*	China
Symmetrospora vermiculata	S42T_46	UC	KU164500	Seawater	China
Symmetrospora vermiculata	JCM10221	C	AB030332	Phylloplane	Thailand
Symmetrospora vermiculata	JCM10222	C	AB030333	Phylloplane	Thailand

SPECIES	STRAIN	TYPE	ACCESSION NUMBER	HOST/SUBSTRATE	COUNTRY/ LOCATION
Symmetrospora vermiculata	JCM10223	C	AB030334	Phylloplane	Thailand
Symmetrospora vermiculata	DMKU-CP558	C	LC430216	Corn leaf (*Zea mays*)	Thailand
Symmetrospora vermiculata	TY-209	C	AY313067	Phylloplane	Thailand
Symmetrospora vermiculata	S35PN09	UC	KM114661	Sugar cane leaf	Thailand
Symmetrospora vermiculata	S41PB11	UC	KM114665	Sugar cane leaf	Thailand
Symmetrospora vermiculata	11SC20	UC	KP866688	Sugar cane leaf	Thailand
Symmetrospora vermiculata	12SC21	UC	KP866689	Sugar cane leaf	Thailand
Symmetrospora vermiculata	DMKU-SE60	C	LC176978	Sugar cane leaf	Thailand
Symmetrospora vermiculata	DMKU-SE75	C	LC176993	Sugar cane leaf	Thailand
Symmetrospora vermiculata	CBS9092^{T}	C	AB030335	Dead leaf of *Pennisetum pediocellatum*	Thailand

Note: C: Cultured fungus: Strain in private or public culture collection. UC: Uncultured fungus: Environmental sampling S: Specimen. Type strain species for each genus appears in bold. Accession numbers in bold were generated in The Aime Lab. Other accession numbers were retrieved from the NCBI.

Figure A 1 Phylogenetic reconstruction for *Bannoa* inferred from combined sequences of ITS, D1–D2 domains of nuclear 28S and nuclear 18S rDNA using ML and BI analysis. New species indicated in bold. Posterior Probability (PP > 0.7) and Bootstrap support (BP > 50%) values are shown above nodes. ns: not supported. Bar 0.01 substitutions per nucleotide position. ^{T} denotes type species strain.

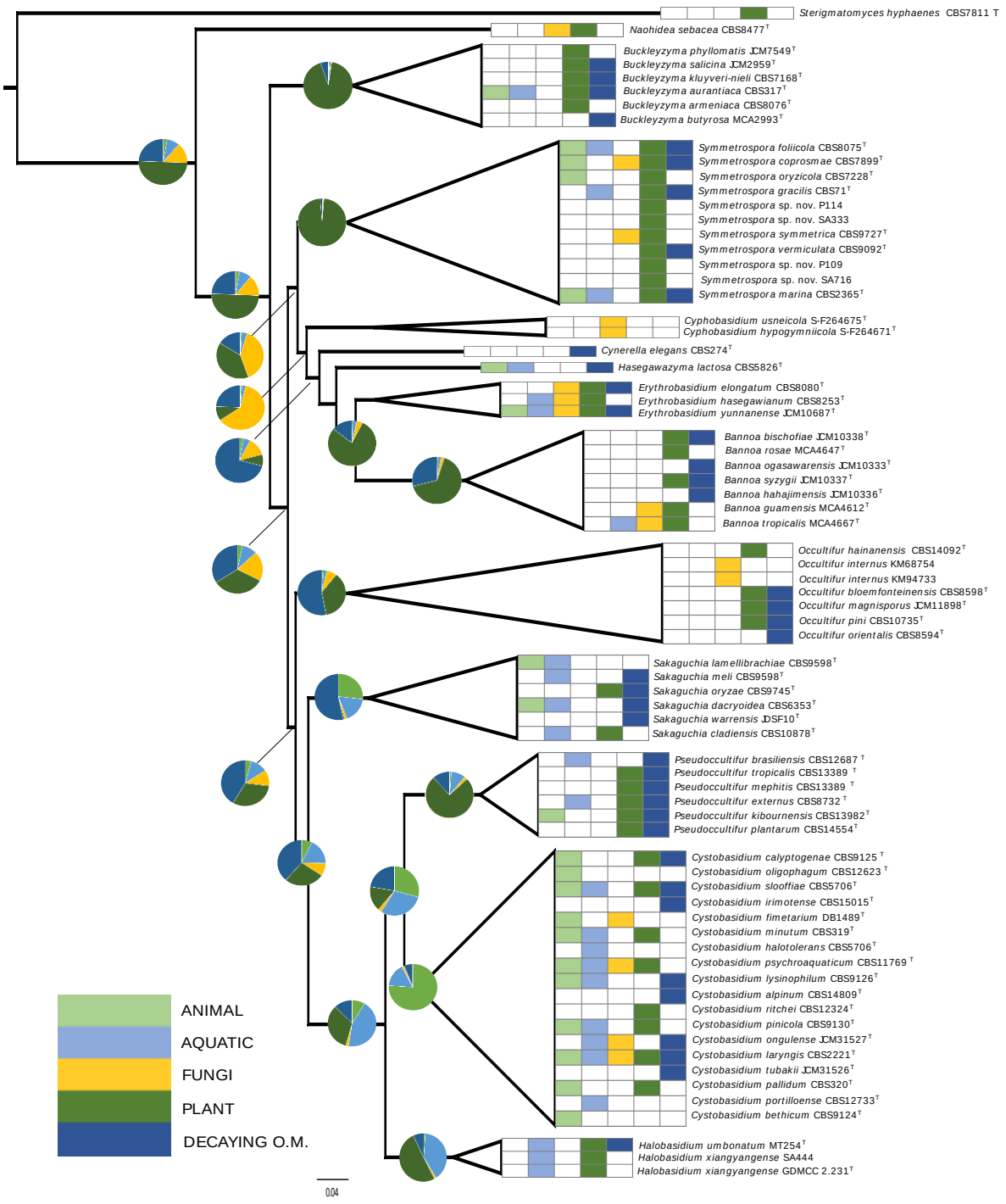


Figure A 2 Ancestral character reconstruction using ML (Maximum Likelihood). Colors represent the different categories of habitat preference of species in the Cystobasidiomycetes. Backbone was built based on the ITS, D1–D2 domains of nuclear 28S, nuclear 18S rDNA, *tef1*, *rpb1*, *rpb2* and *cytb* using ML.

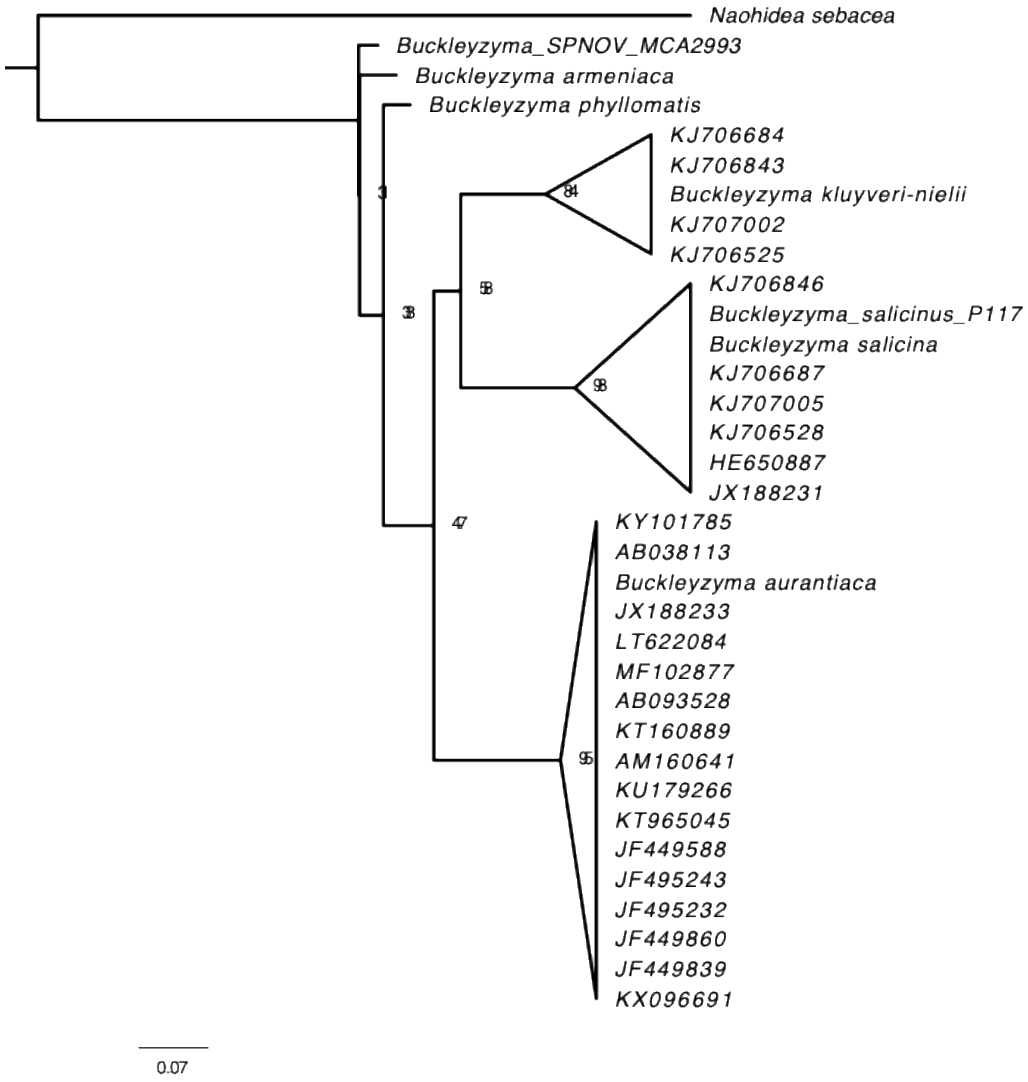


Figure A 3 Phylogenetic reconstruction for *Buckleyzyma* inferred from the ITS using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree. Scale bar equals expected number of substitutions per site.

Figure A 4 Phylogenetic reconstruction for *Buckleyzyma* inferred from the D1–D2 domains of nuclear 28S using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree. Scale bar equals expected number of substitutions per site.

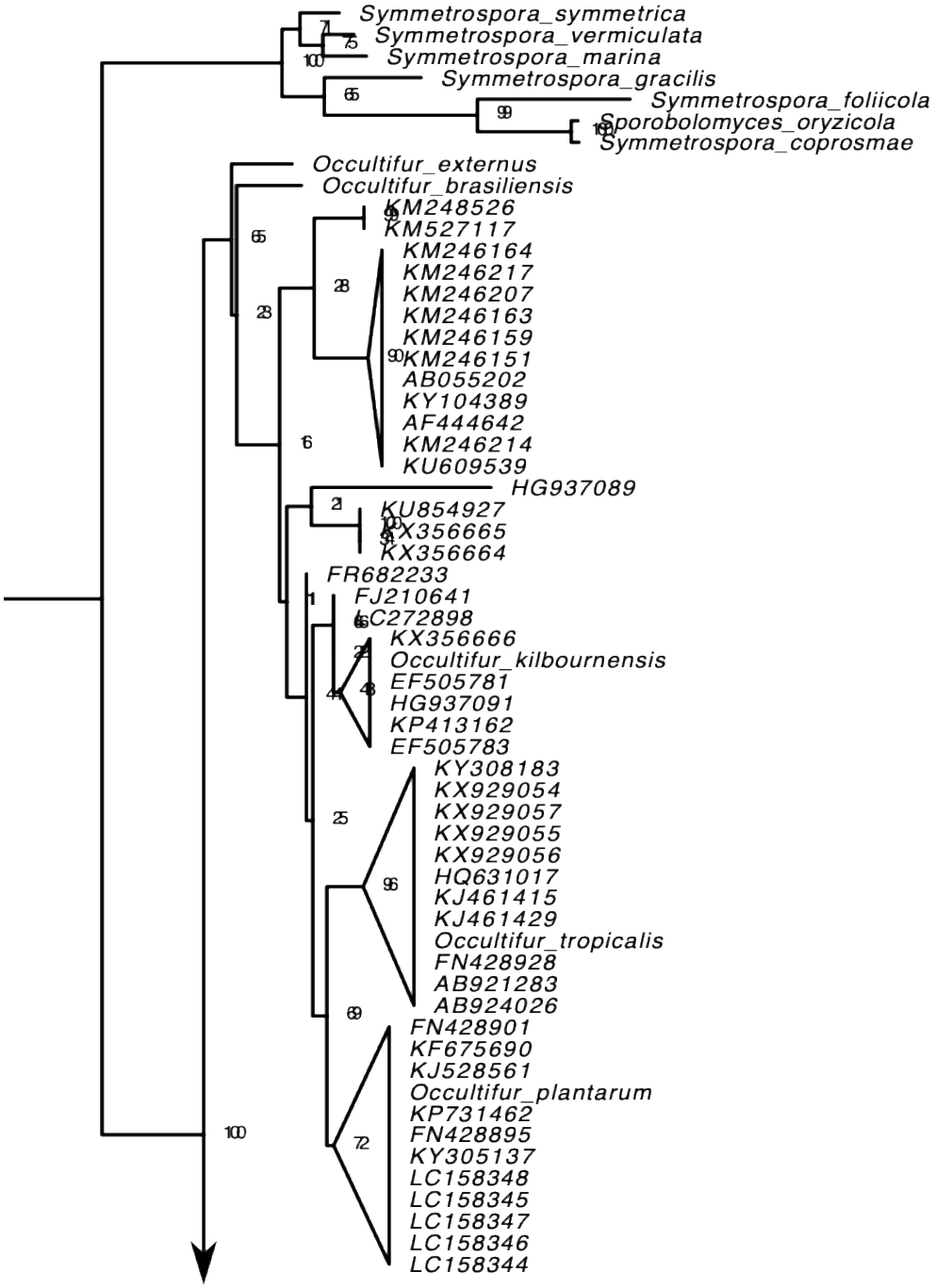


Figure A 5 Phylogenetic reconstruction of the Cystobasidiales inferred from the ITS using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree.

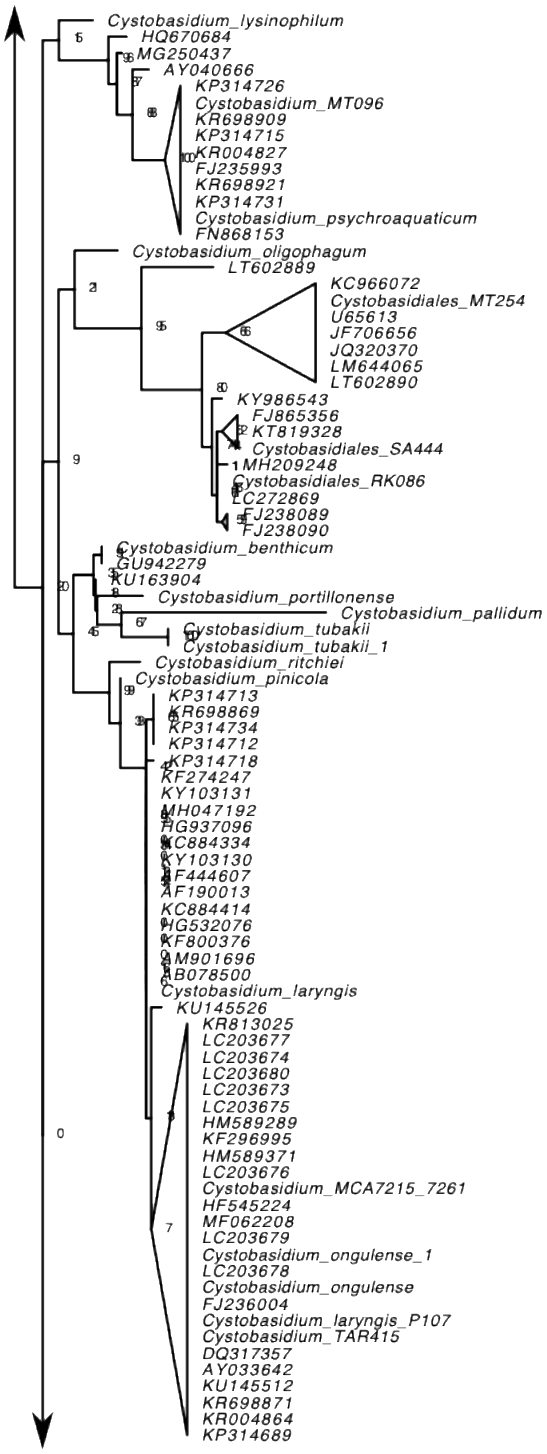


Figure A 5 Continued Phylogenetic reconstruction of the Cystobasidiales inferred from the ITS using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree.

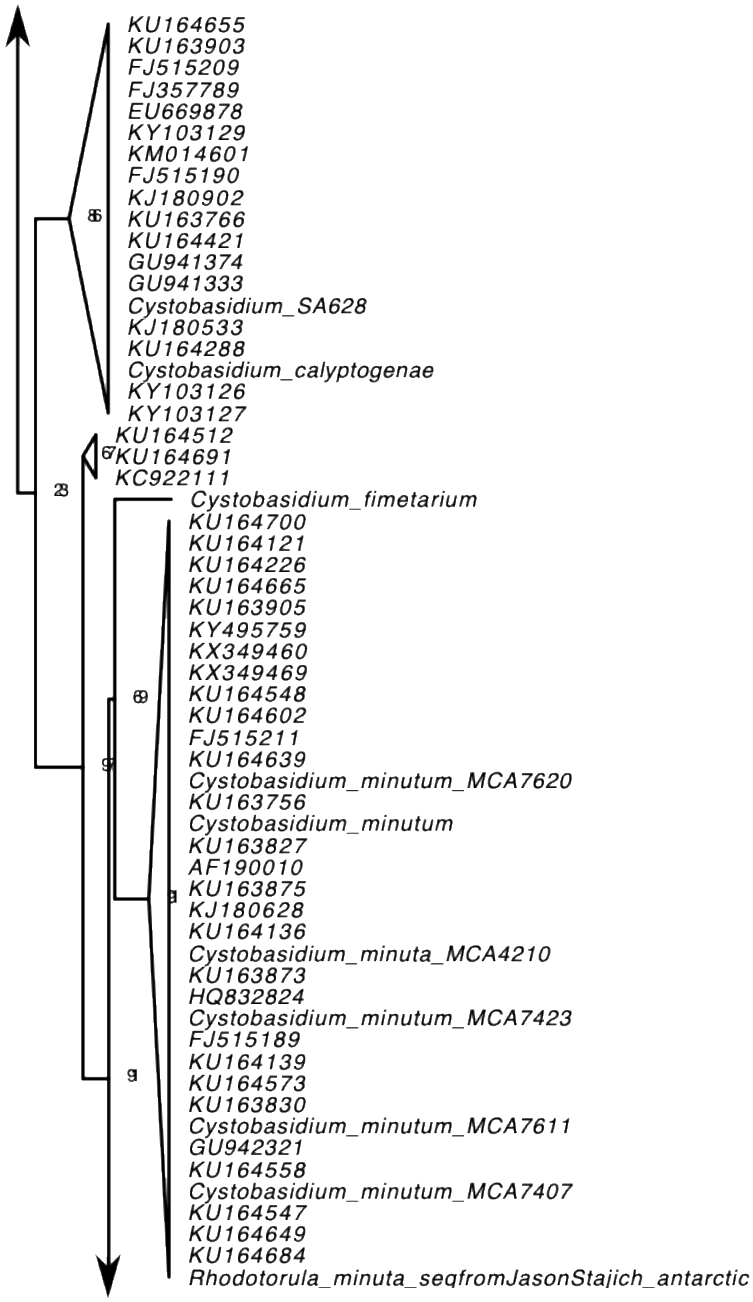


Figure A 5 Continued Phylogenetic reconstruction of the Cystobasidiales inferred from the ITS using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree.

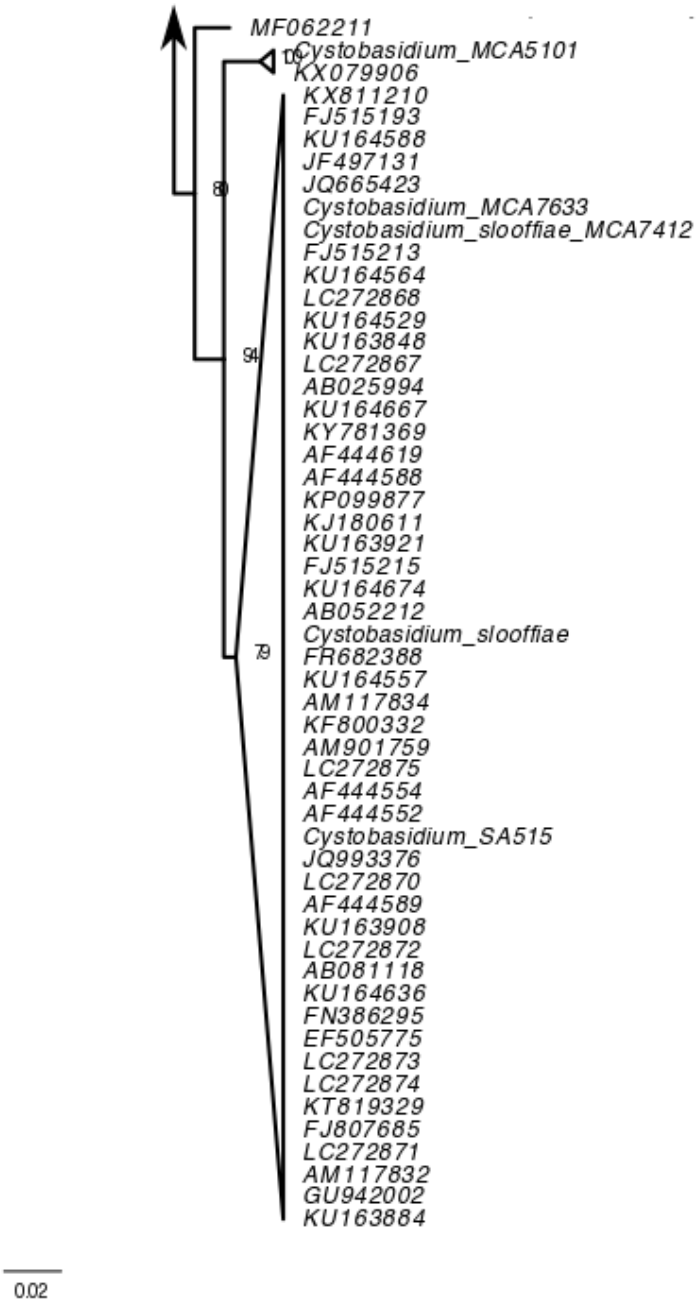


Figure A 5 Continued Phylogenetic reconstruction of the Cystobasidiales inferred from the ITS using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree. Scale bar equals expected number of substitutions per site.

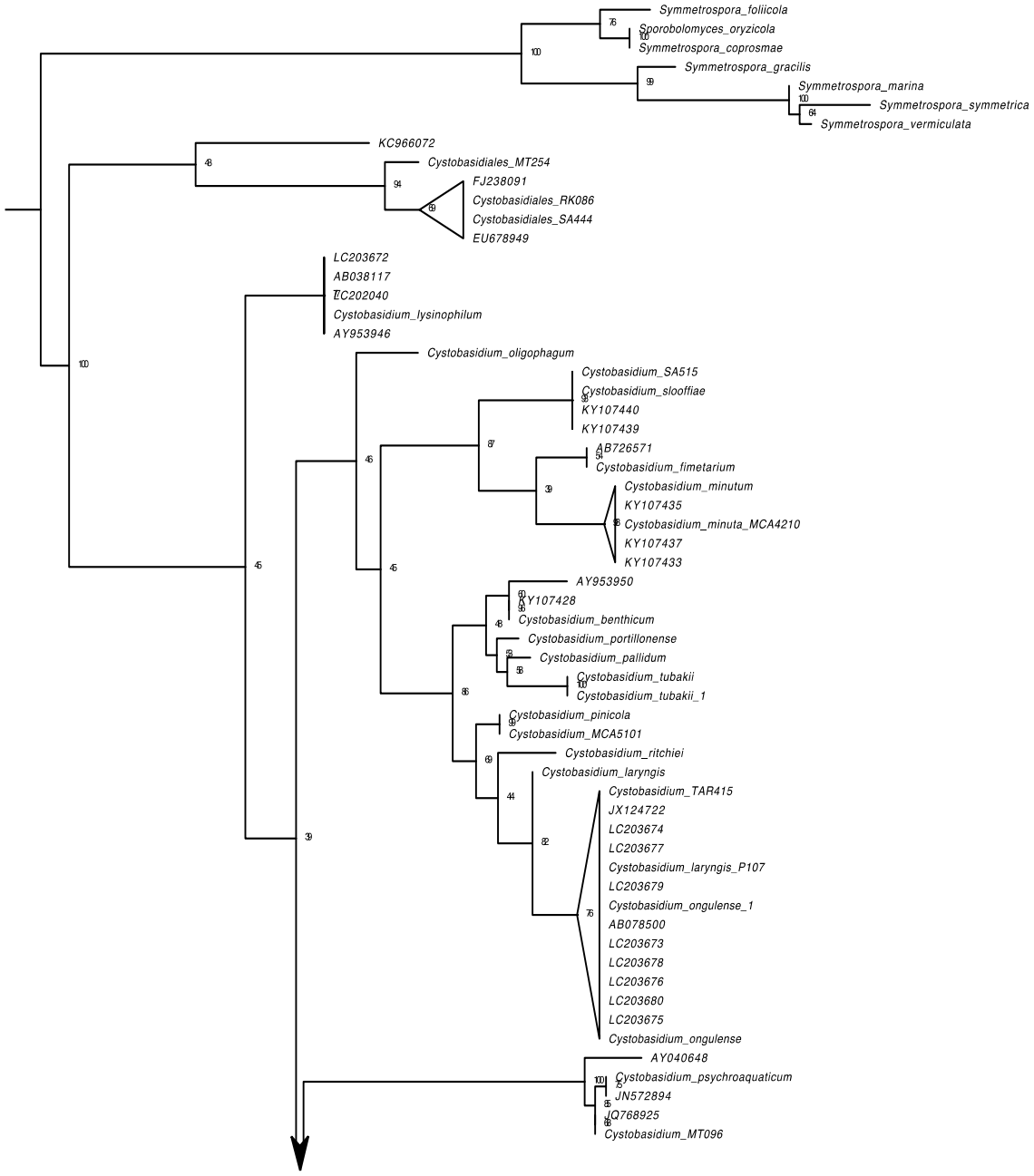


Figure A 6 Phylogenetic reconstruction of the Cystobasidiales inferred from the D1–D2 domains of nuclear 28S using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree.

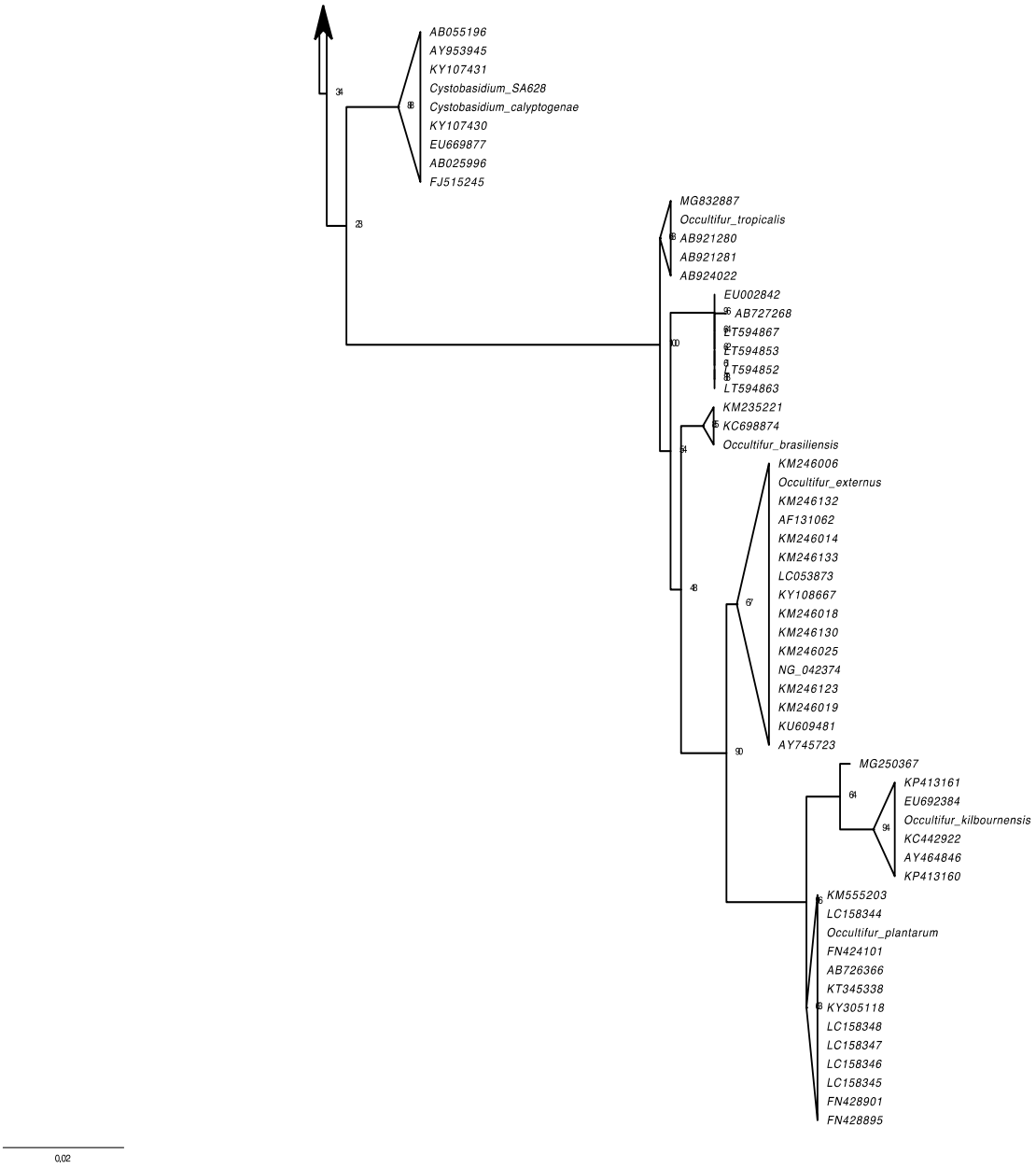


Figure A 6 Continued Phylogenetic reconstruction of the Cystobasidiales inferred from the D1–D2 domains of nuclear 28S using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree. Scale bar equals expected number of substitutions per site.

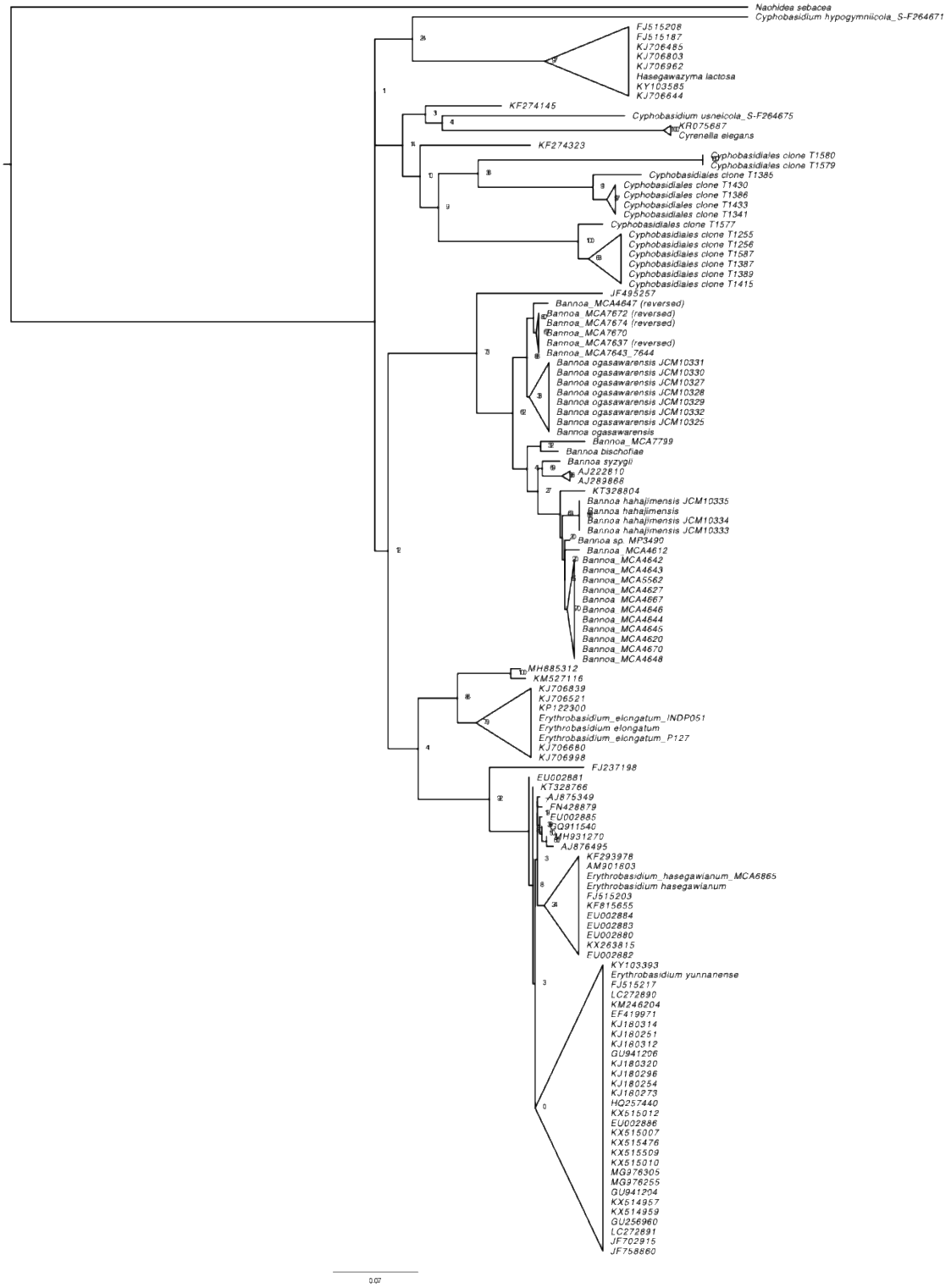


Figure A 7 Phylogenetic reconstruction of the Erythrobasidiales and the genus *Cyphobasidium* inferred from the ITS using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree. Scale bar equals expected number of substitutions per site.

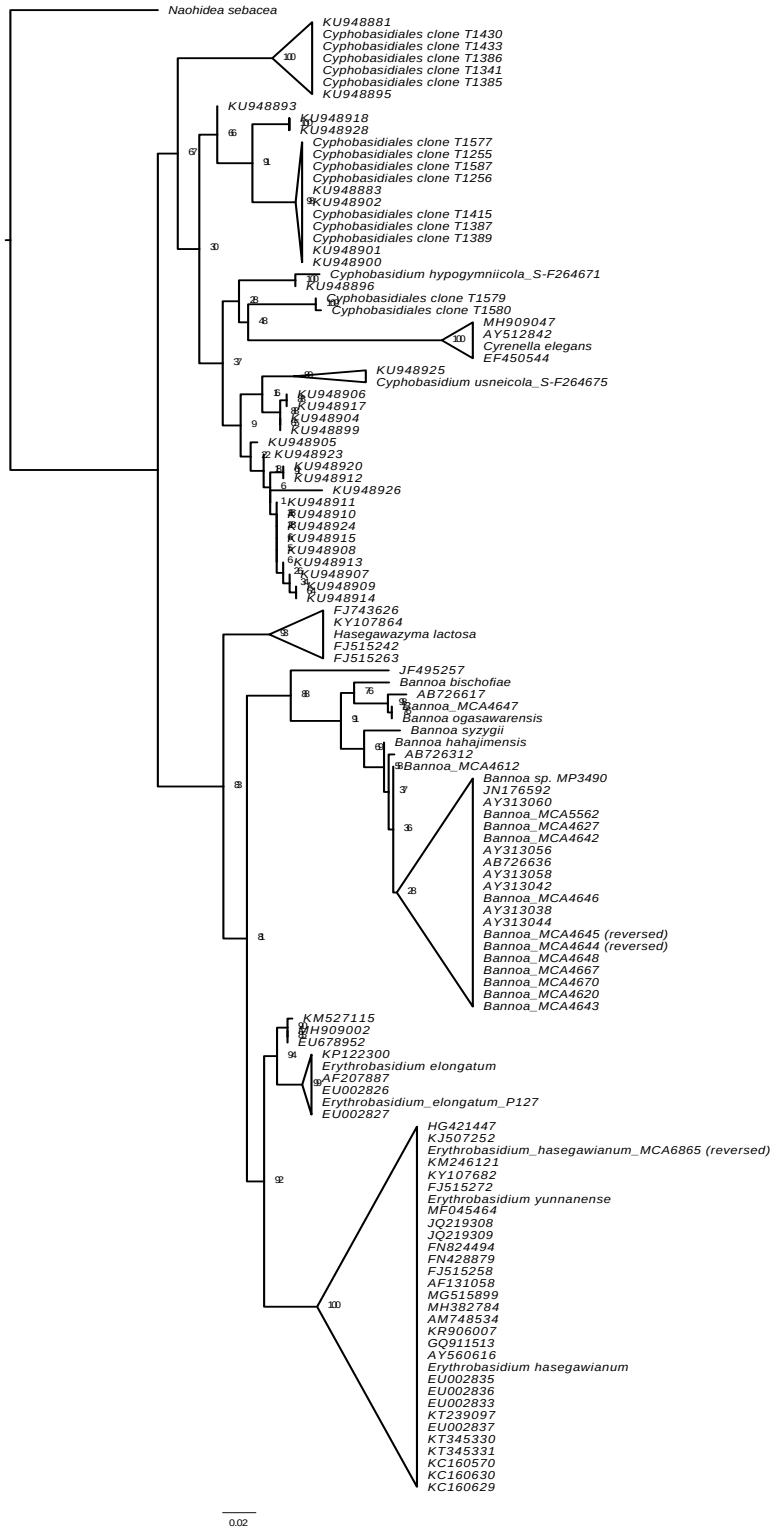


Figure A 8 Phylogenetic reconstruction of the Erythrobasidiales and the genus *Cyphobasidium* inferred from the ITS using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree. Scale bar equals expected number of substitutions per site.

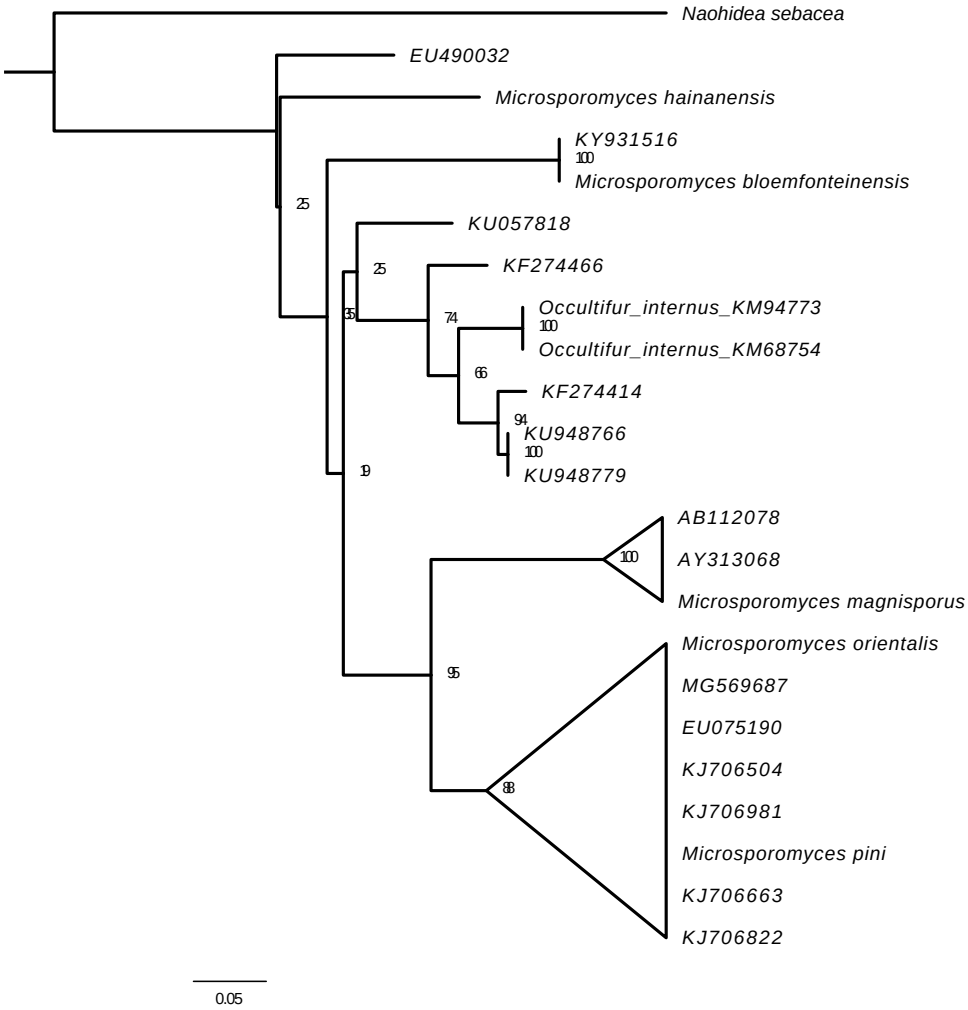


Figure A 9 Phylogenetic reconstruction of *Microsporomyces* inferred from the ITS using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree. Scale bar equals expected number of substitutions per site.

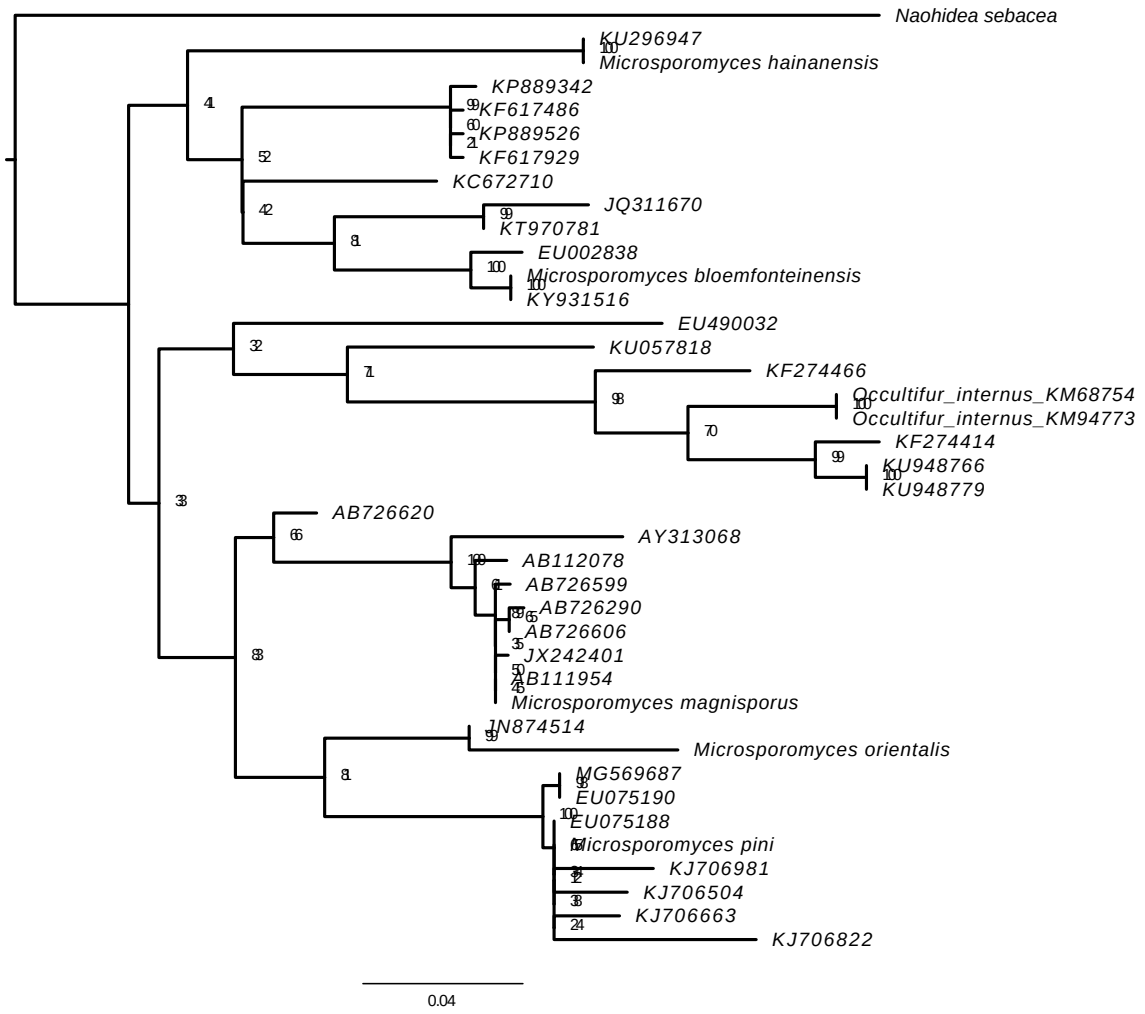


Figure A 10 Phylogenetic reconstruction of *Microsporomyces* inferred from the D1–D2 domains of nuclear 28S using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree. Scale bar equals expected number of substitutions per site.

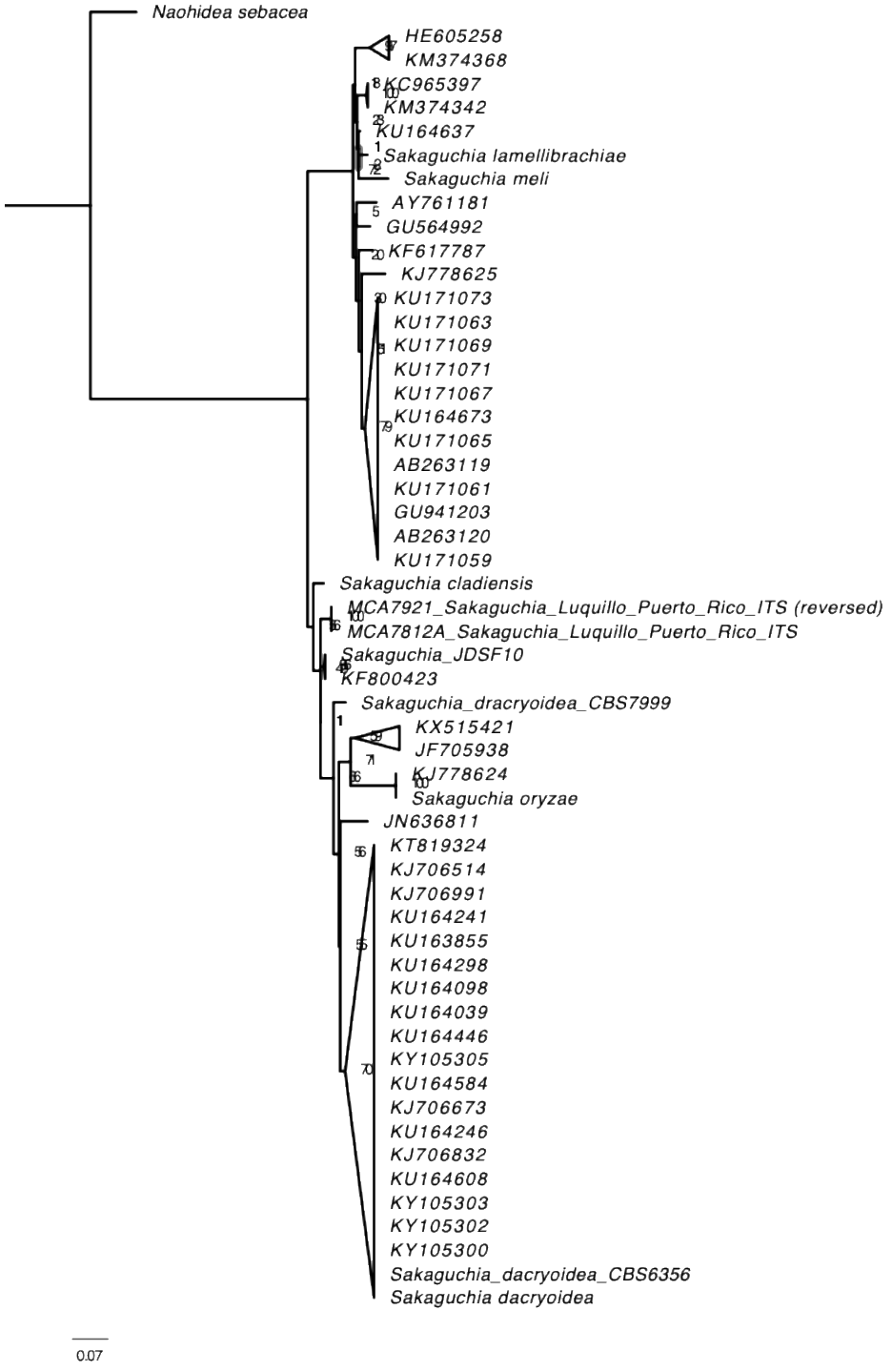
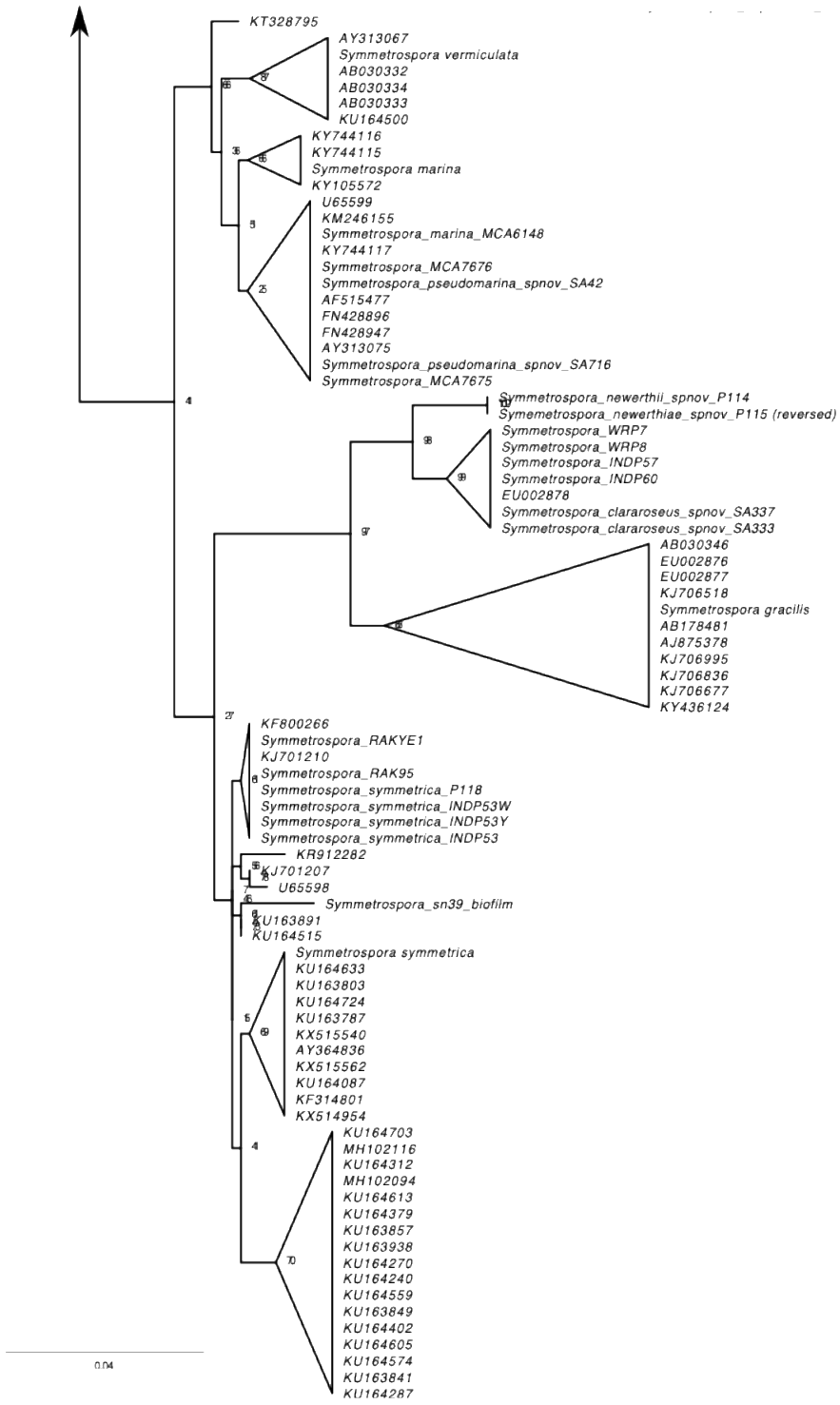


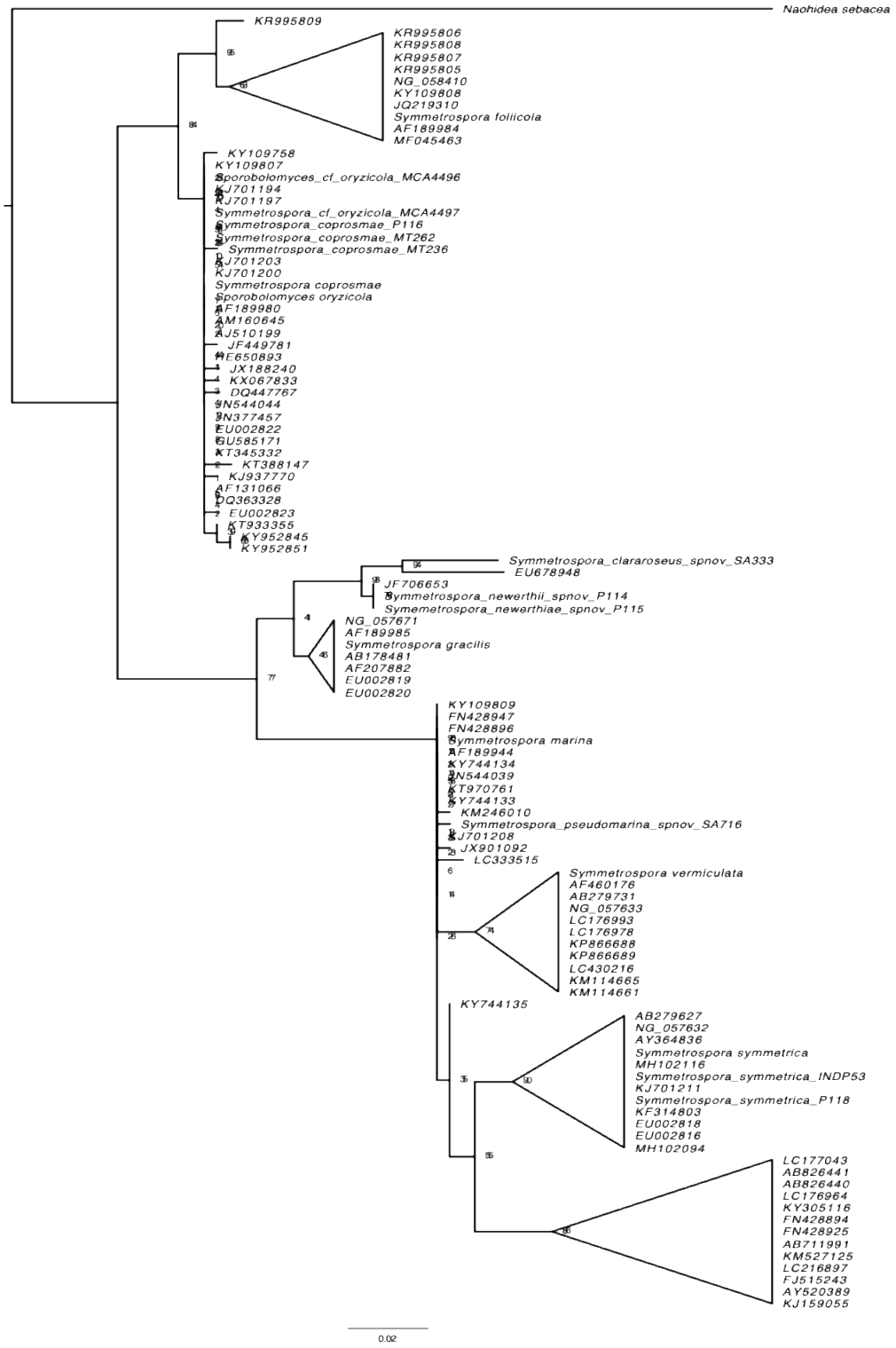
Figure A 11 Phylogenetic reconstruction of *Sakaguchia* inferred from the ITS using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree. Scale bar equals expected number of substitutions per site.

Figure A 12 Phylogenetic reconstruction of *Sakaguchia* inferred from the D1–D2 domains of nuclear 28S using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree. Scale bar equals expected number of substitutions per site.

Phylogenetic reconstruction of *Symmetrospora* inferred from ITS using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree. Scale bar equals expected number of substitutions per site.



Continued Phylogenetic reconstruction of *Symmetrospora* inferred from ITS using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree. Scale bar equals expected number of substitutions per site.



Phylogenetic reconstruction of *Symmetrospora* inferred from D1–D2 domains of nuclear 28S using ML. Bootstrap proportions are shown for each node. The tree is midpoint rooted and was used for species delimitation. Tree. Scale bar equals expected number of substitutions per site.

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