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Hector Urbina & M. Catherine Aime

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Hector Urbina [1] and M. Catherine Aime

Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907

ABSTRACT

Carotenoid-containing yeasts in Sporidiobolales (Microbotryomycetes, Pucciniomycotina, Basidiomycota) have been reported from contrasting ecosystems, including marine, soil, phylloplane, polar ice, and many others. Here, we present several analyses drawing on 583 new isolates collected from various substrates around the globe and publicly available sequences from numerous published environmental studies. We provide a multilocus phylogenetic reconstruction of the order, estimates for total species richness, a snapshot of global distribution patterns, and analysis of niche preferences in Sporidiobolales, emphasizing their occurrence in commercial crops and food products. We evaluated loci commonly used in fungal phylogenetics, finding that RNA polymerase II subunits 1 and 2 (*RPB1, RPB2*) are of little utility in this group. We have reconfirmed the monophyly of Sporidiobolales with three well-supported genera, which are, in descending order of number of species, *Rhodotorula, Sporobolomyces*, and *Rhodosporidiobolus*. From our data, we estimate ca. 260 species in Sporidiobolales, of which 42 are described, and ca. 52,000 species in Pucciniomycotina. The majority of data regarding Sporidiobolales are from North America and Europe, highlighting severe knowledge gaps for most of South and Central America and Africa.

INTRODUCTION

The order Sporidiobolales Doweld (Microbotryomycetes, Pucciniomycotina) originally accommodated ballistosporic, red-pigmented basidiomycete yeasts classified in Sporidiobolaceae R.T. Moore by Moore (1980), with most modern concepts expanding to include members of the genera *Rhodosporidium* I. Banno, *Rhodotorula* F. C. Harrison, *Sporidiobolus* Nyland, and *Sporobolomyces* Kluyver & C.B. Niel. Modern molecular systematic studies demonstrated the polyphyly of most of these genera (Fell et al. 2001; Aime et al. 2006) while recognizing that the type strains belong to Sporidiobolales. Most recently, Wang et al. (2015b), based on multilocus phylogenetic reconstructions, erected a new genus, *Rhodosporidiobolus* Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout, and reduced *Rhodosporidium* and *Sporidiobolus* to synonymy with *Rhodotorula* and *Sporobolomyces*, respectively. The 42 species now accepted in Sporidiobolales are distributed as follows: 9 in *Rhodosporidiobolus*, 15 in *Rhodotorula*, and 18 in *Sporobolomyces* (Wang et al. 2015b). Sporidiobolales species are commonly known as "red" or "carotenoid" yeasts because of their production of

lipid droplets full of carotenoid pigments that impart a rich pink to orange or red color to their colonies (Ratledge 1991; Valadon 1976; Zoz et al. 2015). These pigments are believed to protect the yeasts against ultraviolet (UV) radiation and also provide them with antimicrobial activity, although the mechanisms for this last ability remain poorly characterized (Davoli et al. 2004; Manimala and Murugesan 2014; Konuray and Erginkaya 2015). Red yeasts are reported from a broad array of environments ranging from freshwater and marine ecosystems, soils, and plant tissues to Antarctic permafrost. A few are implicated as emerging human pathogens, and several are proposed as low-cost alternatives for biotechnological production of carotenoids for cosmetics (Mannazzu et al. 2015). Some species of Sporidiobolales are capable of producing carotenoids with anticancer and antiaging activities, vitamin A, and hormone precursors (reviewed by Mata-Gomez et al. 2014; Konuray and Erginkaya 2015; Zoz et al. 2015).

Sporidiobolales yeasts are reported in several independent investigations as members of plant epiphytic

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and phylloplane communities (e.g., Kachalkin et al. 2008), as well as from various plant products including food, e.g., lettuce (Hunter et al. 2015), grapes and wine (Barata et al. 2012), and strawberries (Debode et al. 2013). A few studies indicate that soil-inhabiting Sporidiobolales appear to be richer at lower latitudes (Carrasco et al. 2012; Tedersoo et al. 2014) and in marine environments (Zaky et al. 2014), but distributional data are woefully lacking and there are no investigations of global distribution or species diversity of Sporidiobolales.

Biogeographical studies in fungi are challenging for several reasons. For instance, the kingdom is species-rich with high diversity, but the majority of species remain undescribed, and phylogenetic reconstructions often lack the robust resolution necessary for biogeographical studies. However, biogeographical investigations have been possible for a few well-studied fungal groups, such as *Saccharomyces cerevisiae* (Tofalo et al. 2013), *Cyttaria* (Peterson et al. 2010), *Armillaria* (Koch et al. 2017), and *Inocybaceae* (Matheny et al. 2009), works that continue to improve our understanding of distribution dynamics, speciation history, and actual global distribution of fungi.

We selected the order Sporidiobolales to perform a global distribution and species estimation study given the following characteristics: (i) the confirmed monophyly of the order was established by many previous independent studies (Sampaio et al. 2003; Aime et al. 2006; Wang et al. 2015a, 2015b); (ii) Sporidiobolales data are available from many next-generation sequencing (NGS) studies from a variety of soil and marine ecosystems; (iii) the designated barcode for fungi, the internal transcribed spacer (ITS1-5.8S-ITS2 = ITS) region of the nuc rDNA genes (Schoch et al. 2012), broadly used in environmental metagenomics studies, seems to perform satisfactorily for species delimitation in this group; and (iv) we have built a copious Sporidiobolales yeast collection with molecular data for 583 isolates collected during 32 collecting trips/studies in 11 countries and 10 US states over the past 12 y and isolated from a diverse array of substrates (e.g., endophytes, spoiled food, phylloplane of crop plants, and others). Additionally, over the last year, we completed an exhaustive fungal survey of commercial romaine lettuce (*Lactuca sativa*) obtained from several establishments in five different states in USA that yielded numerous Sporidiobolales isolates.

The main objectives of this study are (i) to assemble a global data set from all available sources and to establish a robust phylogeny for Sporidiobolales; (ii) to estimate the total species number in Sporidiobolales using ITS meta-analysis in conjunction with our unpublished data set; and (iii) to determine the biogeographical distribution of Sporidiobolales, with emphasis on uncharacterized ecosystems and their presence in food products.

MATERIALS AND METHODS

Strain collection and storage of isolates.—The Sporidiobolales isolates employed in this study were obtained over 12 y of collecting during different projects. Appropriate research/collecting permits were granted by the following agencies: The Environmental Protection Agency of Guyana; Ministry of Industry, Commerce, Agriculture & Fisheries, Jamaica; Dirección General de Gestión Sostenible del Património Forestal y Fauna Silvestre, República del Peru; Division of Nature Preserves, Indiana; and Institute of Biology and Soil Science, Far East Branch of Russian Academy of Sciences, Valdivostok, Russia; and Department of Forests, Republic of Vanuatu. The majority of cultures in our collection were obtained using the spore drop technique previously described in Kijpornyongpan and Aime (2017) and Toome et al. (2013), or by direct culturing from a broad variety of substrates/hosts (SUPPLEMENTARY TABLE 1). Isolates from food were made from spoiled food surfaces via direct swabbing and dilution plating, except those from lettuce.

As part of an ongoing study of the microbiome of romaine lettuce, systematic sampling was conducted from 45 lettuce heads obtained from commercial vendors in West Lafayette, Lafayette, and Indianapolis (Indiana), Champaign and Chicago (Illinois), Springfield (Virginia), and Washington, DC. A 25-g sample from different leaves (young and old) was obtained from each plant and blended in 225 mL of sterile 100 μM phosphate buffer. A 1-mL subsample was superficially plated in serial dilutions on yeast extract, peptone, glucose (YPG) medium with 2% agar and amended with chloramphenicol 25 μg/mL and ampicillin 50 μg/mL (all components obtained from BD, Franklin Lakes, New Jersey). Two samplings were performed, one oriented to describe the species richness of culturable fungi and the other to establish the abundance of red yeast in lettuce. For the second, up to 45 yeast isolates were randomly selected, where possible, for purification and ITS sequencing.

Axenic cultures obtained after several rounds of subculturing are kept in 40% glycerol at −80 C in the culture collection of M. C. Aime (MCA), Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana, USA; dried culture vouchers are maintained in the Kriebel Herbarium (PUL), located in the same university.

DNA sequencing, polymerase chain reaction amplification, and DNA sequencing.—Initial DNA for ITS sequencing was extracted using Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin) or amplified directly using the modified colony polymerase chain reaction (PCR) method recommended by Albu et al. (2015) using primers ITS1F and ITS4B (Gardes and Bruns 1993). For the multilocus phylogenetic reconstruction of Sporidiobolales, 64 uncharacterized strains from the MCA collection were selected based on results from ITS sequencing. DNA was reextracted from fresh cultures following the methods of Goodwin and Lee (1993). Amplification of the ITS locus was carried out using primers ITS1F and ITS4 (Bruns et al. 1992) and following the mastermix and PCR amplification protocol of Aime and Phillips-Mora (2005). Amplification of the small subunit (18S) and large subunit (28S) nuc rRNA genes, elongation factor 1α (*EF1a*), and cytochrome *b* (*Cytb*) followed the primer combinations and PCR protocols of Wang et al. (2015a, 2015b). PCR products were sequenced at GeneWiz (https://www.genewiz.com/en). Contig sequences were edited in Sequencher 5.2.1 (http://www.genecodes.com) and sequences were deposited in GenBank, with accession numbers listed in SUPPLEMENTARY TABLES 1 and 2.

Collection of environmental sequencing data.—We assembled a database of ITS sequences generated from type strains of all 42 accepted species of Sporidiobolales sensu Wang et al. (2015b) and combined this with the ITS sequences from our global collection of Sporidiobolales isolates. This data set was blasted against the entire GenBank collection to retrieve similar deposited sequences and their respective accession numbers, resulting in a set of 3846 sequences. Accession numbers for all unique sequence records were used to acquire the respective submission data using the GenBank software EDirect (https://www.ncbi.nlm.nih.gov/books/NBK179288/) and the ecological data associated with the sequences, such as isolation source, locality, database, and source of isolation. This process was repeated over two consecutive months to retrieve newly deposited sequences. The entire data set was inspected for chimeras using the UCHIME script (Edgar et al. 2011), and a final ITS data set of 1170 sequences from unique accessions was retained (SUPPLEMENTARY TABLE 1).

This final ITS data set was clustered again using UCLUST (Edgar 2010) and the script pick_otus.pyin QIIME (Caporaso et al. 2010), using genetic distance values of 0.99, 0.98, and 0.97 and sequence assignment to the best-matching seed rather than the first matching.

To determine the best distance value and evaluate the power of species discrimination of the ITS locus, we searched for values that assigned our 42 type species sequences to individual operational taxonomic units (OTUs). Only at 0.99 and 0.98 distance values were we able to obtain a high number of clusters (41 of 42) to which only one type species was assigned, with only *Sporobolomyces roseus* and *S. metaroseus* clustered together; hence, clustering results at the more conservative 0.98 sequence distance were selected for downstream analyses. After taxonomic assignment of the OTU representative set using BLASTn 2.6.0+ (Altschul et al. 1990), we performed a phylogenetic reconstruction using maximum likelihood (ML) with bootstrap node support values computed after 1000 trees.

Sequence manipulation and phylogenetic and statistical analyses.—Multilocus alignments were curated and concatenated using Mesquite 3.2 (Maddison and Maddison 2017). Maximum likelihood (ML) phylogenetic inference was performed in raxmlGUI (Silvestro and Michalak 2012) under the GTRGAMMA model of sequence evolution and 1000 bootstrap trees. For the ITS analysis, non-Sporidiobolales sequences, identified as those that were difficult to align or with long branches outside of the core order, were discarded. This step was repeated several times until the basal node of Sporidiobolales was recovered by midpoint rooting of the consensus tree. Phylogenetic trees were edited using PHYLOTOOLS in R (https://cran.r-project.org). Trees and alignments were deposited in TreeBASE under the accession number S22154.

We noticed that the phylogenetic reconstruction performed by Wang et al. (2015a) for the order Sporidiobolales lacked support for the internal nodes that define the genera and species and that sequences obtained from this study were often problematic in our reconstructions. For this reason, we conducted a robust analysis of those molecular data under the genealogical concordance phylogenetic species recognition criterion proposed by Taylor et al. (2000). The entire multilocus sequence data set generated for the type species in Sporidiobolales by Wang et al. (2015a) was aligned using the same methods outlined above. Sequences that were difficult to align were re-blasted against the GenBank collection; most of these lacked identity with other Sporidiobolales and belonged to other Pucciniomycotina orders. Through this process, we excised a set of misannotated sequences (SUPPLEMENTARY TABLE 3), resulting in a much more comprehensive and robust mutilocus phylogeny

for this group. Additionally, we were unable to obtain any reliable alignments with any of the RNA polymerase II subunits 1 and 2 (*RPB1, RPB2*) sequences generated by (Wang et al. 2015a); hence, they were not used in our study.

Richness and diversity indices, ordination analysis (principal component analysis [PCA] based on the most common 100 species/out, using Bray-Curtis distance), and species accumulation curves were computed in the R package PHYLOSEQ (Callahan et al. 2016) and the Kruskal-Wallis test to address significance of species/OTU and post hoc analyses (Benjamini-Hochberg false discovery rate and Bonferroni-corrected *P* value) were carried out using the GROUP_SIGNIFICANCE.PY script in QIIME (Caporaso et al. 2010). Tukey test and species accumulation curves based on collector method and Coleman richness were computed using the R package VEGAN (Oksanen et al. 2017).

RESULTS

Sporidiobolales multilocus phylogeny.—A robust phylogenetic reconstruction was necessary to better understand Sporidiobolales species distribution and richness. Our multilocus analyses support the three well-delimited clades in Sporidiobolales as *Sporobolomyces, Rhodotorula*, and *Rhodosporidiobolus* (FIG. 1).

Sporidiobolales estimated species numbers.—The complete ITS data set containing 1755 sequences from 353 studies clustered into 234 OTUs. After normalization based on species richness, the data set was reduced to 860 sequences. Of the 234 OTUs, 41 were assigned to previously described species based on clustering with the type species sequence; however, even at a genetic distance value of 0.98, one pair of sister species could not be resolved (*S. roseus/metaroseus*), species that only have one nucleotide difference in the ITS locus (Valério et al. 2008). The remaining 193 OTUs representing putatively uncharacterized species are distributed among the genera as follows: 27 in *Rhodosporidiobolus* (14% total OTUs), 120 in *Rhodotorula* (62%), and 46 in *Sporobolomyces* (24%) (FIG. 2). The majority of OTUs were recovered once (157 OTUs). However, 82 of these OTUs were deposited in the GenBank plant and fungal database (PLN) and probably represent sequences obtained using Sanger sequencing technology; 7 of the singleton OTUs were from the MCA collection, which were obtained from pure cultures. The remaining 68

OTUs were from the GenBank environmental sequencing database (ENV).

The numbers of observed species and OTUs and the Chao1 and Faith's phylogenetic diversity indices predict differences in diversity among the genera in Sporidiobolales (TABLE 1). Based on species accumulation curves, we predict *Rhodotorula* (ca. 150 spp.) as the most species-rich genus, followed by *Sporobolomyces* (ca. 60 spp.) and *Rhodosporidiobolus* (ca. 50 spp.), giving a total of ca. 260 predicted species for the entire order (FIG. 3). Another remarkable result is that among the most commonly sequenced species/OTUs, there are many undescribed species, which is most conspicuous in *Rhodosporidiobolus* (FIG. 4). From the number of described Pucciniomycotina species now estimated at ca. 8400 (Aime et al. 2014), in which the 42 known Sporidiobolales species account for 0.5% the total diversity, we can extrapolate the known/expected ratio of Sporidiobolales species (42/260) to predict ca. 52 000 total species of Pucciniomycotina.

Sporidiobolales global distribution and occurrence.— Our data set clearly indicates that Sporidiobolales are globally distributed, inhabiting a variety of environments, with the majority of known occurrences recorded from Asia, North America, and Europe, and lower recorded numbers in Africa, Australia, the Caribbean, Central America, and South Asia (FIG. 5A). Sporidiobolales are abundant in plants (flowers, leaves, roots, and wood, including crops), marine environments, and soils, with common life strategies ranging from endophytes, epiphytes (phylloplane), to mycorrhizal associates and saprobes (soil saprobes) (FIG. 5B). The PCA explained 21.4% of the total variation in the distribution of Sporidiobolales (FIG. 6), variation that is explained primarily by the source of isolation ($r^2 = 0.17$, $P = 0.026$), followed by geographical region ($r^2 = 0.09$, $P = 0.008$) and database ($r^2 = 0.03$, $P = 0.001$). These results are interpreted as indicative of substrate and geographical region preference by Sporidiobolales species and bias in the collection efforts, given that the MCA data set mainly comprised isolates from plants and the uneven geographical distribution of collections.

Sporidiobolales in food products and crops.— During our investigations of the mycobiota associated with commercial romaine lettuce, we identified 680 ascomycete and basidiomycete strains (Urbina et al. 2016). Of these, one of the most commonly isolated fungi from lettuce is an apparently undescribed sister species of *Sporobolomyces roseus* (*Sporobolomyces* sp. 1; FIG. 1). When we examined the occurrence of

Figure 1. Midpoint-rooted ML multilocus (18S-ITS-28S nuc rRNA loci, *Cytb*, and *EF1a*) phylogenetic tree of Sporidiobolales. ML score = −26886.79. Nucleotide model used GTRGAMMA. Type strains are denoted with "T." Notice the large number of possible uncharacterized species, indicated by "sp." and in bold. Numbers below branches indicate bootstrap value.

Table 1. Diversity indexes and Tukey's test results.

Index	Sum of squares	Residuals	Tukey test Rhodotorula	Rhodosporidiobolus	Sporobolomyces
Observed species	178.361	4415.50	a	c	b
Chao 1	5944.10	1020251.20	a	b	ab
Faith's phylogenetic	2.72	36.60	a	b	c

Variation in multiple range test results using letter codes to illustrate groupings a, b, and c.

Sporidiobolales in food products and crops, we found at least 30 species that have yet to be characterized (FIG. 2).

DISCUSSION

The evolutionary history of Sporidiobolales was shaped, at least in part, by the novelty of producing ballistospores (Nakase and Suzuki 1986; Kurtzman and Fell 1991; Kurtzman et al. 2011). Ballistospory is not uniform in the order, however, being present in Sporobolomyces species but absent in Rhodotorula and some species of Rhodosporidiobolus. This morphological novelty no doubt contributes significantly to aerial dispersal and colonization of plant leaves in higher strata by members of Sporobolomyces, as elegantly calculated for S. salmonicolor, a species capable of discharging ballistospores to a horizontal distance of ca. 0.5 mm (Stolze-Rybczynski et al. 2009). Our collection of 583 Sporidiobolales isolates includes 17 undescribed species of Sporobolomyces and 7 undescribed species of Rhodotorula (FIG. 1), most isolated using the spore drop method (e.g., Albu et al. 2015), demonstrating that culturing uncharacterized species in Sporobolomyces and Rhodotorula does not require an intense or specialized effort. On the other hand, we have not isolated any uncharacterized species in Rhodosporidiobolus, suggesting that recovering species of this genus may require more specialized methods. They may also have more restricted distributions, because most of the known species do not produce ballistospores and the genus has the lowest number of recognized species and estimated species richness (FIG. 2).

Sporidiobolales sequence data and phylogenetic reconstruction.—The use of traditional ribosomal molecular makers (18S, ITS, and 28S nuc rRNA genes) concatenated with protein coding genes Cytb and EF1a are sufficient to produce a robust, well-supported phylogeny for this group (FIG. 1). Although RPB1 and RPB2 sequences were analyzed in other studies (Wang et al. 2015a), we were unable to obtain any reliable alignments with those data; hence, they were not used in our study. The problem of

misannotated sequence data (Bidartondo 2008) is pervasive within this group; for instance, we found 8 Cytb sequences out of 44 total sequences examined to represent incorrectly identified species (SUPPLEMENTARY TABLE 2).

Estimated number of total Sporidiobolales and Pucciniomycotina species.—To date, 42 species of Sporidiobolales are accepted. However, our analyses, based on more than a decade of new isolates from poorly sampled regions and ecosystems combined with NGS studies, allow robust estimations of ca. 260 species in the order. In other words, thus far global sampling efforts have recovered <16% of predicted species of Sporidiobolales (FIG. 2). We also conservatively estimate the total number of Pucciniomycotina at ca. 52 000 species. Although the vast majority of described Pucciniomycotina belong to a single order, the rust fungi (Pucciniales), we anticipate that much of the undescribed diversity within the subphylum may reside in yeast-like and other microscopic forms (Aime et al. 2006, 2014; Spribille et al. 2016).

Based on our analysis, the use of 97%, 98%, or 99% cutoff for sequence similarity to establish OTUs as a proxy for species in Sporidiobolales slightly underestimates the number of species in this group. This lack of resolution of the ITS has been observed in other fungal groups, e.g., Glomeromycota (House et al. 2016) and Aspergillus and Fusarium (Balajee et al. 2009). Thus, our estimates based on 98% cutoff are likely to be conservative and may underestimate total richness in Sporidiobolales and Pucciniomycotina.

Why do Sporidiobolales appear to be profoundly abundant?.—The combination of morphological and physiological adaptations developed in the evolution of the Sporidiobolales allowed this group to successfully colonize a variety of contrasting environments, hence the large species diversification estimates in this study. The production of carotenoid compounds that give Sporidiobolales members their distinctive colony coloration is one of their most obvious adaptations. It confers the evolutionary advantage of survival under high ultraviolet B (UV-B) radiation, thanks to the
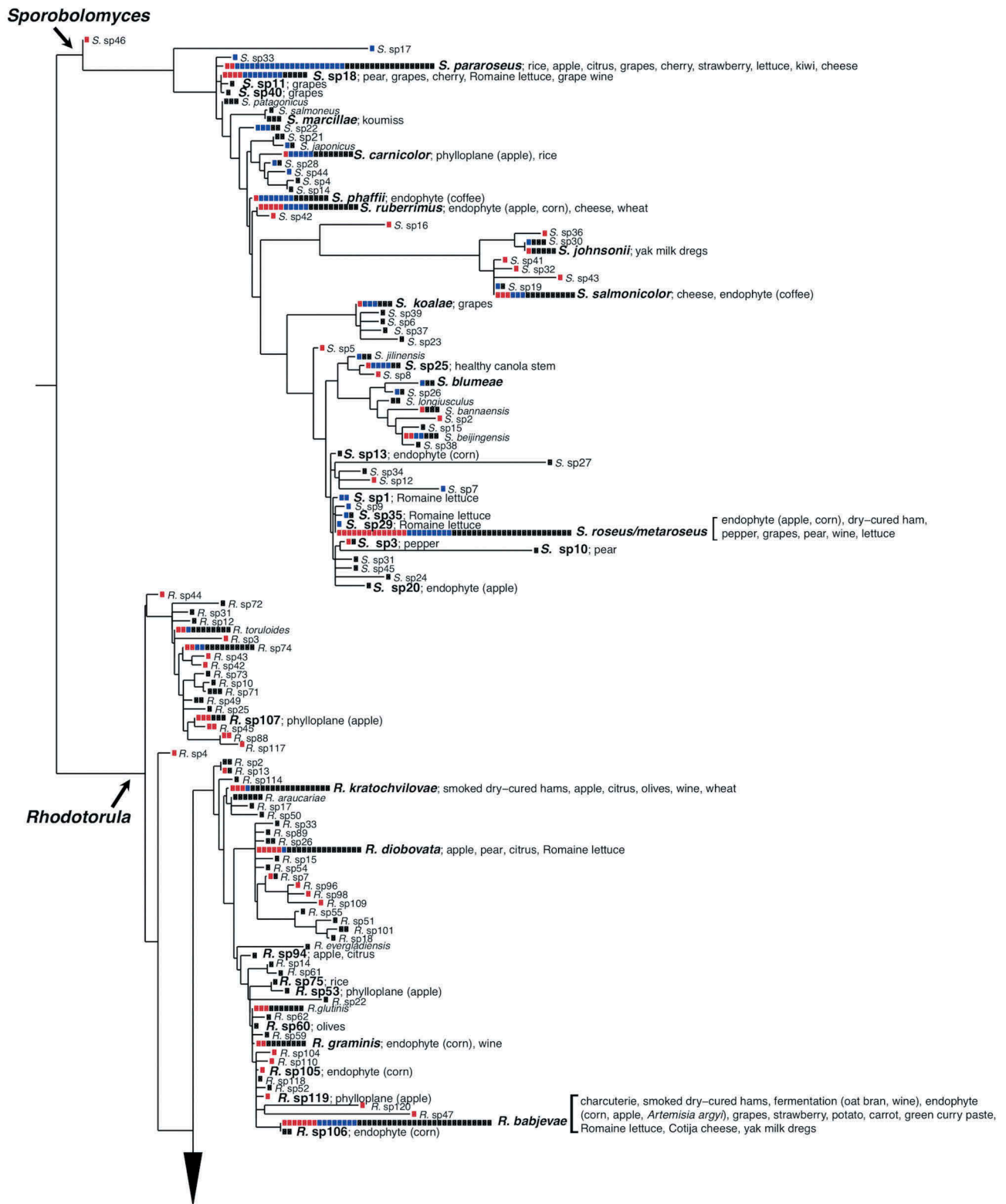
Figure 2. Midpoint-rooted ML phylogenetic tree based on ITS locus for Sporidiobolales emphasizing species/OTUs found in association with plants, crops, and food (in bold). ML score = −13751.42. Nucleotide model used GTRGAMMA with model nucleotide evolution calculated per single locus (ITS1, 5.8S, and ITS2). Number of studies that have reported each branch tip/species denoted by squares using a presence/absence data set.
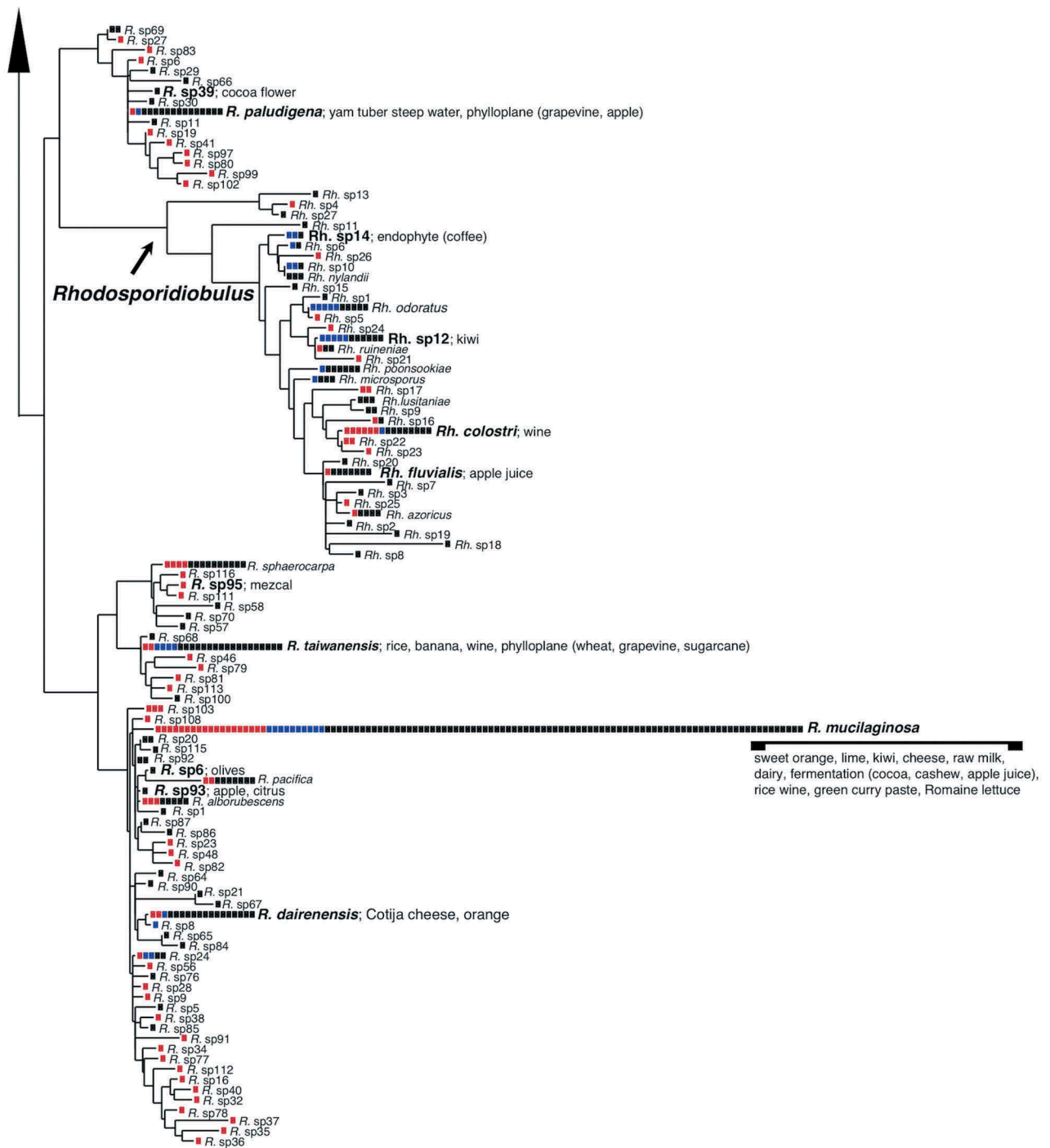
Figure 2. (Continued).

production of torularhodin, as specifically demonstrated in *R. mucilaginosa* (Moline et al. 2010) and *Sporobolomyces* sp. (Manimala and Murugesan 2014). In turn, this adaptation may allow Sporidiobolales to colonize the surface of leaves, flower petals, and fruits, among others, where exposure to high UV-B may otherwise be considered a constraint (Fonseca and Inácio 2006). Other adaptive traits in Sporidiobolales are (i) an ability to survive in high-salt conditions,

thanks to the accumulation of torulene, as demonstrated in *Sporobolomyces* (as *Sporidiobolus*) *pararoseus* (Li et al. 2017); (ii) an ability to survive cold weather conditions; Sporidiobolales are among the most common basidiomycetous yeasts isolated from permafrost, sea water, and Artic ice (see review in Zalar and Gunde 2014); and (iii) antimicrobial activity, as demonstrated in some strains of *Rhodotorula glutinis* (Mutlu et al. 2013) and *Sporobolomyces* sp. (Manimala
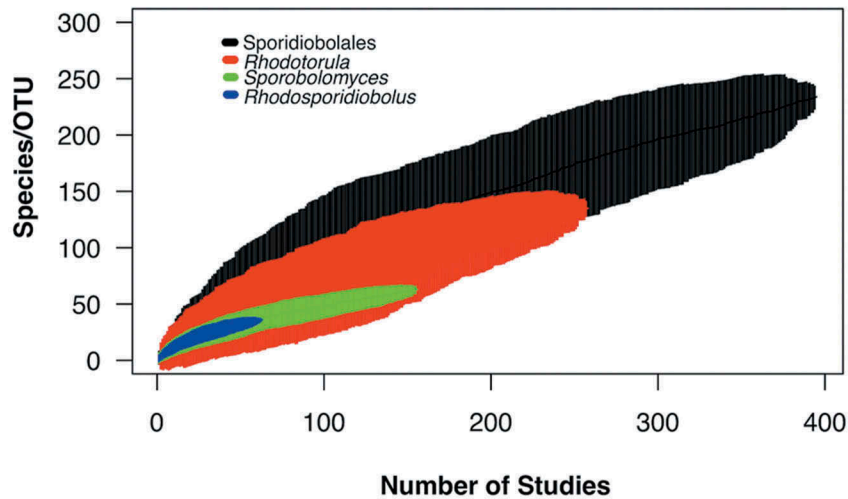
Figure 3. Species accumulation curves based on random sampling of the global data set.
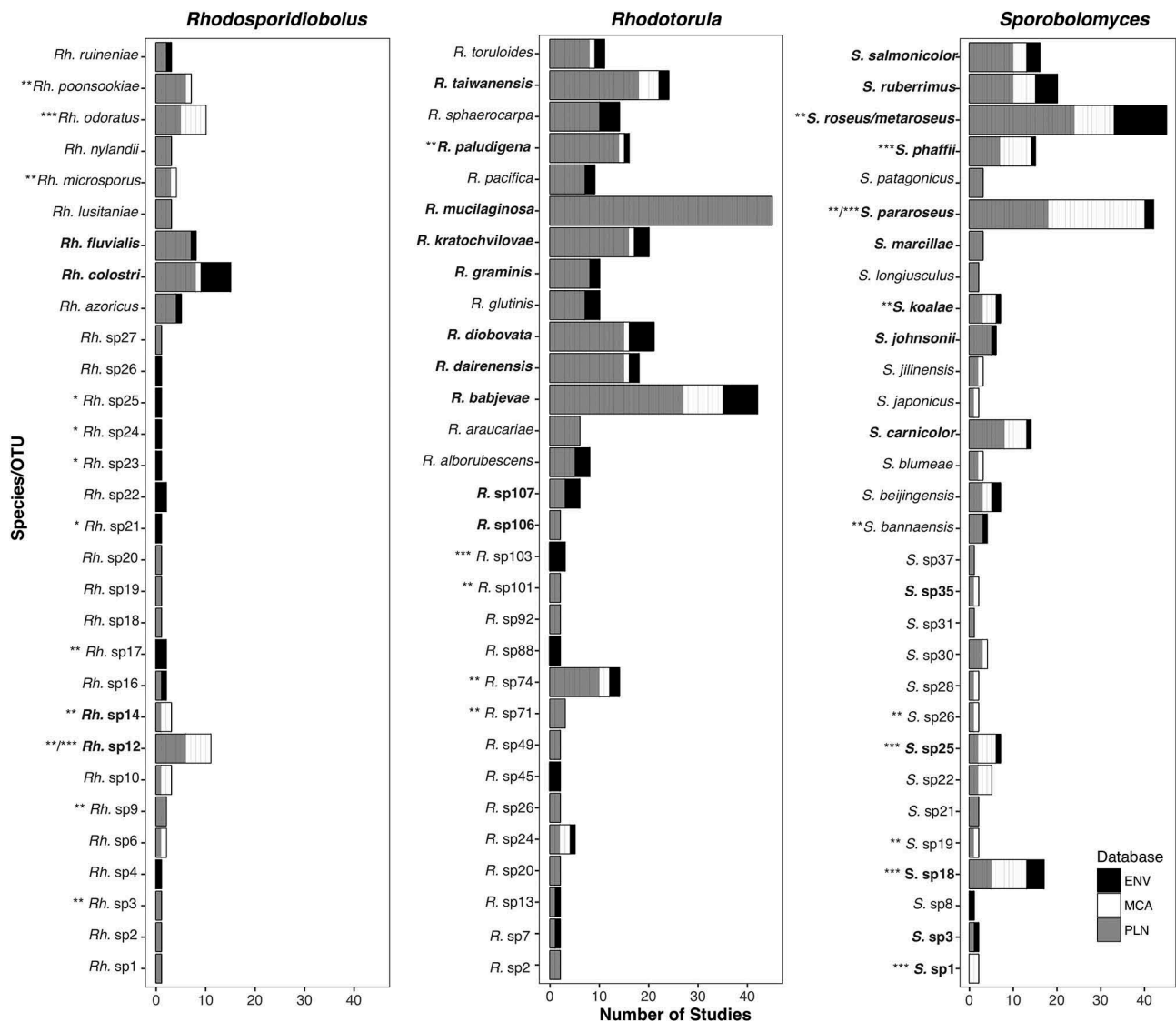

Figure 4. The 30 most commonly reported Sporidiobolales species and putative undescribed species emphasizing species/OTUs found in association with plants, crops, and food (in bold). Databases: Environmental (ENV) and plant (PLN) GenBank databases; MCA collection (MCA). Significant difference in richness of species/OTUs denoted by asterisks as source of isolation (*), geographical region (**), and/or database (***). See SUPPLEMENTARY TABLE 4 for details.

and Murugesan 2014). Additional characteristics of Sporidiobolales with biotechnological applications were recently described: the production of exopolysaccharides in heavy metal–resistant *R. mucilaginosa* in Mexico (Garza-Gonzalez et al. 2016), and tolerance to Hg^{2+} demonstrated in other strains of *R. mucilaginosa* isolated from China (Liu et al. 2017).

Sporidiobolales are a constant presence in food products.—Sporidiobolales species are capable of colonizing many contrasting niches/environments (FIG. 5B). Specifically, with their abilities to tolerate high salinity, high UV radiation, desiccation, and extreme temperatures, they are well suited for establishment on the surface of fruits and vegetables, and they may even have an ability to survive common postharvest sanitation

and storage procedures. The presence of commensal and spoilage red yeasts in vegetables, fruits, and processed food products has been reported by several independent researchers (Barth et al. 2009). Our data set contains at least 41 undescribed Sporidiobolales yeasts from many food products (FIG. 2). One of the most frequent species associated with romaine lettuce in North America appears to be an undescribed species of *Sporobolomyces* (FIG. 1), closely related to *S. roseus*. These results agree with previous studies of greenhouse lettuce (two cultivars of *L. sativa* and one of *L. serricola*) in Europe, which reported *S. roseus* as a significant part of the microbial flora (Hunter et al. 2015). This presents an urgent need for the formal description and full characterization of undescribed species that are constantly consumed together with food products, especially if they have
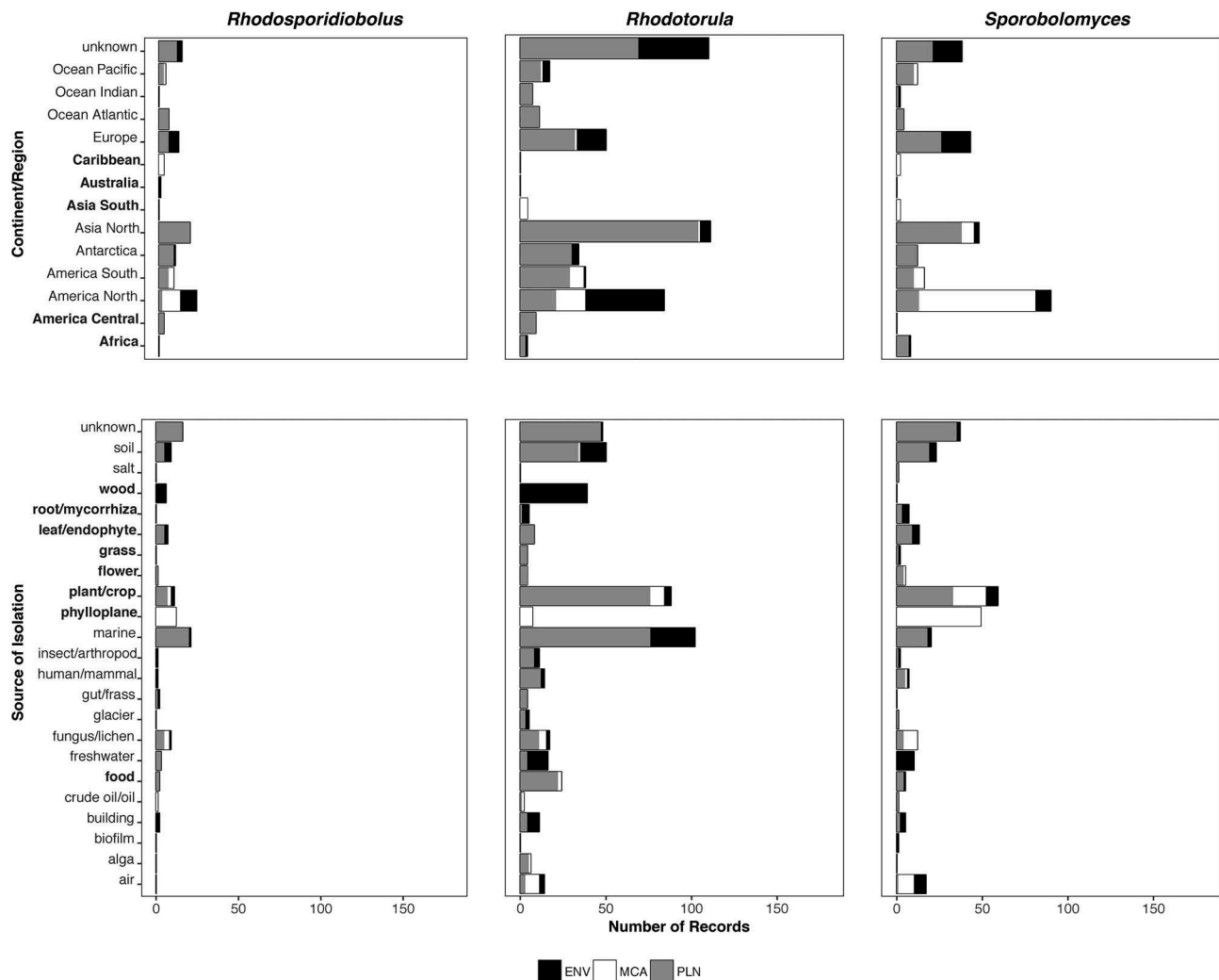
Figure 5. Global distribution of Sporidiobolales. A. Geographical regions (regions relatively lacking in Sporidiobolales data, in bold). B. Sources of isolation (plant, crop, and food sources in bold). Databases: Environmental (ENV) and plant and fungal (PLN) GenBank databases; MCA collection (MCA).
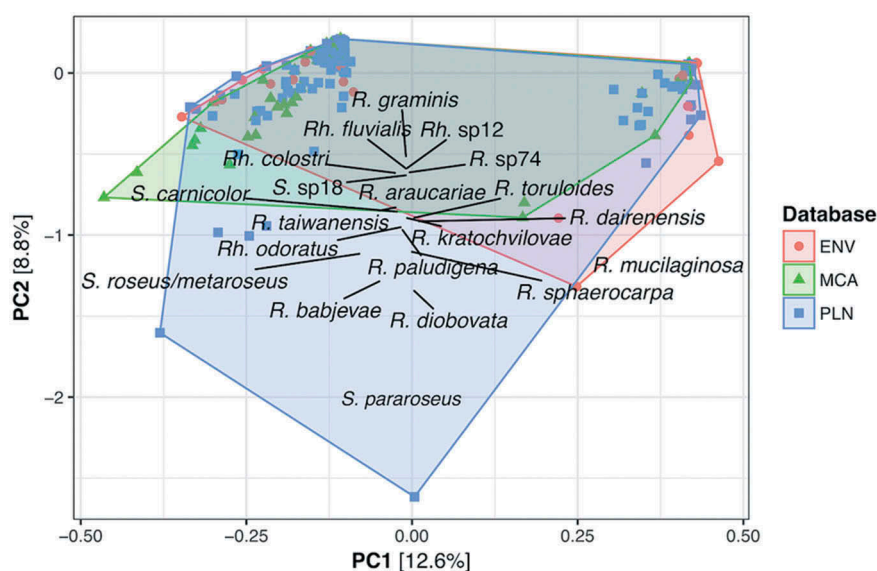
Figure 6. Principal component analysis of Sporidiobolales collections in different databases showing the 20 species present near the extreme of each axis. Databases: Environmental (ENV), and plant (PLN) GenBank databases; MCA collection (MCA).

any pathogenic tendencies (e.g., growth at 37 C, or resistance to antifungal agents and/or fungicides), given that several *Rhodotorula* species (*R. glutinis, R. mucilaginosa*) recently were reported as emerging human and animal pathogens (Tuon and Costa 2008; Alvarez-Perez et al. 2010; Wirth and Goldani 2012).

Mora et al. (2011) emphasize that the estimation of species diversity should be considered a primary research question. Here, we report the apparent under-description of one of the more readily cultured and sequenced, globally ubiquitous, orders of fungi and provide conservative estimates that indicate that less than 16% of extant Pucciniomycotina have been described to date. This number is especially astounding considering the relative abundance of Sporidiobolales in most ecosystems from marine and arctic environments to soils and a variety of fresh and processed foods.

ORCID

Hector Urbina http://orcid.org/0000-0002-5570-4537
M. Catherine Aime http://orcid.org/0000-0001-8742-6685

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