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# Red yeasts from leaf surfaces and other habitats: three new species and a new combination of *Symmetrospora* (*Pucciniomycotina*, *Cystobasidiomycetes*)

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**Abstract:** Our understanding of the systematics of red yeasts has greatly improved with the availability of sequence data and it is now clear that the majority of these fungi belong to three different classes of *Pucciniomycotina* (*Basidiomycota*): *Agaricostilbomycetes*, *Cystobasidiomycetes*, and *Microbotryomycetes*. Despite improvements in phylogenetic placement, the taxonomy of these fungi has long been in need of revision and still has not been entirely resolved, partly due to missing taxa. In the present study, we present data of culture-based environmental yeast isolation, revealing several undescribed species of *Symmetrospora*, which was recently introduced to accommodate six species previously placed in the asexual genera *Sporobolomyces* and *Rhodotorula* in the *gracilis*/*marina* clade of *Cystobasidiomycetes*. Based on molecular phylogenetic analyses of three rDNA loci, morphology, and biochemical studies, we formally describe the following new species: *Symmetrospora clarorosea sp. nov*. from leaf surfaces in Portugal and the USA; *S. pseudomarina sp. nov*. from leaf surfaces in Brazil, and the USA and decaying wood in the USA; and *S. suhii sp. nov*. from a beetle gut in the USA, leaf surfaces in Brazil and marine water in the Taiwan and Thailand. Finally, we propose a new combination for *Sporobolomyces oryzicola* based on our molecular phylogenetic data, *Symmetrospora oryzicola comb. nov*.

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## INTRODUCTION

The red-pigmented yeasts are ubiquitous microbes that often occur on the surfaces of plant material (Phaff 1990, Takashima & Nakase 2000, Fell *et al*. 2001, Inácio *et al*. 2002). In the past, these were grouped artificially into asexual genera—primarily *Sporobolomyces* and *Rhodotorula*—based on morphology and physiology (Boekhout 1991, Hamamoto *et al*. 2011, Sampaio 2011). Although DNA sequence data have demonstrated repeatedly that these asexual genera are polyphyletic (*e.g.*, Hamamoto & Nakase 2000, Aime *et al*. 2006, Wang *et al*. 2015a, b), taxonomic revision has been long overdue. Within *Pucciniomycotina*, *Sporobolomyces*, and *Rhodotorula* species have been placed in several orders within *Agaricostilbomycetes*, *Cystobasidiomycetes*, and *Microbotryomycetes* in the past (*e.g.*, Fell *et al*. 2000, Aime *et al*. 2006, Bauer *et al*. 2006). In the seven-locus phylogeny of Wang *et al*. (2015a), representatives of *Rhodotorula* and *Sporobolomyces* occurred in 17 and 23 clades, respectively. Wang *et al*. (2015b) revised five polyphyletic genera (*Bensingtonia*, *Rhodosporidium*, *Rhodotorula*, *Sporidiobolus* and *Sporobolomyces*). These authors proposed new combinations for 27 species of *Rhodotorula* (in 15 genera) and for 40 species of *Sporobolomyces* (in 16 genera).

Our study focuses on one of the groups within *Cystobasidiomycetes* that is known as the *gracilis* lineage (Scorzetti *et al*. 2002) or *marina* clade (Nagahama *et al*. 2006, Kurtzman *et al*. 2011, Wang *et al*. 2015a). No sexual morph is known for any species in this lineage and all the species have been described as either *Sporobolomyces* or *Rhodotorula*. The *gracilis*/*marina* clade contains seven species originating from various parts of the world. The first species that was described is *S. gracilis*, isolated from a decaying leaf in western Europe (Derx 1930). Other known species are: *S. foliicola* isolated from the leaf surface of *Banksia collina* in Australia (Shivas & Miranda 1983); *S. oryzicola* from a dead *Oryza sativa* leaf in Japan (Nakase & Suzuki 1986); *S. coprosmae* from dead leaves and fruit of *Coprosma tenuifolia* in New Zealand (Hamamoto & Nakase 1995); *S. vermiculatus* from a dead leaf of *Pennisetum pedicellatum* in Thailand (Takashima & Nakase 2000); and *S. symmetricus* from a *Betula platyphylla* leaf in China (Wang & Bai 2004). The only species not originally described from plant material is *R. marina*, a yeast isolated from shrimp (*Penaeus setiferus*) wash water in Texas, USA (Phaff *et al.* 1952).

All seven species mentioned above form smooth, butyrous, somewhat shiny colonies on agar medium. The colonies produce entire margins and colony color varies from pink to brick-red. None of these species have been observed to form hyphae or pseudohyphae, but most of them do form ballistoconidia (Hamamoto *et al*. 2011, Sampaio 2011). These characters are

shared with many other species in *Cystobasidiomycetes* and *Microbotryomycetes*, and modern generic circumscriptions of these yeasts are mainly based on molecular phylogenetic data (Wang *et al*. 2015b). Using phylogenetic inference analyses of a seven-locus dataset and an extended LSU rDNA locus dataset, Wang *et al*. (2015b) proposed the genus *Symmetrospora* to accommodate six of the seven species in the *gracilis*/*marina* clade. In this study, we reveal and formally describe three new species of *Symmetrospora* based on culture studies, physiological characterization, and rDNA sequence data (Kurtzman *et al.* 2011) from newly and previously collected material. In addition, according to the results of our phylogenetic reconstruction, we propose a new combination.

## MATERIALS AND METHODS

### Sample collection and isolations

Eighteen new yeast strains were examined during this study. Fifteen of these were obtained from various leaf surfaces in Illinois, Indiana, Louisiana, and Michigan in the USA and in Taiwan. Leaves were cut into small pieces that then were attached using Vaseline Petroleum Jelly to the inner lid of a Petri dish containing agar media. Chloramphenicol (1 mL L21) was added to the media to limit bacterial growth. One strain (BG 02-5-27-3-2-2) was isolated from the gut of a *Staphylinidae* beetle as described in Suh *et al*. (2005) and one strain (SA42) was isolated from a small piece of decaying wood using the spore-drop method as outlined above. Strain SA107 was obtained from indoor air by exposing a media plate on a lab bench top for 1 h.

All pure cultures were maintained on potato dextrose agar (PDA); long-term preservation of isolates was accomplished in the Aime Lab at Purdue University on PDA slants at 4 °C and as glycerol stocks at -80 °C. Holotypes are deposited at PUL (Kriebel Herbarium, Purdue University, West Lafayette, Indiana, USA) as dried inert material; ex-type and other cultures are deposited at the CBS culture collection (Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands).

### Morphological and physiological characteristics

Colony morphology was described by examining 10-d-old cultures on Yeast Malt extract Agar (YMA). Corn Meal Agar (CMA) and Dalmau plates were used to test for the formation of pseudohyphae and/or true hyphae. Culture colors were described subjectively and coded from 10-d-old cultures on YMA and CMA. Color codes were assigned following the Online Auction Color Chart (Kramer 2004). Microscopic characters were examined with a Nikon Eclipse 80i microscope with standard differential interference contrast (DIC) settings and with 40× and 100× objectives. Cell measurements from 20 cells grown in YM broth for 5 d were taken with an ocular micrometer using 100× oil-immersion objective. Images were taken with Nikon Digital Sight DS-Fi1 camera setup and measurements were calibrated with a stage micrometer.

Assimilation of various single carbon sources was determined for yeast species using Biolog YT microplates (Biolog Inc., Hayward, California, USA). Two-d-old cultures on YMA were used to inoculate BUY agar plates (Biolog Inc.). After 48 h of growth, these plates were used to prepare cell suspensions for inoculating the microplates. The optical density of the cell suspension in sterile water was adjusted to 0.04 (= 91 % transmittance) and 100 µL of that suspension was transferred to each microplate well. Measurements were performed at 1, 2, 5, 10, and 14 d post inoculation (dpi) using the ELx800 Universal Microplate Reader (Bio-Tek Instruments Inc., Winooski, Vermont, USA). The turbidity in the wells of each microplate was determined separately and the well with the highest reading value per each plate was determined (considered as 100 %). The wells with turbidity values lower than 20 % of the maximum value were recorded as negative and higher than 50 % were recorded as positive. The assimilation ability of the wells that had turbidity values between 20 % and 50 % was considered uncertain. The data from each plate were used only after the turbidity values for both negative control wells of the plate remained below 20 % which in most cases was around 10 dpi.

The assimilation of nitrogen compounds, fermentation ability, and the ability to grow in highly osmotic environment were tested on agar media as described in Suh *et al*. (2008). The maximum growth temperature was determined on YMA plates at 30 °C, 35 °C, and 37 °C.

### PCR, sequencing and phylogenetic inference

The small and large subunits (SSU, LSU) of the nuclear ribosomal DNA (rDNA) and the internal transcribed spacer (ITS) region were amplified by colony PCR. The LSU D1/D2 region is the DNA barcode for yeasts (Kurtzman & Robnett 1998), whereas the ITS is the default barcode for the Kingdom Fungi (Schoch *et al.* 2012). One colony of cells from a 2-d-old culture was eluted in 100 µL of sterile water, 5 µL of which was used as template for PCR. PCRs were carried out in 25 µL reactions containing 12.5 µL Apex Taq RED Master Mix (Genesee Scientific, San Diego, California, USA), 1.25 µL of each 10 µM primer, and 5 µL of $ddH_2O$. Primer pairs were NS1/NS4 and NS3/NS8 for SSU (White *et al*. 1990), ITS1F/ITS4 for ITS (White *et al*. 1990, Gardes & Bruns 1993), and LR0R/LR6 for LSU (Vilgalys & Hester 1990, Rehner & Samuels 1994). An Eppendorf Mastercycler EP Gradient Thermal Cycler was used for amplifications. Cycling conditions for the ITS locus were initial denaturation at 95 °C for 5 min; followed by 35 cycles of denaturing at 95 °C for 30 s, annealing at 45 °C for 45 s and elongation at 72 °C for 45 s; and a final elongation step of 72 °C for 7 min. Cycling conditions were the same for SSU and LSU, except for an extended extension up to 1 min for both loci, and annealing at 55 °C for 45 s for the SSU locus. Purification of PCR products and sequencing using the same primers were outsourced to Genewiz (South Plainfield, New Jersey, USA). Generated sequences were assembled, edited and trimmed using Sequencher v. 5.2.3 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and are deposited in GenBank (accession numbers in Table 1).

A Nucleotide BLAST search (https://blast.ncbi.nlm.nih.gov/) confirmed that all strains belonged to *Symmetrospora*. Datasets were constructed for individual SSU, ITS, and LSU loci by downloading sequences of type strains of described *Symmetrospora* species (accession numbers in Table 1). Alignments were constructed using MUSCLE v. 3.7 (Edgar 2004), which is available on the CIPRES Science Gateway v. 3.3 (Miller *et al*. 2010). Ambiguously aligned regions and uninformative positions were removed by using trimAl v. 1.2 (Capella-Gutiérrez *et al*. 2009) with a gap threshold of 60 % and coverage of 50 %. We constructed a concatenated SSU+ITS+LSU dataset of 31 isolates in MEGA v. 7 (Kumar *et al*. 2016). Phylogenetic relationships were inferred by analyzing the combined three-

**Table 1.** Species used in phylogenetic analysis, with strain information, type status (indicated by T), GenBank accession numbers of rDNA sequences (SSU, ITS, LSU), and references. Accession numbers of sequences generated during this study are in boldface.

| Species | Strain | Status | SSU | ITS | LSU | References |
|---|---|---|---|---|---|---|
| *Naohidea sebacea* | CBS 8477 | – | KP216515 | DQ911616 | DQ831020 | Wang *et al*. (2015a), P.B. Matheny & D.S. Hibbett unpubl. data, Matheny *et al*. (2006) |
| *Buckleyzyma armeniaca* | CBS 8076 | T | – | AF444523 | AF189920 | Fell *et al*. (2000), Scorzetti *et al*. (2002) |
| *Buckleyzyma aurantiaca* | CBS 317 | T | KJ708436 | AF444538 | AF189921 | Fell *et al*. (2000), Scorzetti *et al*. (2002), Wang *et al*. (2015a) |
| *Buckleyzyma kluyveri-nielii* | CBS 7168 | T | AB021674 | AF444544 | AF189988 | Fell *et al*. (2000), Hamamoto & Nakase (2000), Scorzetti *et al*. (2002) |
| *Erythrobasidium hasegawianum* | CBS 8253 | T | D12803 | AF444522 | AF189899 | Suh & Sugiyama (1993), Fell *et al*. (2000), Scorzetti *et al*. (2002) |
| *Erythrobasidium yunnanense* | JCM 10687 | T | AF229176 | AB030353 | AB127358 | Bai *et al*. (2001), Nagahama *et al*. (2006) |
| *Symmetrospora clarorosea* | SA308 | – | **KJ701227** | **KJ701225** | **KJ701226** | This study |
| | SA333 | – | **KJ701230** | **KJ701228** | **KJ701229** | This study |
| | CBS 14055 (WRP 7) | T | **KJ701233** | **KJ701231** | **KJ701232** | This study |
| | WRP 8 | – | **KJ701236** | **KJ701234** | **KJ701235** | This study |
| *Symmetrospora coprosmae* | CBS 7899 | T | D66880 | KY105570 | KY109807 | Hamamoto & Nakase (2000), Vu *et al*. (2016) |
| | MT 262 | – | **KJ701201** | **KJ701199** | **KJ701200** | This study |
| | MT 264 | – | – | **KJ701205** | – | This study |
| | P 116 | – | **KJ701204** | **KJ701202** | **KJ701203** | This study |
| *Symmetrospora cf. coprosmae* | HU9059 | – | – | **MN586903** | – | This study |
| | HU9256 | – | – | **MN586904** | **MN586902** | This study |
| *Symmetrospora foliicola* | CBS 8075 | T | AB021671 | KY105571 | KY109808 | Hamamoto & Nakase (2000), Vu *et al*. (2016) |
| *Symmetrospora gracilis* | CBS 71 | T | D10788 | AF444578 | AF189985 | Nakase *et al*. (1993), Fell *et al*. (2000), Scorzetti *et al*. (2002) |
| *Symmetrospora marina* | CBS 2365 | T | AB126645 | AF444504 | AF189944 | Fell *et al*. (2000), Scorzetti *et al*. (2002), Nagahama *et al*. (2006) |
| *Symmetrospora* sp. | P 114 | – | **KJ701221** | **KJ701219** | **KJ701220** | This study |
| | P 115 | – | **KJ701224** | **KJ701222** | **KJ701223** | This study |
| *Symmetrospora oryzicola* | CBS 7228 | T | AB021677 | AF444546 | AF189990 | Fell *et al*. (2000), Hamamoto & Nakase (2000), Scorzetti *et al*. (2002) |
| | MCA4496 | – | **KJ701195** | **KJ701193** | **KJ701194** | This study |
| | MCA4497 | – | **KJ701198** | **KJ701196** | **KJ701197** | This study |
| *Symmetrospora pseudomarina* | SA42 | – | **KJ701215** | **KJ701213** | **KJ701214** | This study |
| | CBS 14057 (SA716) | T | **KJ701218** | **KJ701216** | **KJ701217** | This study |
| *Symmetrospora suhii* | CBS 14094 (BG 02-5-27-3-2-2) | T | AY520260 | **KJ701206** | AY520389 | Su *et al*. (2005), this study |
| *Symmetrospora symmetrica* | CBS 9727 | T | KJ708350 | KY105573 | KY109810 | Wang *et al*. (2015a), Vu *et al*. (2016) |
| | P 118 | – | **KJ701212** | **KJ701210** | **KJ701211** | This study |
| | SA107 | – | **KJ701209** | **KJ701207** | **KJ701208** | This study |
| *Symmetrospora vermiculata* | CBS 9092 | T | | KY105574 | AF460176 | Scorzetti *et al*. (2002), Vu *et al*. (2016) |

locus dataset by maximum likelihood (ML). We used the command-line version of IQ-TREE (Nguyen *et al*. 2015) under partitioned models (Chernomor *et al*. 2016). Appropriate models of nucleotide substitution were selected according to the corrected Akaike Information Criterion (AICc) through the built-in ModelFinder (Kalyaanamoorthy *et al*. 2017). Selected models were TIM2+F+R2 (SSU, -lnL = 6946.074), TIM2+F+G4 (ITS1, -lnL = 2010.895), K3P (5.8S, -lnL = 554.742), SYM+G4 (ITS2, -lnL = 3158.263), and TIM2+F+I+G4 (LSU, -lnL = 4498.519). Ultrafast bootstrapping was done with 1 000 replicates (Hoang *et al*. 2018). The final tree with ML bootstrap support values (BS) was visualized in FigTree v. 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) and edited in Adobe Illustrator CC 2018.

In keeping with Kurtzman & Robnett (1998), we calculated the % similarity and number of nt differences between ex-type rDNA sequences (LSU, ITS) of our new species and their closest related relatives. These numbers are given in the respective diagnoses.

## RESULTS

### Nucleotide alignment dataset & phylogenetic inference

During this study, we generated 47 rDNA sequences (14 SSU, 18 ITS, 15 LSU) for 18 examined strains of *Symmetrospora*. The SSU section of our concatenated rDNA sequence dataset comprised 1 664 characters, of which 1 523 were constant and 56 were parsimony-informative. The ITS (partitioned into ITS1, 5.8S, and ITS2) comprised 173+158+249 characters, of which 92+151+117 were constant and 49+2+73 were parsimony-informative. Finally, the LSU comprised 728 characters, of which 585 were constant and 99 were parsimony-informative. Maximum likelihood of the combined SSU+ITS+LSU dataset allowed comparisons with reference sequences from ex-type strains (Fig. 1).

Of the 18 strains isolated in this study, nine represented four undescribed species of which three are formally described below. The remaining nine isolates were *Symmetrospora* (cf.) *coprosmae* (MT 262, MT 264, P 116, HU9059, HU9256), *S. oryzicola* (MCA4496, MCA4497), and *S. symmetrica* (P 118, SA107). Characteristics of colony and cell morphology were not sufficient to differentiate among the new *Symmetrospora* species. The colony pigmentation for most of the studied strains was dark pink to orange-red, whereas the color of *S. pseudomarina* strains varied from salmon pink (SA716) to red (SA42). All strains had butyrous colonies with entire margins, only presenting some variation in the shiny or dull appearance. However, results from assimilation studies (Table 2) supported the delimitation of species based on our molecular phylogenetic data.

### Taxonomy

***Symmetrospora*** Q.M. Wang *et al.*, *Stud. Mycol.* **81**: 175. 2015.
*Type species*: *Symmetrospora gracilis* (Derx) Q.M. Wang *et al.*, *Stud. Mycol.* **81**: 176. 2015.

***Symmetrospora clararosea*** Toome, Albu & Aime, ***sp. nov.*** MycoBank MB809695. Fig. 2A.

*Etymology*: Referring to the color of the colonies on solid media (*clarus* = bright, *roseus* = pink).

*Diagnosis*: LSU shares 97.84 % identity with ex-type sequence of *S. gracilis* (13 nt different); ITS shares 96.15 % identity with ex-type sequence of *S. gracilis* (23 nt different). Different from *S. gracilis* by the ability to assimilate sucrose, galactose, melezitose, and L-arabinose.

*Typus*: **USA**, Louisiana, Florida Parishes Region, East Baton Rouge Parish, Baton Rouge, Louisiana State University campus, 30.407817N, 91.176187W, 27 Jan. 2009, *W.R. Pilcher*, surface of *Quercus virginiana* leaf (*Fagales*, *Fagaceae*), WRP 7 (dried inert material at PUL **holotype**), ex-type culture at CBS (CBS 14055), GenBank accession nos. KJ701233 (SSU), KJ701231 (ITS), KJ701232 (LSU).

*Description*: Colonies on YMA are butyrous, smooth, with entire margins, shiny or dull, pink (oac573 on YMA but oac574 on CMA). Growth at 20–25 °C (optimal); no growth at 30 °C. Yeast cells after 5 d in YM broth ellipsoid, 2–5 × 5–10 µm (av. 3.4 × 7.2 µm), with length/width ratio of 1.4–3.5 (av. 2.2); polar budding from a narrow base, generally only one bud per cell; ballistosporic. Fermentation ability absent. Carbon compounds assimilated: D-cellobiose, gentiobiose, maltotriose, melezitose, sucrose, turanose, D-glucose, galactose, methyl-α-D-glucoside, D-mannitol, D-sorbitol, adonitol, D-arabitol, ribitol, glycerol, L-arabinose, and D-xylose. No growth on melibiose, D-glucosamine, amygdalin, and erythritol. Nitrogen compounds assimilated: nitrate, D-tryptophan, L-lysine (variable), and cadaverine (variable). Additional compounds assimilated: D-gluconic acid and 2-keto-D-gluconic acid. Osmotic stress: no growth in the presence of 10 % NaCl or 50 % glucose. No pseudohyphae or hyphae observed. Sexual morph unknown.

*Additional material examined*: **USA**, Louisiana, Florida Parishes Region, East Baton Rouge Parish, Baton Rouge, Louisiana State University campus, 30.407817N, 91.176187W, 13 Apr. 2011, *S. Albu*, surface of *Lygopodium japonicum* leaf (*Schizaeales*, *Lygodiaceae*), SA333, referred to as *Sporobolomyces* sp. *cf. gracilis* 1 in Albu (2012), culture at CBS (CBS 14085), GenBank accession nos. KJ701230 (SSU), KJ701228 (ITS), KJ701229 (LSU); *Ibid.*, Jan. 2009, *W.R. Pilcher*, surface of *Salix* sp. leaf (*Malpighiales*, *Salicaceae*), WRP 8, culture at CBS (CBS 14093), GenBank accession nos. KJ701236 (SSU), KJ701234 (ITS), KJ701235 (LSU); Louisiana, Florida Parishes Region, East Baton Rouge Parish, Baton Rouge, Louisiana State University campus, 30.409093N, 91.176428W, 18 Mar. 2011, *S. Albu*, surface of *Dryopteris erythrosora* leaf (*Polypodiales*, *Dryopteridaceae*), SA308, referred to as *Sporobolomyces* sp. *cf. gracilis* 2 in Albu (2012), GenBank accession nos. KJ701227 (SSU), KJ701225 (ITS), KJ701226 (LSU).

*Habitat and distribution*: On leaf surfaces in North America (USA, Louisiana).

*Notes*: A Portuguese strain, CBS 10200 (Inácio *et al*. 2009), appears to be conspecific to *S. clararosea* based on its published ITS and LSU rDNA sequences (Table 3). These sequences were submitted to GenBank as *Symmetrospora* sp.

***Symmetrospora oryzicola*** (Nakase & M. Suzuki) Haelew. & Aime, ***comb. nov.*** MycoBank MB833757. Fig. 2B.
*Basionym*: *Sporobolomyces oryzicola* Nakase & M. Suzuki, *J. Gen. Appl. Microbiol., Tokyo* **32**: 152. 1986.

*Description*: Colonies on YMA butyrous, smooth but becoming verrucose in age, with entire margins, dull, dark pink to red (oac588 on YMA but oac577 on CMA). Growth at 20–25 °C (optimal) and at 30 °C (weak). Yeast cells after 5 d in YM broth subglobose, 4–6 × 6–8 µm (av. 4.9 × 7.2 µm), with length/width ratio of 1.2–1.75 (av. 1.5); polar budding from a narrow base; ballistosporic. No pseudohyphae or hyphae observed. Sexual morph unknown.

*Materials examined*: **Taiwan**, Southern Taiwan Region, Tainan City, Shanhua District, World Vegetable Center (Asian Vegetable Research and Development Center), 23.115782N, 120.298994E, 25 Jul. 2011, *M.C. Aime*, surface of *Vigna* sp. leaf (*Fabales*, *Fabaceae*), MCA 4496, culture at CBS (CBS 14050), GenBank accession nos. KJ701195 (SSU), KJ701193 (ITS), KJ701194 (LSU); *Ibid.*, MCA 4497, GenBank accession nos. KJ701198 (SSU), KJ701196 (ITS), KJ701197 (LSU).

*Habitat and distribution*: On leaf surfaces in Asia (Japan, Taiwan).

*Notes*: The sister species of *S. oryzicola* is *S. coprosmae*, which is reported from various substrates in Europe (Austria, Molnár *et*

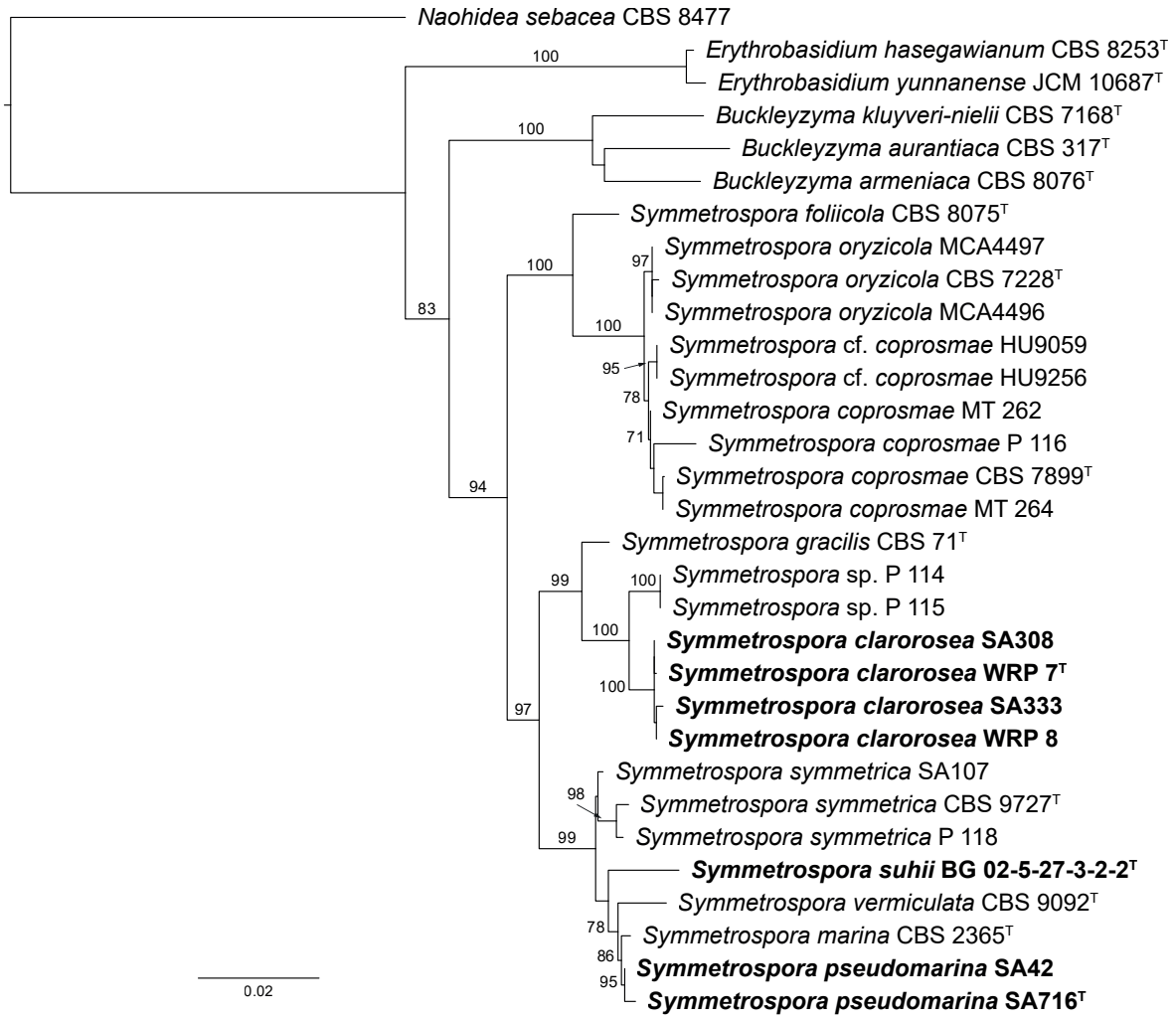


**Fig. 1.** Phylogenetic placement of *Symmetrospora clarorosea sp. nov.*, *S. oryzicola comb. nov.*, *S. pseudomarina sp. nov.*, and *S. suhii sp. nov.*, reconstructed from a combined dataset of SSU, ITS, and LSU sequences. The topology is the result of maximum likelihood inference performed with IQ-TREE. For each node, ML BS support values ≥ 70 % are presented above/below the branch leading to that node.

**Table 2.** Physiological differences between *Symmetrospora clarorosea sp. nov.*, *S. pseudomarina sp. nov.*, *S. suhii sp. nov.*, and their closest related species.

| Compound | *S. clarorosea* | *S. gracilis* | *S. marina* | *S. pseudomarina* | *S. suhii* | *S. symmetrica* | *S. vermiculata* |
|---|---|---|---|---|---|---|---|
| Sucrose | + | - | + | + | + | + | l |
| Melibiose | - | - | - | - | u | - | - |
| Galactose | + | - | + | u | u | - | + |
| Lactose | u | + | - | + | + | + | + |
| Trehalose | u | + | - | + | + | + | + |
| Maltose | u | - | + | - | + | - | - |
| Melezitose | + | - | w/s | + | u | + | + |
| Cellobiose | + | + | + | + | u | + | l |
| D-Xylose | + | + | s | + | + | - | + |
| L-Arabinose | + | - | s | + | u | - | + |
| L-Rhamnose | u | - | s | +[a] | - | - | - |
| L-Sorbose | u | - | w/s | - | - | - | l/w |
| Ribitol | + | + | w/s | + | + | + | l/w |
| Nitrate | + | - | - | u | w | - | - |

+, growth; -, no growth; l, latent; s, positive but slow; v, variable; w, weak; u, uncertain. Data for the new species taken from the ex-type strains (*S. clarorosea*, WRP 7; *S. pseudomarina*, SA716; *S. suhii*, BG 02-5-27-3-2-2). Data for the reference species taken from Kurtzman *et al*. (2011).

[a] The assimilation of L-rhamnose was uncertain for the ex-type strain but positive for the other strain tested (SA42).

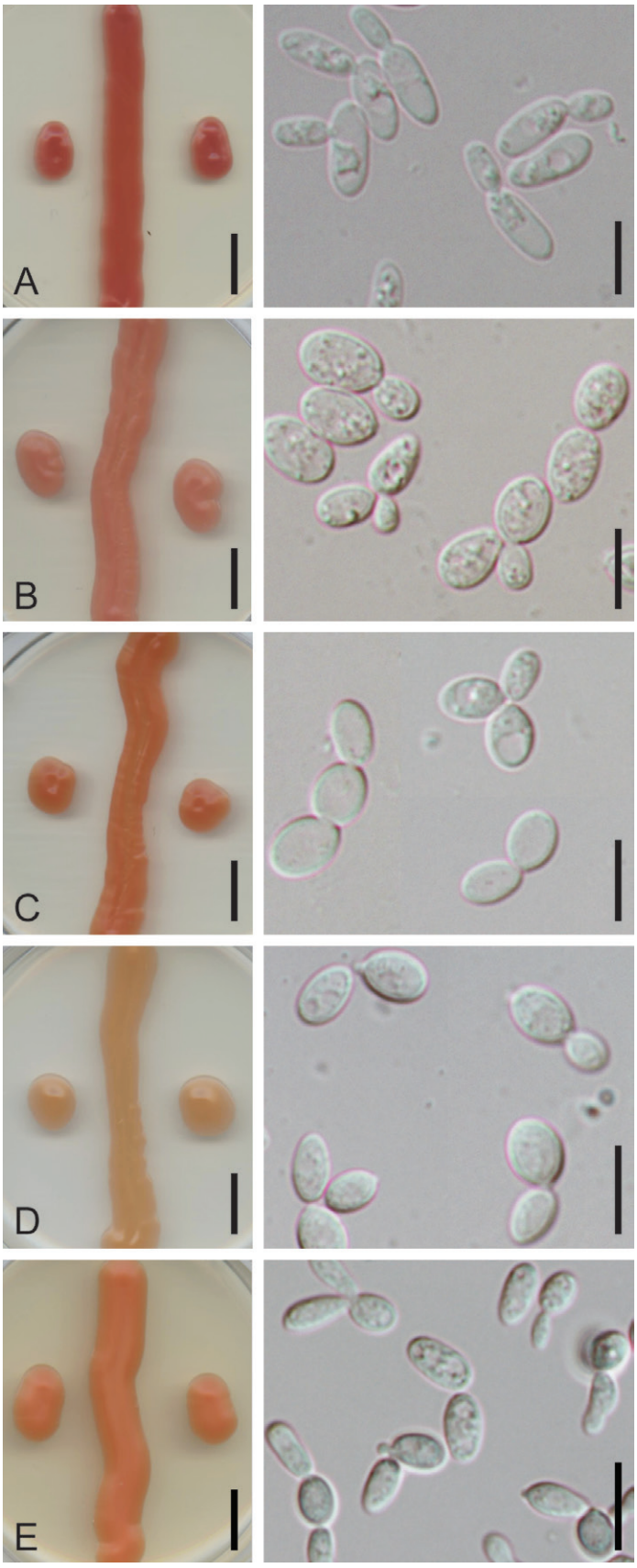


**Fig. 2.** Colony and cell morphology of *Symmetrospora* species on YMA (left panels) and YM broth (right panels): **A.** *Symmetrospora clarorosea* strain SA308. **B.** *Symmetrospora oryzicola* strain MCA 4496. **C–D.** *Symmetrospora pseudomarina* strains SA42 (C) and SA716 (D, ex-type), showing the variation in colony color between the two strains. **E.** *Symmetrospora suhii* strain BG 02-5-27-3-2-2 (ex-type). Scale bars = 1 cm in culture images (left panels), 10 µm in cell images (right panels).

*al*. 2008), North America (USA, Indiana & Michigan, this study), and Oceania (New Zealand, ex-type strain, Hamamoto & Nakase 1995). In contrast, *S. oryzicola* is much less commonly found; in addition to the two strains from Taiwan presented here, only the Japanese ex-type strain of *S. oryzicola* is known (Nakase & Suzuki 1986).

***Symmetrospora pseudomarina*** Haelew., Albu & Aime, ***sp. nov.*** MycoBank MB809701. Fig. 2C–D.

*Etymology*: Referring to similarities and past confusion with *S. marina*.

*Diagnosis*: LSU shares 99.83 % identity with ex-type sequence of *S. marina* (1 nt different) and 99.49 % with ex-type sequence of *S. vermiculata* (3 nt different); ITS shares 98.24 % identity with ex-type sequence of *S. marina* (10 nt different) and 97.18 % with ex-type sequence of *S. vermiculata* (15 nt different). Different from *S. marina* by the ability to assimilate lactose and D-trehalose, and the inability to assimilate maltose and L-sorbose. Different from *S. vermiculata* by the ability to assimilate L-rhamnose and the inability to assimilate L-sorbose.

*Typus*: **USA**, Louisiana, Florida Parishes Region, East Baton Rouge Parish, Baton Rouge, Louisiana State University campus, 30.409093N, 91.176428W, 1 Nov. 2011, S. Albu, surface of *Dryopteris erythrosora* leaf (*Polypodiales*, *Dryopteridaceae*), SA716 (dried inert material at PUL **holotype**), referred to as *Rhodotorula marina* 3 in Albu (2012), ex-type culture at CBS (CBS 14057), GenBank accession nos. KJ701218 (SSU), KJ701216 (ITS), KJ701217 (LSU).

*Description*: Colonies on YMA butyrous, smooth, with entire margins, shiny, salmon pink to red (culture SA42 oac617 on YMA but oac618 on CMA; culture SA716 oac619 on YMA and CMA). Growth at 20–25 °C (optimal) and at 30 °C (variable); no growth at 35 °C. Yeast cells after five days in YM broth globose to ovoid, 3–5 × 4–8 µm (av. 3.8 × 5.5 µm), with length/width ratio of 1–2.3 (av. 1.5); polar budding from a narrow base, generally 1–2 buds per cell; ballistosporic. Fermentation ability absent. Carbon compounds assimilated: D-cellobiose, gentiobiose, melezitose, sucrose, trehalose, D-glucose, methyl-β-D-glucoside, arbutin, D-mannitol, D-sorbitol, adonitol, D-arabitol, ribitol, glycerol, D-arabinose, L-arabinose, and D-xylose. Variable growth on maltotriose, palatinose, turanose, D-psicose, and L-rhamnose. No growth on maltose, melibiose, stachylose, D-glucosamine, L-sorbose, and erythritol. Nitrogen compounds assimilated: L-lysine and D-tryptophan. Additional compounds assimilated: fumaric acid, L-malic acid, bromosuccinic acid, L-glutamic acid, and D-gluconic acid. Osmotic stress: no growth in the presence of 10 % NaCl or 50 % glucose. No pseudohyphae or hyphae observed. Sexual morph unknown.

*Additional material examined*: **USA**, Louisiana, Rapides Parish, Lena, Kisatchie National Forest, in the vicinity of 31.583217N, 92.544855W, 9 Oct. 2010, *S. Albu*, decaying wood, SA42, culture at CBS (CBS 14084), GenBank accession nos. KJ701215 (SSU), KJ701213 (ITS), KJ701214 (LSU).

*Habitat and distribution*: On leaf surfaces in North America (USA) and South America (Brazil) and on decaying wood in North America (USA).

**Table 3.** Additional strains downloaded from NCBI GenBank of *Symmetrospora clarorosea sp. nov*., *S. pseudomarina sp. nov*., and *S. suhii sp. nov*., with original identification in GenBank, accession numbers for ITS and LSU, BLAST results, and references.

| Strain | ID in GenBank | Species | Origin | ITS | ITS BLAST | LSU | LSU BLAST | References |
|---|---|---|---|---|---|---|---|---|
| CBS 10200 | *Symmetrospora* sp. | *Symmetrospora clarorosea* | Portugal | EU002879 | 98.91 % | EU002821 | 99.65 % | Inácio *et al*. (2009) |
| A31 | *Symmetrospora marina* | *Symmetrospora pseudomarina* | Brazil | KM246155 | 98.42 % | KM246010 | 99.67 % | T.S. Leite *et al*. unpubl. data |
| DMKU5-4 | *Symmetrospora* sp. | *Symmetrospora suhii* | Thailand | LC216897 | 100 % | LC216897 | 99.83 % | S. Limtong & C. Kaewkrajay unpubl. data |
| IMUFRJ 52025 | *Symmetrospora* aff. *marina* | *Symmetrospora suhii* | Brazil | FN428894 | 99.33 % | FN428894 | 99.84 % | J.R.A. Ribeiro unpubl. data |
| IMUFRJ 52026 | *Symmetrospora* aff. *marina* | *Symmetrospora suhii* | Brazil | FN428925 | 99.48 % | FN428925 | 99.67 % | J.R.A. Ribeiro unpubl. data |
| SM10 | *Symmetrospora* sp. | *Symmetrospora suhii* | Taiwan | FJ515188 | 100 % | FJ515243 | 99.66 % | Chang *et al*. (2016) |

*Notes*: One strain for which ITS and LSU sequences have been published (T.S. Leite *et al*. unpubl. data) is conspecific with *S. pseudomarina*. This strain, accessioned as *S. marina*, was isolated from *Coffea arabica var.* Catucaí Amarel (*Gentianales*, *Rubiaceae*) in Brazil (details in Table 3). *Symmetrospora pseudomarina* is distinguished from sister species *S. marina* by several characteristics: rDNA sequence data (Fig. 1), habitat (marine in *S. marina* versus phylloplane in *S. pseudomarina*), assimilation profiles (Atkin *et al*. 1970, Sampaio 2011), and colony color (pink in *S. marina* versus variable in *S. pseudomarina*).

***Symmetrospora suhii*** Toome & Aime, ***sp. nov.*** MycoBank MB809699. Fig. 2E.

*Etymology*: Named after Dr. Sung-Oui Suh, scientist at the American Type Culture Collection, who isolated and partially characterized the ex-type strain of this species.

*Diagnosis*: LSU shares 98.67 % identity with ex-type sequence of *S. marina* (8 nt different), 98.47 % with ex-type sequence of *S. vermiculata* (9 nt different), and 98.43 % with ex-type sequence of *S. pseudomarina* (9 nt different); ITS shares 95.18 % identity with ex-type sequence of *S. marina* (28 nt different), 95.29 % with ex-type sequence of *S. pseudomarina* (27 nt different), and 94.74 % with ex-type sequence of *S. vermiculata* (29 nt different). Different from *S. vermiculata* by the ability to assimilate maltose and the inability to assimilate L-sorbose. Different from *S. marina* by the ability to assimilate lactose, trehalose, and nitrate, and the inability to assimilate L-sorbose and L-rhamnose. Different from *S. pseudomarina* by the ability to assimilate maltose and the inability to assimilate L-rhamnose.

*Typus*: **USA**, Louisiana, Florida Parishes Region, East Baton Rouge Parish, Baton Rouge, Rural Life Museum, 27 May 2002, *S.-O. Suh*, gut of staphylinid beetle (*Coleoptera*, *Staphylinidae*) collected from mushroom, BG 02-5-27-3-2-2 (dried inert material at PUL **holotype**), ex-type culture at CBS (CBS 14094), GenBank accession nos. AY520260 (SSU), KJ701206 (ITS), AY520389 (LSU).

*Description*: Colonies on YMA butyrous, smooth, with entire margins, shiny or dull, occasionally elevated in the center, red-orange (oac649 on YMA but oac650 on CMA). Growth at 20–25 °C (optimal), and at 30 °C (weak); no growth at 35 °C. Yeast cells after 5 d in YM broth ellipsoid, 3–4 × 4–7 µm (av. 3.7 × 5.4 µm), with length/width ratio of 1–1.75 (av. 1.5); polar budding from a narrow base, occasionally more than one bud per cell; ballistosporic. Fermentation ability absent. Carbon compounds assimilated: gentiobiose, maltose, palatinose, sucrose, trehalose, maltitol, D-mannitol, D-sorbitol, adonitol, D-arabitol, glycerol, D-ribose, and D-xylose. No growth on L-rhamnose, L-sorbose, and erythritol. Nitrogen compounds assimilated: ethylamine, cadaverine, creatine, D-tryptophan, and nitrate (weak). Osmotic stress: no growth in the presence of 10 % NaCl or 50 % glucose. No pseudohyphae or hyphae observed. Sexual morph unknown.

*Habitat and distribution*: In beetle gut in North America (USA); on leaf surfaces in South America (Brazil); in marine water in Asia (Taiwan, Thailand).

*Notes*: Four strains appear to be conspecific with *S. suhii* based on their published ITS and LSU rDNA sequences (Table 3). Their sequences were submitted to GenBank under different names. These strains are: DMKU 5-4 (from a sea sponge/marine water in Thailand, S. Limtong & C. Kaewkrajay unpubl. data); IMUFRJ 52025 and IMUFRJ 52026 (from sugarcane leaves in Brazil, as *S. aff. marina*, J.R.A. Ribeiro unpubl. data); SM10 (in marine water in Taiwan, Chang *et al*. 2016).

## Additional materials examined

***Symmetrospora coprosmae*** (Hamam. & Nakase) Q.M. Wang *et al.*, *Stud. Mycol.* **81**: 175. 2015.
*Basionym*: *Bullera coprosmae* Hamam. & Nakase, *Antonie Leeuwenhoek* **69**: 281. 1996.

*Materials examined*: **USA**, Illinois, Cook County, Chicago, 18 May 2016, *H. Urbina*, surface of *Lactuca sativa* leaf (*Asterales*, *Asteraceae*), lettuce head no. L30, HU9256, GenBank accession nos. MN586904 (ITS), MN586902 (LSU), as *S. cf. coprosmae*; Indiana, Tippecanoe County, Wabash Township, West Lafayette, 40.455385N, 86.917498W, 5 May 2016, *H. Urbina*, surface of *Lactuca sativa* leaf, lettuce head no. L13, HU9059, GenBank accession no. MN586903 (ITS), as *S. cf. coprosmae*; Louisiana, Orleans Parish, New Orleans, Audubon Park, 29.924645N, 90.129245W, 13 Nov. 2010, *S.L. Newerth*, surface of *Cyrtomium falcatum* leaflet (*Polypodiales*, *Dryopteridaceae*), P 116, GenBank accession nos. KJ701204 (SSU), KJ701202 (ITS), KJ701203 (LSU); Michigan, Emet County, Cross Village Township, 45.645097N, 85.039827W, 1 Sep. 2013, *M. Toome-Heller*, surface of *Salix* sp. leaf infected with *Melampsora* sp. (*Pucciniales*, *Melampsoraceae*), MT 262, GenBank accession

nos. KJ701201 (SSU), KJ701199 (ITS), KJ701200 (LSU); *Ibid.*, MT 264, GenBank accession no. KJ701205 (ITS).

***Symmetrospora symmetrica*** (F.Y. Bai & Q.M. Wang) Q.M. Wang *et al.*, *Stud. Mycol.* **81**: 176. 2015.
*Basionym*: *Sporobolomyces symmetricus* F.Y. Bai & Q.M. Wang, *FEMS Yeast Res.* **4**: 584. 2004.

*Materials examined*: **USA**, Indiana, Tippecanoe County, Wabash Township, West Lafayette, Purdue University campus, 40.422300N, 86.917383W, 14 Jun. 2013, *S.L. Newerth*, surface of *Pinus nigra* leaf (*Pinales*, *Pinaceae*), P 118, culture at CBS (CBS 14058), GenBank accession nos. KJ701212 (SSU), KJ701210 (ITS), KJ701211 (LSU); Louisiana, Florida Parishes Region, East Baton Rouge Parish, Baton Rouge, Louisiana State University campus, 30.411000N, 91.177300W, 11 Nov. 2010, *S. Albu*, indoor air contaminant, SA107, culture at CBS (CBS 14059), GenBank accession nos. KJ701209 (SSU), KJ701207 (ITS), KJ701208 (LSU).

## DISCUSSION

The asexual red-yeast genera *Sporobolomyces* and *Rhodotorula* are polyphyletic (*e.g.*, Nakase *et al*. 1993, Fell *et al*. 2000, Hamamoto & Nakase 2000, Aime *et al*. 2006, Boekhout *et al*. 2011, Wang *et al*. 2015a). Wang *et al*. (2015b) provided a taxonomic infrastructure for the *gracilis*/*marina* clade of the *Cystobasidiomycetes* and introduced the genus *Symmetrospora* with six species. Even though *Sporobolomyces oryzicola* was part of the maximally supported *Symmetrospora* clade in their LSU rDNA phylogenetic reconstruction, the authors did not introduce the new combination. In our three-locus phylogeny, *S. oryzicola* was maximally supported as a sister species of *S. coprosmae*. Hence, we formally include it in the genus *Symmetrospora*.

This study reveals that *Symmetrospora* is more diverse and more broadly distributed than currently recognized. Our opportunistic collecting, mostly from university campuses, increased the number of known species in the group by two thirds and expanded known geographic ranges for previously described species. Our records of *S. coprosmae* and *S. symmetrica* are the first ones for the USA, and our strains of *S. oryzicola* represent the first report of this species outside Japan. Overall, we present the first reports of any species in this genus for North America. We also consider sequences from strains previously isolated by other researchers in Table 3, which represent additional isolates of the species described here. These isolates have identical or near-identical ITS and/or LSU sequences to our type strains. By considering these isolates, we were able to reveal broader occurrences of the new species. For example, we collected *S. suhii* from Louisiana, USA, but sequence data from GenBank revealed that this species is likely more broadly distributed – with isolates from South America and Asia (Chang *et al*. 2016, S. Limtong & C. Kaewkrajay unpubl. data, J.R.A. Ribeiro unpubl. data).

In addition to expanding both the number of species in the genus and distributional ranges, our study also reveals a diverse array of habitats for *Symmetrospora* species. Whereas most species were isolated from the phylloplane, several strains were from the air, marine water, the beetle gut, and a sea sponge. This is in addition to previous work, which reported the isolation of a strain of *S. symmetrica* from *Pleurotus eryngii*, causing red spot disease (Xu *et al*. 2014). When isolated from the phylloplane, no preference for host plants can be detected. As an example, *S. clarorosea* was isolated from leaves of two unrelated fern species and two unrelated species of eudicots. We also found more than one species of yeast from a single host plant; strains of both newly described species *S. clarororea* and *S. pseudomarina* were isolated from leaf surfaces of *D. eyrthrosora*.

We confirm previous findings that culture-based sampling from the surface of leaves, referred to as the phylloplane, effortlessly results in the discovery of undescribed species. During an ongoing study of the fungal microbiome of romaine lettuce, we sequenced the ITS region of 330 strains, resulting in 63 species of which 11 are undescribed (Urbina & Aime 2018, D. Haelewaters & M.C. Aime, unpubl. data). Two of these strains isolated from romaine lettuce leaves, HU9059 and HU9256, were identified as *S.* cf. *coprosmae* and are reported here. Whereas a number of these new species have been described over the last years (*e.g.*, Inácio *et al*. 2002, 2005, Péter *et al*. 2007, Golubev & Scorzetti 2010, Toome *et al*. 2013, Wang *et al*. 2016, Limtong *et al*. 2017), many remain undescribed even though they have been recognized as new. One of such examples was a two-year survey of phylloplane yeasts at Nature Park of Arrábida in Portugal that resulted in over 850 isolates representing 70 species, half of which may be new to science (Inácio *et al*. 2002). Likewise, a survey targeting phylloplane-colonizing basidiomycete yeasts reported 29 potential new species from a collection of 463 isolates, including the type strain of S. pseudomarina (Albu 2012). It seems that the extent of species diversity in the leaf habitat is not fully understood yet, highlighting the importance of further studies to capture the hidden fungal biodiversity.

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