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Large biodiversity of yeasts in French Guiana and the description of *Suhomyces coccinellae* f.a. sp. nov. and *Suhomyces faveliae* f.a. sp. nov.

Noémie Jacques† and Serge Casaregola*

Abstract

The extent of the diversity of yeasts in tropical rain forest and different environments from French Guiana was investigated. A total of 365 samples were collected from various substrates, such as plants, fruits and insects, at 13 locations, yielding 276 pure yeast isolates. Sequence analysis of the D1/D2 domains of the large subunit rRNA gene indicated that 210 isolates out of 276 belonged to 82 described species (67 Saccharomycotina, 14 Basidiomycota and 1 Pezizomycotina). In addition to these, a total of 54 Saccharomycotina isolates could not be assigned to a known species. These belonged to 14 genera and should be studied further from a taxonomic point of view. In addition, among the 43 Basidiomycotina isolates found, 12 could not be assigned to a known species. This report shows an unexpected biodiversity and indicates that oversea territories, such as French Guiana, constitute a largely unexplored reservoir for yeast diversity. Two Saccharomycotina strains, CLIB 1706 and CLIB 1725, isolated from an insect and from a fern respectively, were characterized further and were shown to belong to the *Suhomyces* clade on the basis of the rDNA sequence comparison. CLIB 1706^{T}rDNA sequences showed nine substitutions and three indels out of 556 bp (D1/D2 domains) and 32 substitutions and 12 indels out of 380 bp [internal transcribed spacer (ITS)] with that of the most closely related species *Suhomyces guaymorum* CBS 9823^T. CLIB 1725^T rDNA sequences presented 18 substitutions and one indel out of 549 bp (D1/D2 domains) and 48 substitutions and 11 indels out of 398 bp (ITS) with that of its closest relative *Suhomyces vadensis* CBS 9454^T. Two novel species of the genus *Suhomyces* were described to accommodate these two strains: *Suhomyces coccinellae* f.a. sp. nov. (CLIB 1706^T=CBS 14298^T) and *Suhomyces faveliae* f.a. sp. nov. (CLIB 1725^T=CBS 14299^T).

INTRODUCTION

French Guiana is located in the northern part of South America; it borders Brazil to the east and south, and Suriname to the west. The Amazonian forest is located in the most remote part of the country. French Guiana is a region that offers great potential for the study of microbial biodiversity due to its unique and important ecosystems, such as tropical rainforests and coastal mangroves. The presence of primeval forests, which are biodiversity hotspots, provides this country with one of the highest levels of diversity in terms of flora and fauna [1].

Recent studies on yeast isolated from rainforests have concentrated on the search for new species, and several have been described, mainly from samples collected in Brazil [2–8], China [9–15]) or Thailand ([16–21]).

This is in contrast with French Guiana where few species have been described recently. These include *Candida tallmaniae*, *Candida vaughaniae* [22], *Wickerhamomyces chaumierensis*, *Candida robnettiae*, *Candida pseudoflosculorum*, *Candida eppingiae* [23], *Saccharomycopsis guyanensis* [24], *Hyphopichia buzzinii* [25] and two species of the genus *Starmerella*, *Starmerella reginensis* and *Starmerella kourouensis* [26]. Incidentally, the isolates which led to the description of the species *S. guyanensis*, *H. buzzinii*, *S. reginensis* and *S. kourouensis* had been collected during the field collections described here. Therefore, the small number of species discovered in French Guiana is an indicator of the little attention received by this region of the world. In addition to the cited works, two studies of yeast communities in French Guiana have focused on microorganisms involved in human infections [27, 28].

Author affiliation: CIRM-Levures, INRA, Micalis Institute, Jouy-en-Josas, France.
***Correspondence:** Serge Casaregola, serge.casaregola@inra.fr
Keywords: novel yeast species; French Guiana; *Suhomyces coccinellae*; *Suhomyces faveliae*.
Abbreviations: D1/D2, D1/D2 domains of the large subunit rRNA gene; ITS, internal transcribed spacer; YEA, 0.5 % yeast extract, 1 % glucose; YPD, 1 % yeast extract, 1 % peptone, 1 % glucose.
†Present address: Bioger, INRA, Thiverval-Grignon, France.
The GenBank/EMBL/DDBJ accession numbers for the sequences determined in this study are listed in Table 1. The Mycobank (http://www.mycobank.org) accession numbers for *Suhomyces coccinellae* sp. nov. and *Suhomyces faveliae* sp. nov. are, respectively, MB 815607 and MB 815608. One supplementary figure and two supplementary tables are available with the online version of this article.

In 2016, Kurtzman, Robnett and Blackwell [29] placed 24 species of the *Candida tanzawaensis* clade, a clade that was created to accommodate the species *Candida tanzawaensis* and six related species [30], in the new genus *Suhomyces* [29]. The original clade was increased with the description of 16 novel species [31]. It is interesting to note that the vast majority of the species of this clade have been isolated from beetles that feed on fungi [31, 32].

In this study, a total of 276 isolates are described. They were isolated from 365 samples of natural substrates, such as plants, fruits and insects. Among these 276 isolates, 54 Saccharomycotina and 12 Basidiomycota, corresponded to novel species on the basis of DNA comparison analysis. This very high proportion of novel yeast species, amounting to 23.9 % of the total yeast isolates collected confirms that a large part of the biodiversity of yeasts remains to be discovered and shows that French Guiana is an important reservoir of this biodiversity. In the present study, we also report the description of two novel yeast species belonging to the *Suhomyces* clade, *Suhomyces coccinellae* f.a. sp. nov. and *Suhomyces faveliae* f.a. sp. nov.

METHODS

Yeast isolation

A total of 365 samples were collected in May 2008 and in April 2010 from various substrates on seven major sites in French Guiana. These major sites are Kourou, Sinnamary, Saül, Cacao, Matoury, Saint-Elie and Regina, as indicated in Fig. 1. The distance between the most remote collection areas, Sinnamary and Saül, is 177 km. For three of these major sites, Kourou, Saül and Regina, sampling was carried out in different places: two for Kourou ('Montagne des Singes' and the town of Kourou itself), three for Saül ('boucle des gros arbres' path, 'layon des eaux claires' path and Saül village) and four for Regina ('savane roche' path, 'inn' area, the banks of the river Approuage and the cacao factory 'Cacao d'Amazonie'). In addition, three field collections were carried out at different times in both the 'Montagne des singes' path in the Kourou area and in the town of Kourou, and two field collections were carried out at different times in both major sites Cacao and Sinnamary. In total, 19 collections were performed in this area. The distribution of isolates per sampling site is shown in Fig. S1 (available in the online version of this article).

Samples were aseptically collected using sterile plastic tubes or sterile swab. Each sample was then plated either by direct streak inoculation on YPD (1 % yeast extract, 1 % peptone, 1 % glucose) agar medium supplemented with 200 µg streptomycin or after dissection of a piece of the sample placed directly on the Petri dish. Plates were incubated at room temperature until yeast colonies appeared. Two or three representatives of each colony morphotype were then purified by repeated streaking on YPD agar plates. Purified yeast strains were suspended in YEA (0.5 % yeast extract, 1 % glucose) supplemented with 10 % glycerol (vol/vol) and maintained at −80 °C for later identification. The strains described in this work are listed Table 1; they have been deposited in the Biological Resource Centre CIRM-Levures (http://www.inra.fr/cirm/Levures, Jouy en Josas, France).

Sequencing and phylogenetic analysis

Nucleic acids were extracted and purified following the procedures of Hoffman [33]. The D1/D2 large subunit (LSU) rRNA gene (D1/D2 domains) and the internal transcribed spacer (ITS), which includes ITS1–5.8S–ITS2 and actin (*ACT1*) genes, were amplified by PCR in a final reaction mixture of 50 µl containing between 25 and 50 ng genomic DNA, 0.8 mM dNTPs, 0.4 µM forward and reverse primers in the recommended buffer and 1 U Takara ExTaq. Primers used for symmetrical amplifications were NL1 (5′-GCATATCAATAAGCGGAGGAA) and NL4 (5′-GGTCCGTGTTTCAAGACGG) [34] for the D1/D2 domains, ITS1 (5′-TCCGTAGGTGAACCTGCGG) and ITS4 (5′-TCCTCCGCTTATTGATATGC) [35] for the ITS region and CA14-DEHA (5′-CTGGGACGATATGGAAAAGATCTGGC) and CA5R-DEHA (5′-GAACAATTGAAGGTCCAGATTCATC) for the *ACT1* exon 2. Amplification reactions were run on a 2720 thermal cycler (Applied Biosystems) as follows: 4 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 40 s at the annealing temperature (54 °C for the D1/D2 domains, 48 °C for ITS, 54 °C for *ACT1* exon 2) and 90 s at 72 °C, with a final extension step of 7 min at 72 °C. PCR products were separated by electrophoresis using a 1 % agarose gel. The resulting amplicons

Fig. 1. Map of the seven sites of French Guiana sampled in this study: Kourou [(montagne des singes" path, Kourou town); Sinnamary (Paracou area); Saül (Saül village, "boucle des gros arbres" path, "layon des eaux claires" path); Cacao, (Molokoi path); Matoury, (Mirande path); Saint Elie; Regina ("savane roche" path, inn area, Approuage banks, cacao factory).

Table 1. Yeast strains isolated and identified to the species level

Species	Clade	Strain number	Location	Substrate	GeneBank accession number	
Saccharomycotina					**D1/D2**	**ITS**
Blastobrotrys sp.		CLIB 1738	Kourou	Ladybird	LT160950	LT160958
Brettanomyces naardenensis		CLIB 1177	Kourou	Awara		
Brettanomyces naardenensis		CLIB 1178	Saul	Flower		
Candida boidinii	*Ogatae*	CLIB 1425	Regina	Cocoa		
Candida boidinii	*Ogatae*	CLIB 1448	Saint Elie	Flower		
Candida cerambycidarum	*Yamadazyma*	CLIB 1183	Saul	Berry		
Candida cylindracea	*Ogatae*	CLIB 1168	Kourou	Fruit		
Candida eppingiae	*Metschnikowia*	CLIB 1723	Saul	Flower		
Candida intermedia	*Clavispora*	CLIB 1420	Kourou	Pineapple		
Candida intermedia	*Clavispora*	CLIB 1548	Saint Elie	Flower		
Candida intermedia	*Clavispora*	CLIB 1611	Kourou	Flower		
Candida intermedia	*Clavispora*	CLIB 1612	Regina	Cocoa	LN870344	LN870325
Candida intermedia	*Clavispora*	CLIB 1944	Saul	Fruit		
Candida intermedia	*Clavispora*	CLIB 1947	Regina	Fig		
Candida intermedia	*Clavispora*	G240A	Kourou	Corn		
Candida jaroonii	*Yamadazyma*	CLIB 1430	Regina	Flower		
Candida jaroonii	*Yamadazyma*	CLIB 1444	Saul	Animal		
Candida jaroonii	*Yamadazyma*	CLIB 1549	Regina	Fruit		
Candida leandrea	*Kodamae*	CLIB 1614	Regina	Flower	LN870345	LN870326
Candida leandrea	*Kodamae*	CLIB 1746	Saul	Flower		
Candida melibiosica	*Metschnikowia*	CLIB 1185	Kourou	Wasp		
Candida melibiosica	*Metschnikowia*	CLIB 1186	Cacao	Flower		
Candida melibiosica	*Metschnikowia*	CLIB 1187	Matoury	Flower		
Candida michaelii	*Yamadazyma*	CLIB 1418	Saint Elie	Flower		
Candida mycetangii	*Cyberlindnera*	CLIB 1616	Cacao	Animal	LN870346	LN870327
Candida natalensis	*Kurtzmaniella*	CLIB 1188	Saul	Snail		
Candida natalensis	*Kurtzmaniella*	CLIB 1189	Saul	Fruit		
Candida natalensis	*Kurtzmaniella*	CLIB 1190	Matoury	Fruit		
Candida natalensis	*Kurtzmaniella*	CLIB 1194	Kourou	Fruit		
Candida natalensis	*Kurtzmaniella*	CLIB 1195	Kourou	Fruit		
Candida natalensis	*Kurtzmaniella*	CLIB 1551	Saint Elie	Cobweb		
Candida natalensis	*Kurtzmaniella*	CLIB 1552	Kourou	Flower		
Candida natalensis	*Kurtzmaniella*	CLIB 1554	Kourou	Flower		
Candida natalensis	*Kurtzmaniella*	CLIB 1622	Kourou	Flower		
Candida natalensis	*Kurtzmaniella*	G238	Kourou	Brinjal		
Candida natalensis	*Kurtzmaniella*	G241B	Kourou	Mango		
Candida natalensis	*Kurtzmaniella*	G332A	Regina	Fig		
Candida natalensis	*Kurtzmaniella*	G257	Kourou	Plant		
Candida orthopsilosis	*Lodderomyces*	CLIB 1191	Saul	Insect	LN909490	LN909476
Candida parapsilosis	*Lodderomyces*	CLIB 1550	Saint Elie	Cobweb		
Candida peltata	*Nakazawaea*	CLIB 1192	Kourou	River water		
Candida peltata	*Nakazawaea*	CLIB 1553	Kourou	Fruit		
Candida pseudointermedia	*Clavispora*	CLIB 1193	Kourou	Banana		
Candida pseudointermedia	*Clavispora*	CLIB 1438	Kourou	Flower		
Candida pseudointermedia	*Clavispora*	CLIB 1439	Saul	Flower		
Candida pseudointermedia	*Clavispora*	CLIB 1566	Kourou	Corn		
Candida quercitrusa	*Kurtzmaniella*	CLIB 1432	Kourou	Papaya		
Candida railenensis	*Kurtzmaniella*	CLIB 1423	Regina	Papaya		
Candida saopaulonensis	*Metschnikowia*	CLIB 1424	Regina	Flower		
Candida sorboxylosa		CLIB 1440	Regina	Ramboutan		

Table 1. cont.

Species	Clade	Strain number	Location	Substrate	GeneBank accession number	
Saccharomycotina					**D1/D2**	**ITS**
Candida sorboxylosa		CLIB 1449	Regina	Flower	LN909489	LN909475
Candida sp. 1		CLIB 1735	Kourou	Berry	LN875210	LN875188
Candida sp. 2		CLIB 1740	Kourou	Fungi	LN909487	LN909473
Candida sp. 3		CLIB 1710	Kourou	Papaya	LT883653	LN875173
Candida sp. 4		CLIB 1736	Saul	Fungi	LN875211	LN875189
Candida sp. 5		CLIB 1741	Saul	Flower	LT160951	
Candida sp. 6		CLIB 1743	Matoury	Fruit	LT160952	
Candida sp. 7		CLIB 1954	Saint Elie	Plant	LN909495	LN909481
Candida sp. 8		CLIB 1709	Regina	Butterfly	LT160949	LT160957
Candida sp. 9		CLIB 1744	Kourou	Fruit	LN875214	LN875193
Candida tallmaniae	*Yamadazyma*	CLIB 1617	Cacao	Flower		
Candida tallmaniae	*Yamadazyma*	CLIB 1724	Kourou	Flower		
Candida tropicalis	*Lodderomyces*	CLIB 1196	Kourou	Banana		
Clavispora sp. 1		CLIB 1717	Kourou	Fruit	LN875200	LN875178
Clavispora sp. 2		CLIB 1728	Kourou	Fruit	LN909486	LN909472
Clavispora sp. 3		CLIB 1715	Cacao	Fig	LN875198	LN875176
Clavispora sp. 4		CLIB 1705	Saint Elie	Flower	LN875194	LN875171
Clavispora sp. 5		CLIB 1716	Saint Elie	Plant	LN875199	LN875177
Clavispora sp. 6		CLIB 1733	Saint Elie	Plant	LN875208	LN875186
Cyberlindnera fabianii		CLIB 1431	Regina	Environment		
Cyberlindnera subsufficiens		CLIB 1228	Kourou	River water		
Debaryomyces hansenii		CLIB 1143	Saul	Insect		
Debaryomyces hansenii	Clade 3	CLIB 1604	Matoury	Flower	LN870340	LN870319
Debaryomyces nepalensis		CLIB 1142	Saul	Fly		
Debaryomyces polymorphus		CLIB 1555	Regina	Insect		
Debaryomyces polymorphus		CLIB 1556	Kourou	Insect		
Diutina catenulata		CLIB 1179	Saul	Flower		
Geotrichum candidum		CLIB 1154	Cacao	Flower		
Geotrichum phurueaensis		CLIB 1232	Regina	–		
Hanseniaspora guilliermondii		CLIB 1559	Regina	Lemon		
Hanseniaspora guilliermondii		G 236B1	Kourou	Pineapple		
Hanseniaspora guilliermondii		G 241A2	Kourou	Mango		
Hanseniaspora guilliermondii		G 316B	Regina	Ramboutan		
Hanseniaspora meyeri		CLIB 1206	Kourou	Insect		
Hanseniaspora opuntiae		CLIB 1200	Kourou	Plant		
Hanseniaspora opuntiae		CLIB 1201	Kourou	Mango		
Hanseniaspora opuntiae		CLIB 1202	Saul	Flower		
Hanseniaspora opuntiae		CLIB 1203	Saul	Plant		
Hanseniaspora opuntiae		CLIB 1204	Kourou	Fruit		
Hanseniaspora opuntiae		CLIB 1205	Kourou	Fruit		
Hanseniaspora opuntiae		CLIB 1229	Matoury	Fruit	LT883654	
Hanseniaspora opuntiae		CLIB 1442	Regina	Fruit		
Hanseniaspora opuntiae		CLIB 1450	Regina	Fig		
Hanseniaspora opuntiae		CLIB 1451	Regina	Cocoa		
Hanseniaspora opuntiae		CLIB 1557	Kourou	Papaya		
Hanseniaspora opuntiae		CLIB 1558	Regina	Cocoa		
Hanseniaspora opuntiae		CLIB 1560	Regina	Flower		
Hanseniaspora opuntiae		CLIB 1561	Regina	Flower		
Hanseniaspora opuntiae		CLIB 1562	Regina	Cocoa		
Hanseniaspora opuntiae		CLIB 1624	Cacao	Flower	LN870350	LN870331

Table 1. cont.

Species	Clade	Strain number	Location	Substrate	GeneBank accession number	
Saccharomycotina					**D1/D2**	**ITS**
Hanseniaspora opuntiae		CLIB 1635	Kourou	Awara	LN870358	LN870320
Hanseniaspora opuntiae		CLIB 1945	Regina	Fruit		
Hanseniaspora opuntiae		G 340B	Regina	Carapa seed		
Hanseniaspora opuntiae		G 343	Regina	Flower		
Hanseniaspora opuntiae		G 349B	Regina	Papaya		
Hanseniaspora opuntiae		G 250	Kourou	Fruit		
Hanseniaspora opuntiae		G 285B	Cacao	Fig		
Hanseniaspora opuntiae		G 313	Regina	Fruit		
Hanseniaspora opuntiae		G 342	Regina	Flower		
Hanseniaspora pseudoguilliermondii		CLIB 1441	Regina	Orange		
Hanseniaspora sp.		CLIB 1623	Kourou	Flower	LN870349	LN870330
Hanseniaspora thailandica		CLIB 1437	Regina	Cocoa		
Hanseniaspora thailandica		CLIB 1443	Regina	Flower		
Hanseniaspora thailandica		CLIB 1625	Regina	Fish	LN870351	LN870332
Hanseniaspora uvarum		CLIB 1207	Cacao	Berry		
Hanseniaspora uvarum		CLIB 1208	Kourou	Papaya		
Hanseniaspora uvarum		CLIB 1209	Saul	Flower		
Hanseniaspora uvarum		CLIB 1210	Saul	Flower		
Hanseniaspora uvarum		CLIB 1563	Regina	Insect		
Hanseniaspora uvarum		CLIB 1564	Regina	Insect		
Hanseniaspora uvarum		CLIB 1626	Sinnamary	Fruit	LN870352	LN870333
Hanseniaspora uvarum		CLIB 1627	Saul	Flower	LN870353	LN870334
Hyphopichia burtonii		CLIB 1436	Regina	Fish		
Hyphopichia buzzinii		CLIB 1739	Cacao	Berry	LN875215	LN875191
Kodamaea ohmeri		CLIB 1214	Kourou	Papaya		
Kodamaea ohmeri		CLIB 1215	Kourou	Ramboutan		
Kodamaea ohmeri		CLIB 1216	Kourou	Fruit		
Kodamaea ohmeri		CLIB 1565	Regina	Insect		
Kodamaea ohmeri		CLIB 1567	Regina	Cocoa		
Kodamaea ohmeri		CLIB 1703	Saul	Insect		
Kodamaea ohmeri		CLIB 1948	Saul	Berry		
Kodamaea ohmeri		CLIB 1952	Kourou	Banana		
Kodamaea ohmeri		CLIB 1957	Saul	Flower		
Kodamaea ohmeri		CLIB 1961	Saul	Flower		
Kodamaea ohmeri		G 241C	Kourou	Mango		
Kodamaea ohmeri		G 341C	Regina	Cocoa		
Kurtzmaniella sp. 1		CLIB 1609	Kourou	Fruit	LN909493	LN909479
Kurtzmaniella sp. 1		CLIB 1720	Kourou	Berry	LT160954	
Kurtzmaniella sp. 2		CLIB 1620	Cacao	Flower	LN870347	LN870328
Kurtzmaniella sp. 2		CLIB 1621	Saint Elie	Flower	LN870348	LN870329
Kurtzmaniella sp. 2		CLIB 1949	Sinnamary	Flower		
Kurtzmaniella sp. 2		CLIB 1950	Sinnamary	Flower		
Kurtzmaniella sp. 2		CLIB 1951	Saint Elie	Fruit		
Kurtzmaniella sp. 2		CLIB 1953	Kourou	Flower		
Kurtzmaniella sp. 2		CLIB 1956	Saint Elie	Flower		
Kurtzmaniella sp. 2		CLIB 1958	Saint Elie	Flower		
Kurtzmaniella sp. 2		CLIB 1636	Saint Elie	Environment	LN870359	LN870321
Kurtzmaniella sp. 2		CLIB 1943	Cacao	Flower		
Martiniozyma asiatica		CLIB 1447	Kourou	Environment		
Martiniozyma asiatica		CLIB 1608	Regina	Flower	LN870342	LN870323

Table 1. cont.

Species	Clade	Strain number	Location	Substrate	GeneBank accession number	
Saccharomycotina					**D1/D2**	**ITS**
Metschnikowia cerradonensis		CLIB 1628	Regina	Flower		
Metschnikowia koreensis		CLIB 1217	Kourou	Flower		
Metschnikowia koreensis		CLIB 1218	Kourou	Flower		
Metschnikowia koreensis		CLIB 1219	Saul	Flower		
Metschnikowia koreensis		CLIB 1568	Cacao	Flower		
Metschnikowia koreensis		CLIB 1630	Cacao	Flower	LN870355	LN870336
Metschnikowia koreensis		CLIB 1959	Saul	Flower		
Metschnikowia lochheadii		CLIB 1220	Saul	Flower		
Metschnikowia peoriensis		CLIB 1629	Saul	Flower	LN870354	LN870335
Metschnikowia peoriensis		CLIB 1704	Saul	Flower	LT160955	
Metschnikowia peoriensis		CLIB 1946	Saul	Flower	LN909494	LN909480
Metschnikowia sp. 1		CLIB 1730	Kourou	Flower	LN875207	LN875185
Metschnikowia sp. 1		CLIB 1731	Kourou	Plant		
Metschnikowia sp. 2		CLIB 1742	Kourou	Fruit	LN875213	LN875192
Metschnikowia sp. 2		CLIB 1745	Kourou	Fruit		
Metschnikowia sp. 3		CLIB 1712	Saul	Flower		
Metschnikowia sp. 3		CLIB 1737	Kourou	Banana	LN875212	LN875190
Metschnikowia sp. 5		CLIB 1721	Saul	Flower	LN909485	LN909471
Metschnikowia sp. 6		CLIB 1747	Saul	Flower	LT160953	
Metschnikowia sp. 7		CLIB 1719	Matoury	Flower	LN875202	LN875180
Metschnikowia sp. 8		CLIB 1734	Sinnamary	Insect	LN875209	LN875187
Metschnikowia viticola		CLIB 1632	Matoury	Flower	LN909491	LN909477
Meyerozyma caribbica		CLIB 1223	Kourou	Papyrus		
Meyerozyma caribbica		CLIB 1224	Sinnamary	Ramboutan		
Meyerozyma caribbica		CLIB 1963	Regina	Cocoa		
Meyerozyma guilliermondii		CLIB 1433	Regina	Cocoa		
Pichia bruneiensis		CLIB 1453	Regina	Butterfly	LN909488	LN909474
Pichia chibodasensis		CLIB 1633	Cacao	Flower	LN870356	LN870337
Pichia kluyveri		CLIB 1225	Saul	Fruit		
Pichia kluyveri		CLIB 1569	Regina	Plant		
Pichia kluyveri		CLIB 1570	Kourou	Fruit		
Pichia kudriavzevii		CLIB 1212	Kourou	Mango		
Pichia kudriavzevii		CLIB 1419	Regina	Leech		
Pichia kudriavzevii		CLIB 1427	Regina	Insect		
Pichia kudriavzevii		CLIB 1434	Regina	Cocoa		
Pichia kudriavzevii		CLIB 1571	Regina	Cocoa		
Pichia kudriavzevii		CLIB 1572	Regina	Cocoa		
Pichia kudriavzevii		G 318B	Regina	Cocoa		
Pichia kudriavzevii		G 320-1B	Regina	Cocoa		
Pichia occidentalis		CLIB 1211	Saul	Coconut		
Pichia occidentalis		CLIB 1435	Regina	Fish		
Pichia sporocuriosa		CLIB 1631	Regina	Ramboutan	LN909482	LN909468
Pichia terricola		CLIB 1213	Saul	Flower		
Pichia terricola		CLIB 1416	Regina	Flower		
Priceomyces sp.		CLIB 1603	Kourou	Flower	LN870339	LN870318
Saccharomycopsis crataegensis		CLIB 1426	Regina	Fruit		
Saccharomycopsis guyanensis		CLIB 1454	Kourou	Flower	HG764731	HG939420
Saccharomycopsis guyanensis		CLIB 1455	Kourou	Flower	HG328370	HG328369
Saturnispora diversa		CLIB 1184	Saul	Snail		
Saturnispora hagleri		CLIB 1227	Saul	Fruit		

Table 1. cont.

Species	Clade	Strain number	Location	Substrate	GeneBank accession number	
Saccharomycotina					**D1/D2**	**ITS**
Saturnispora silvae		CLIB 1429	Regina	–		
Saturnispora silvae		CLIB 1452	Regina	Cocoa		
Saturnispora sp.		CLIB 1708	Regina	Flower	LN909484	LN909470
Schwanniomyces polymorphus		CLIB 1198	Sinnamary	Cobweb		
Schwanniomyces polymorphus		CLIB 1199	Matoury	Flower		
Schwanniomyces polymorphus		CLIB 1605	Sinnamary	Flower		
Schwanniomyces polymorphus		CLIB 1606	Sinnamary	Flower		
Schwanniomyces polymorphus		CLIB 1638	Kourou	Flower		
Schwanniomyces sp.		CLIB 1711	Saint Elie	Flower	LN875196	LN875174
Schwanniomyces vanrijiae		CLIB 1607	Sinnamary	Cobweb	LN909492	LN909478
Starmera stellimalicola		CLIB1428	Regina	Cocoa		
Starmerella sp. 1 *(kourouensis)*		CLIB 1707	Kourou	Flower	LN909483	LN909469
Starmerella sp. 2 *(reginensis)*		CLIB 1634	Regina	Flower	LN870357	LN870338
Suhomyces sp. 1 *(coccinellae)*		CLIB 1706	Saint Elie	Ladybird	LN875195	LN875172
Suhomyces sp. 2 *(faveliae)*		CLIB 1725	Saul	Plant	LN875204	LN875182
Suhomyces xylopsoci		CLIB 1197	Saul	Fly		
Wickerhamiella parazyma		CLIB 1445	Saul	Flower		
Wickerhamiella parazyma		CLIB 1446	Regina	Flower		
Wickerhamiella parazyma		CLIB 1960	Saint Elie	Fly	LT160956	LT160959
Wickerhamiella sp. 1		CLIB 1727	Kourou	Flower	LN909497	
Wickerhamiella sp. 2 *(kurtzmanii)*		CLIB 1732	Kourou	Flower	LN909498	MH396622
Wickerhamiella sp. 3		CLIB 1718	Saint Elie	Flower	LN875201	LN875179
Wickerhamomyces anomalus		CLIB 1221	Kourou	Papaya		
Wickerhamomyces anomalus		CLIB 1222	Kourou	Papaya		
Wickerhamomyces rabaulensis		CLIB 1226	Saul	Berry		
Wickerhamomyces sp. 1		CLIB 1722	Kourou	Berry	LN875203	LN875181
Wickerhamomyces sp. 2		CLIB 1714	Regina	Cocoa	LN909496	
Yamadazyma akitaensis		CLIB 1417	Saint Elie	Flower		
Yamadazyma sp. 1		CLIB 1610	Kourou	Berry	LN870343	LN870324
Yamadazyma sp. 2		CLIB 1729	Regina	Plant	LN875206	LN875184
Yamadazyma sp. 3		CLIB 1713	Regina	Butterfly	LN875197	LN875175
Pezizomycotina						
Aureobasidium pullulans		CLIB 1176	Kourou	Flower		
Aureobasidium pullulans		CLIB 1422	Regina	Flower		
Basidiomycota						
Colacogloea sp.		CLIB 1726	Regina	Flower	LN875205	LN875183
Cryptococcus sp.		CLIB 1995	Kourou	Berry	LT627368	
Cystofilobasidium sp.		CLIB 3011	Saul	Lemon	LT627385	
Dirkmeia churashimaensis		CLIB 3006	Saul	Flower	LT627380	
Fereydounia khargensis		CLIB 3022	Kourou	Plant	LT627396	
Moesziomyces parantarcticus		CLIB 3004	Saul	Flower	LT627378	
Moesziomyces parantarcticus		CLIB 3005	Saul	Flower	LT627379	
Moesziomyces parantarcticus		CLIB 3030	Regina	Flower	LT627404	
Moesziomyces parantarcticus		CLIB 3034	Regina	Lemon	LT627408	
Moesziomyces antarcticus		CLIB 3003	Saul	Flower	LT627377	
Moesziomyces antarcticus		CLIB 3033	Regina	Flower	LT627407	
Moesziomyces antarcticus		CLIB 3035	Regina	Lemon	LT627409	
Moniliella sp. 1		CLIB 3017	Saint Elie	Flower	LT627391	
Moniliella sp. 2		CLIB 3026	Regina	Flower	LT627400	
Naganishia diffluens		CLIB 3001	Kourou	Mango	LT627375	

Table 1. cont.

Species	Clade	Strain number	Location	Substrate	GeneBank accession number	
Saccharomycotina					**D1/D2**	**ITS**
Papiliotrema flavescens		CLIB 1993	Kourou	Flower	LT627366	
Papiliotrema flavescens		CLIB 2000	Sinnamary	Flower	LT627373	
Papiliotrema flavescens		CLIB 3002	Saul	Insect	LT627376	
Papiliotrema flavescens		CLIB 3032	Regina	Flower	LT627406	
Papiliotrema laurentii		CLIB 1997	Kourou	Flower	LT627370	
Papiliotrema laurentii		CLIB 1998	Kourou	Soil	LT627371	
Papiliotrema sp. 1		CLIB 3012	Cacao	Plant	LT627386	
Papiliotrema sp. 2		CLIB 3014	Matoury	Flower	LT627388	
Papiliotrema nemorosus		CLIB 3018	Saint Elie	Flower	LT627392	
Pseudozyma hubeiensis		CLIB 1994	Kourou	Berry	LT627367	
Pseudozyma hubeiensis		CLIB 1996	Kourou	Flower	LT627369	
Pseudozyma hubeiensis		CLIB 1999	Sinnamary	Spider	LT627372	
Pseudozyma hubeiensis		CLIB 3009	Saul	Plant	LT627383	
Pseudozyma hubeiensis		CLIB 3013	Matoury	Insect	LT627387	
Rhodotorula mucilaginosa		CLIB 3020	Kourou	Ladybird	LT627394	
Rhynchogastrema complexa		CLIB 3007	Saul	Insect	LT627381	
Sporisorium sp.		CLIB 3008	Saul	Plant	LT627382	
Sympodiomycopsis sp.		CLIB 3024	Regina	Flower	LT627398	
Trichosporon asahii		CLIB 3010	Saul	Flower	LT627384	
Trichosporon asahii		CLIB 3016	Saint Elie	Pomelo	LT627390	
Trichosporon asahii		CLIB 3019	Kourou	Sugar apple	LT627393	
Trichosporon asahii		CLIB 3028	Regina	Environment	LT627402	
Trichosporon asahii		CLIB 3029	Regina	Fish	LT627403	
Trichosporon coremiiforme		CLIB 3027	Regina	Insect	LT627401	
Trichosporon sp.		CLIB 3023	Cacao	Flower	LT627397	
Ustilaginomycotina sp.		CLIB 3031	Regina	Flower	LT627405	
Vanrija humicola		CLIB 1615	Sinnamary	Insect	LT627374	
Xenoacremonium sp.		CLIB 3015	Saint Elie	Plant	LT627389	

were sequenced on both strands by Eurofins MWG Operon (Ebersberg, Germany). Sequencing primers for D1/D2 domains, ITS and ACT1 were those used for PCR amplification. Sequences were assembled with the phred/phrap/consed package and compared with sequences in databases, such as Genbank (http://www.ncbi.nlm.nih.gov) and YeastIP [36]; http://genome.jouy.inra.fr/yeastip) using the BLAST program. Sequence alignments were generated using ClustalX2 [37] and were adjusted manually. Phylogenetic trees were reconstructed with the maximum-likelihood program implemented in MEGA6 [38, 39]. Phylogenetic trees were visualized with NJplot [40].

Phenotypic characterization

Morphological observations and metabolic tests were performed on CLIB 1706^T and CLIB 1725^T according to established methods [41, 42]. For the phenotypic tests, sugar fermentations were carried out in Durham tubes on media containing 0.5 % yeast extract and 1 % of each tested sugar. The ID 32 C systems (bioMérieux) were used to assess growth on various carbon sources after incubation at 28 °C for 2 days. Assimilation of nitrogen compounds was assessed on Yeast Carbon Base minimal medium (Difco) supplemented with 1 % standard nitrogen sources [43]. Growth at various temperatures was determined by cultivation of the strains in YPD. Sporulation capacity was assessed on 5 % malt agar and Yeast Mold (YM) agar after incubation for 3 weeks at 25 °C. Mycelium formation was investigated on cornmeal agar (Becton, Dickinson and Company) in slide culture at 25 °C for up to seven days. Morphological properties were studied under a Laborlux S light microscope (Leica Microsystems) coupled to a digital camera.

RESULTS AND DISCUSSION

Isolation and identification to the species level

Yeasts were isolated from a large, diversity of samples, such as plants, flowers, fruits, insects, environment, animal and fungi in order to explore biodiversity. The largest part of the isolates was collected from plants and flowers (122 isolates) and from fruits and berries (103 strains) (Fig. S1).

An initial sequencing of the D1/D2 domains was performed as described in the methods for the 276 strains listed in Table 1. These sequences were compared with those present in international databases to identify the strains to the species level. According to Vu *et al.* [44], yeast species may be discriminated with sequence divergence values as low as 0.49 % divergence for the D1/D2 domains. In addition, it is known that divergence in sequences of the D1/D2 domains may not correctly discriminate different species [3, 45] therefore, when strains had D1/D2 domain sequences diverging by more than three bp (whatever the length of the sequence) in the comparison with the respective type strain, ITS sequencing was performed to remove any ambiguity when assigning a strain to a species. In some instances, ITS sequencing was performed systematically. Finally, the cut-off chosen for the identification to the species level was 5 bp divergence in the D1/D2 domains. This sequence analysis led to the unambiguous identification of 210 yeast isolates assigned to described species from the phylum Basidiomycota (31 isolates), and the two subphyla Saccharomycotina and Pezizomycotina (177 isolates and 2 isolates, respectively). Among them, 42 species were represented by a single isolate, and 40 species were represented by at least two isolates. In total, the 210 isolated strains belonged to 32 genera and 82 species. On the basis of the sequence comparison analysis described, a total of 66 isolates presented sequence divergence indicating that they may belong to undescribed species (Table S1, available in the online version of this article). These potential novel species will be described later in this paper. There is another exception, strain CLIB 1604 (Table 1), which belongs to the genus *Debaryomyces*. In this genus, divergence at the level of the rDNA gene may be highly reduced, for example in the *Debaryomyces hansenii*/*Debaryomyces fabryi* species complex [46, 47] therefore our threshold does not apply. Indeed, the partial sequence for the actin coding gene was obtained for CLIB 1604 and it differed by six bp plus one indel (out of 818 bp) from that of the *D. hansenii* type strain CBS 767^T. It is most related to *D. hansenii* CBS 1795, with two substitutions and one indel out of 818 bp, indicating that CLIB 1604 belongs to clade 3 of *D. hansenii* [45].

Distribution of species and isolates in Saccharomycotina clades

Given the heterogeneous nature of the genus *Candida*, providing the number of strains belonging to this genus is not very informative. We have therefore compiled the distribution of strains and species by clades (Table S2). By doing so, we identified species of the genus *Candida*, including those 'not assigned to a clade', as defined in the YeastIP webservice (http://genome.jouy.inra.fr/yeastip). These species, i.e. species which have not been assigned to a clade yet, turned out to be very rare, 11 isolates belonging to 10 species. Very interestingly, nine of these species were undescribed species identified in this work, the described species of the genus *Candida* being *Candida sorboxylosa*, with two isolates.

Of the defined clades, the *Metschnikowia* clade is the most highly represented on the basis of the number of species, 15 for *Metschnikowia* vs. eight for the closely related *Clavispora* clade and eight for *Yamadazyma*. If we consider the number of isolates per clade, the most frequently encountered clade is *Hanseniaspora* with 43 isolates, followed by *Kurtzmaniella* and *Metchnikowia* (27 isolates), *Pichia* (18 isolates) and *Clavispora* (17 isolates) (Table S2).

The most commonly found yeast species were *Hanseniaspora opuntiae* with 25 isolates, *Candida natalensis* with 13 isolates, *Kodamaea ohmeri* with 12 isolates and *Pichia kudriavzevii* with eight isolates (Table 1). The *H. opuntiae* isolates were collected from fruits and plants, including flowers. This is in accordance with the ecology of this species, for which strains have been isolated from Cactaceae in the Hawaiian Islands and from grape berries in Australia and in Greece [48]. *H. opuntiae* isolates were collected in five of the seven major sites of collection and were most prevalent in the Regina area (52 %). The *C. natalensis* isolates were collected from different type of samples, such as fruits, flowers, an eggplant, a snail and a cobweb in five of the seven major collection sites; 61.5 % of *C. natalensis* isolates were collected in the 'Montagne des Singes' in Kourou. The 12 *K. ohmeri* isolates were isolated from fruits, flowers and insects in three of the seven major collection sites. The major part of these *K. ohmeri* strains (41.6 %) was isolated from fruits in the Kourou market and originating from various parts of the region. The eight *P. kudriavzevii* isolates have been collected from fruit, insect and from the environment of the cocoa factory in Regina (62.5 %). In fact, this species has already been described as being involved in the cocoa bean fermentation for chocolate production [49].

Yeasts associated with cocoa fermentation

Yeasts associated with the cocoa bean fermentation environment in Regina were found to belong to 13 species *C. boidinii* (one strain), *C. intermedia* (one strain), *C. silvae* (one strain), *C. stellimalicola* (one strain), *Cyberlindnera fabianii* (one strain), *H. opuntiae* (three strains), *H. thailandica* (one strain) *K. ohmeri* (two strains), *Meyerozyma caribbica* (one strain), *M. guilliermondii* (one strain), *P. kudriavzevii* (five strains), *Trichosporon asahii* (one strain) and one as yet undescribed species of the genus *Wickerhamomyces* (one strain).

The species *P. kudriavzevii*, *H. opuntiae*, *H. thailandica*, *C. stellimalicola*, *Cyberlindnera fabianii*, *Meyerozyma caribbica*, *Trichosporon asahii*, *C. intermedia* and *K. ohmeri* have been found in various cocoa fermentation all over the world [51–61]. Five of these species have been isolated during the fermentation process of Ghanaian cocoa bean [60], whereas *C. boidinii*, *C. silvae*, *M. guilliermondii* and one as yet not described species were found for the first time in this type of fermentation. However, we do not know whether the strains collected in this work participate in the fermentation process. One of the major species involved in cocoa fermentation, *H. opuntiae*, was not over-represented in our sampling compared with aprevious

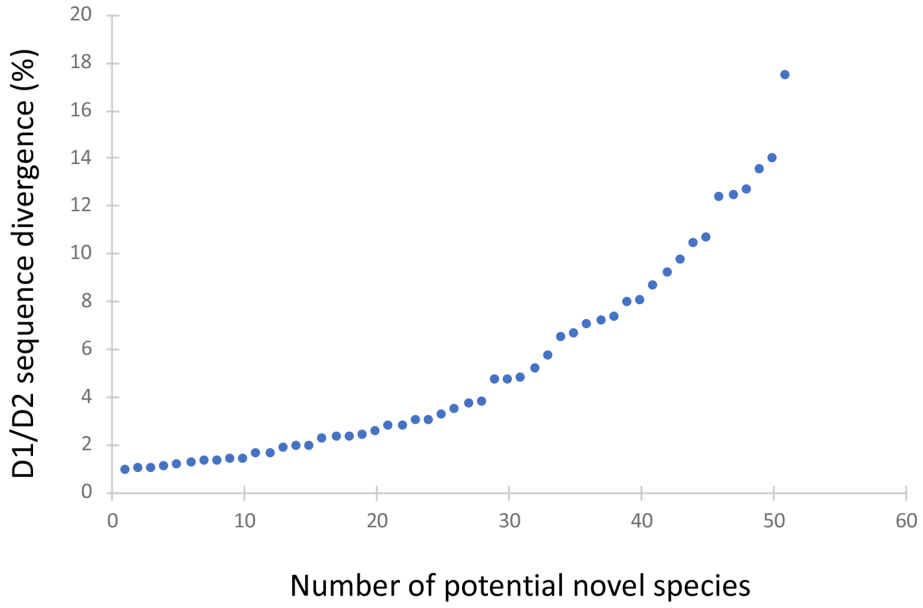

Fig. 2. Extent of the genetic diversity within the D1/D2 sequence in 51 potential novel species. The sequence divergence (%) of the D1/D2 sequence of 51 potential novel species with those of their respective closest neighbours was plotted against the number of potential novel species.

metagenomic analysis [55], in which DNA of this species represented over 10 % of all yeast DNAs. It is also worth mentioning that the major species *H. guillermondii* and *S. cerevisiae,* 42.5 and 9.5 % of all yeast DNA in the study of Illeghems *et al.* [55], respectively were not present in our samples. This may be due to the different processes analyzed, heap fermentation for French Guiana and box fermentation for Brazil.

Extent of biodiversity

Comparison of the sequences isolated in this work with sequences in the international repositories revealed that 66 isolates carried divergent sequences at the level of the D1/D2 domains and/or the ITS region, which could indicate that these isolates represent undescribed species. It has been suggested [62] that 1 % of divergence within the D1/D2 domains could differentiate species in Saccharomycotina yeasts. Since this pioneering study, [63] it has been deduced from the comparison of sequences from around 9000 strains that yeast species could be discriminated with as little as 0.49 % sequence divergence in the D1/D2 domains [63], and also a potential threshold of 1.59 % divergence in the ITS for species discrimination has been considered. Here, we considered that 66 isolates could not be classified as representing known species on the basis of sequence divergence from those of known species from 0.91 % up to 17.41 % in the D1/D2 LSU domains. Fig. 2 indicates the extent of genetic diversity within the D1/D2 domains by providing the divergence in percentage of 51 D1/D2 domain sequences of 64 isolates, when compared with the most closely related species. For two isolates, CLIB 3008 and CLIB 3023, sequence divergence was too high due to poor sequence alignment and they were not included in Fig. 2. It is noteworthy that only one isolate, *Priceomyces* sp. CLIB 1603, displayed a sequence divergence that amounts to less than 1 %, the threshold proposed previously [62]. Sequence divergence of the ITS of this isolate (16 bp out of 610, i.e. 2.62 %, when compared with that of *Priceomyces fermenticarens* type strain CBS 7040^T) confirmed that strain CLIB 1603 represents an undescribed species.

For ITS, the minimal divergence observed for these 66 isolates was 2.24 %. It must be noted that levels of divergence varied between the D1/D2 domains and ITS for strains CLIB 1708 and CLIB 1711, which displayed two nucleotides divergence at the level of ITS and 12.68 and 1.23 % at the level of D1/D2 domains, respectively.

We therefore consider that our work revealed 53 novel species represented by 66 isolates. The isolates and their geographic location of isolation are listed in Table S1. Interestingly, no novel species were identified among the 20 isolates from the banks of the Approuage river of Regina, while in Saint Elie, 60.9 % of the isolates (14 isolates out of 23) represented novel species. We found more generally that the number of novel species was not proportional to the number of isolates collected, since in Kourou for instance, field collection led to a larger number of novel species, 23 compared with the 10 novel species isolated in Regina, whereas the number of isolates collected was similar between the two areas, 84 and 80 respectively (Fig. S1).

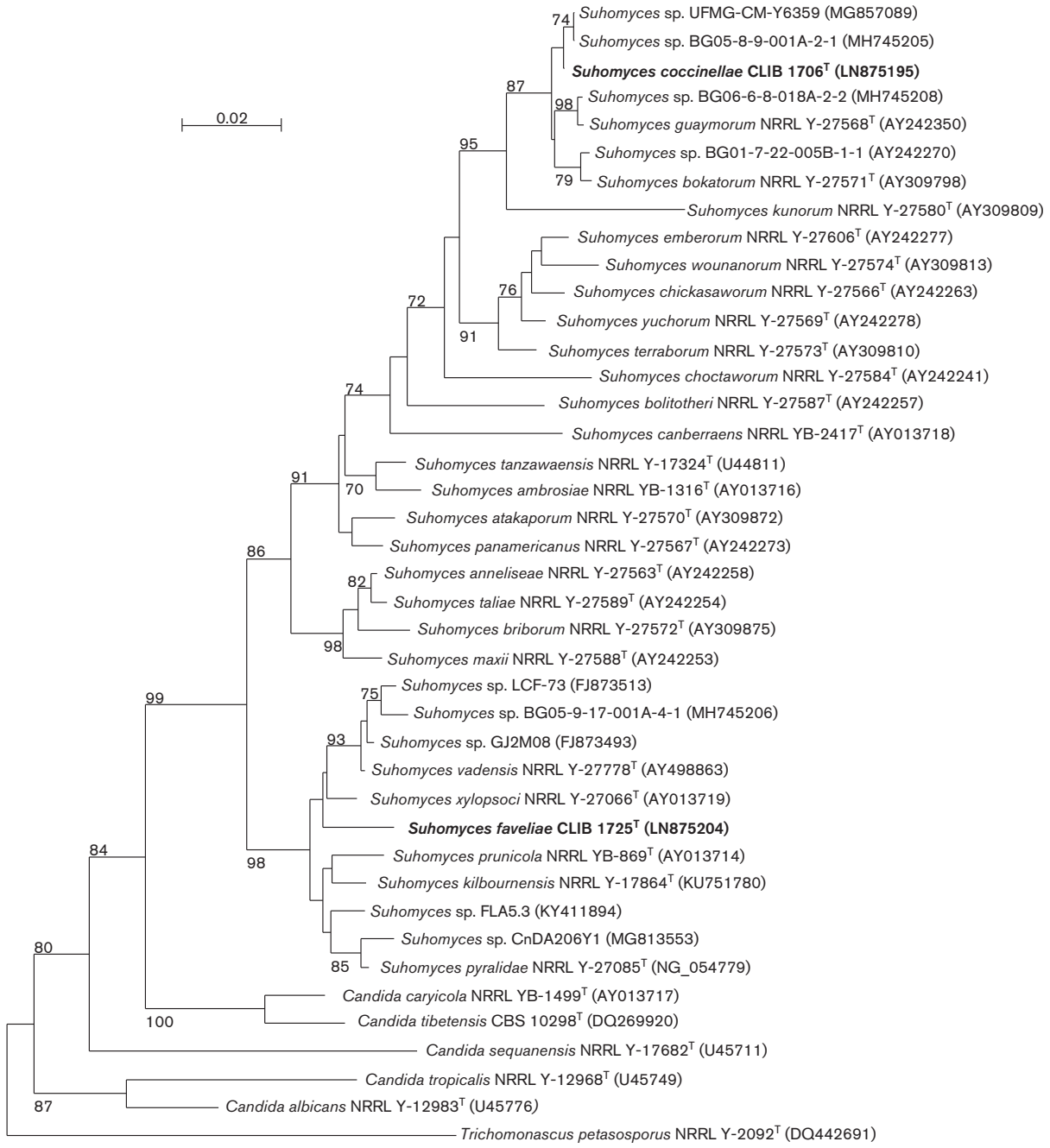

Fig. 3. Phylogenetic placement of *Suhomyces coccinellae* sp. nov. and *Suhomyces faveliae* sp. nov. based on the alignment of sequences of the D1/D2 domains of the LSU rRNA gene (sequence accession numbers are shown in parentheses). The tree was reconstructed using the neighbor-joining method for 472 aligned positions with the Kimura two-parameter method and Gamma-distributed rates. The analysis involved 41 nucleotide sequences. All positions containing gaps and missing data were eliminated. Bootstrap values were determined from 1000 replications. Bar, 0.02 substitutions per site.

Most of the novel species were found to belong to the genus *Metschnikowia*, with ten isolates, and *Candida,* with nine isolates. Interestingly, two isolates, CLIB 1730 and CLIB 1731 representing an undescribed species *Metschnikowia* sp. 1, and two other isolates CLIB 1742 and CLIB 1745 representing another undescribed species *Metschnikowia* sp. 2, isolated in the Montagne des Singes of Kourou, were found repeatedly in 2008 and in 2010. This indicates that these strains are endemic to the 'Montagne des Singes' and perhaps also to their respective substrates, flowers and fruits.

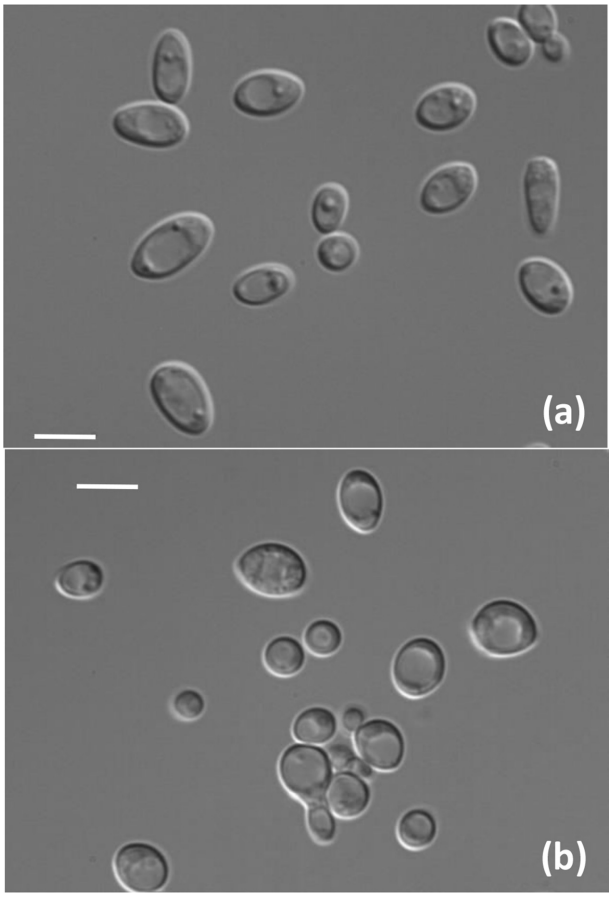

Fig. 4. Light micrographs of vegetative cells cultivated on YPD agar plates for 3 days at 28 °C. (a) *Suhomyces coccinellae* f.a. sp. nov. CLIB 1706^T; (b) *Suhomyces faveliae* f.a. sp. nov. CLIB 1725^T. Bars, 10 µm.

Among the 365 samples collected, one of these samples, a flower collected in the 'Montagne des Singes' in Kourou, was associated with five strains, that represented different species. This turned out to be the highest number of different isolates obtained in one sample. Among these five isolates three represented novel species (CLIB 1707, CLIB 1727 and CLIB 1732 represented the taxa, *Starmerella* sp. 1, *Wickerhamiella* sp. 1 and *Wickerhamiella* sp. 2, respectively). We also observed that four different yeast species were isolated from three other substrates, a flower collected in Saül, a papaya sold on Kourou market and a butterfly in Regina. For the butterfly two out of the four associated isolates represented novel species (CLIB 1709 to *Candida* sp. 8 and CLIB 1713 to *Yamadazyma* sp. 3), isolates from the two other samples, flower and papaya, represented known species.

Out of the 43 basidiomycota isolates collected, the most prevalent represent the described species *Pseudozyma hubeiensis* and *Trichosporon asahii* with five isolates each. *P. hubeiensis* isolates have been found in four categories of substrates (fruit, flower, insect and plant) and *Trichosporon asahii* isolates have been collected from four categories of substrates (fruit, flower, environment and animal). A total of 12 basidiomycota isolates (27.9 %) were found to represent undescribed species, with sequence divergence varying between 1 and 8.65 % at the level of D1/D2 domains; among them, two isolates represented members of the genus *Papiliotrema* and two isolates represented members of the genus *Moniliella*. As mentioned earlier, there were also two basidiomycetous isolates, CLIB 3008 and CLIB 3023, for which no BLAST results were obtained because of an important sequence divergence.

The 66 strains representing the potential novel species are listed in Table S1, they represent 23.9 % of the isolates recovered during this investigation. This table also gives information about the substrates of isolation and the sampling sites. Table S1 allows an analysis of the geographical distribution of the species. The ten isolates of *Kurtzmaniella* sp. 2 have been recovered at four different locations: Kourou, Sinnamary, Cacao and St Elie. In addition, the two isolates of *Metschnikowia* sp.3 were from Kourou and from Saul. Representatives of these two undescribed species appear to be widespread in this region (Table S1).

CONCLUSIONS

This study has provided information on the type and the extent of the diversity of yeast species found in the natural environment of French Guiana. We show that the number of novel species can increase drastically as soon as intensive work on collecting and isolating yeast strains is done. When one considers Ascomycotina yeasts alone, over 23 % of the isolates identified here represented undescribed yeast species. This work is reminiscent of the diversity found in the gut of insects [31, 64–66], in which an unexpectedly high number of novel species were discovered. On the other hand, recent field collection targeted to Dutch soils in a large number of locations reveals less diversity, with 4 % novel species among the 386 isolates collected [67]. As pointed out by these authors, the relatively poor occurrence of novel taxa may be due to the fact that the soils of isolation were managed [68]. In this respect, our results are less surprising, since unexplored areas may be a more favorable environment for biodiversity discovery. It must also be stressed that in our study, isolation of yeasts was performed by simply streaking the samples on a rich YPD medium agar plate only and that potential variations in optimal growth temperature were not accounted for. Considering the average temperature in French Guyana, the fact that temperatures was not controlled may not affect our results. However, one may expect to obtain an even a larger wealth of biodiversity by using advanced culturomics methods in well controlled conditions.

Since the search for yeast biodiversity is increasingly undertaken (Suh *et al*, [65, 67], we now have a better idea of the specificities of yeasts in terms of geographical locations and substrates, which allows targeted field collection. However, areas such as the north of South America or South East Asia

need more exploration. The recent work by Groenewalld *et al*. [65] confirms that substrates such as soils are a very rich source of yeasts. However, as pointed out by Yurkov [66], a lot of work is needed to have a better picture of the extant of the diversity of these biotopes. But, yeasts from soil seem to be taxonomically distinct from those isolated from traditional fermentation or substrates, such as plants (this work) or the insect gut [66].

Beyond the important biodiversity reported here, our work indicates that biodiversity in many parts of the world has been largely overlooked and that it is necessary to have more systematic approaches from a geographical point of view to bring this biodiversity to light. Our work is consistent with the existence of allopatric speciation as demonstrated by Kuehne *et al*. [68] and corroborated by the numerous studies on biodiversity analysis in Asia or South America.

Finally, this work provides 276 isolates that are maintained at the CIRM-Levures Biological Resource Centre (Jouy-en-Josas, France). Isolates are fully available for research; a declaration of access for research use of resources can simply be sent to the French Ministry of the Environment to comply with the French Biodiversity Act which entered into force on 1 July 2017 to accompany the European Regulation on the Nagoya Protocol.

TWO NOVEL SPECIES OF THE GENUS *SUHOMYCES*

Pairwise comparisons of CLIB 1706^T sequence of the D1/D2 domains with that of the type strain of the most similar species, *Suhomyces guaymorum* CBS 9823^T, showed nine substitutions and three indels out of 556 bp. Comparison of the ITS sequences between CLIB 1706^T and the species of the genus *Suhomyces* revealed a divergence of 32 out of 380 bp and 12 indels. The results of those sequence comparisons indicated that CLIB 1706^T represented a taxon clearly distinct from the other species of the genus *Suhomyces* and the species *Suhomyces coccinellae* was created to accommodate CLIB 1706^T. In particular, two sequences of the D1/D2 domains from two *Suhomyces* sp. strains BG05-8-9-001A-2–1 (accession number MH745205) and UFMG-CM-Y6359 (accession number MG857089), isolated from beetle gut in the USA and from fruiting body of basidiomycete fungi in Brazil, respectively, were found to be quite similar to that of CLIB 1706^T. Sequence MH745205 from BG05-8-9-001A-2–1 showed one substitution and two indels over 549 bp, when compared with the CLIB 1706 sequence. Sequence MG857089 from UFMG-CM-Y6359 showed one substitution over 507 bp, when compared with the CLIB 1706 sequence. These sequence comparison results indicate that strains CLIB 1706^T, BG05-8-9-001A-2–1 and UFMG-CM-Y6359 are very likely to be conspecific. Unfortunately, ITS sequences for these two strains were not available and no further sequence comparison could be performed to confirm this proposition.

Pairwise comparisons of the sequence of the D1/D2 domains of CLIB 1725^T with those of the type strains of the most similar species showed 18 substitutions and one indel out of 549 bp with *Suhomyces vadensis* CBS 9454^T, 17 substitutions and one indel out of 549 bp with *Suhomyces xylopsoci* CBS 6037^T. Comparison of the ITS sequences between CLIB 1725^T and *Suhomyces vadensis* CBS 9454^T gave an alignment of 350 out of 398 bp with 48 substitutions and 11 indels. These sequence comparisons indicated that CLIB 1725^T represented a taxon clearly distinct from *S. vadensis* and the species *Suhomyces faveliae* was created to accommodate this strain. The phylogenetic placement of the two novel species of the genus *Suhomyces*, as well as strains not assigned to a species yet, within the genus *Suhomyces* is shown in Fig. 3.

Ecology of *Suhomyces* isolates

CLIB 1706^T and CLIB 1725^T were found on an insect and on a fern, respectively. The strains conspecific to CLIB 1706^T were isolated from fungi and from beetle gut. Out of 24 already described species of the genus *Suhomyces* [29], 19 have been isolated from the guts of insects in a variety of countries and have been extensively described [32]. There are some exceptions, such as the strains isolated from soil, moss, gum exudates, maize kernels and rotted mushroom. Strain *S. faveliae* CLIB 1725^T, which has been isolated from fern, therefore increases the numbers of non-insect-isolated species of the genus *Suhomyces*. Our two strains originated from a tropical country, but a number of isolates representing members of the genus *Suhomyces* have been collected in warm areas with high humidity, such as Japan, Panama and the south of the USA [29]. In contrast to most species of the genus *Suhomyces*, *S. faveliae* does not ferment glucose or trehalose and utilizes nitrate as a sole source of nitrogen (with a delay).

Description of *Suhomyces coccinellae* Jacques and Casaregola f.a. sp. nov.

Suhomyces coccinellae (coc.ci.nel'lae N.L. gen. n. *coccinellae* of *Coccinella* the name of the insect on which the type strain was isolated).

After two days on YPD agar at 25 °C, the colonies are cream colored, convex, circular, smooth and semi-glossy. Cells are globose to ellipsoid (5–8×7–13 µm) and occur singly or in mother-pairs (Fig. 4). Budding is unipolar. After 7 days at 25 °C on corn meal agar, pseudohyphae and true mycelium with lateral conidia are formed. Ascospores are not observed on Mac Clary's acetate agar and malt extract agar after 20 days at 25 °C.

Glucose and trehalose are fermented but cellobiose, galactose, lactose, maltose, melezitose, melibiose, raffinose and sucrose are not fermented. Cellobiose, D-galactose, D-glucose, 2-keto-D-gluconate, D-gluconate, D-glucosamine (weakly), *N*-acetyl-glucosamine, maltose, melezitose, methyl α-D-glucoside, sucrose, D-sorbitol, trehalose, D-xylose and mannitol are assimilated. L-arabinose, erythritol, glycerol, inositol, DL-lactate, lactose, melibiose, raffinose, L-

rhamnose, D-ribose, sodium glucuronate and L-sorbose are not assimilated. Potassium nitrate (weakly), sodium nitrite (weakly), L-lysine, cadaverine, creatine and creatinine are assimilated. Ethylamine is not assimilated. Growth in 10 % NaCl (w/v) and in 50 % glucose is positive. Growth in 0.01 % cycloheximide and in 60 % glucose is negative. Production of acid acetic is negative. Urease hydrolysis is negative. Maximum growth temperature is 30 °C.

The holotype CLIB 1706 was isolated from the surface of a ladybird in the forest of Saint Elie. It is preserved in a metabolically inactive state by lyophilization at the Biological Resource Centre CIRM-Levures, INRA/AgroParisTech, 78350 Jouy-en-Josas, France. Ex type culture has also been deposited in the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, under the accession number CBS 14298^T. MycoBank accession number: MB 815607.

The GenBank/EMBL/DDBJ accession numbers for the sequence of the D1/D2 region of the large subunit rRNA gene and the ITS region are LN875195 and LN875172, respectively.

Description of *Suhomyces faveliae* Jacques and Casaregola f.a. sp. nov.

Suhomyces faveliae (fa.ve'li.ae. N.L. gen. n. *faveliae* in acknowledgement of Dr Anne Favel, University of Aix-Marseille, France, for her assistance in collecting samples in French Guyana).

After four days on YPD agar at 25 °C, the colonies are off-white, dull, hirsute, butyrous and rough with undulating margins. Cells are ovoid or globose (5–9×5–12 µm) and occur singly (Fig. 4). Budding is multipolar. After 7 days at 25 °C on corn meal agar, pseudohyphae with lateral conidia are formed. Ascospores are not observed on Mac Clary's acetate agar and malt extract agar after 20 days at 25 °C.

Galactose is fermented (delayed). Cellobiose, glucose, lactose, maltose, melezitose, melibiose, raffinose and trehalose are not fermented. D-Galactose, D-glucose, D-gluconate, 2-keto-D-gluconate, D-glucosamine, glycerol, D-sorbitol, trehalose and mannitol are assimilated. L-arabinose, cellobiose, erythritol, *N*-acetyl-glucosamine, inositol, DL-lactate, lactose, maltose, melibiose, melezitose, methyl α-D-glucoside, sucrose, D-xylose, raffinose, L-rhamnose, D-ribose, sodium glucuronate and L-sorbose are not assimilated. Potassium nitrate (weakly), sodium nitrite, L-lysine, cadaverine, creatine (weakly) and creatinine are assimilated. Ethylamine is not assimilated.

Growth in 10 % NaCl (w/v) and in 50 % glucose is positive. Growth in 0.01 % cycloheximide and in 60 % glucose is negative. Production of acid acetic is negative. Urease hydrolysis is negative. Maximum growth temperature is 35 °C.

The holotype CLIB 1725 was isolated from the surface of a fern in the forest of Saül (*Layon des eaux claires* path). It is preserved in a metabolically inactive state by lyophilization at the Biological Resource Centre CIRM-Levures, INRA/AgroParisTech, 78350 Jouy-en-Josas, France. Ex type culture has been deposited in the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, under the accession number CBS 14299^T. MycoBank accession number: MB 815608.

The GenBank/EMBL/DDBJ accession numbers for the sequence of the D1/D2 region of the large subunit rRNA gene and the ITS region are LN875204 and LN875182, respectively.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Gargominy O. Biodiversité et conservation dans les collectivités françaises d'outre-mer. *Paris: Comité français pour l'UICN* 2003.
2. Sena LM, Morais CG, Lopes MR, Santos RO, Uetanabaro AP *et al.* D-Xylose fermentation, xylitol production and xylanase activities by seven new species of *Sugiyamaella*. *Antonie van Leeuwenhoek* 2017;110:53–67.
3. Jacques N, Sarilar V, Urien C, Lopes MR, Morais CG *et al.* Three novel ascomycetous yeast species of the *Kazachstania* clade, *Kazachstania saulgeensis* sp. nov., *Kazachstania serrabonitensis* sp. nov. and *Kazachstania australis* sp. nov. Reassignment of *Candida humilis* to *Kazachstania humilis* f.a. comb. nov. and *Candida pseudohumilis* to *Kazachstania pseudohumilis* f.a. comb. nov. *Int J Syst Evol Microbiol* 2016;66:5192–5200.
4. Lopes MR, Morais CG, Kominek J, Cadete RM, Soares MA *et al.* Genomic analysis and D-xylose fermentation of three novel *Spathaspora* species: *Spathaspora girioi* sp. nov., *Spathaspora hagerdaliae* f. a., sp. nov. and *Spathaspora gorwiae* f. a., sp. nov. *FEMS Yeast Res* 2016;16:fow044.
5. Dayo-Owoyemi I, Rosa CA, Rodrigues A, Pagnocca FC. *Wickerhamiella kiyanii* f.a., sp. nov. and *Wickerhamiella fructicola* f.a., sp. nov., two yeasts isolated from native plants of Atlantic rainforest in Brazil. *Int J Syst Evol Microbiol* 2014;64:2152–2158.
6. Morais CG, Lara CA, Marques S, Fonseca C, Lachance MA *et al.* *Sugiyamaella xylanicola* sp. nov., a xylan-degrading yeast species isolated from rotting wood. *Int J Syst Evol Microbiol* 2013;63:2356–2360.
7. Barbosa AC, Morais CG, Morais PB, Rosa LH, Pimenta RS *et al.* *Wickerhamiella pagnoccae* sp. nov. and *Candida tocantinsensis* sp. nov., two ascomycetous yeasts from flower bracts of *Heliconia psittacorum* (Heliconiaceae). *Int J Syst Evol Microbiol* 2012;62:459–464.

8. Santos RO, Cadete RM, Badotti F, Mouro A, Wallheim DO *et al.* *Candida queiroziae* sp. nov., a cellobiose-fermenting yeast species isolated from rotting wood in Atlantic Rain Forest. *Antonie van Leeuwenhoek* 2011;99:635–642.
9. Zheng J, Lu YF, Liu XJ, Hui FL. *Cyberlindnera xishuangbannaensis* f.a., sp. nov., a yeast isolated from rotting wood. *Int J Syst Evol Microbiol* 2017;67:5051–5055.
10. Zhang CY, Liu XJ, Yi ZH, Ren YC, Li Y *et al.* *Starmerella anomalae* f. a., sp. nov., *Starmerella asiatica* f.a., sp. nov., *Starmerella henanensis* f.a., sp. nov. and *Starmerella scarabaei* f.a., sp. nov., four yeast species isolated from scarab beetles. *Int J Syst Evol Microbiol* 2017;67:1600–1606.
11. Liu XJ, Yi ZH, Ren YC, Li Y, Hui FL. Five novel species in the *Lodderomyces* clade associated with insects. *Int J Syst Evol Microbiol* 2016;66:4881–4889.
12. Ren YC, Liu XJ, Yi ZH, Hui FL. *Nematodospora anomalae* sp. nov., a novel and D-xylose-fermenting yeast species in the *Lodderomyces* clade. *Int J Syst Evol Microbiol* 2016;66:4046–4050.
13. Liu XJ, Wang Y, Ren YC, Hui FL. *Wickerhamiella brachini* f.a., sp. nov., *Wickerhamiella pterostichi* f.a., sp. nov. and *Wickerhamiella qilinensis* f.a., sp. nov., three yeast species isolated from insects. *Int J Syst Evol Microbiol* 2016;66:3995–4001.
14. Zhu XF, Zhang DP, Yang S, Zhang QW. *Candida xinjiangensis* sp. nov., a new anamorphic yeast species isolated from *Scolytus scheryrewi* Semenov in China. *Arch Microbiol* 2017;199383:377.
15. Alimadadi N, Soudi MR, Wang SA, Wang QM, Talebpour Z *et al.* *Starmerella orientalis* f.a., sp. nov., an ascomycetous yeast species isolated from flowers. *Int J Syst Evol Microbiol* 2016;66:1476–1481.
16. Polburee P, Lertwattanasakul N, Limtong P, Groenewald M, Limtong S. *Nakazawaea todaengensis* f.a., sp. nov., a yeast isolated from a peat swamp forest in Thailand. *Int J Syst Evol Microbiol* 2017;67:2377–2382.
17. Jindamorakot S, Am-In S, Kaewwichian R, Limtong S. *Yamadazyma insecticola* f.a., sp. nov. and *Yamadazyma epiphylla* f.a., sp. nov., two novel yeast species. *Int J Syst Evol Microbiol* 2015;65:1290–1296.
18. Surussawadee J, Khunnamwong P, Srisuk N, Limtong S. *Papiliotrema siamense* f.a., sp. nov., a yeast species isolated from plant leaves. *Int J Syst Evol Microbiol* 2014;64:3058–3062.
19. Polburee P, Yongmanitchai W, Ohashi T, Fujiyama K, Limtong S. *Barnettozyma siamensis* f.a., sp. nov., a lipid-accumulating ascomycete yeast species. *Int J Syst Evol Microbiol* 2014;64:3053–3057.
20. Kaewwichian R, Limtong S. *Nakazawaea siamensis* f.a., sp. nov., a yeast species isolated from phylloplane. *Int J Syst Evol Microbiol* 2014;64:266–270.
21. Junyapate K, Jindamorakot S, Limtong S. *Yamadazyma ubonensis* f.a., sp. nov., a novel xylitol-producing yeast species isolated in Thailand. *Antonie van Leeuwenhoek* 2014;105:471–480.
22. Groenewald M, Robert V, Smith MT. The value of the D1/D2 and internal transcribed spacers (ITS) domains for the identification of yeast species belonging to the genus *Yamadazyma*. *Persoonia* 2011;26:40–46.
23. Groenewald M, Robert V, Smith MT. Five novel *Wickerhamomyces*- and *Metschnikowia*-related yeast species, *Wickerhamomyces chaumierensis* sp. nov., *Candida pseudoflosculorum* sp. nov., *Candida danieliae* sp. nov., *Candida robnettiae* sp. nov. and *Candida eppingiae* sp. nov., isolated from plants. *Int J Syst Evol Microbiol* 2011;61:2015–2022.
24. Jacques N, Louis-Mondesir C, Coton M, Coton E, Casaregola S. Two novel *Saccharomycopsis* species isolated from black olive brines and a tropical plant. Description of *Saccharomycopsis olivae* f. a., sp. nov. and *Saccharomycopsis guyanensis* f. a., sp. nov. Reassignment of *Candida amapae* to *Saccharomycopsis amapae* f. a., comb. nov., *Candida lassenensis* to *Saccharomycopsis lassenensis* f. a., comb. nov. and *Arthroascus babjevae* to *Saccharomycopsis babjevae* f. a., comb. nov. *Int J Syst Evol Microbiol* 2014;64:2169–2175.
25. Ribeiro LR, Santos ARO, Groenewald M, Smith MTH, Lara CA *et al.* Description of *Hyphopichia buzzinii* f.a., sp. nov. and *Hyphopichia homilentoma* comb. nov., the teleomorph of *Candida homilentoma*. *Antonie van Leeuwenhoek* 2017;110:985–994.
26. Amoikon TLS, Grondin C, Djeni TN, Jacques N, Casaregola S *et al.* *Starmerella reginensis* f.a., sp. nov. and *Starmerella kourouensis* f. a., sp. nov., isolated from flowers in French Guiana. *Int J Syst Evol Microbiol* 2018;68:2299–2305.
27. Simonnet C, Berger F, Gantier JC. Epidemiology of superficial fungal diseases in French Guiana: a three-year retrospective analysis. *Med Mycol* 2011;49:608–611.
28. Debourgogne A, Iriart X, Blanchet D, Veron V, Boukhari R *et al.* Characteristics and specificities of *Cryptococcus* infections in French Guiana, 1998-2008. *Med Mycol* 2011;49:864–871.
29. Kurtzman CP, Robnett CJ, Blackwell M. Description of *Teunomyces* gen. nov. for the *Candida kruisii* clade, *Suhomyces* gen. nov. for the *Candida tanzawaensis* clade and *Suhomyces kilbournensis* sp. nov. *FEMS Yeast Res* 2016;16.
30. Kurtzman CP. Six new anamorphic ascomycetous yeasts near *Candida tanzawaensis*. *FEMS Yeast Res* 2001;1:177–185.
31. Suh SO, McHugh JV, Blackwell M. Expansion of the *Candida tanzawaensis* yeast clade: 16 novel *Candida* species from basidiocarp-feeding beetles. *Int J Syst Evol Microbiol* 2004;54:2409–2429.
32. Kijpornyongpan T, Urbina H, Suh SO, Luangsa-Ard J, Aime MC *et al.* The *Suhomyces* clade: from single isolate to multiple species to disintegrating sex loci. *FEMS Yeast Res* 2019;19.
33. Hoffman CS, Winston F. A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. *Gene* 1987;57:267–272.
34. O'Donnell K. *Fusarium* and its relativees. In: Reynolds DR and Taylor JW (editors). *The Fungal Holomorph: Mitotc, Meiotic and Pleomorphic Speciation in Fungal Systematics*. Wallingford, UK: CAB International; 1993. pp. 225–233.
35. White TJ, Bruns TD, Lee S, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis DH, Sninsky J and White TJ (editors). *PCR Protocols: a Guide to Methods and Applications*. San Diego: Academic press; 1990. pp. 315–322.
36. Weiss S, Samson F, Navarro D, Casaregola S. YeastIP: a database for identification and phylogeny of *Saccharomycotina* yeasts. *FEMS Yeast Res* 2013;13:117–125.
37. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA *et al.* Clustal W and Clustal X version 2.0. *Bioinformatics* 2007;23:2947–2948.
38. Tamura K, Peterson D, Peterson N, Stecher G, Nei M *et al.* MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;28:2731–2739.
39. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
40. Perrière G, Gouy M. WWW-query: an on-line retrieval system for biological sequence banks. *Biochimie* 1996;78:364–369.
41. Yarrow D. Methods for the isolation, maintenance and identification of yeasts. In: Kurtzman CP and Fell JW (editors). *The Yeasts, a Taxonomic Study*. Amsterdam: Elsevier; 1998. pp. 77–100.
42. Barnett JA, Payne RW, Yarrow D. *Yeasts: Characteristics and Identification*, 3rd ed. Cambridge: Cambridge University Press; 2000.
43. Van der Walt JP, Yarrow D. Methods for the isolation, maintenance, classification and identification of yeasts. In: Kreger-van Rij NJW (editor). *The Yeasts, A Taxonomic Study*, 3rd ed. Amsterdam: Elsevier; 1984.
44. Vu D, Groenewald M, Szöke S, Cardinali G, Eberhardt U *et al.* DNA barcoding analysis of more than 9000 yeast isolates contributes to quantitative thresholds for yeast species and genera delimitation. *Stud Mycol* 2016;85:91–105.

45. Jacques N, Mallet S, Casaregola S. Delimitation of the species of the Debaryomyces hansenii complex by intron sequence analysis. *Int J Syst Evol Microbiol* 2009;59:1242–1251.
46. Groenewald M, Daniel HM, Robert V, Poot GA, Smith MT. Polyphasic re-examination of *Debaryomyces hansenii* strains and reinstatement of *D. hansenii*, *D. fabryi* and *D. subglobosus*. *Persoonia* 2008;21:17–27.
47. Nguyen HV, Gaillardin C, Neuvéglise C. Differentiation of *Debaryomyces hansenii* and *Candida famata* by rRNA gene intergenic spacer fingerprinting and reassessment of phylogenetic relationships among *D. hansenii*, *C. famata*, *D. fabryi*, *C. flareri* (=*D. subglobosus) and D. prosopidis*: description of *D. vietnamensis* sp. nov. closely related to *D. nepalensis*. *FEMS Yeast Res* 2009;9:641–662.
48. Cadez N, Smith MTH. Hanseniaspora Zikes (1912). In: Kurtzman CP, Boekhout P (editors). *The Yeasts, a Taxonomic Study*, vol. 2. Amsterdam: Elsevier; 1912. pp. 421–434.
49. Ho VT, Zhao J, Fleet G. Yeasts are essential for cocoa bean fermentation. *Int J Food Microbiol* 2014;174.
50. Samagaci L, Ouattara H, Niamké S, Lemaire M. *Pichia kudrazevii* and *Candida nitrativorans* are the most well-adapted and relevant yeast species fermenting cocoa in Agneby-Tiassa, a local Ivorian cocoa producing region. *Food Res Int* 2016;89:773–780.
51. Miescher Schwenninger S, Freimüller Leischtfeld S, Gantenbein-Demarchi C. High-throughput identification of the microbial biodiversity of cocoa bean fermentation by MALDI-TOF MS. *Lett Appl Microbiol* 2016;63:347–355.
52. Visintin S, Alessandria V, Valente A, Dolci P, Cocolin L. Molecular identification and physiological characterization of yeasts, lactic acid bacteria and acetic acid bacteria isolated from heap and box cocoa bean fermentations in West Africa. *Int J Food Microbiol* 2016;216:69–78.
53. Papalexandratou Z, Lefeber T, Bahrim B, Lee OS, Daniel HM *et al.* *Hanseniaspora opuntiae*, *Saccharomyces cerevisiae*, *Lactobacillus fermentum*, and *Acetobacter pasteurianus* predominate during well-performed Malaysian cocoa bean box fermentations, underlining the importance of these microbial species for a successful cocoa bean fermentation process. *Food Microbiol* 2013;35:73–85.
54. Illeghems K, De Vuyst L, Papalexandratou Z, Weckx S. Phylogenetic analysis of a spontaneous cocoa bean fermentation metagenome reveals new insights into its bacterial and fungal community diversity. *PLoS One* 2012;7:e38040.
55. Papalexandratou Z, De Vuyst L. Assessment of the yeast species composition of cocoa bean fermentations in different cocoa-producing regions using denaturing gradient gel electrophoresis. *FEMS Yeast Res* 2011;11:564–574.
56. Papalexandratou Z, Falony G, Romanens E, Jimenez JC, Amores F *et al.* Species diversity, community dynamics, and metabolite kinetics of the microbiota associated with traditional Ecuadorian spontaneous cocoa bean fermentations. *Appl Environ Microbiol* 2011;77:7698–7714.
57. Nielsen DS, Snitkjaer P, van den Berg F. Investigating the fermentation of cocoa by correlating denaturing gradient gel electrophoresis profiles and near infrared spectra. *Int J Food Microbiol* 2008;125:133–140.
58. Meersman E, Steensels J, Struyf N, Paulus T, Saels V *et al.* Tuning chocolate flavor through development of thermotolerant *Saccharomyces cerevisiae* starter cultures with increased acetate ester production. *Appl Environ Microbiol* 2016;82:732–746.
59. Daniel HM, Vrancken G, Takrama JF, Camu N, De Vos P *et al.* Yeast diversity of Ghanaian cocoa bean heap fermentations. *FEMS Yeast Res* 2009;9:774–783.
60. Jespersen L, Nielsen DS, Hønholt S, Jakobsen M. Occurrence and diversity of yeasts involved in fermentation of West African cocoa beans. *FEMS Yeast Res* 2005;5:441–453.
61. Kurtzman CP, Robnett CJ. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek* 1998;73:331–371.
62. Suh SO, McHugh JV, Pollock DD, Blackwell M. The beetle gut: a hyperdiverse source of novel yeasts. *Mycol Res* 2005;109:261–265.
63. Suh SO, Nguyen NH, Blackwell M. A yeast clade near *Candida kruisii* uncovered: nine novel *Candida* species associated with basidioma-feeding beetles. *Mycol Res* 2006;110:1379–1394.
64. Suh SO, Nguyen NH, Blackwell M. Yeasts isolated from plant-associated beetles and other insects: seven novel *Candida* species near *Candida* albicans. *FEMS Yeast Res* 2008;8:88–102.
65. Groenewalld M, Lombard L, De Vries M, Lopez AG, Smith M *et al.* Diversity of yeast species from Dutch garden soil and the description of six novel Ascomycetes. *FEMS Yeast Res* 2018;18.
66. Yurkov AM. Yeasts of the soil - obscure but precious. *Yeast* 2018;35:369–378.
67. Bing J, Han PJ, Liu WQ, Wang QM, Bai FY. Evidence for a far East Asian origin of lager beer yeast. *Curr Biol* 2014;24:R380–R381.
68. Kuehne HA, Murphy HA, Francis CA, Sniegowski PD. Allopatric divergence, secondary contact and genetic isolation in wild yeast populations. *Curr Biol* 2007;17:407–411.