Cryptogamie, Mycologie, 2017, 38 (4): 469-483
© 2017 Adac. Tous droits réservés

The genus *Resinicium* in French Guiana and the West Indies: a morphological and molecular survey, revealing *Resinicium grandisporum sp. nov.*

Gérald GRUHN[a], Sylvain DUMEZ[b], Pierre-Arthur MOREAU[b], Mélanie ROY[c], Orinne MORREALE[c], Heidy SCHIMANN[d] & Régis COURTECUISSE[b]*

[a]Office National des Forêts, 5 avenue Mirandol, 48000, Mende, France

[b]Université de Lille, Faculté de Pharmacie, Laboratoire des Sciences Végétales et Fongiques, EA 4483 IMPECS, 59000, Lille, France

[c]Laboratoire Évolution et Diversité Biologique, Université Paul Sabatier (UPS) – CNRS, Toulouse, France, 118 route de Narbonne, 31062, Toulouse, France

[d]INRA – UMR Écologie des Forêts de Guyane (AgroParisTech, CIRAD, CNRS, Université de Guyane, Université des Antilles), 97310, Kourou, France

Abstract – A revision of *Resinicium* collections (Basidiomycota, Hymenochaetales) from French Guiana and French West Indies is provided, and a new species, *Resinicium grandisporum sp. nov*., supported by morphological as well as phylogenetic analyses based on ITS rDNA sequences, is described and illustrated. An updated key of the genus *Resinicium* is also provided, which includes species previously described from outside of the studied area.

Basidiomycota / French Guiana / Hymenochaetales / Martinique Island / phylogeny / taxonomy

INTRODUCTION

The genus *Resinicium* Parmasto, mainly known to mycologists through its common and widespread type species, *R. bicolor* (Alb. & Schwein.: Fr.) Parmasto, is currently placed in the Hymenochaetales, and belongs to Repetobasidiaceae (Larsson *et al.*, 2006; Moreau & Courtecuisse, 2015). Originally based on morphological features (Parmasto, 1968), i.e. corticioid basidiomata forming astrocystidia and halocystidia (Eriksson *et al.*, 1981; Ginns & Lefebvre, 1993), this genus has been recently delineated on the basis of molecular data (Nakasone, 2007) and currently encompasses eight morphologically and molecularly identified species. A synapomorphic character, i.e. the presence of astrocystidia, supports the monophyly of the genus *Resinicium*, while species with only halocystidia are placed in the unrelated genera *Phlebia* Fr.: Fr. (Phlebioid clade; Binder *et al.*, 2005, Larsson *et al.*,

* Corresponding author: gerald.gruhn@onf.fr

doi/10.7872/crym/v38.iss4.2017.469

2004, 2006), or *Mycoacia* Donk (sister to *Resinicium*; Nakasone, 2007, as an alternative to "*Resinicium s.l.*").

Apart for the pan-temperate *R. bicolor* (Eriksson *et al.*, 1981; Nakasone, 2007), the geographical distribution of the genus looks fragmentary. For instance, *R. friabile* and *R. saccharicola* are documented as pantropical species, although only known from few collections each. A recent synonym *of R. friabile, Gloeoradulum luteosulphureum* Rick from Southern Brazil, was recently reinstated (Baltazar *et al.*, 2016). However, this synonym is invalid as it was published in the invalid genus *Gloeoradulum* Lloyd (McNeill *et al.* 2012, art. 35.1). The five other species cited by Nakasone (2007) were collected from the North Caribbean area, considered as a hot-spot for the genus. Species of *Resinicium* have not yet been reported from the Lesser Antilles (Minter *et al.*, 2001; Courtecuisse & Welti, 2013) or the Guiana shield (Courtecuisse *et al.*, 1996). Novel data from these areas are expected to reveal more basal lineages and to confirm tropical America as a possible origin for the radiation of the genus, as suggested by Nakasone (2007).

Several recent mycological surveys in French Guiana and Martinique (French West Indies) that focused on corticioid species yielded ten samples of *Resinicium*. Morphological data and ITS sequences revealed three distinct species, here compared to available data from literature and public sequence databases.

A novel species, *Resinicium grandisporum* G. Gruhn, S. Dumez & H. Schimann, as well as new collections attributed to *R. saccharicola* and to *R. mutabile* are described and illustrated, compared to related species, and their phylogenetic placement is provided. An update of Nakasone's (2007) worldwide identification key of *Resinicium* is proposed.

MATERIALS AND METHODS

Morphological studies

Macroscopic and microscopic studies were based on fresh and dried material. Sections were prepared with a razor blade and observed in several aqueous solutions: 10% ammonia Congo Red, 3% potassium hydroxide with addition of 1% phloxine B, Melzer's reagent, and Cotton Blue. Measurements were made from microphotographs under 1000× magnification, using the software Mycomètre (Fannechère, 2011), Q or the average spore length/spore witdth ratio were also provided. The number of measured spores is indicated in brackets. Spore measurements were based on spores obtained from spore prints, observed in Melzer in side view with apiculus excluded (Duhem, 2010). Specimens are conserved in the Herbarium of the Faculty of Pharmacy of Lille, France (LIP), with duplicates in the private herbarium of G. Gruhn.

DNA extraction and PCR amplifications

Each sample was incubated at 65°C in 500 µL of extraction buffer (100 mM Tris/HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2% (w/v) CTAB, 0.2% (v/v) 2-mercaptoethanol and 0.1 mg.mL^{-1} proteinase K) for 1 h. One volume of chloroform/isoamylic alcohol (24/1; v/v) was added after the incubation. The mixture was gently mixed and centrifuged at 9500 g for 10 min. The upper phase was placed in a new

tube and gently mixed with one volume of isopropanol before centrifugation. The resulting pellet was rinsed in 70% (v/v) ethanol and centrifuged again. The ethanol was then removed and the pellet was air dried in order to remove any trace of ethanol. The pellet was finally dissolved in TE buffer (10 mM Tris, bring to pH 8.0 with HCl, 1 mM EDTA) at 4°C overnight.

PCR reactions were performed in a final volume of 50 µL containing 1 µL of DNA and 49 µL solution of 80 mM Tris·HCl pH 9.4, 20 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.2% (w/v) Tween-20, 0.2 µM dNTPs, 0.2 µM of each primer (ITS-1F: CTTGGTCATTTAGAGGAAGTAA (Gardes & Bruns, 1993) and ITS4:TCCTCCGCTTATTGATATGC (White *et al.*, 1990) and 1.25U Taq DNA Polymerase (Euromedex, Souffelweyersheim, France). PCR reactions were conducted as follow: an initial denaturation at 95°C for 2 minutes following by 40 cycles of 30 s at 95°C; 30 s at 53°C; 1 min at 72°C followed by a final extension step at 72°C for 5 min. Aliquots of 5 µL PCR products were resolved at 8 $V.cm^{-1}$ on a 1.5% (w/v) agarose gel with 0.5 $\mu g.mL^{-1}$ ethidium bromide in TAE buffer (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA). PCR products were then sequenced by Sanger method with ITS-1F as sequencing primer.

Phylogenetic studies

In addition to the ten newly obtained sequences (Genbank accession numbers reported in Table 1), 27 sequences of *Resinicium* available in GenBank (*https://www.ncbi.nlm.nih.gov/genbank*) were downloaded and aligned using BioEdit (*http://www.mbio.ncsu.edu/BioEdit*). Analyses were conducted online at *www.phylogeny.lirmm.fr* (Dereeper *et al.*, 2008). Multiple sequence alignments were carried out with MUSCLE 3.7 (Edgar, 2004) using full processing mode and 16 iterations. All positions were included in the analysis. Maximum likelihood phylogenetic analyses were performed with PhyML 3.0 aLRT (Zwickl, 2006), using the GTR + I + Γ model of evolution. Branch support was assessed using the non-parametric version of the approximate likelihood-ratio test, implemented in PhyML (SH-aLRT; Anisimova *et al.*, 2006). Two sequences selected amongst external branches close to *Resinicium* according to Larsson (2007): *Rickenella fibula* and *Globulicium hiemale*, were selected as outgroups in the phylogenetic tree presented in Fig. 1.

Table 1. GenBank accession numbers (ITS sequences) and herbarium numbers of sequenced collections

Species	*Herbarium*	*Collection n°*	*GenBank*
R. grandisporum	LIP 0001287 (holotype)	GUY13-030	KY995326
		GUY13-008	KY995325
		GUY13-031	KY995327
	LIP 0001294	MAR12-326	KY995329
R. mutabile		GUY12-087	KY995322
		MAR15-174	KY995330
	LIP 0001288	MAR15-175	KY995331
R. saccharicola	LIP 0001289	GUY12-118	KY995323
	LIP 0001290	GUY12-158	KY995324
	LIP 0001291	MAR12-230	KY995328

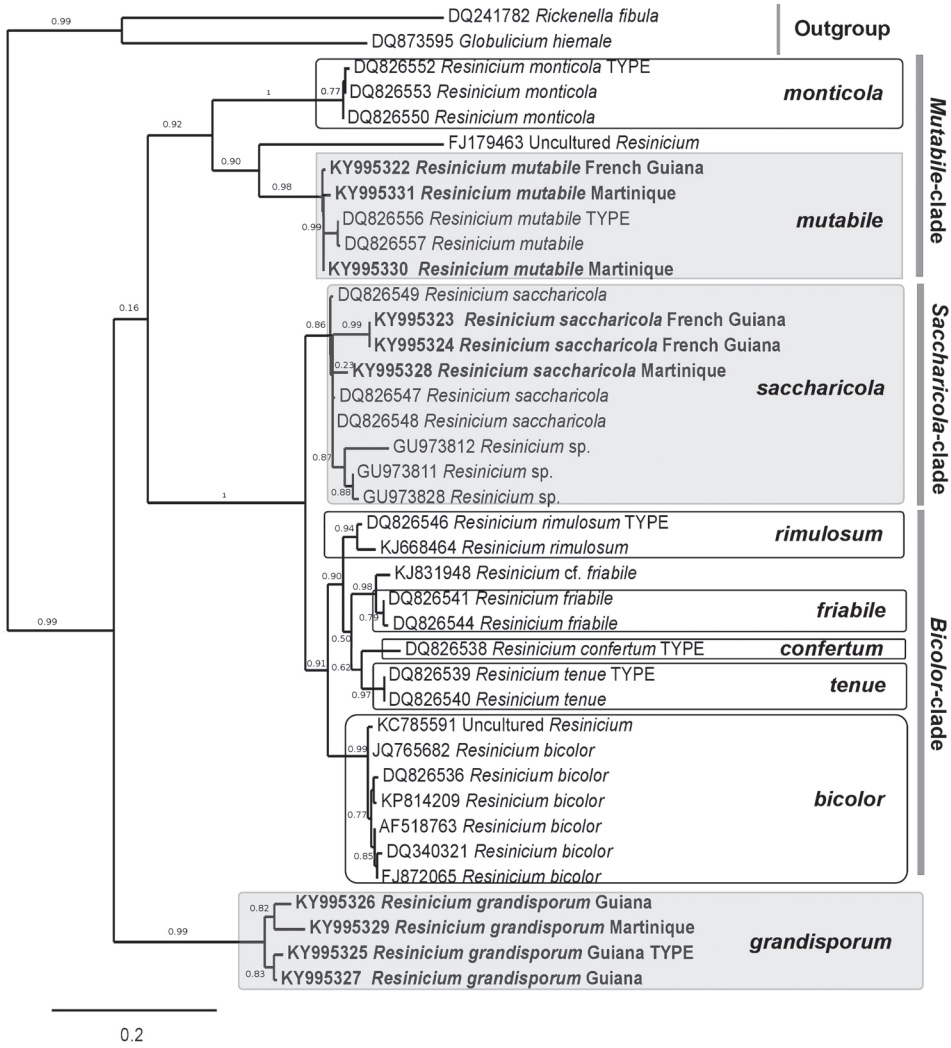

Fig. 1. ML phylogenetic reconstruction of the *Resinicium*-clade based on ITS sequences. Bootstrap values (%) are shown when > 70%.

TAXONOMY

Resinicium grandisporum G. Gruhn, S. Dumez & E. Schimann, ***sp. nov.*** **Figs 2-3**

Mycobank: MB 821864

Diagnosis: differs from *Resinicium bicolor* by spores mostly longer than 8 µm.

Holotype: FRANCE: French Guiana, Saül, B. Diadema trail, on unidentified dead trunk lying on the ground, 19 Oct 2013, LIP 0001287; isotype priv. herb. Gruhn GUY 13-030.

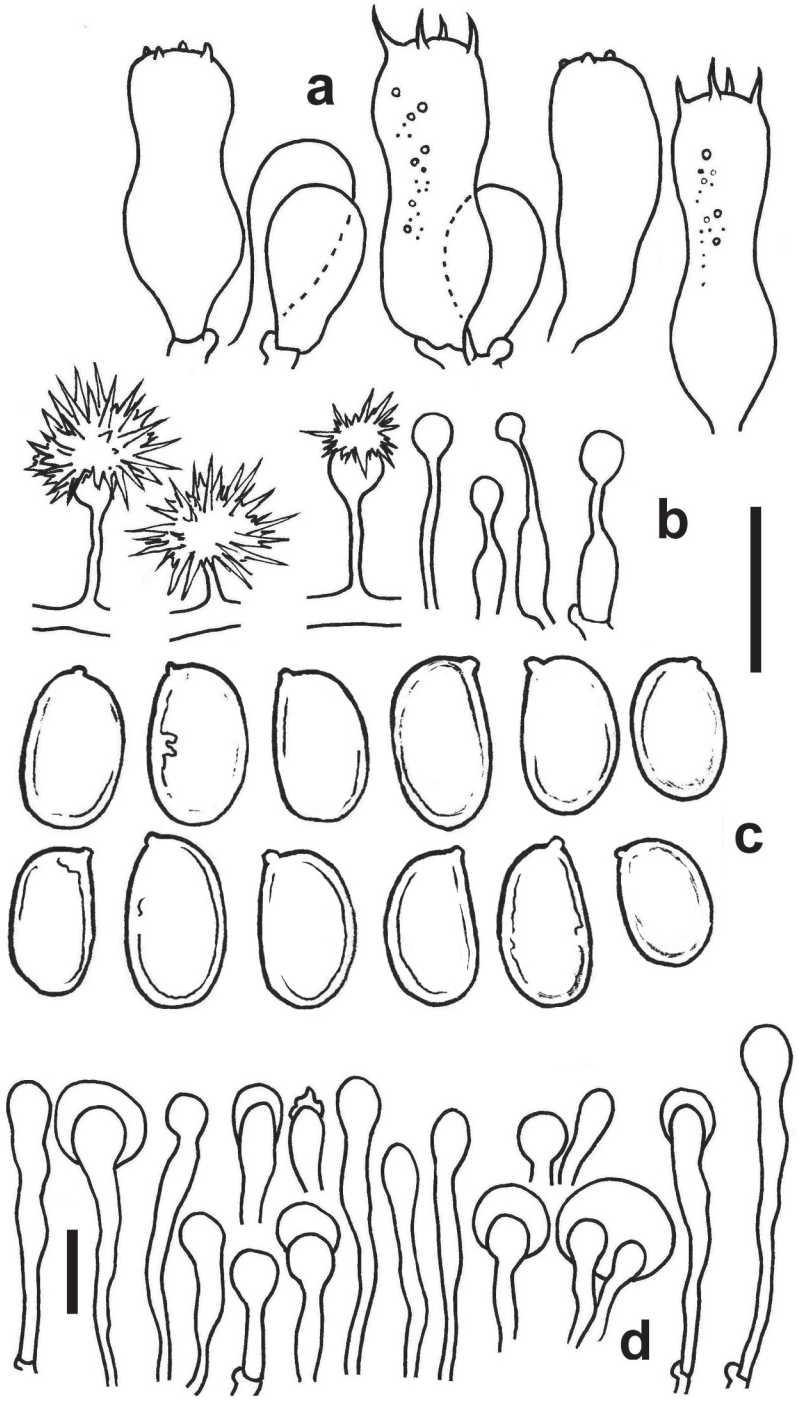

Fig. 2. ***Resinicium grandisporum*** sp. nov. (holotype). **a.** basidia, **b.** astrocystidia, **c.** spores, **d.** halocystidia. Scale: bar = 10 μm.

Fig. 3. ***Resinicium grandisporum*** sp. nov. (holotype). Dried basidiome, macromorphology. Credit: G. Gruhn. Scale: bar = 1 cm.

Etymology: *grandisporum*: refers to the spores characterizing this species, the largest known in the genus.

Basidiome annual, widely effused (up to 20 × 10 cm), adnate, strongly attached to the substrate. *Hymenium* odontoid, cream-colored when fresh, dark yellowish in herbarium; aculei rather distant (3 to 4 per mm), single, with round apex, sometimes slightly spinose, the higher ones slightly attenuate with a short fimbriate sterile apex, 150-400 µm high. *Context* farinaceous, not stratified, white when fresh, light cream-coloured and partly yellowish in herbarium, ochraceous in old parts, smooth between aculei, 50-100 µm thick, rarely cracked into polygons, substrate easily seen through thinner parts of the context. *Margin* gradually thinning out, minutely farinaceous, white, early garnished with small aculei; hyphal cords sometimes present in substrate, concolourous with the hymenium, 100-150 µm diameter, sometimes flat with a fertile farinaceous white surrounding are. *Spore print* white.

Hyphal system monomitic; hyphae 2-3 µm wide, thick walled in subiculum, walls about 0.5 µm thick, thin walled in hymenium; rounded clamp connections present at all septa. Context of aculei composed of vertical, partially agglutinated hyphae; apex of aculei are non-differentiated hyphae mixed with halocystidia. Hyphidia not seen. *Astrocystidia* abundant in hymenium and subiculum, often pleural and originating from thin-walled hyphae, otherwise terminal and clamped, core 0.5-1 µm wide, usually regular and cylindrical in the central, mostly straight part, when terminal with enlarged base, ending in a bulbous apex, 2.5-3 µm diam., either entirely or apically covered by star-shaped crystals, 7-9 µm wide, greenish in KOH. Capitate *halocystidia* located on aculei, measuring (15-)25-40 × 6 µm, clamped, deeply pedunculate, broadened at one third of the length, with constrictions, apex 7-10 µm wide, inflate to vesiculose, sometimes largely clavate, always rounded in shorter halocystidia, smooth, covered by a large hyaline amorphous mass sometimes

embedding several halocystidia. *Basidia* at first globose to pyriform, when mature measuring (15-)19-22 × 6.5-7.5 µm, 4-spored with thin sterigmata, stout, cylindrical to clavate with a median constriction, thin-walled, with granular content, clamped. *Spores* ellipsoid, [n = 40] (7.7-)8.0-9.6(-10.7) × (4.0-)4.3-5.5 µm, Q = 1.8, slightly thick-walled, wall smooth, non cyanophilous, inamyloid; content slightly dextrinoid.

Material studied: **FRANCE**: French Guiana: Saül, B. Diadema trail, on slope, on undefined dead hardwood, 19 Oct 2013, leg. & det. G. Gruhn priv. herb. Gruhn GUY13-008 (LIP 0001287, holotype); *idem*, Crique Limonade, trail to the Diadema field station, on undefined dead hardwood, 19 Oct 2013, leg. & det. G. Gruhn, priv. herb. Gruhn GUY13-031; Martinique, Le Prêcheur, Anse Lévrier, sentier de Trois-Rivières, on undefined dead hardwood, 17 Aug 2012, leg. & det. G. Gruhn, priv. herb. Gruhn MAR12-326 (LIP 0001294).

Observations: this new species fits with the recently revised definition of the genus (Nakasone, 2007). It is well characterized by the presence of numerous astrocystidia and pedunculate halocystidia, always clamped hyphae, stout basidia, and spores longer than 8 µm. All *Resinicium* species known hitherto have shorter basidia and spores. The spore sizes of *R. grandisporum* specimens are given in Table 2. All spores show a slight dextrinoid reaction, an unusual feature never noticed within *Resinicium* species.

The micro- and macromorphology of *R. grandisporum* do not substantially differ from those of *R. bicolor*, a well-known cosmopolitan species that is usually recognized in the field (Eriksson *et al.*, 1981) and grows in Gymnosperm logs in the Northern hemisphere, but has not been confirmed from the Southern hemisphere (Hjortstam & Larsson, 2007; Nakasone, 2007). *Resinicium grandisporum* shares the main features of *R. bicolor*, including spore shape, but the width of the latter is less than 3.5 µm, and the length rarely more than 8 µm (Nakasone, 2007; Eriksson *et al.*, 1981). The morphology of halocystidia also differs between those two species: the bulb diameter is 6 µm wide in *R. grandisporum*, much bigger in *R. bicolor* (7-11 µm).

In *R. grandisporum*, astrocystidia are numerous, and halocystidia can be easily observed, as also in *R. rimulosum*, *R. tenue*, *R. bicolor*, and all other species of the core *Resinicium*-clade. These microscopic features would indicate a greater affinity with the latter species than those of the *Mutabile*-clade, which lack true halocystidia but rather have vesicular cystidia without an interior apical bulb according to Nakasone (2007). Our phylogenetic results (Fig. 1) actually place *R. grandisporum* in a third monospecific lineage, and affinities with the two other clades are not resolved by ITS sequences.

Table 2. Spore sizes of *R. grandisporum* and *R. saccharicola*. Q = average ratio L/w

Collection n°	*Spore size*	*Q*
R. grandisporum		
MAR12-326	(7.4-)7.5-9.1(-9.6) × (3.5-)3.9-5.1(-5.3) µm	1.8
GUY13-008	(5.8-)6.2-7.3(-7.8) × (3.4-)3.6-4.6(-4.9) µm	1.7
GUY13-030 (typus)	(7.7-)8.0-9.6(-10.7) × (4.0-)4.3-5.5 µm	1.8
GUY13-031	(8.2-)8.9-10.8(-11.5) × (4.2-)4.4-5.5(-5.8) µm	2.0
R. saccharicola		
GUY12-118	(4.5-)5.2-6.3(-7.1) × (3.1-)3.4-4.4(-4.5) µm	1.5
GUY12-158	(4.8-)5.0-6.1(-6.9) × (3.2-)3.3-4.2(-4.5) µm	1.5

Resinicium mutabile K.K. Nakasone, Canadian Journal of Botany 85: 430 (2007)
Fig. 4

Basidiome annual, effused, forming confluent patches, adnate, loosely attached to the substrate. *Hymenium* odontoid, smooth at margin, cream coloured when fresh, ochraceous to light brown in herbarium, cracked and porulose between aculei under lens. Aculei crowded (6 to 10 per mm), dark brown, with rounded apex, up to 100 µm long, fragile, surface partly covered by chocolate-coloured granules. *Context* white to cream, fragile, farinaceous, under lens a loose hyphal structure including white granular elements, about 70 µm thick. Margin white to cream, minutely farinaceous in thinnest parts, with hyphal cords in substratum. *Spore print* white.

Hyphal system monomitic, made of thin-walled hyphae in subiculum and subhymenium, 1.5-2 µm wide, clamped at all septa. Core of the aculei composed of parallel, agglutinate hyphae, embedded in a brown resinoid matrix; apex of aculei made of non-differentiated hyphae mixed with halocystidia and sparse hyphidiae measuring 18-22 × 2-2.5 µm. Hymenial cystidia of 3 kinds: 1) *astrocystidia* irregularly sparse, numerous to absent in hymenium, either pleural on clamped hyphae, less than 1.5 µm wide, or terminal and clamped, core thin 0,5-1 µm, cylindrical, inflated at apex, 2-3 µm wide, entirely covered by long bipyramidal star-shaped crystals, 6-7 µm diam.; also present at margin in clusters, with larger crystals 6-15 µm diam; 2) *capitate halocystidia* (10-)20 × 5-6 µm, numerous in the hymenium, stalked (base 5 to 15 µm long), cylindrical to broadened and constricted with a rounded inflated apex, clamped, smooth, non-cyanophilous, always seen without apical deposits; 3) *vesicular cystidia* slender with obtuse apex, 1-2 µm wide, hardly differentiated, bearing a wide mucoid globule visible in Cotton Blue and in distilled water, 5-18 µm diam., light brown to golden yellow. *Basidia* (11-)15 × 5-5.5 µm, 4-spored with thin sterigmata, clavate with a median constriction, thin-walled, with granular contents clamped. *Spores* [n = 36] (4.0-)4.2-5.0(-5.3) × (2.9-)3.0-3.9(-4.0) µm, Q = 1.3, shortly ellipsoid, thin-walled and smooth, slightly cyanophilous, inamyloid.

Material studied: **FRANCE**, Martinique, Fort-de-France, RBI des Pitons du Carbet, lieu-dit sentier du Plateau Perdrix, on *Heliconia* sp. stem, 1 Jun 2015, leg. & det. G. Gruhn, priv. herb. Gruhn MAR15-175 (LIP 0001288); *idem*, a few metres far from priv. herb. Gruhn MAR15-175, on hardwood, 6 Jun 2015, leg. & det. G. Gruhn, priv. herb. Gruhn MAR15-174; French Guiana: Régina, access path to the Nouragues inselberg, on hardwood, 20 Jun 2012, leg. & det. G. Gruhn, priv. herb. Gruhn GUY12-087. **PUERTO RICO**: BPI FP-150015-Sp, 1996/06/30, Ridge above chicken farm above Rio Sabana, Luquillo Municipio, on decorticated hardwood branch, leg. & det. K.K. Nakasone; BPI FP-102989-Sp (isotype) 1996/06/28 Rio Grande Municipio County, El Verde Field Station, 400m, Caribbean Natl. Forest, on corticated angiosperm, well decayed, leg. & det. K.K. Nakasone; BPI PR-1366, 1993/11/16, Trail to Rio Sonadora, El Verde, Luquillo Mts Nakasone, on *Guarea guidonia* 6 inches branch, leg. Lodge/Burdsall/Boyd, det. K.K. Nakasone. **USA**: Florida: BPI HHB-6952, 1972/08/07, Gumbo Limbo Trail, Everglades Natl.. Park Dade County, leg. H.H. Burdsall, det. K.K. Nakasone; BPI HHB-7140, 1972/08/14, Gumbo Limbo Trail, Everglades Natl. Park Dade County, on *Ficus aurea*, leg. H.H. Burdsall, det. K.K. Nakasone; BPI FP-150720, 2001/11/15, Humminbird loop Trail, Blue Hole Natl. Park, on decorticated hardwood, leg. & det. K.K. Nakasone; Hawaii: BPI RLG-18649, 1991/10/22, Kalopa State Park, Hamakua District County, on old stump of hardwood (probably *Casuarina* sp.), leg. R.L. Gilbertson, det. K.K. Nakasone.

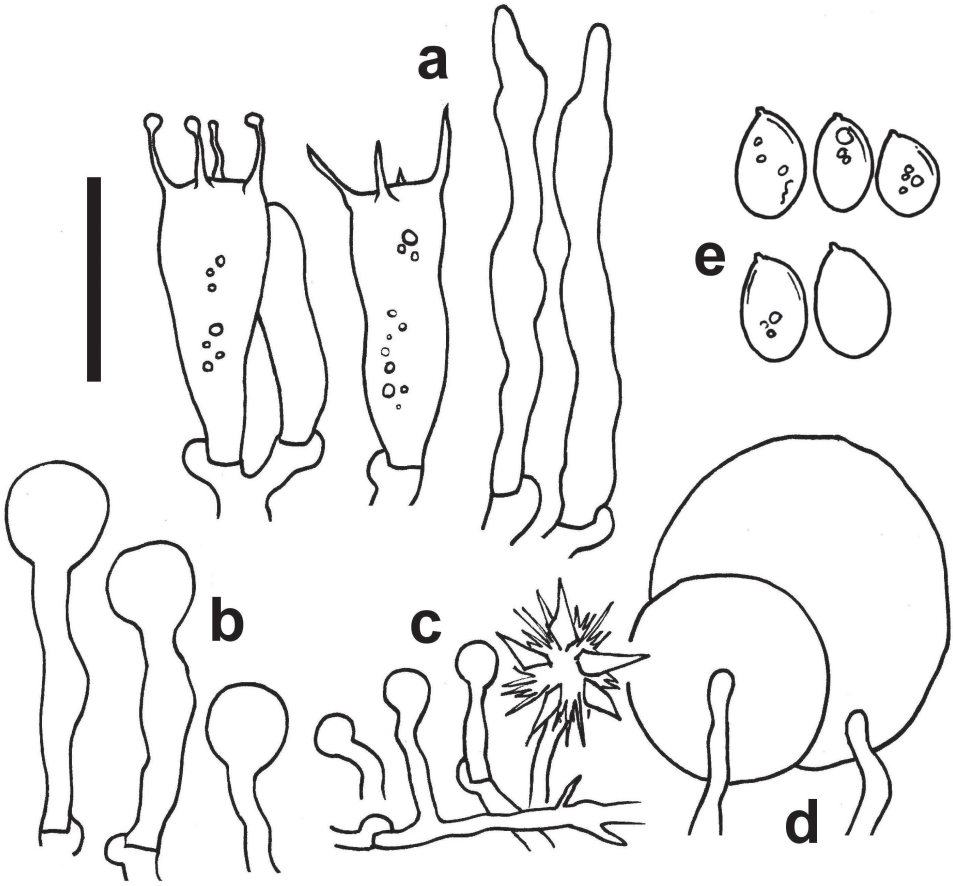

Fig. 4. ***Resinicium mutabile*** (MAR15-175). **a.** basidia and non-differentiated hyphae in aculei, **b.** halocystidia, **c.** astrocystidia (without and with crystals), **d.** vesicular cystidia, **e.** spores observed in Cotton blue. Scale: bar = 10 µm.

Observations: *Resinicium mutabile*, so far only known from the Neotropics, is described with vesicular cystidia with an undifferentiated stalk and a lack of interior apical bulb (Nakasone, 2007). Some macroscopic and microscopic features of two collections from Martinique are identical to this description of *R. mutabile*, i.e. resinous vesicles are numerous in all collections, and the basidiome is soft, fragile and brown. However, our two Martinique collections differ in the presence of true halocystidia, the length of their aculei, and their spore size.

We confirmed the absence of true halocystidia in the holotype of *R. mutabile*, but they are present in our collections as true capitate halocystidia with stalk and rounded bulb, and without vesicles. Our collection from Martinique, made of variously mature basidiomata, suggests that halocystidia develop with maturity and may be lacking or being easily overlooked at young stages.

As stated by Nakasone (2007), we confirm that basidia and spores are absent in many of the herbarium collections studied. In comparison, our specimens from Martinique are mature and well developed, producing abundant spore prints with spores slightly shorter and ellipsoid (4.2-5.0 × 3.0-3.9 µm and Q = 1.31) for our collections compared to the original description of *R. mutabile* [(4.5-)5.0-5.5 × 3.0-3,5(-4) µm and Q = 1.42-1.65]. Indeed, the isotype has been examined and spores longer than 5.0 µm were rarely observed. Such a difference is possible between measurements based on mature spores from spore prints and on spores sampled directly from the basidiome, as the latter are usually shorter (B. Duhem and G. Trichies com. pers.).

Resinicium saccharicola (Burt) K.K. Nakasone, Karstenia 40: 113 (2000) **Fig. 5**

Basidiome annual, effused, very thin, adnate, loosely attached to the substrate. *Hymenium* grandinioid when fresh, colliculose in herbarium, cream coloured when fresh, slightly darkening in herbarium; under lens, porulose in thinner parts between aculei, otherwise context stretched. Aculei flattened in herbarium, crowded (8 to 10 per mm), concolourous, up to 50 µm long. *Context* white to cream, fragile, about 30 µm thick. Margin concolourous, thinning out, little aculei easily seen beyond the margin. No cords seen. *Spore print* hyaline.

Hyphal system monomitic, made of slightly thick-walled intricate hyphae in subiculum and thin-walled in subhymenium, 2-2.5 µm wide, mostly unclamped, some rare single clamps seen. Core of the grandinioid elements undifferentiated. No intrahymenial hyphidiae. Hymenial cystidia of 2 kinds: 1) *astrocystidia* rare to absent in hymenium, on capillaceous fibers diam. < 0,5 µm, without clamps, ending with an acute or bulbous apex, in such cases 1.5-2 µm diam., star-shaped crystals usually free in the hymenium, rarely covering the astrocystidia apex, 5-6 µm diam., easily crushed by tapping; 2) *capitate halocystidia* 15-20 × 5-6 µm, common, stalked, short (base 5 to 15 µm long), cylindrical with a rounded inflate apex, neck constricted below the apex, diam. 5-6 µm, unclamped, smooth, slightly thick-walled subicular hyphae, slightly cyanophilous, sometimes covered by a large hyaline guttulate mass, diam. (13-)15-19 µm, mostly seen as an empty and wrinkled wall on the head of the halocystidia. Basidioles globose, then pyriform, developing in stout mature *basidia* 13-17 × 5.5-6.0 µm, 4-spored with thin sterigmata, long 4-5 µm, clavate with a median constriction, thin-walled, with granular contents, fragile when tapped, unclamped. *Spores* [42] (4.8-)5.0-6.1(-6.9) × (3.2-)3.3-4.2(-4.5) µm, Q = 1.5, broadly ellipsoid to ovoid, thin-walled and smooth, slightly cyanophilous, inamyloid.

Material studied: **FRANCE**: French Guiana: Sinnamary, close to Paracou camp, on white sands, sampled on a palm rachis, 25 Jun 2012, leg. & det. G. Gruhn, priv. herb. Gruhn GUY12-158 (LIP 0001290); Paracou camp, parcelle 10, near tree n°5, on palm rachis, 23 Jun 2012, leg. & det. G. Gruhn, priv. herb. Gruhn GUY12-118 (LIP 0001289); Martinique: Le Prêcheur, Anse Couleuvre, sentier de la Chute de la rivière Couleuvre, on dead *Bambusa vulgaris*, 9 Aug 2012, leg. & det. G. Gruhn, priv. herb. Gruhn MAR12-230 (LIP 0001287).

Additional material studied for Resinicium aculeatum: **EQUATORIAL GUINEA**: Rio Muni, San Joaquin de Ndgiacon, 50 m, on *Oryternntheira abyssinica*, 4 Dec 1990, leg. M.T. Tellería, M. Carvalho, C. Lado & F. Pando, det. M.T. Tellería, I. Melo & M. Dueñas, MA-Fungi 73568.

Observations: Our specimens of *R. saccharicola* have been collected on *Bambusa vulgaris* or palm rachis in Martinique and French Guiana respectively. In all cases, basidioma are thin and grandinioid, and cystidioles are rare, cylindrical to obclavate (as described in Nakasone, 2000), but contrary to previous descriptions

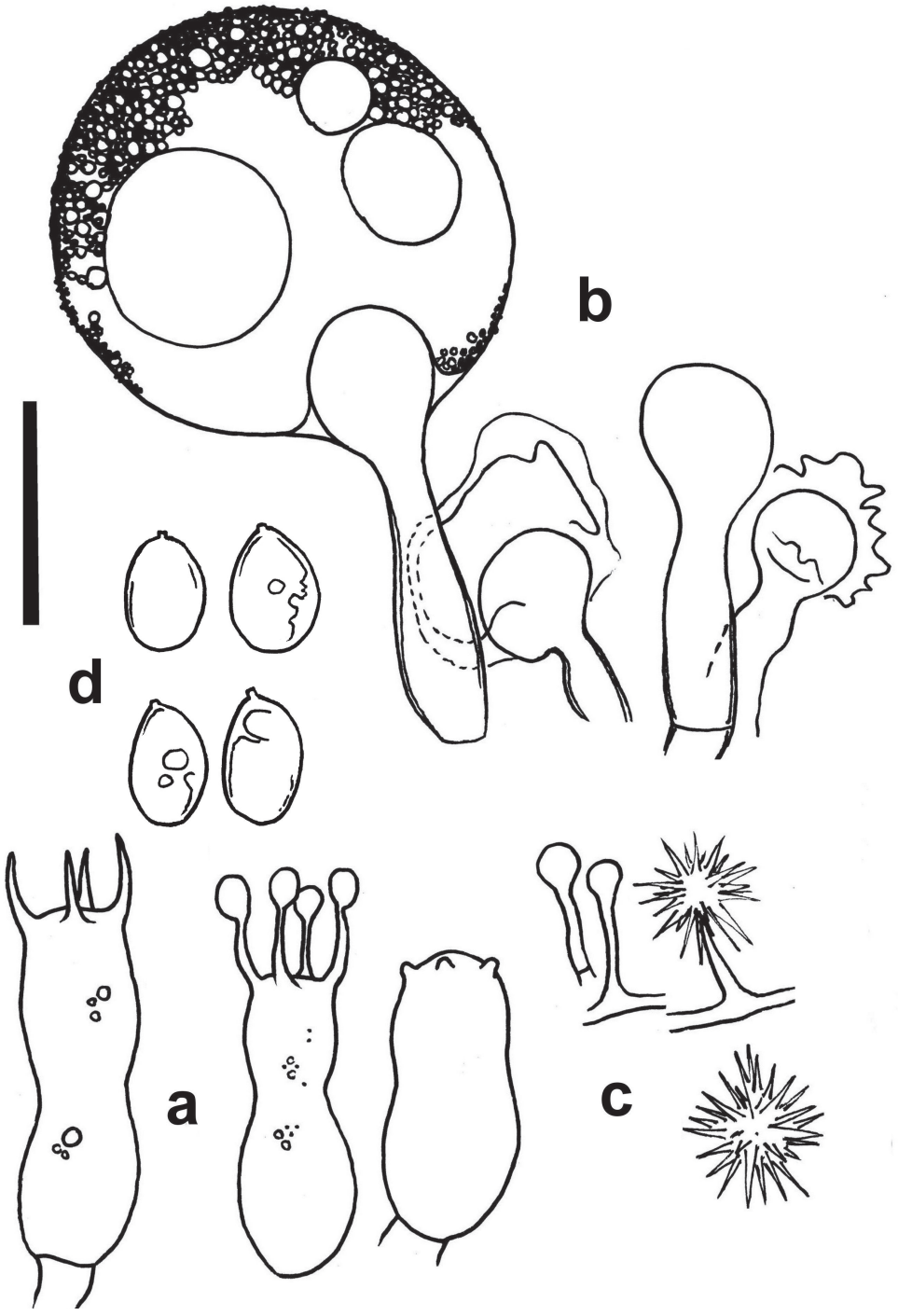

Fig. 5. ***Resinicium saccharicola*** (GUY12-158). **a.** basidia, **b.** halocystidia, **c.** astrocystidia, **d.** spores observed in Cotton blue. Scale: bar = 10 µm.

astrocystidia are rare and sometimes not seen in the French Guianan collections. Spores are usually shorter than 7 µm (see Table 2) as for Caribbean collections of *R. saccharicola*: 4.5-6.0(-7.0) × (3.0-)3.5-4.0(-4.5) µm (Nakasone, 2000).

RESULTS AND DISCUSSION

A revised ITS-based phylogeny of Resinicium

The alignment of 37 sequences of *Resinicium* spp. and two outgroup sequences encompassed 732 positions analyzed by ML phylogenetic reconstruction. The topology of the tree (Fig. 1) is congruent with the ITS and LSU trees published by Nakasone (2007). The phylogenetic analysis reveals a new, basal and well-supported lineage represented by four sequences of *R. grandisporum* described below. The four sequences (3 from Guiana and 1 from Martinique) are slightly divergent without geographical pattern: MART12-326 and GUY13-030 differ from the two others by 1 substitution on ITS2; on ITS1 a "TAT" series is repeated 4 times on the two same sequences, while GUY13-008 has only 3 "TAT" series, and Guy13-031 is interpreted as heterozygotic on this series (double-peak chromatograms after the 123th position suggest that PCR products are either 3- or 4-"TAT" long).

The position of *R. grandisporum* in this phylogeny is not strongly supported within the *Resinicium*-clade. Its peculiarities such as spore size and dextrinoidity are unique in the genus. However, its features are more similar to the core *Resinicium*-clade including *R. bicolor*, rather than the *Monticola/Mutabile*-clades (in which halocystidia are replaced by vesicular cystidia) and the *Saccharicola*-clade (clampless). An analysis of a more conserved region than the ITS barcode should help to resolve the phylogenetic relationships of this genus in future studies.

The other Caribbean collections studied here cluster in two well-supported branches (Fig. 1): 1) a *Mutabile*-clade encompassing sequences of *R. monticola* (including type) and *R. mutabile* (including type), the two sequences from French Guiana and Martinique being nested into the *R. mutabile* clade; 2) a *Bicolor*-clade representing the 6 other species of the genus, with *R. saccharicola* as the basal group.

The *Mutabile*-clade is paraphyletic in Nakasone's (2007: Fig. 1A) ITS reconstruction, as it appears in one of our analyses (not shown) that applied the same parameters as Nakasone (2007) but excluding the environmental sequence FJ179463 (isolated from an orchid root, Bourbon Isl.). This sequence generated by Martos *et al.* (2009) should be carefully checked, but the presence of vesicular cystidia, instead of halocystidia, support the hypothesis of a unique lineage encompassing *R. mutabile* and *R. monticola*.

The situation of *Resinicium saccharicola*

The three last sequences (2 from Guiana and 1 from Martinique) cluster together in a clade representing *R. saccharicola* sensu Nakasone (2000), encompassing 6 other sequences from tropical America. The sequence MAR12-230 (Martinique) is identical to 3 sequences from Puerto Rico generated by Nakasone (2007). The two sequences from Guiana (GUY12-118 and GUY12-158) differ from the former (95.5% of similarity with MAR12-230, from a 608 bp-long pairwise alignment, i.e. 3 substitutions/deletions on ITS1, 6 on ITS2). When other available sequences from GenBank (including 3 from Brazil) are included in the analysis, no phylogenetic pattern can be found to support a specific distinction.

Resinicium saccharicola, studied from two French Guianan and one Caribbean collections, confirms the variability and the broad distribution already documented by Hjortstam & Melo (1997) and Nakasone (2000). The three ITS sequences cluster in a strongly supported clade with sequences from Puerto Rico, Costa Rica, Taiwan and Hawaii. This cluster shows an intraspecific variability that does not correlate with morphological variations. All specimens of this species lack clamp connections, which distinguishes this species from other *Resinicium* spp. sequenced. Morphologically, the French Guianan collections differ from the Caribbean collection MAR12-230 by the scarcity of astrocystidia, found to be abundant by Nakasone (2000) and in the Martinique collection. More specimens and genetic markers are needed to investigate further morphological and geographic variations in a biogeographic context.

Several features distinguish *R. saccharicola* from other *Resinicium* species. *R. saccharicola* has tapering cystidioles (Nakasone, 2000), while *R. grandisporum* has none. *Resinicium aculeatum*, another unclamped species from Equatorial Guinea, has halocystidia with aculeolate apical bulbs (Tellería *et al.*, 2008), while apical bulbs are smooth in *R. grandisporum*. Moreover, spore shape and size are different in each species. Unfortunately, molecular data are still missing for *R. aculeatum*.

Nakasone (2000) proposed a synonymy between *R. granulare* and *R. saccharicola,* both clampless species. In our phylogeny (Fig. 1), all clampless collections cluster in a single clade, with some intraspecific variability. To test this pattern, we would need more molecular data on other clampless species, such as *R. aculeatum*, described from tropical Africa. Its micromorphological characters (Tellería *et al.*, 2008) are very close to *R. saccharicola,* from which it only differs by the aculeolate bulb of their halocystidia.

CONCLUSION

Nakasone (2007) hypothesized that the Caribbean area was a hot-spot of diversity for the genus *Resinicium*. Our data from French Guiana and the West Indies confirm this hypothesis, but also reveal a strong connection between the Caribbean and the Guiana Shield. Indeed, the three species detected were equally found in the two regions. It cannot be excluded that further investigations in the Amazonian basin would reveal more species of *Resinicium*. Interestingly, all extra-American sequences belong to species in derived position in the phylogeny (*R. saccharicola* from Taiwan or Hawaii; *R. bicolor* from the Northern Hemisphere), as well the West African *R. aculeatum* if confirmed as related to *R. saccharicola*. Their position suggests that the original diversification of the genus probably took place where most basal species are now observed, i.e. in the Amazonian and Caribbean areas, where further species are likely to be discovered.

Key to the species in *Resinicium* s. str.

This worldwide key is adapted from Nakasone (2007), with inclusion of *R. grandisporum* and *R. aculeatum.*

1b. Clamp connections frequent 2
1a. Clamp connection absent or very scattered (*Saccharicola*-clade).....9

2a (1b). Halocystidia rare or absent, replaced by vesicular cystidia (*Mutabile*-clade).....3
2b. Halocystidia numerous 4

3a (2a). Basidiomata tuberculate to papillose, white to yellow.....*R. monticola*
3b. Basidiomata spinose, brown .....*R. mutabile*

4a (2b). Basidiospores cylindrical to narrowly ellipsoid, Q = 1.6-2.4.....*8*
4b. Basidiospores ellipsoid, Q = 1.4-1.6.....5

5a (4b). Basidiomata subceraceous to ceraceous.....6
5b. Basidiomata soft to subceraceous.....7

6a (5a). Basidiomata strongly rimose, white to yellow .....*R. rimulosum*
6b. Basidiomata rarely with cracks, yellow to yellowish brown .....*R. friabile*

7a (5b). Aculei single, up to 100 µm long .....*R. tenue*
7b. Aculei aggregated, up to 1 mm long.....*R. confertum*

8a (4a). Spore width in average < 3.5 µm, Northern hemisphere.....*R. bicolor*
8b. Spore width in average > 4.0 µm, Neotropics .....*R. grandisporum*

9a (1a). Halocystidia with bulb smooth in KOH.....*R. saccharicola*
9b. Halocystidia with bulb finely aculeolate in KOH.....*R. aculeatum*

Acknowledgments. The authors gratefully thank Dr. N. Hallenberg, B. Duhem (†) and G. Trichies who provided valuable advice, and Dr. B. Paulus for her revision of our manuscript. Drs. K. K. Nakasone and B. Ortiz-Santana at CFMR, and M. Dueñas at MA are acknowledged for arranging specimen loans. Specimens were collected in French Guiana during field-trips funded by the FUNGUY and DIADEMA projects, Laboratoire d'Excellence CEBA (*http://www.labex-ceba.fr/*, CEBA, ref. ANR-10-LABX-25-01) financed by "Investissements d'Avenir" and managed by the French "Agence Nationale de la Recherche", and in Guadeloupe and Martinique islands through the program « Inventaire mycologique des champignons des Petites Antilles" coordinated by the Société Mycologique de France (SMF) and supported by the Office National des Forêts (ONF), Direction de l'Environnement, de l'Aménagement et du Logement (DEAL), Parc Naturel Régional de Martinique (PNRM) and the Région Martinique. Molecular analyses were financially supported by the University Lille2 (laboratory IMPECS – EA 4483), and partly by G. Gruhn's private contribution.

REFERENCES

ANISIMOVA M., GASCUEL O. & SULLIVAN J., 2006 — Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. *Systematic Biology* 55: 539-552. doi: 10.1080/10635150600755453

BALTAZAR J., SILVEIRA R. & RAJCHENBERG M., 2016 — Type studies of J. Rick's corticioid homobasidiomycetes (Agaricomycetes, Basidiomycota) housed in the Herbarium Anchieta (PACA). *Phytotaxa* 255(2): 101-132. doi: 10.11646/phytotaxa.255.2.1

BINDER M., HIBBETT D.S., LARSSON K.-H., LARSSON E., LANGER E. & LANGER G., 2005 — The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (Homobasidiomycetes). *Systematics and Biodiversity* 3(2): 113-157.

COURTECUISSE R. & WELTI S., 2013 — Preliminary check-list of the Fungi recorded from French West Indies (Lesser Antilles): Martinique, Guadeloupe and connected territories. II – Non-gilled Basidiomycota (gasteroid species, rusts and smuts excluded). *Documents mycologiques* XXXV: 47-173.

COURTECUISSE R., SAMUELS G.J., HOFF M., ROSSMAN A.Y., CREMERS G., HUHNDORF S.M. & STEPHENSON S.L., 1996 — Check-list of fungi from French Guiana: studies in the flora of the Guianas n° 80. *Mycotaxon* 57: 1-85.

DEREEPER A., GUIGNON V., BLANC G., AUDIC S., BUFFET S., CHEVENET F. & DUFAYARD J.-F., 2008 — *Phylogeny.fr*: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research* 36: 465-469.

DUHEM B., 2010 — Éléments pour une approche de l'étude des corticiés. I. La sporée. *Bulletin de la Société Mycologique de France* 126 (2): 143-160.

EDGAR R.C., 2004 — MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5:113. doi: 10.1186/1471-2105-5-113

ERIKSSON J., HJORTSTAM K. & RYVARDEN L., 1981 — The Corticiaceae of the North Europe, 6. *Phlebia – Sarcodontia*. Oslo: Fungiflora, pp. 1049-1276.

FANNECHÈRE G., 2011 — Mycomètre 2.05 *http://mycolim.free.fr/sml/gener/logiciels1.html*

GARDES M. & BRUNS T.D., 1993 — ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113-118.

GINNS J. & LEFEBVRE M.N.L., 1993 — Lignicolous corticioid fungi (Basidiomycota) of North America. *Mycologia Memoirs* 19: 1-247.

HJORTSTAM K. & LARSSON K.-H., 2007 — Checklist of corticioid fungi (Basidiomycota) from the tropics, subtropics, and the southern hemisphere. *Synopsis Fungorum* 22: 27-146.

HJORTSTAM K. & MELO I., 1997 — *Resinicium friabile* sp. nov. and a survey of the genus *Resinicium* (Basidiomycotina, Aphyllophorales). *Mycotaxon* 65: 323-329.

LARSSON K.-H., PARMASTO E., FISCHER F.M., LANGER E., NAKASONE K.K. & REDHEAD S.A., 2006 — Hymenochaetales: a molecular phylogeny for the hymenochaetoid clade. *Mycologia* 98(6): 926-936.

LARSSON K.-H., 2007 — Re-thinking the classification of corticioid fungi. *Mycological Research* 111(9): 1040-1063.

LARSSON K.-H., LARSSON E. & KÕLJALG U., 2004 — High phylogenetic diversity among corticioid homobasidiomycetes. *Mycological Research* 108 (9): 983-1002.

MARTOS F., DULORMNE M., PAILLER T., BONFANTE P., FACCIO A., FOURNEL J., DUBOIS M.-P. & SELOSSE M.-A., 2009 — Independent recruitment of saprotrophic fungi as mycorrhizal partners by tropical achlorophyllous orchids. *New Phytologist* 184 (3): 668-681.

MCNEILL J., BARRIE F.R., BUCK W.R., DEMOULIN V., GREUTER W., HAWKSWORTH D.L., HERENDEEN P.S., KNAPP S., MARHOLD K., PRADO J., PRUD'HOMME VAN REINE W.F., SMITH G.F., WIERSEMA J. & N.J. TURLAND, 2012 — International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). [Regnum vegetabile no. 154.] Königstein: Koeltz Scientific Books.

MINTER D.W., HERNÁNDEZ M.R. & PORTALES J.M., 2001 — Fungi of the Caribbean: An Annotated Checklist. PDMS publ., 946 p.

MOREAU P.-A. & COURTECUISSE R., 2015 (publ. 2016) — Les noms qui changent… (3): le point sur les « omphaloïdes ». *Documents Mycologiques* XXXVI: 102-106.

NAKASONE K.K., 2000 — Re-examination of *Odontia sacchari*, *O. saccharicola*, and *Corticium granulare*. *Karstenia* 40: 111-116.

NAKASONE K.K., 2007 — Morphological and molecular studies on *Resinicium* s. str. *Canadian Journal of Botany* 85(4): 420-436.

PARMASTO E., 1968 — Conspectus Systematis corticiacearum. Tartu: Academie Scientiarum R.P.S.S Estonicae.

TELLERÍA M.T., MELO I. & DUEÑAS M., 2008 — *Resinicium aculeatum*, a new species of corticioid fungi from Equatorial Guinea. *Nova Hedwigia* 87 (1-2): 195-200.

WHITE T.J., BRUNS T., LEE S. & TAYLOR J.W., 1990 — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M.A., D. H. Gelfand, J.J. Sninsky & T. J. White (eds.), PCR *Protocols: A Guide to Methods and Applications.* New York: Academic Press, pp. 315-322.

ZWICKL D.J., 2006 — Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. Dissertation. Austin: The University of Texas.