



A new species and a new combination in *Waitea* (Corticiales, Basidiomycota) and the phylogenetic affinity of *Disporotrichum*

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Abstract: The new corticioid species *Waitea guianensis* was described and illustrated from French Guiana, based on morphological and molecular phylogenetic evidence. It was characterized by thin, resupinate basidiomata on wood, isodiametric subhymenial hyphae, lack of clamps, tetrasporic clavate basidia with median constriction, and ellipsoid basidiospores. Ex-holotype DNA sequences from the nuclear ribosomal regions ITS, nLSU, and nSSU were obtained. Phylogenetic analyses confirmed a close relationship of the new species to *Waitea circinata*. In addition, the new combination *Waitea arvalis* is proposed, based on *Laetisaria arvalis*. The monotypic genus *Disporotrichum* is revealed to belong in the Corticiales, Corticiaceae. A key to the accepted species in *Waitea* is provided.

Keywords: Corticiaceae, *Laetisaria*, neotropic fungi, phylogeny, phytopathogens

INTRODUCTION

Waitea Warcup & P.H.B. Talbot is a corticioid genus typified with *W. circinata* Warcup & P.H.B. Talbot (Warcup & Talbot 1962). This genus is known as saprotroph or plant pathogen of legumes, cereals and turf grasses, and is hitherto monotypic [see Jayawardena et al. (2019), also Roberts (1999) put *W. nuda* under synonymy of *W. circinata*]. *Waitea circinata* produces thin, pinkish white basidiomata with hypochnoid consistency, occasional small pink-orange sclerotia, wide, short-celled and contorted hyphae without clamps, 'rhizoctonia-like' hyphae in subiculum, cylindrical to uniform basidia, rounded

probasidia, and oblong to broadly ellipsoid basidiospores (Warcup & Talbot 1962, Diederich et al. 2003, Gorjón 2020). The asexual morph of *W. circinata*, *Rhizoctonia zae* Voorhees, is a widespread soil-borne plant pathogen of mainly cereals (Jayawardena et al. 2019). The basidiomata are rare in nature, and also have been found on dead wood (Roberts 2003).

In the study by DePriest et al. (2005), *Waitea* was placed in the Corticiales and this classification was later confirmed by other researchers (Larsson 2007, Diederich & Lawrey 2007, Lawrey et al. 2007, Lawrey et al. 2008, Ghobad-Nejhad et al. 2010, Diederich et al. 2018, He et al. 2019). Ghobad-Nejhad et al. (2010) showed that *Waitea* was in the Corticiaceae s.s.

During a field trip to French Guiana sponsored by DIADEMA (DIsecting Amazonian Diversity by Enhancing a Multiple taxonomic-groups Approach) to improve the knowledge of biodiversity in this South American territory of France (Orivel & Baraloto 2018, Roy et al. 2018), a conspicuous wood-inhabiting corticioid fungus was collected. Morphological features and preliminary blastn searches (Altschul et al. 1990) suggested this taxon was close to *Waitea*. In this study, we use molecular analyses and morphological methods to describe a new species of *Waitea* from French Guiana.

MATERIALS AND METHODS

Morphological characterization

Macroscopic and microscopic studies were done based on hand-made sections from basidiomata. 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 in lactic acid. Measurements were made from micrographs under 1000× magnification, using the software Mycomètre (Fannechère 2020). Basidiospores obtained from spore prints were measured in side view, in Melzer's reagent, excluding the apiculus (Duhem 2010). Q is the ratio of spore length/spore width. Specimens were preserved in the

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Herbarium of the Faculty of Pharmacy of Lille, France (LIP), with duplicates at KAS and ICH. Herbaria acronyms follow Thiers (2021).

Molecular analyses

Total DNA was extracted from a small piece of dried basidiomata based on Murray & Thompson (1980) protocol with a few modifications. PCR procedure included 35 cycles with an annealing temperature of 54°C (Mullis & Faloona 1987). The ITS1F and ITS4 primers (White et al. 1990, Gardes & Bruns 1993) were employed to amplify the rDNA ITS region, LR0R and LR5 primers (Vilgalys & Hester 1990, Cubeta et al. 1991) for the 28S rDNA region (nLSU), and NS19b and NS41 primers (Hibbett 1996) for the 18S rDNA (nSSU) amplification. PCR products were analyzed on 1% agarose gel and sequenced with PCR primers. Chromatograms were considered for reading errors which then were corrected. Newly generated sequences from the holotype of *W. guianensis* were submitted to GenBank with the accession numbers of MW449090 (ITS), MW449101 (nLSU), and MW449084 (nSSU).

The phylogenetic position of the new species was determined by analysis of ITS and nLSU regions. These regions are widely used to examine relationships at the species level and higher taxonomic levels, respectively (Vu et al. 2019). Taxa for the phylogenetic

analyses were sampled from the Corticiales with special focus on Corticiaceae s.s. (Ghobad-Nejhad et al. 2010, Diederich et al. 2018, Jayawardena et al. 2019), and with particular attention to generic types. Sequences were aligned in MUSCLE (Edgar 2004) and trimmed with Gblocks (Castresana 2000). The datasets were analyzed using MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003), implementing the best-fit model of nucleotide evolution as inferred from MrModeltest 2.3 (Nylander 2004), with Akaike information criterion (AIC) estimator. The nLSU dataset was analyzed with GTR+I+G model, and *Gloeophyllum abietinum* (Bull.) P. Karst. was selected as an outgroup following Ghobad-Nejhad et al. (2010).

For a more detailed view on the positioning of the undescribed taxon within *Waitea* compared to closely related genera, an ITS dataset was created to include additional *Waitea* sequences. The ITS dataset was analyzed with GTR+I+G model, as suggested by MrModeltest. *Punctularia strigosozonata* (Schwein.) P.H.B. Talbot was used as the outgroup, following the results from nLSU analyses. Both nLSU and ITS datasets were analyzed with 20 M generations and 5000 sample frequencies. Full accession number and isolate number of all isolates used in the phylogenetic analyses are shown at the terminals in the phylogenetic trees (Fig. 1-2).

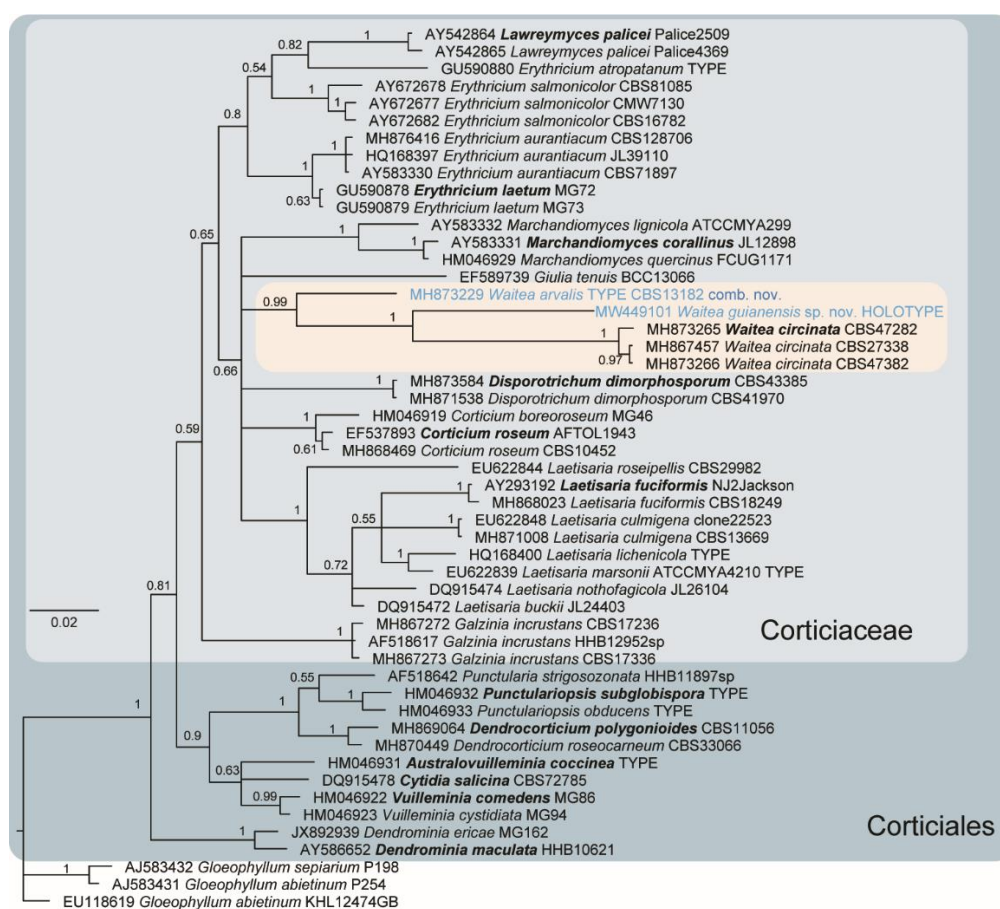


Fig. 1. Phylogenetic position of *Waitea guianensis* sp. nov. based on the Bayesian phylogram of *LSU* rDNA sequences. The light-yellow box defines the *Waitea* clade. Each terminal contains GenBank accession number, species name, and isolate number. Generic types are shown in bold. Posterior probabilities are shown above the branches. The tree is rooted with *Gloeophyllum abietinum*.

RESULTS

Morphological features as well as the blastn searches of nuclear ribosomal ITS, nLSU, and nSSU regions suggested the placement of the undescribed French Guiana sample in the Corticiaceae family and close to the genus *Waitea*. This was confirmed by phylogenetic analyses of ITS and nLSU regions (Figs. 1–2).

The nLSU dataset consisted of 51 taxa and 936 characters of which, 672 characters were constant, and 205 characters informative. The nLSU Bayesian phylogram is shown in Fig. 1. The French Guianan sample appeared in a well-supported clade (Posterior Probability = 1.00) that included three isolates of *Waitea circinata*. The ITS dataset consisted of 43 taxa and 546 characters, with 281 constant and 216 informative characters. The ITS phylogram is presented in Fig. 2. The new species was nested in *Waitea* clade, containing five isolates of *Waitea circinata* (PP = 1.00) as well as *W. arvalis*. The French Guianan sample is a new taxon and is described and

illustrated below.

Taxonomy

Waitea guianensis G. Gruhn & Ghobad-Nejhad *sp. nov.*
Figs. 3–4

Mycobank number: MB 838351

Diagnosis. The species differs from *Waitea circinata* by its narrower subicular hyphae, non-contorted subhymenial hyphae, and its shorter, ellipsoid basidiospores.

Etymology. refers to the collection place, in French Guiana.

Typus. French Guiana, Saül, “A” Diadema trail, lat. 3.587, long. -53.225, alt. 208 m, on a slope in a tropical rain forest, on bark of a dead hardwood branch, 23.X.2013, leg. G. Gruhn, LIP-GG-GUY13-110 (herb. LIP, holotype), isotypes at KAS and ICH. Ex-holotype DNA sequences: MW449090 (ITS), MW449101 (nLSU), and MW449084 (nSSU).

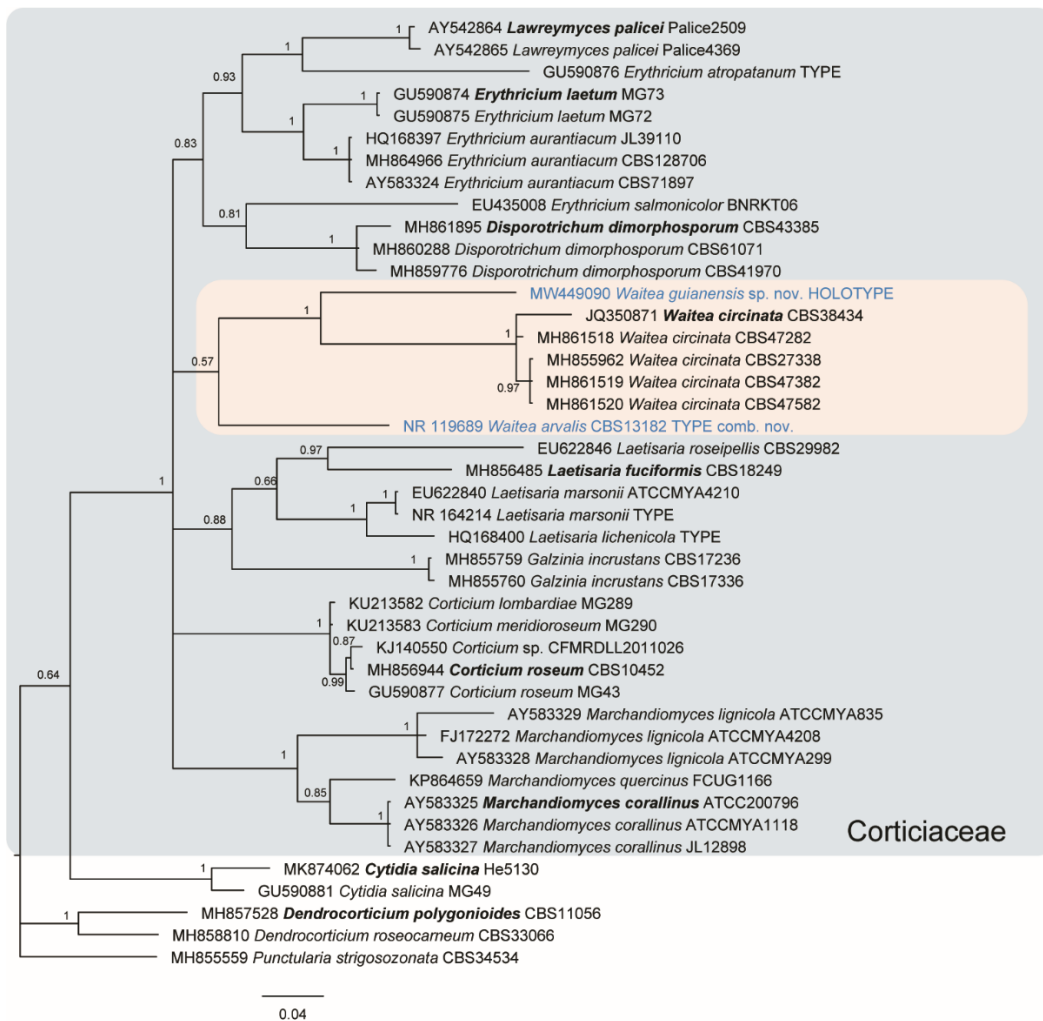


Fig. 2. Phylogenetic position of *Waitea guianensis* sp. nov. based on the Bayesian phylogram of ITS rDNA sequences. The light-yellow box shows the *Waitea* clade. Each terminal contains GenBank accession number, species name, and isolate number. Generic types are shown in bold. Posterior probabilities are shown above the branches. The tree is rooted with *Punctularia strigosozonata*.

Basidiomata annual, widely effused, up to 25 × 7 cm, adnate, pellicular, 30–50 µm thick. *Hymenium* smooth, white to whitish-gray and porulose in dried state. *Margin* concolorous with the hymenium, with thin hyphal stands. *Hyphal system* monomitic, all hyphae without clamps, smooth, colorless. Hyphae in strands and subiculum branching at right angles, thick-walled, walls 1–1.5 µm thick, hyphae 5–7 µm wide. Subhymenial hyphae with firm walls, more or less compact, isodiametric, short-celled, 5–7(12) µm wide. *Hyphidia* and *cystidia* none. *Basidia* at first subglobose (probasidia), becoming elongate to cylindrical-suburniform with constriction, 15–22 × 4–5 µm, with four sterigmata, thin-walled, contents with fine acyanophilous granules. *Basidiospores* ellipsoid, (4.5) 5–6.5 (7) × 3.5–4 (5) µm [33 spores measured], Q = 1.3–1.7 (1.8), aseptate, colorless, smooth, thin-walled, inamyloid, indextrinoid, and acyanophilous.

Ecology and habitat. Known only from the type locality. Saprotrophic on wood, in a rain forest in French Guiana.

Notes. *Waitea guianensis* resembles *W. circinata* in its thin, soft, pellicular basidiomata, lack of clamps, thick-walled basal hyphae, short-celled subhymenial hyphae, urniform to cylindrical, constricted basidia, lack of hyphidia and cystidia, and ellipsoid

basidiospores (Warcup & Talbot 1962, Roberts 2003, Gorjón 2020). *Waitea guianensis* differs from *W. circinata* by its narrower subicular hyphae (11–16 µm wide in *W. circinata*), non-contorted subhymenial hyphae, and its shorter basidiospores. Septate basidiospores were not observed in *W. guianensis*. Some straight conidiogenous hyphae at the basidiomata margin, bearing fusiform conidia at apex, resembling asexual *Botryobasidium*, were observed, but we were unable to confirm their association with *W. guianensis* basidiomata.

Isodiametric subhymenial hyphae observed in *Waitea guianensis* are also found in *Brevicellicium* K.H. Larss. & Hjortstam (Hjortstam & Larsson 1978). *Brevicellicium* species, however, have clamped hyphae (except one species), subglobose to ellipsoid basidiospores (Gorjón 2020), and belong in the Trechisporales (Tellería et al. 2013). *Brevicellicium permodicum* (H.S. Jacks.) Ginns & M.N.L. Lefebvre lacks clamps, and its placement in the genus is questionable according to Tellería et al. (2013); it differs from *Waitea guianensis* by its very thin, almost invisible basidiomata, short, obovate basidia, and subglobose to pyriform basidiospores, 6.5–7.5 × 5.5–6 µm (Jackson 1950, Ginns & Lefebvre 1993).

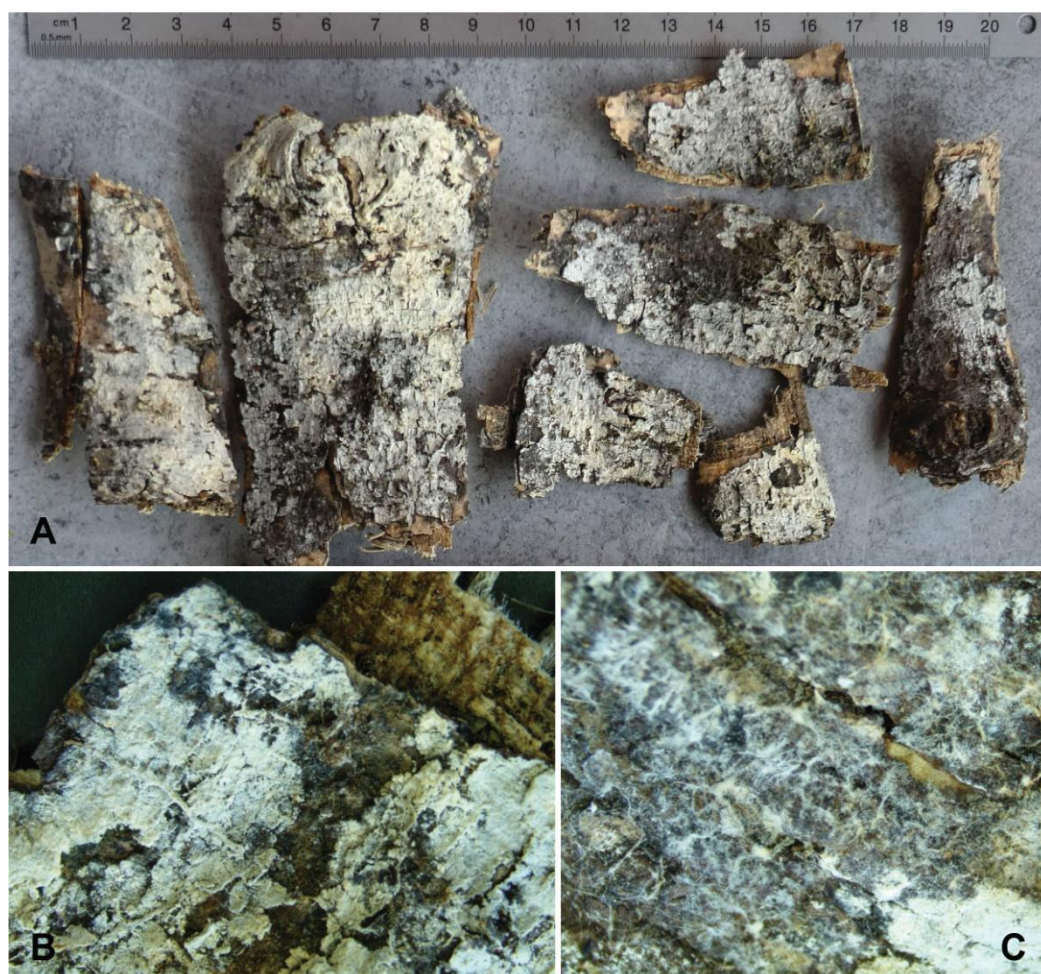
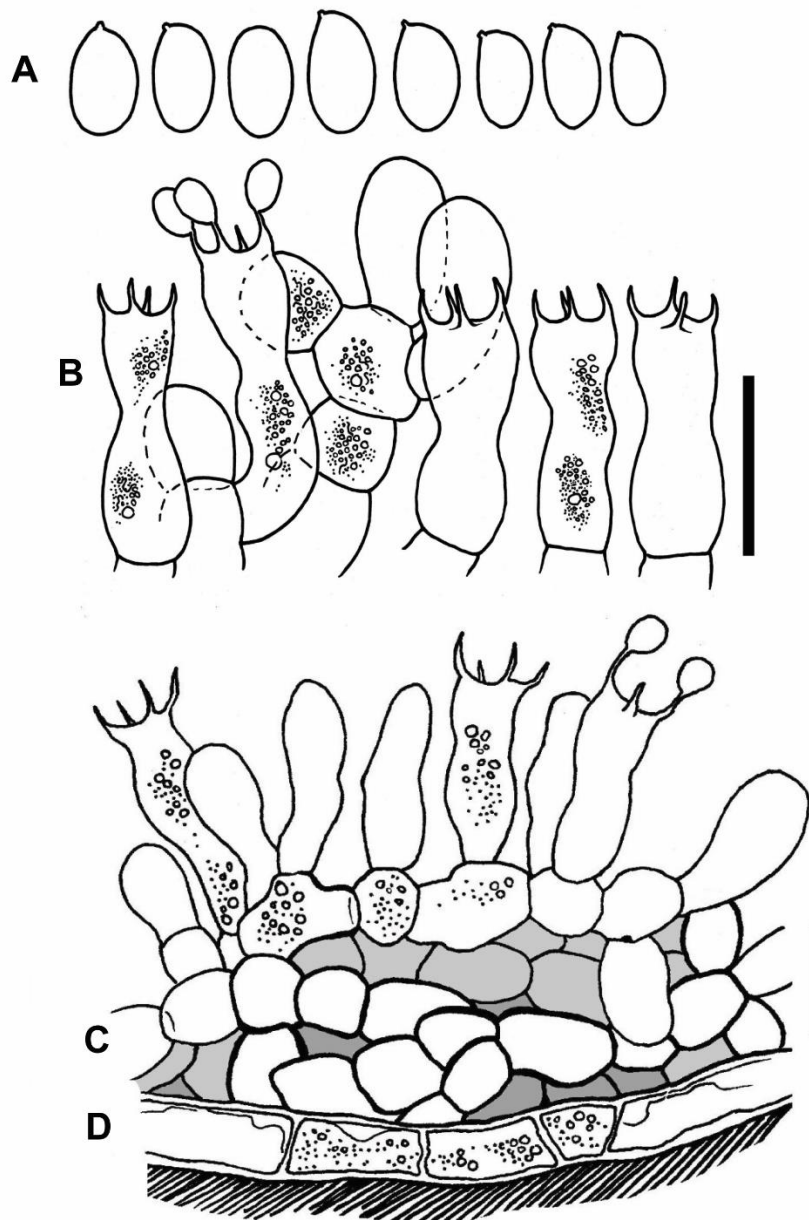


Fig. 3. *Waitea guianensis* basidiomata from the holotype. a. basidiomata. b. close-up of basidiomata. c. close-up of hyphal strands.

Fig. 4. *Waitea guianensis* line drawings from the holotype. a. basidiospores. b. basidia. c. isodiametric subhymenial hyphae. d. subicular hyphae. Bars = 10 μ m.



Waitea arvalis (Burds.) Ghobad-Nejhad **comb. nov.**
Mycobank number: MB 838361

Basionym. *Laetisaria arvalis* Burds., in Burdsall, Hoch, Boosalis & Setliff, *Mycologia* 72(4): 729 (1980).

Notes. The genus *Laetisaria* Burds., typified with *L. fuciformis* (Berk.) Burds., is a corticioid genus with thin, pink to pale red, resupinate basidiomata, and diverse nutritional habits (Burdsall 1979, Diederich et al. 2018). Recently, Diederich et al. (2018) provided phylogenetic evidence that *Laetisaria* is a well-supported monophyletic clade that did not include *L. arvalis*. This is in keeping with the morphological disparity between *L. arvalis* and the generic type *L. fuciformis* observed by Burdsall et al. (1980) and Stalpers & Loerakker (1982). The close phylogenetic relationship between *L. arvalis* and *W. circinata* has been shown in several studies (Lawrey et al. 2008,

Ghobad-Nejhad et al. 2010, Ghobad-Nejhad & Hallenberg 2011, Diederich et al. 2018, Jayawardena et al. 2019, page 79, Fig. 5). Similar to *W. circinata*, *L. arvalis* is isolated from soil, has thin, pink basidiomata, has an asexual form, develops thick-walled basal hyphae (mainly) lacking clamps, and has ellipsoid spores (Burdsall et al. 1980).

Note on phylogenetic placement of *Disporotrichum*

Disporotrichum Stalpers is a monotypic, asexual genus typified by *D. dimorphosporum* (Arx) Stalpers and is characterized by dimorphic conidia, and simple septate hyphae with occasional verticillate clamps (Stalpers 1984). The species is used in the food industry to produce non-alcoholic beverages, soft drinks, and bakery ingredients (Jeurissen et al. 2016, Silano et al. 2020).

Phylogenetic position of *Disporotrichum* in the Basidiomycota was unknown because of the lack of

sequence data and noted as *incertae sedis* in Agaricomycetes (He et al. 2019). However, recently available ITS and nLSU sequences provided by Vu et al. (2019), appeared in blastn searches with sequences of *W. guianensis*, and were included in the ITS and

nLSU phylogenetic analyses herein. The ITS and LSU phylograms (Figs. 1–2) place *Disporotrichum* among Corticiaceae genera, establishing that *Disporotrichum* belongs in the Corticiaceae s.s., Corticiales.

Key to the accepted *Waitea* species

1. Basidiospores > 8 µm long 2
 Basidiospores ≤ 7 µm long *W. guianensis*
2. Subhymenial hyphae convoluted, basidiospores oblong, 8–12 × 3.5–5 (6) µm, basal hyphae
 11–16 µm wide *W. circinata*
 Subhymenial hyphae not convoluted, basidiospores broadly ellipsoid, 9.5–12.5 (13.5) × 5.5–
 7 µm, basal hyphae (3–)5–9(–13) µm wide *W. arvalis*

Iconography for *Waitea* species:

Waitea arvalis: Burdsall et al. 1980: 732.

Waitea circinata: Warcup & Talbot 1962: 504; Talbot 1965: 393; Tu & Kimbrough 1978: 456; Cléménçon 1990: 56; Roberts 2003: 63; Oberwinkler et al. 2013: 766.

Waitea guianensis: this study.

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معرفی یک گونه و یک ترکیب جدید در جنس *Waitea* (Corticiales, Basidiomycota) و موقعیت
فیلوژنتیکی جنس *Disporotrichum*

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چکیده: در این مقاله گونه جدیدی به نام *Waitea guianensis* از گویان فرانسه بر اساس شواهد مورفولوژیکی و فیلوژنی مولکولی شرح داده شده و تصاویر آن ارائه می‌شود. مشخصات مهم این گونه جدید عبارتند از: بازیدیوماتای نازک مسطح روی چوب، هیف‌های ایزودیامتریک، فقدان قوس کلامپ، بازیدی‌های چماقی با قوس میانی و چهار استریگماتا، و بازیدیوسپورهای بیضوی. از نمونه هولوتایپ، توالی‌های DNA ریبوزومی از نواحی ITS، nLSU و nSSU بدست آمد. آنالیزهای فیلوژنی قرابت گونه جدید را به *Waitea circinata* تایید نمود. ترکیب جدید *Waitea arvalis* بر اساس *Laetisaria arvalis* معرفی می‌شود. همچنین نتایج فیلوژنی نشان می‌دهد که جنس مونوتیپیک *Disporotrichum* به خانواده Corticiaceae تعلق دارد. در انتها کلید شناسایی گونه‌های *Waitea* نیز ارائه می‌شود.

کلمات کلیدی: Corticiaceae، *Laetisaria*، فیلوژنی، بیمارگر گیاهی، قارچ‌های نئوتروپیک