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New records of three *Ramichloridium* species on banana leaves in Panama and Taiwan

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ABSTRACT

Although bananas are economically important crops and many fungi are recorded from these plants, detailed data about the fungi are scarce, e.g. with respect to their distribution and morphology in the field and in culture. Three hyphomycetes (anamorphic Dothideomycetes) known as potential pathogens on leaves of *Musa* species were identified based on morphology and DNA sequences. For the first time, *Ramichloridium biverticillatum* and *R. musae* are recorded from Panama and *R. biverticillatum* and *R. ducassei* from Taiwan and new hosts. The fungi are described and illustrated in detail from observation in situ and compared to their known morphology in vitro, in order to clarify some deviating molecular and morphological data from different sources. An updated preliminary key to *Ramichloridium* species on banana leaves is provided.

1. Introduction

Ramichloridium species are morphologically characterized by macronematous, pigmented, unbranched or profusely branched conidiophores and one-celled conidia produced from minute conidiogenous scars scattered along the apex or sides of terminal and intercalary conidiogenous cells. These species have recently been revealed as closely related to cercosporoid fungi, i.e. anamorphs of *Mycosphaerella* and related Dothideomycetes, bitunicate Ascomycota (Arzanlou et al. 2007). Within these Dothideomycetes, *Ramichloridium* species are scattered among at least two clades, with the type species being nested in a clade together with *Dissoconium* species and the majority of species in a clade comprising also species of *Periconiella*, *Zasmidium*, and other genera (Arzanlou et al. 2007). Undescribed *Ramichloridium*-like anamorphs are scattered among even more diverse groups of Pezizomycotina (Seifert et al. 2011).

Recently, more than ten species of fungi have been newly described from diseased banana species worldwide (Arzanlou et al. 2007, 2008; Shivas et al. 2011; Van Hove et al. 2011; Wong et al. 2012; Hao et al. 2013). These recent new findings indicate that many more species await discovery and that little is known about the geographic distribution of these fungi and their specificity on banana species and other hosts. Among the species on banana, two species named *Periconiella musae* Stahel ex M.B. Ellis and *Veronaea musae* M.B. Ellis were described by Ellis (1967, 1976). Both species were considered

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synonyms by de Hoog (1977), as *Ramichloridium musae* (Stahel ex M.B. Ellis) de Hoog, but then separated again as *R. biverticillatum* Arzanlou & Crous (for *P. musae*) and *R. musae* (Stahel ex M.B. Ellis) de Hoog (for *V. musae*). Presently, five *Ramichloridium* species are known on banana, namely *R. australiense* Arzanlou & Crous, *R. biverticillatum*, *R. ducassei* R.G. Shivas, Grice & A.J. Young, *R. musae*, and the anamorph of *Mycosphaerella mozambica* Arzanlou & Crous (Arzanlou et al. 2007, 2008; Shivas et al. 2011).

Several of the newly described fungi on banana have been characterized only from culture but not in situ on the natural host (Arzanlou et al. 2007, 2008; Hao et al. 2013), and on the contrary, few species have been described in situ, but not in culture (Shivas et al. 2011). During identification of our *Ramichloridium* specimens collected in Panama and Taiwan, we encountered problems, because morphologies can differ considerably in nature and in vitro and often only one growth form is known for a certain species, and the other growth form only for another species. Therefore, the aim of our study was to identify *Ramichloridium* species on banana in Panama and Taiwan, and to provide complementary information to avoid confusion caused by different morphologies in culture and on the natural substrate.

2. Materials and methods

2.1. Morphological studies

Senescent or diseased leaves of banana (*Musa* spp.) were collected in February 2012 in the field in Panama and in March and June 2012 and May 2013 in Taiwan and taken to the laboratory for cultivation and morphological and molecular analyses. The leaves were dried and Panamanian specimens deposited at the Herbario Nacional of the Universidad de Panamá (PMA) and the Herbario de la Universidad Autónoma de Chiriquí, Panama (UCH); Taiwanese specimens were deposited at the herbarium of the National Museum of Natural Science, Taichung, Taiwan (TNM). Duplicate specimens of *Ramichloridium* species on banana leaves from Australia obtained from the Plant Pathology Herbarium, Department of Primary Industries, Indooroopilly, Queensland, Australia (BRIP), were investigated for morphological comparison and deposited at TNM. Further specimens from former IMI were loaned from Royal Botanic Gardens, Kew, UK (K). Microscopic characteristics were observed using fungal material mounted in 5–10% (w/v) aqueous KOH solution or cotton blue in lactophenol. Measurements were based on data from 20 or 30 examples of the respective structure and are presented as mean value ± standard deviation with extreme values given in brackets, and separate mean values. Drawings were made freehand on scaled paper. Fungi were isolated by transfer of conidia with a flamed needle onto 2% malt-extract agar (MEA) with 0.2% chloramphenicol and cultivated at approx. 25 °C at diffuse daylight. The cultures of the Panamanian strains were lost due to contamination, the cultures of Taiwanese strains were deposited at the Bioresource and Collection Center, Hsinchu, Taiwan (BCRC). For characterization of the strains, MEA (Scharlau, Sentmenat, Spain), potato dextrose agar (PDA, Scharlau), and corn meal agar (CMA, Fluka, St. Louis, USA; Steinheim, Germany) were used.

2.2. DNA comparison

A culture sample of R. Kirschner & O. Cáceres 3680 from Panama was crushed and smeared on a FTA Whatman card and DNA isolation was processed according to the manufacturer's protocol. Fresh material from cultures from Taiwan was crushed by shaking the sample at 30 Hz (MiniBeadBeater-8) in a 1.5 ml tube together with glass beads 2.5 mm in diameter. For isolating nucDNA and purification of PCR products the Genomic DNA Spin Kit (Plant), Bioman Scientific Co., Ltd., Taiwan, and illustra GFX PCR DNA and Gel Band Purification Kit, GE Healthcare, UK, were used, respectively, according to the manufacturers' protocols. For amplification of sequences of rRNA genes, primers ITS1F and ITS4 were used (Gardes and Bruns 1993) for the Taiwanese specimens and primers NL1 and NL4 for the Panamanian specimen (White et al. 1990). Success of the amplification was assessed with 0.7% agarose gel electrophoresis followed by staining with GelRed™ (Biotium, Hayward, CA, USA) visualized under UV light (312 nm). Sequencing of DNA was done by Mission Biotech (Nankang, Taipei) with the same primers as for the PCR. Sequences were edited with CodonCode Aligner version 4.0.1 (CodonCode Corporation) and consensus sequences deposited at GenBank. Related DNA sequences were retrieved with the help of the BLAST function of GenBank.

3. Results

Ramichloridium biverticillatum Arzanlou & Crous, in Arzanlou et al., Stud. Mycol. 58: 72 (2007). Fig. 1.

≡ *Periconiella musae* Stahel ex M.B. Ellis, Mycol. Pap. 111: 5 (1967).

rRNA gene sequences: The nuclear ribosomal internal transcribed spacer (ITS) sequences of *R. biverticillatum* TNM F0026790 (=R. Kirschner 3896; 561 bp, GenBank accession no. KF286985) and TNM F0026791 (=R. Kirschner 3897; 558 bp, GenBank accession no. KF286986) were 100% identical and showed 99% and 100% identity (505 of 510 positions and 510 of 510 positions; respectively) with those of *R. biverticillatum* from Australia (HQ149689, HQ149688, respectively, Shivas et al. 2011) and 99% identity (487 of 492 positions) with that of *R. biverticillatum* from Surinam on which the description by Arzanlou et al. (2007) was based (EU041796, from CBS 335.36). The identity with other species was 95% and lower, when sequences exceeding 440 bp were compared.

Specimen from Panama (R. Kirschner 3686 as a representative): Leaf yellowing due to senescence, without discrete spots. Hyphae external (internal hyphae not checked), hyaline or pale brown, smooth or covered with minute plate-like, flat and round ornamentation, 2 μm wide. Conidiophores arising singly from external mycelium, erect, medium brown, becoming paler towards the apex, unbranched or with 2–6 conidiogenous cells in alternate or opposite lateral position at the apex, stipe smooth, with basal septum 0–5 μm apart from the surface of subtending hypha, distances between septa