Take-all or nothing

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Abstract: Take-all disease of *Poaceae* is caused by *Gaeumannomyces graminis* (*Magnaporthaceae*). Four varieties are recognised in *G. graminis* based on ascospore size, hyphopodial morphology and host preference. The aim of the present study was to clarify boundaries among species and varieties in *Gaeumannomyces* by combining morphology and multi-locus phylogenetic analyses based on partial gene sequences of ITS, LSU, *tef1* and *rpb1*. Two new genera, *Falciphoriella* and *Gaeumannomycella* were subsequently introduced in *Magnaporthaceae*. The resulting phylogeny revealed several cryptic species previously overlooked within *Gaeumannomyces*. Isolates of *Gaeumannomyces* were distributed in four main clades, from which 19 species could be delimited, 12 of which were new to science. Our results show that the former varieties *Gaeumannomyces graminis* var. *avenae* and *Gaeumannomyces graminis* var. *tritici* represent species phylogenetically distinct from *G. graminis*, for which the new combinations *G. avenae* and *G. tritici* are introduced. Based on molecular data, morphology and host preferences, *Gaeumannomyces graminis* var. *maydis* is proposed as a synonym of *G. radicicola*. Furthermore, an epitype for *Gaeumannomyces graminis* var. *avenae* was designated to help stabilise the application of that name.

Key words: Cryptic species, *Gaeumannomyces graminis*, *Magnaporthaceae*, Phylogeny, *Triticum*.
Taxonomic novelties: New genera: *Falciphoriella* M. Hern.-Restr. & Crous, *Gaeumannomycella* M. Hern.-Restr. & Crous; **New species:** ***Falciphoriella*** *solaniterrestris* M. Hern.-Restr. & Crous, ***Gaeumannomycella*** *caricis* M. Hern.-Restr. & Crous, ***Gaeumannomyces*** *arxii* M. Hern.-Restr. & Crous, *G. australiensis* M. Hern.-Restr. & Crous, *G. californicus* M. Hern.-Restr. & Crous, *G. ellisiorum* M. Hern.-Restr. & Crous, *G. floridanus* M. Hern.-Restr. & Crous, *G. fusiformis* M. Hern.-Restr. & Crous, *G. glycinicola* M. Hern.-Restr., G. Canning & Crous, *G. graminicola* M. Hern.-Restr. & Crous, *G. hyphopodioides* M. Hern.-Restr. & Crous, *G. oryzicola* M. Hern.-Restr. & Crous, *G. setariicola* M. Hern.-Restr. & Crous, *G. walkeri* M. Hern.-Restr. & Crous; **New combinations:** ***Gaeumannomyces tritici*** (J. Walker) M. Hern.-Restr. & Crous, ***Gaeumannomyces avenae*** (E. M. Turner) M. Hern.-Restr. & Crous; **Typification: Epitypification:** ***Gaeumannomyces graminis* var. *avenae*** (E. M. Turner) Dennis.

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INTRODUCTION

Take-all is one of the most important root diseases in cereal crops and grasses, caused by *Gaeumannomyces graminis*. Taxonomic placement of *Gaeumannomyces graminis* at the variety level has been a research topic for many decades. Based on morphology, pathogenicity and host preference, four varieties of this species can be recognised (Turner 1940, Walker 1972, Yao *et al.* 1992). The type variety *Gaeumannomyces graminis* var. *graminis* (*Ggg*) causes crown (black) sheath rot of rice, dieback in Bermuda grass, take-all root rot of St. Augustine grass or root decline of other warm-season turf grasses (Walker 1972, 1981, Elliott 1991, Ward & Bateman 1999). It is the least aggressive and is also often found as a weak pathogen or saprobe on cereals, grasses and soybeans (Walker 1980, Roy *et al.* 1982, Ward & Bateman 1999). *Gaeumannomyces graminis* var. *avenae* (Turner 1940, Dennis 1960) (*Gga*) causes take-all of oats and take-all patch of turfgrasses, although it can also infect wheat, rye and barley. *Gaeumannomyces graminis* var. *tritici* (Walker 1972) (*Ggt*) is the most aggressive variety and is known as the wheat take-all fungus. It infects mainly wheat but can also infect triticale, barley and rye as well as other cereals and grasses (Walker 1980, Ward & Bateman 1999, Freeman & Ward 2004). Take-all of wheat is the most important root disease of wheat worldwide. *Gaeumannomyces graminis* var. *maydis* (Yao *et al.* 1992) (*Ggm*) is the most recently described variety and causes take-all of maize but also can slightly infect *Sorghum* and other cereals.

The sexual morph in *Gaeumannomyces* is characterised by the production of globose or pyriform, immersed ascomata with a conical to cylindrical neck, and fusiform, multiseptate and hyaline ascospores. Asexual morphs are characterised by phialidic conidiogenous cells with refractive collarettes and lunate or phialophora-like conidia. For a long time the asexual morphs in *Gaeumannomyces* were referred to *Phialophora*, but based on morphology, Gams (2000) proposed the genus *Harpophora* to accommodate the phialidic asexual morphs in *Magnaporthaceae*. However, *Harpophora* became the later synonym of *Gaeumannomyces*, following the Melbourne code (Luo *et al.* 2015c).

Hyphopodia are commonly found in this genus and in other members of *Magnaporthaceae*. This feature has been used as a taxonomic character to differentiate some of the varieties in *G. graminis*. The asexual morph of *Ggg* has been reported to have lobed hyphopodia (Walker 1980, Ward & Bateman 1999, Freeman & Ward 2004). On the other hand *Ggt*, *Gga* and *Ggm* are characterised by the production of simple hyphopodia in the substrate (Walker 1972, Yao *et al.* 1992).

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However, differentiation among isolates of *Gaeumannomyces* based on disease symptoms, host range, cultural and/or morphological characteristics is difficult, time consuming and is in many cases inconclusive (Ulrich *et al.* 2000, Freeman & Ward 2004). Different molecular techniques have been used to identify species and varieties in *Gaeumannomyces*, for example RAPD (Wetzel *et al.* 1996, Augustin *et al.* 1999, Ulrich *et al.* 2000), RFLP (Bateman *et al.* 1992, Tan *et al.* 1994, Ward & Akrofi 1994), amplification of specific gene sequences within the ITS nrDNA (Bryan *et al.* 1995, Ward & Bateman 1999, Ulrich *et al.* 2000), or avenacinase-like genes (Rachdawong *et al.* 2002). Those studies revealed that *Ggt* and *Gga* form a monophyletic clade, whereas *Ggg* appears to be polyphyletic, with high variability among isolates (Elliott *et al.* 1993, Ward & Akrofi 1994, Fouly *et al.* 1996, Tan 1997, Ward & Bateman 1999, Fouly & Wilkinson 2000, Saleh & Leslie 2004, Sadeghi *et al.* 2012). In addition, *Ggm* is related to another maize root pathogen named *G. radicicola* (Luo *et al.* 2015c), formerly recognised as *Harpophora radicicola* and *H. zeicola* (Ward & Bateman 1999, Gams 2000). Phylogenetic studies also revealed new lineages in *Gaeumannomyces* referred to as "*Phialophora* sp. GP57" (Ward & Bateman 1999) and "group E" (Ulrich *et al.* 2000). Nevertheless, no formal names or combinations have been proposed.

The genus *Gaeumannomyces* (*Magnaporthaceae*, *Magnaporthales*), was established by von Arx & Olivier (1952) to accommodate *Ophiobolus graminis*, formerly described as *Rhaphidophora graminis*. Besides *G. graminis* and *G. radicicola*, this genus includes other root-infecting pathogens such as *G. wongoonoo*; the cause of a patch disease of *Stenotaphrum secundatum* (buffalo grass) (Wong 2002) and *G. caricis* occurring on *Carex* spp. (*Cyperaceae*) (Walker 1980). Endophytic and saprobic fungi have been found in this genus as well, for example *G. amomi*, described as endophytic in *Amomum* and *Alpinia* (*Zingiberaceae*) (Bussaban *et al.* 2001), and the saprobic *G. licualae*, an unusual *Gaeumannomyces* species collected from palm (*Licuala* sp.), known only from the type locality; Brunei Darussalam (Fröhlich & Hyde 2000).

The number of taxa in *Magnaporthaceae* with phialophora-, and harpophora-like asexual morphs has been increasing in the past 20 years, together with the introduction of new genera, e.g. *Falciphora* (Yuan *et al.* 2010, Luo *et al.* 2015c), *Magnaporthiopsis* (Luo & Zhang 2013), and *Pseudophialophora* (Luo *et al.* 2014, 2015b), with a high number of cryptic species among those genera.

Other studies relocated some species previously accommodated in *Gaeumannomyces* for example; *G. incrustans* was transferred to *Magnaporthiopsis* (Luo & Zhang 2013). *Slopeiomyces* and *Kohlmeyeriopsis* were proposed as new genera to accommodate *G. cylindrosporus* and *G. medullaris* respectively (Klaubauf *et al.* 2014).

The aims of the present study were: (1) to explore the diversity of *Gaeumannomyces* isolates, collected from diverse geographic origins and from different hosts; (2) to determine the phylogenetic relationships of the isolates using a multi-locus sequence alignment consisting of partial gene sequences of LSU (28S nrDNA), ITS (internal transcribed spacers and intervening 5.8S nrRNA gene), *tef1* (translation elongation factor 1-alpha) and *rpb1* (RNA polymerase II large subunit); (3) to resolve the taxonomy of *Gaeumannomyces* by adopting a polyphasic approach; and (4) to designate epitypes and reference sequences for species of *Gaeumannomyces*.

MATERIALS AND METHODS

Isolates and morphological analysis

A total of 83 strains identified as *Gaeumannomyces* or *Harpophora* (*Phialophora*) from different localities and hosts were examined (Table 1). Specimens were obtained from the culture collection of the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands, the Monica Elliott personal collection, University of Florida, USA, the working collection of P.W. Crous (CPC) housed at CBS, and the Rothamsted plant pathology culture collection, Department of Plant Biology and Crop Science, Rothamsted Research, Harpenden, Herts, UK.

Isolates were cultured on 2 % potato dextrose agar (PDA), 2 % malt extract agar (MEA; Oxoid) and oatmeal agar (OA; Crous *et al.* 2009), and incubated at 25 °C under daylight conditions for 1–3 wk; UV light conditions were used for some isolates to induce sporulation. After 7 d of incubation the colony diameters were measured and the colony morphologies described. Colony colours on the surface and reverse of inoculated media were assessed according to the colour charts of Rayner (1970). Micromorphological descriptions and 30 measurements of relevant features were carried out from mature cultures mounted in clear lactic acid. For ascomata, measurements were taken from 5 to 10 structures depending on availability. Observations and photomicrographs were made with a Nikon SMZ1500 stereo-microscope, and with a Nikon Eclipse N*i* microscope, using a DS-Ri2 digital camera (Nikon, Tokyo, Japan) and NIS-Elements imaging software v. 4.20. Reference strains were deposited in the CBS culture collection. Taxonomic information and nomenclature for new species were deposited in MycoBank (www.MycoBank.org; Crous *et al.* 2004).

DNA isolation, amplification and sequences alignment

Genomic DNA was extracted from fungal colonies growing on MEA using the Wizard® Genomic DNA purification kit (Promega, Madison, USA), according to the manufacturer's protocols. Procedures for amplifying and sequencing the internal transcribed spacer nrDNA including the intervening 5.8S nrDNA (ITS) and partial large subunit nrDNA (28S nrDNA; LSU), were performed as described in Hernández-Restrepo *et al.* (2016). Part of the largest subunit of the RNA polymerase II gene (*rpb1*) was amplified and sequenced as described in Klaubauf *et al.* (2014). Translation elongation factor 1-α gene (*tef1*), corresponding to the section 983–1567 bp, was amplified and sequenced as described in Rehner & Buckley (2005). Sequences were edited and consensus sequences constructed using SeqMan Pro (DNASTAR, Madison, WI, USA) and deposited in GenBank (Table 1).

To further study the phylogenetic relationships, additional homologous sequences of members of *Magnaporthales* were retrieved from GenBank and combined with those generated during the present study (Table 1). Sequence alignments were performed with MAFFT v. 7 (Katoh & Standley 2013) using the defaults settings and adjusted by hand in MEGA v. 6.06 (Tamura *et al.* 2013).