Grass Research

ARTICLE

Open Access

https://doi.org/10.48130/GR-2023-0020

Grass Research **2023**, 3:20

Candidacolonium agrostis, a novel species associated with summer patch-like disease on *Agrostis stolonifera* in East China

Jiamei Geng[1], Yuxin Zhou[2*], Yinglu Dong[1], Kurt Lamour[3], Zhimin Yang[1], Jun Liu[1] and Jian Hu[1*]

[1] *College of Agro-grassland Science, Nanjing Agricultural University, Nanjing 210095, China*
[2] *Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing 210014, China*
[3] *Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996, USA*
* Corresponding authors, E-mail: rita1720@126.com; jaffyhu@njau.edu.cn

Abstract

Creeping bentgrass (*Agrostis stolonifera*) is one of the most preferred turfgrass species for putting greens on golf courses throughout China. In autumn 2020 and summer 2021, a summer patch-like disease was observed on creeping bentgrass putting greens at two golf courses in East China. Fungal isolates with similar morphology were consistently isolated from the dark root tissues. Four representative isolates from the two golf courses were identified through morphological, biological, and phylogenetic analyses. Bayesian methods and maximum likelihood were used to construct phylogenetic trees based on both multiple and single loci of partial sequences of the 18S nuc rDNA (SSU), ITS1-5.8S-ITS2 nuc rDNA internal transcribed spacer (ITS), and 28S nuc rDNA (LSU) regions, and of the largest subunit of RNA polymerase II (*RPB1*), minichromosome maintenance complex 7 (*MCM7*), and translation elongation factor 1-alpha (*TEF1*) genes, respectively. The isolates consistently formed a highly supported clade within the genus *Candidacolonium*, which was further supported by distinctive morphological characters. In pathogenicity tests, the fungus produced slightly lobed or mitten-shaped hyphopodia, colonized roots of creeping bentgrass *via* ectotrophic, dark runner hyphae, and caused disease symptoms with root discoloration, root and shoot mass reduction, and yellow leaf spots. This pathogenic fungus is described as a new species, *Candidacolonium agrostis* sp. nov., and is most likely the cause of a summer patch-like disease on creeping bentgrass in East China.

Citation: Geng J, Zhou Y, Dong Y, Lamour K, Yang Z, et al. 2023. *Candidacolonium agrostis*, a novel species associated with summer patch-like disease on *Agrostis stolonifera* in East China. *Grass Research* 3:20 https://doi.org/10.48130/GR-2023-0020

Introduction

Creeping bentgrass (*Agrostis stolonifera* L.), one of the major cool-season turfgrass species, is widely planted in temperate and subtropical regions of China[1,2]. It exhibits fine leaf textures, high shoot densities, short internodes, prostrate growing habits and tolerance to extremely close mowing[3]. However, creeping bentgrass is not well adapted to the persistent hot and humid conditions during summer months, which often results in reduced plant vigor and increased susceptibility to diseases[4]. The root disease, summer patch, caused by *Magnaporthiopsis poae*, was originally isolated from symptomatic roots of Kentucky bluegrass in the USA[5]; it has been documented in creeping bentgrass in China, recently[2,6]. Take-all patch, caused by *Gaeumannomyces graminis* var. *avenae*, pythium root rot or dysfunction (*Pythium* spp.) were common root diseases of creeping bentgrass in the USA[7,8], but have not been documented in China. Among the root associated pathogenic fungi, there are a few fungi that colonize plant roots *via* darkly pigmented, ectotrophic, runner hyphae, which were named ectotrophic root-infecting (ERI) fungi[9]. Members of the ERI fungi were soil-borne, and some of them were the causal agents of destructive turfgrass diseases[10]. Several well-known genera of ERI fungi have been described on turfgrass, including *Gaeumannomyces*, *Ophiosphaerella*, and *Magnaporthiopsis*[11–13]. Recently, a new genus of ERI fungi, *Candidacolonium*, was described by Vines[14], and was associated with the decline of ultradwarf bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) in late summer and early fall in the Deep South of the USA[14,15]. Virulence tests revealed that *C. cynodontis* was one of the most destructive and aggressive ERI fungi and the main cause of summer decline observed on ultradwarf bermudagrass[14]. *C. cynodontis* readily produced round to mitten-shaped hyphopodia, which were frequently observed on roots as well as stolons[14].

In autumn 2020 and summer 2021, a summer patch-like disease emerged on creeping bentgrass greens at two golf courses in East China. Initially, the symptom of the disease showed multiple irregular yellowing spots (3–10 cm in diameter). As the disease progressed, the spots coalesced into larger irregular patches. Roots of the creeping bentgrass were sparse, shortened and dark colored. Dark ectotrophic runner hyphae and slightly lobed or mitten-shaped hyphopodia were observed inside and on the surface of brown or black roots under the microscope. The symptomatology, time of occurrence, as well as presence of slightly lobed or mitten-shaped hyphopodia, suggested that the disease on creeping bentgrass may be caused by an ERI fungal species[9].

Accurate identification of ERI fungi is generally difficult and time consuming by traditional morphological methods[10]. With the exception of the different shapes of hyphopodia and the occasional production of ascocarps and ascospores, the ERI fungi do not produce distinguishable features that allow for precise and timely identification[14,16,17]. In addition, production

of ascocarps was often difficult due to attenuated biotypes or incompatible mating types[10]. According to only morphological characters, accurate identification of ERI fungi was often hampered by highly similar structures, especially conidia, among close related species[18,19]. Multi-locus molecular phylogenetic analysis had been successfully used for the identification of ERI fungi, especially for new species[18,20].

The objective of this study was to employ molecular as well as morphological methods to identify the fungal isolates recovered from diseased creeping bentgrass roots, and determine the main fungal species that cause the summer patch-like disease on creeping bentgrass by pathogenicity tests. The work will provide important references for the management of creeping bentgrass root diseases at golf courses in East China.

Materials and methods

Fungal isolation

Samples of symptomatic creeping bentgrass were collected by a soil corer (5 cm in diameter and 15 cm in depth) from the putting greens at Taihu golf course in Wuxi city, Jiangsu province and Jianhu golf course in Shaoxing city, Zhejiang province (China). Root materials were rinsed with distilled water to remove organic materials and soil particles, and microscopically checked for the presence of dark and runner hyphae, a characteristic sign of ERI fungi. Dark colored roots were cut into sections with 2−3 cm in length, surface disinfested with a 0.6% sodium hypochlorite solution for 5 min, rinsed two consecutive times with sterile-distilled water, placed on sterile filter paper, and dried in a laminar flow hood. The dried root sections were transferred onto potato dextrose agar (PDA) medium amended with 50 mg·L^{-1} each of streptomycin sulfate, ampicillin and tetracycline. Plates (9 cm in diameter) were incubated at 25 °C until hyphal growth was observed from the discoloring root tissues. Two to three consecutive hyphal tip transfers were conducted to obtain pure cultures. Fungal cultures were stored for the long term as described by Hu et al.[21].

PCR amplification and sanger sequencing

Fungal cultures were grown on PDA at 25 °C for 7−10 d to produce actively growing colonies. Mycelia of each strain were scraped without agar medium using a sterilized scalpel and put into a 2-mL micro-centrifuge tube. The genomic DNA (gDNA) was extracted with the previously described method[22]. Concentration and purity of the extracted gDNA was tested by a NanoDrop 2000/2000c device (Thermo Fisher Scientific, Inc., Waltham, MA, USA). DNA samples of the fungal isolates were stored at −20 °C for further use.

PCR amplifications were conducted on a thermal cycler (Applied Biosystems, USA) in a 30-μL reaction volume. The PCR reaction system and cycling condition were the same as described in a previous study[14]. Six genes were used for multi-locus phylogenetic analysis, including the internal transcribed spacer (ITS), 28S large subunit (LSU) rRNA gene and 18S small subunit (SSU) rRNA gene regions of rRNA gene, the largest subunit of RNA polymerase II gene (*RPB1*), a DNA replication licensing factor gene for mini-chromosome maintenance complex component 7 (*MCM7*) and the translation elongation factor 1-alpha gene (*TEF1*). Partial sequences of ITS were used for preliminary identification through BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Isolates were considered as new fungal species and were further used for multi-locus phylogenetic, morphological and biological analyses, if the percent identity of their ITS sequences was less than 97% as compared with the submitted ITS sequences in GenBank database through BLAST analysis. Primers used for PCR amplifications of the six genes were the same as in Vines' research[14]. PCR products were examined electrophoretically in 1% agarose gels. The PCR fragment was purified and sent to Tsingke Biotechnology Co., Ltd. for sequencing. The obtained sequences of the potential new species were deposited in GenBank, and the specific information of the strains representing different species used for constructing the phylogenetic tree is provided in Table 1.

Sequence data and phylogenetic analyses

Phylogenetic analyses were implemented in PhyloSuite software version 1.2.1[23]. The amplified sequences of the six individual genes were aligned respectively with the MAFFT program by the algorithm G-INS-i[24], alignments were checked with characteristics weighted equally. The incongruence length difference test[25] was implemented to determine if data for the six genes could be combined with the software PAUP 4.0a169[26]. A multi-locus dataset was produced by concatenating the aligned sequences of individual genes with the Concatenate Sequence program[23]. The best nucleotide substitution models for individual genes were selected using the Akaike Information Criterion (AIC) with the ModelFinder program[27]. Individual gene trees were generated for each gene with the above predicted model parameters. Phylogenetic analysis was performed with the multi-locus dataset using maximum likelihood (ML) and Bayesian inference (BI) approaches. BI phylogenies were implemented using MrBayes 3.2.6[28] with partition model (2 parallel runs and 10 million generations), in which 25% of the initially sampled data were discarded as burn-in, the remaining trees were applied to calculate posterior probabilities (PP). ML phylogenies were conducted using IQ-TREE[29] with Edge-linked partition model (5000 ultrafast bootstraps)[30] and Shimodaira-Hasegawa-like approximate likelihood-ratio test[31]. Clades with PP ≥ 0.95 and bootstrap values (BS) ≥ 70% were considered as well supported. Phylograms were annotated and viewed with Interactive Tree Of Life (iTOL) version 6[32].

Cultural characteristics

Cultural characteristics were recorded from PDA. Mycelial plugs (diameter at 5 mm) were taken from the margins of one-week growing colonies, transferred to new PDA plates (diameter at 9 cm), and incubated in the dark at 25 °C. The diameter of the colony for each culture was measured daily for five days. The color of fungal colony was noted according to Rayner[33]. Microscopic measurements and photographs were conducted from slides of fungi mounted in sterile-distilled water with a stereo-microscope (OLYMPUS SZX16), and with a digital microscope (OLYMPUS DP80). The width of the hyphae and the size of the hyphopodia were measured from 20−30 structures depending on availability. The specimens and ex-type culture were deposited and preserved in Fungarium of Guangdong Institute of Microbiology (GDGM) and Guangdong Microbial Culture Collection Center (GDMCC), respectively.

Optimum temperature for *in vitro* growth

Two isolates (13g11 and JH1) of the potential new species were used to estimate the optimal temperature for their

Table 1. Species name, isolate IDs, and GenBank accession numbers for fungal isolates used for phylogenetic analyses in this study.

Species name	Isolate ID	ITS	LSU	SSU	*MCM7*	*RPB1*	*TEF1*
Buergenerula spartinae	ATCC 22848[a]	JX134666	DQ341492	DQ341471	JX134706	JX134720	JX134692
Candidacolonium agrostis[b]	**13g11**	**OM910761**	**OM910782**	**OM900029**	**OM938227**	**OM938229**	**OM938231**
Candidacolonium agrostis	**13g12**	**OM910762**	**OM910783**	**OM900030**	**OM938228**	**OM938230**	**OM938232**
Candidacolonium agrostis	**JH1**	**OP811262**	**OP811312**	**OP811273**	**OP957424**	**OP957426**	**OP957428**
Candidacolonium agrostis	**JH2**	**OP811263**	**OP811313**	**OP811274**	**OP957425**	**OP957427**	**OP957429**
Candidacolonium cynodontis	HP24-3	KJ855497	KM401637	KP129316	KP007344	KP268919	KP282703
Candidacolonium cynodontis	HP38-4	KJ855498	KM401638	KP129317	KP007345	KP268920	KP282704
Gaeumannomyces graminis var. graminis	GSGC15-4	KJ855495	KM401635	KP129314	KP007342	KP268917	KP282701
Gaeumannomyces graminis var. avenae	CBS 187.65	JX134668	JX134680	JX134655	JX134708	JX134722	JX134694
Gaeumannomyces graminis var. tritici	M55	JF414850	JF414900	JF414875	JF710395	JF710445	JF710420
Gaeumannomyces paulograminis	RS7-1	KJ855507	KM401647	KP129326	KP007350	KP268929	KP282713
Magnaporthiopsis cynodontis	RS7-2	KJ855508	KM401648	KP129327	KP007351	KP268930	KP282714
Magnaporthiopsis cynodontis	KR10-6	KJ855499	KM401639	KP129318	KP007357	KP268921	KP282705
Magnaporthiopsis cynodontis	TPC5-3	KJ855514	KM401654	KP129333	KP007356	KP268936	KP282720
Magnaporthiopsis incrustans	WW3-5	KJ855515	KM401655	KP129334	KP007332	KP268937	KP282721
Magnaporthiopsis panicorum	CM10S2	KF689644	KF689634	KF689594	KF689604	KF689614	KF689624
Magnaporthiopsis poae	TAP41	KJ855512	KM401652	KP129331	KP007354	KP268934	KP282718
Magnaporthiopsis rhizophila	M23	JF414834	JF414883	JF414858	JF710384	JF710432	JF710408
Nakataea oryzae	M21	JF414838	JF414887	JF414862	JF710382	JF710441	JF710406
Nakataea oryzae	M69	JX134672	JX134684	JX134658	JX134712	JX134726	JX134698
Nakataea oryzae	M71	JX134673	JX134685	JX134659	JX134713	JX134727	JX134699
Ommidemptus affinis	ATCC 200212	JX134674	JX134686	JX134660	JX134714	JX134728	JX134700
Pseudophialophora cynodontis	RW3-4	KJ855509	KM401649	KP129328	KP007352	KP268931	KP282715
Pseudophialophora eragrostis	CM12M9	KF689648	KF689638	KF689598	KF689608	KF689618	KF689628
Pseudophialophora eragrostis	CM20M5-2	KF689647	KF689637	KF689597	KF689607	KF689617	KF689627
Pseudophialophora panicorum	CM9S6	KF689651	KF689641	KF689601	KF689611	KF689621	KF689631
Pseudophialophora schizachyii	AL2M1	KF689649	KF689639	KF689599	KF689609	KF689619	KF689629
Pyricularia grisea	M83	JX134671	JX134683	JX134657	JX134711	JX134725	JX134697
Slopeiomyces cylindrosporus	CBS 610.75	JX134667	DQ341494	DQ341473	JX134707	JX134721	JX134693

[a] ATCC = American Type Culture Collection, Manassas, Virginia; CBS = Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands. [b] Species names in boldface indicate newly submitted sequences from this study.

growth. The isolates were grown on PDA at 25 °C for 5 d. Mycelial plugs with 5-mm in diameter from the margin of the colony were obtained and transferred onto the center of new PDA plates. The plates were sealed and incubated in the dark at 4, 20, 25, 29, and 37 °C for 5 d. Each isolate had three replications at each temperature, and mycelial growth was measured at the end of the incubation. The effects of temperature treatment were identified by analysis of variance using the general linear model procedure implemented in SAS 9.4 (SAS Institute, Cary, North Carolina, USA). The mean differences were compared with Fisher's protected least significance difference (LSD) test at $\alpha = 0.05$.

Pathogenicity tests

Pathogenicity experiments were conducted by artificial inoculations with the type strain 13g11. Inoculum was prepared through sterilizing oat (*Avena sativa*) seeds in a 50 mL flask. Ten actively growing mycelial plugs with 5-mm in diameter of the strain were added to the sterilized oat substrate, which was shaken and incubated at 28 °C in the dark for one month. Inoculations were conducted in plastic pots (15 cm top diameter × 10 cm bottom diameter × 15 cm height) by placing eight inoculated oat seeds over 100 g of sterilized Pindstrup substrate (Pindstrup Mosebrug, Pindstrup, Denmark) and sand mixture (1:1, v/v), and healthy creeping bentgrass were transplanted to the pots from the Baima research station of Nanjing Agricultural University. The plastic pots inoculated with eight noninfected and sterilized oat seeds served as the control. The inoculated pots were placed in a growth chamber with 95% relative humidity and a 12-h day/night cycle at 26/22 °C. All pots were covered with plastic bags for the first 5 d and watered once daily to favor disease development. The inoculated plants were monitored for development of symptoms weekly, and a final assessment was conducted one month after the inoculations. Re-isolations and identifications were performed as described above to fulfill Koch's postulates.

Genealogical Concordance Phylogenetic Species Recognition analysis

Recombination event between the potential new species and its phylogenetically related species was characterized with the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) model by performing a pairwise homoplasy index (Φw) (PHI) test[34]. The test was performed in SplitsTree4 v.4.16.1. (http://uni-tuebingen.de),[35] and the recombination level within phylogenetically related species was determined. Recombination was considered when the PHI value was below 0.05 (Φw < 0.05). The relationships between closely related species could be visualized by constructing a phylogenetic network with the LogDet transformation and the NeighborNet method, and the network was displayed with the EqualAngle algorithm and bootstrap analysis (1,000 replicates)[36].

Results

Sequence data and phylogeny

Partial sequences of ITS, LSU, SSU, *MCM7, RPBI* and *TEF1* genes were sequenced in four isolates (13g11, 13g12, JH1 and

JH2). The incongruence length difference test generated a *p* value of 0.01 of the concatenated dataset, which indicated that some incongruence was shown in the datasets for the six regions. These regions were still combined, because studies revealed that the threshold of significance at 0.05 might be too conservative, and it had been shown that concatenating incongruent datasets improved the accuracy of phylogeny[37]. The single-locus BI phylogenetic trees for ITS, rDNA (ITS + LSU + SSU), *MCM7*, *RPB1*, and *TEF1* were shown in Supplemental Figs S1–S5. The multilocus dataset comprised 3,778 characters, there were 504 nucleotide characters (including gaps) in the SSU alignment, 510 in ITS, 819 in LSU, 500 in *MCM7*, 612 in *RPB1*, and 833 in *TEF1*. A total of 2,833 characters were conserved, and 689 were parsimony informative.

GTR+F+I+G4 was estimated as the best nucleotide substitution model for ITS, rDNA, *RPB1*, *MCM7* and *TEF1*. The multi-locus phylogenetic tree constructed was similar to those constructed from the datasets of individual markers. The BI and ML phylogenetic trees were constructed similarly in topology (Fig. 1 and Supplemental Fig. S6), and only the BI tree is shown in Fig. 1.

Our four fungal isolates were placed with confidence into the subclade of *Candidacolonium* (PP = 1, BS = 100%), from which a new species in *Candidacolonium* was identified, and found to be distinct from *C. cynodontis* in this genus. A new species of *Candidacolonium* is thereby proposed based on the phylogeny analysis and morphological characteristics (see Taxonomy; Fig. 2).

Growth rate

The two isolates of the potential new species, 13g11 and JH1, exhibited similar growth rates. They all grew well at 20–29 °C, but did not show growth at 4 °C, and reached maximal growth at 26–29 °C (Fig. 3). The two isolates 13g11 and JH1 grew significantly slower ($p < 0.05$) than the two *M. poae* isolates Y15 and S24 at 20, 26 and 29 °C after 5 d of inoculation.

Pathogenicity tests in a growth chamber

The inoculation experiment in the growth chamber led to disease symptoms on creeping bentgrass and fulfilled the Koch's postulates. The roots turned dark-colored and rotted one month after inoculation. Runner hyphae with brown color were apparently observed from roots of all grasses with fungal inoculation (Fig. 2). No symptoms could be found on the grass roots with control treatment. The fungal isolates with similar morphology were consistently recovered from all inoculated samples, and a representative isolate was selected for molecular identification by ITS sequencing. The morphology of the recovered fungal isolates was similar with the isolate 13g11 used for inoculation, and the ITS sequence matched that of the inoculated isolate with 100% identity. Foliar and root health were significantly reduced in inoculated samples compared with uninoculated samples.

GCPSR analysis

A PHI test using a six-locus concatenated dataset (ITS, LSU, SSU, *MCM7, RPBI* and *TEF1*) revealed that the recombination event was not observed between *C. agrostis* and *C. cynodontis*

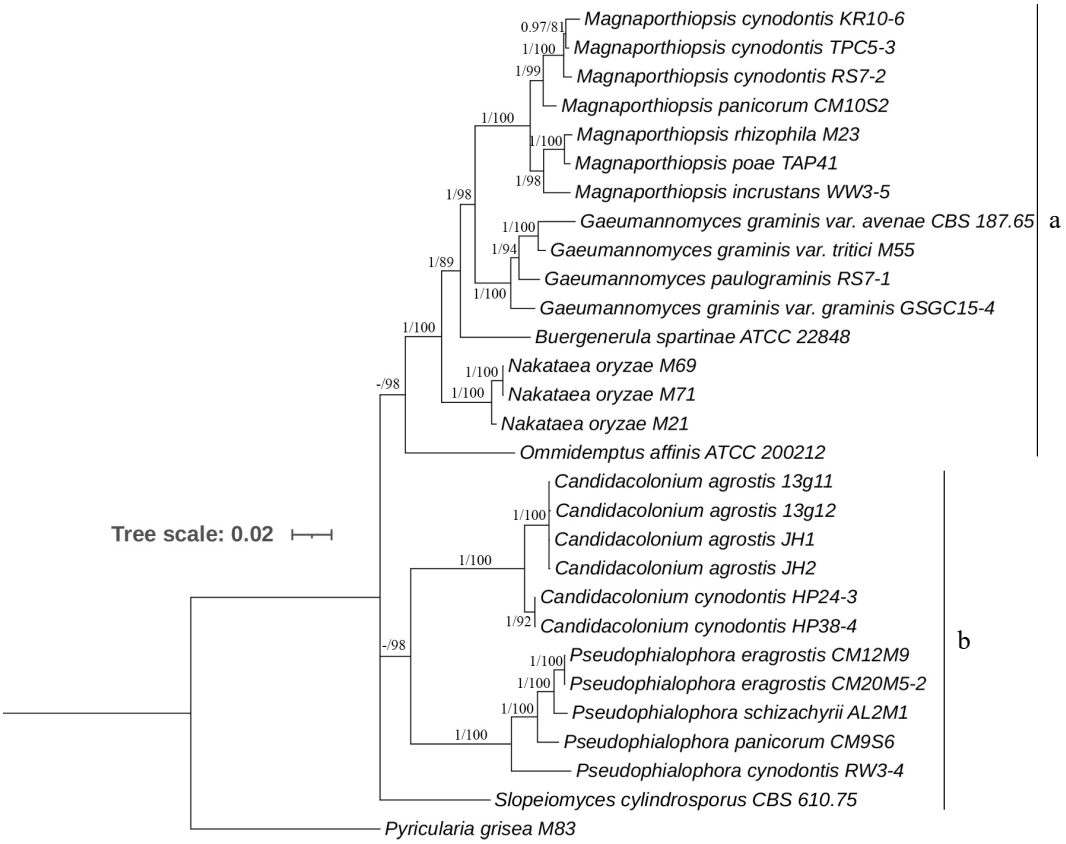

Fig. 1 Majority rule Bayesian phylogenetic tree derived from the combined *ITS*, *LSU*, *SSU*, *MCM7*, *RPB1*, and *TEF1* DNA sequence data sets. Nodes having Bayesian inference posterior probabilities ≥ 0.95 and maximum likelihood bootstrap values ≥ 75% are shown. The tree is rooted with *Pyricularia grisea* M83.

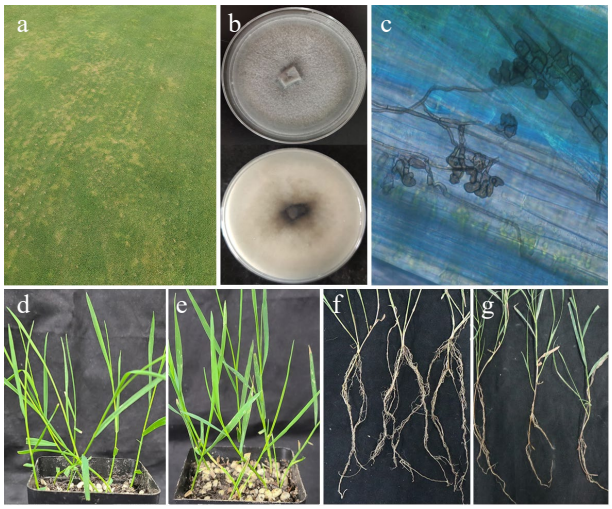

Fig. 2 Disease symptoms, morphological characters and pathogenicity of *Candidacolonium agrostis*. (a) Summer patch-like disease on creeping bentgrass putting green. (b) Front and back of colony on PDA plate after 14-d incubation. (c) Hyphopodia. (d), (e) Creeping bentgrass top growth one month after inoculation with sterile and 13g11 inoculum, respectively. (f), (g) Creeping bentgrass roots one month after inoculation with sterile and 13g11 inoculum, respectively.

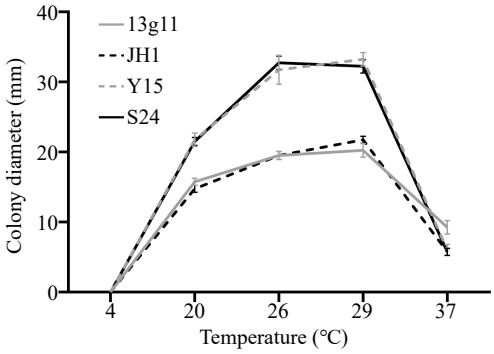

Fig. 3 Total mycelial growth (colony diameter, mm) of *Candidacolonium agrostis* (13g11 and JH1) and *Magnaporthiopsis poae* (Y15 and S24) isolates on PDA after 5 d of incubation in the dark at temperatures of 4, 20, 26, 29 and 37 °C. Error bars represent the mean ± standard deviation.

(Φw = 1.0), indicating that they were two different species. No recombination event was observed among the four isolates (13g11, 13g12, JH1 and JH2) within the new species *C. agrostis* (Φw = 1.0).

Taxonomy

In this study, phylogenetic analyses revealed an undescribed species in the genus *Candidacolonium* causing summer patch-like disease in East China. The proposed new species was isolated from creeping bentgrass roots and is named *Candidacolonium agrostis*.

Candidacolonium agrostis J. Hu and J. M. Geng, sp. nov.

MycoBank: MB849256.

Typification: China, Jiangsu province: Wuxi city, 31°24′38.9″N, 120°07′24.4″E. Roots of *Agrostis stolonifera*, 11 Oct. 2020, J. Hu and J. M. Geng, 13g11 = THAS136. (holotype: not CDGM89573, ex-type culture: GDMCC 3.776.).

Additional specimens examined: China, Jiangsu province: Wuxi city, 31°24′38.9″N, 120°07′24.4″E. Roots of *Agrostis stolonifera*, 11 Oct. 2020, J. Hu and J. M. Geng, 13g12 = THAS137. China, Zhejiang province: Shaoxing city, 118°80′24.2″N, 32°06′46.5″E. Roots of *Agrostis stolonifera*, 15 Jul. 2021, J. Hu and Y. L. Dong, JH1. China, Zhejiang province: Shaoxing city, 118°80′24.2″N, 32°06′46.5″E. Roots of *Agrostis stolonifera*, 15 Jul. 2020, J. Hu and Y. L. Dong, JH2.

Morphological description: Colony diameter was measured approximately 19.0 mm on PDA at 25 °C in the dark after 5 d of incubation. The color of colony was bright white after 7-d growth, and pale grayish buff after 14- to 21-d growth. Reverse of the colony was pigmented dark grayish buff at the center of PDA plate. Hyphae were septate, 2.8-3.6 μm (mean = 3.2; S.D. = 0.3; n = 30) wide, and hyaline to brown in color. Brown stigmatopods measured 5.0-11.8 × 4.6-6.3 (mean = 7.0 × 5.4; S.D. = 1.8, 0.6; n = 30) Brown slightly lobed or mitten-shaped hyphopodia measured 7.9-11.8 × 4.2-8.5 μm (mean = 10.4 × 6.5; S.D. = 1.1, 1.1; n = 30).

Notes: C. agrostis was phylogenetically close to *C. cynodontis*. However, it clearly formed a single clade with high PP/BS values (1/100%). BLAST analyses showed that the ITS, LSU, *MCM7, RPBI* and *TEF1* sequences of *C. agrostis* differed from *C. cynodontis* by 20, 1, 12, 18, 15 bp, respectively. No nucleotide differences were observed in LSU between the two species. *C. agrostis* grew faster than *C. cynodontis* on PDA plate in the dark at 25 °C, and its host was *Agrostis stolonifera*. The two species showed different colony colors[14]. The differences in the sizes of hyphae and hyphopodia were not apparent between the two species[14].

Etymology: The specific epithet refers to its host's generic name.

Distribution: East China

Discussion

In this study, a new species in the genus *Candidacolonium* was identified, causing a summer patch-like disease on creeping bentgrass in East China. The concatenated six-gene and individual gene datasets were highly homologous in topology. BI and ML analyses were able to separate all the taxa into two main clades (A and B). Multiple plant pathogenic species in the genera *Gaeumannomyces*, *Magnaporthiopsis*, *Buergenerula*, *Nakataea* and *Omnidemptus* formed the clade A. Clade B comprised three distinct lineages, the novel species proposed in this study, *C. cynodontis* and the genus *Psedophialophora* belonged to the clade B. The concept of new species is well supported not only by the multi-locus phylogenetic analysis, but also by morphological and biological characteristics, and the root habit of the fungi.

Morphological features such as hyphopodial morphology have traditionally been used as identifying features for ERI fungi[18]. Similar to *C. cynodontis*, *C. agrostis* also produces slightly lobed or mitten-shaped hyphopodia, which are distinct to lobed or simple hyphopodia produced by the genus *Gaeumannomyces*[14,38]. It is appears that the slightly lobed or mitten-shaped hyphopodia may be characteristic of *Candidacolonium* spp. However, other ERI fungi, such as *Slopeiomyces cylindrosporus*, also produce similarly shaped hyphopodia[39]; therefore, these hyphopodia are not diagnostic for *Candidacolonium* spp.

The disease severity caused by *C. agrostis* was significantly different at the two sampled golf courses. This may be because they were sampled at different times, i.e. during a hot summer

and a cool autumn. *C. agrostis* has been observed to cause severe damage on creeping bentgrass putting greens during hot weather. *In vitro* assays of temperature sensitivity also support that *C. agrostis* grows better at higher temperatures. Moreover, creeping bentgrass are weakened by many stressed factors during the hot summer months, and become increasingly vulnerable to ERI fungi[9]. Similar pathogenicity results were observed in other ERI fungi, including *C. cynodontis*, which caused more severe disease at 30 °C than at 20 °C in an *in vivo* evaluation[15]. The pathogenicity analysis in this study successfully recreated the disease symptoms; however, they were not as severe as observed by the superintendents at golf courses. This is probably because turfgrass plants used for the *in vivo* evaluation were grown in a growth chamber with 12-h day/night cycle at 26/22 °C, which was at near optimal conditions, and the plants were not stressed. To gain a better understanding of the role *C. agrostis* play in the summer patch-like disease, field conditions must be considered. A proposed evaluation strategy could be to inoculate *C. agrostis* isolates into established creeping bentgrass turf, and the research plots be managed according to golf course standards.

C. agrostis is demonstrated to be associated with canopy decline symptoms of creeping bentgrass during summer months. The symptoms of affected creeping bentgrass were similar to summer patch disease, which is a common and very destructive root disease of cool-season turfgrasses[5]. To date, the fungus *M. poae* is known as the only pathogen associated with root disease on creeping bentgrass in China. This study reports a new fungal species of *Candidacolonium* associated with summer patch-like symptoms on creeping bentgrass that has a different growth rate, morphology and genetics compared with the causal agent of summer patch *M. poae*. The results from this study confirmed the pathogenicity of *C. agrostis* on creeping bentgrass in the controlled environment and its association with creeping bentgrass at two golf courses in East China. By contrast, summer patch of creeping bentgrass in North China, is caused by *M. poae*. Summer patch-like disease on creeping bentgrass in East China has not been widely studied[2,6]. Further investigation into the distribution and prevalence of *C. agrostis* populations on creeping bentgrass in East China is warranted, and should lead to better understanding of the overall damage caused by this fungus in this region.

In conclusion, a novel ERI fungal species in the genus *Candidacolonium* was obtained and identified from roots of creeping bentgrass that showed summer patch-like disease at golf courses in East China. Prior to this study, *M. poae* was the only known ERI fungus in the Magnaporthaceae associated with summer patch on creeping bentgrass in China. The finding brings about inquiries concerning the role this fungal species plays in the root disease of creeping bentgrass putting greens during summer months in East China. Future studies may include developing the control methods, determining the host range, identifying the dissemination modes, as well as developing rapid diagnostic methods for this fungal species.

Acknowledgments

Jiangsu Institute of Botany Talent Fund, grant number: JIBTF202212. We thank Jiangsu Institute of Botany Talent Fund (grant number: JIBTF202212) for financial support, we also thank Zhang Hongliang from Wuxi Taihu Golf Club for helping collect the diseased samples and providing historical information of the disease.

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary Information accompanies this paper at (https://www.maxapress.com/article/doi/10.48130/GR-2023-0020)

Dates

Received 30 May 2023; Accepted 31 August 2023; Published online 8 October 2023

References

1. Li Q, Li R, He F, Yang Z, Yu J. 2022. Growth and physiological effects of chitosan on heat tolerance in creeping bentgrass (*Agrostis stolonifera*). *Grass Research* 2:6
2. Zhou Y, Yin M, Liu F. 2022. First report of summer patch of creeping bentgrass caused by *Magnaporthiopsis poae* in southeastern China. *Plant Disease* 106:1527
3. Kaminski JE, Dernoeden PH, O'Neill NR, Momen B. 2002. Reactivation of bentgrass dead spot and growth, pseudothecia production, and ascospore germination of *Ophiosphaerella agrostis*. *Plant Disease* 86:1290–96
4. Shi Y, Zhang J, Li H, Li M, Huang B. 2018. Butanediol-enhanced heat tolerance in *Agrostis stolonifera* in association with alteration in stress-related gene expression and metabolic profiles. *Environmental and Experimental Botany* 153:209–17
5. Landschoot PJ, Jackson N. 1989. *Magnaporthe poae* sp. nov., a hyphopodiate fungus with a *Phialophora* anamorph from grass roots in the United States. *Mycological Research* 93:59–62
6. Hu J, Liu Q, Liu M, Zhang F, Xiao Y, et al. 2017. First report of summer patch of creeping bentgrass caused by *Magnaporthe poae* in China. *Plant Disease* 101:634
7. Kerns JP, Tredway LP. 2007. First report of pythium root dysfunction of creeping bentgrass caused by *Pythium volutum* in North Carolina. *Plant Disease* 91(5):632
8. Thomas SL, Bonello P, Lipps PE, Boehm MJ. 2006. Avenacin production in creeping bentgrass (*Agrostis stolonifera*) and its influence on the host range of *Gaeumannomyces graminis*. *Plant Disease* 90:33–38
9. Tredway LP. 2006. Genetic relationships among *Magnaporthe poae* isolates from turfgrass hosts and relative susceptibility of 'Penncross' and 'Penn A-4' creeping bentgrass. *Plant Disease* 90:1531–38
10. Wetzel HC, Dernoeden PH, Millner PD. 1996. Identification of darkly pigmented fungi associated with turfgrass roots by mycelial characteristics and RAPD-PCR. *Plant Disease* 80:359–64
11. Vines PL, Hoffmann FG, Meyer F, Allen TW, Tomaso-Peterson M. 2021. *Gaeumannomyces nanograminis*, sp. nov., a hyphopodiate fungus identified from diseased roots of ultradwarf bermudagrass in the United States. *Mycologia* 113:938–48
12. Câmara MPS, O'neill N, van Berkum P, Dernoeden PH, Palm ME. 2000. *Ophiosphaerella agrostis* sp. nov. and its relationship to other species of *Ophiosphaerella*. *Mycologica* 92:317–25
13. Luo J, Zhang N. 2013. *Magnaporthiopsis*, a new genus in Magnaporthaceae (Ascomycota). *Mycologia* 105:1019–29
14. Vines PL. 2015. *Evaluation of ultradwarf bermudagrass cultural management practices and identification, characterization, and pathogenicity of ectotrophic root-infecting fungi associated with*

summer decline of ultradwarf bermudagrass putting greens. Master of Science Thesis. Mississippi State University, Mississippi. https://scholarsjunction.msstate.edu/td/2079

15. Stephens CM, Gannon TW, Cubeta MA, Sit TL, Kerns JP. 2022. Characterization and aggressiveness of take-all root rot pathogens isolated from symptomatic bermudagrass putting greens. *Pytopathology* 112:811–19
16. Wong PTW, Dong C, Stirling AM, Dickinson ML. 2012. Two new *Magnaporthe* species pathogenic to warm-season turfgrasses in Australia. *Australasian Plant Pathology* 41:321–29
17. Wong PTW, Dong C, Martin PM, Sharp PJ. 2015. Fairway patch - a serious emerging disease of couch (syn. bermudagrass) [*Cynodon dactylon*] and kikuyu (*Pennisetum clandestinum*) turf in Australia caused by *Phialocephala bamuru* P. T. W. Wong & C. Dong sp. nov. *Australasian Plant Pathology* 44:545–55
18. Hernández-Restrepo M, Groenewald JZ, Elliott ML, Canning G, McMillan VE, et al. 2016. Take-all or nothing. *Studies in Mycology* 83:19–48
19. Wong PTW, Tan YP, Weese TL, Shivas RG. 2022. *Magnaporthiopsis* species associated with patch diseases of turfgrasses in Australia. *Mycosphere* 13:602–11
20. Zhang N, Zhao S, Shen Q. 2011. A six-gene phylogeny reveals the evolution of mode of infection in the rice blast fungus and allied species. *Mycologia* 103:1267–76
21. Hu J, Zhou Y, Geng J, Dai Y, Ren H, et al. 2019. A new dollar spot disease of turfgrass caused by *Clarireedia paspali*. *Mycological Progress* 18:1423–35
22. Hulvey J, Popko JT Jr, Sang H, Berg A, Jung G. 2012. Overexpression of *ShCYP51B* and *ShatrD* in *Sclerotinia homoeocarpa* isolates exhibiting practical field resistance to a demethylation inhibitor fungicide. *Applied and Environmental Microbiology* 78:6674–82
23. Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, et al. 2020. PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources* 20:348–55
24. Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30:772–80
25. Farris JS, Källersjö M, Kluge AG, Bult C. 1994. Testing significance of incongruence. *Cladistics* 10:315–19
26. Swofford DL. 2002. *PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4b10*. Sunderland, Massachusetts: Sinauer Associates.
27. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14:587–89
28. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61:539–42
29. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32:268–74
30. Minh BQ, Nguyen MAT, von Haeseler A. 2013. Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* 30:1188–95
31. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, et al. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59:307–21
32. Letunic I, Bork P. 2021. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Research* 49:293–96
33. Rayner RW. 1970. *A mycological colour chart*. Kew, UK: Commonwealth Mycological Institute. 34 pp.
34. Bruen TC, Philippe H, Bryant D. 2006. A simple and robust statistical test for detecting the presence of recombination. *Genetics* 172:2665–81
35. Huson DH, Bryant D. 2006. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 23:254–67
36. Dress AWM, Huson DH. 2004. Constructing splits graphs. *IEEE/ACM Transactions on Computational Biology and Bioinformatics* 1:109–15
37. Cunningham CW. 1997. Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* 14:733–40
38. Freeman J, Ward E. 2004. *Gaeumannomyces graminis*, the take-all fungus and its relatives. *Molecular Plant Pathology* 5:235–52
39. Hornby D, Slope DB, Gutteridge RJ, Sivanesan A. 1977. *Gaeumannomyces cylindrosporus*, a new ascomycete from cereal roots. *Transactions of the British Mycological Society* 69:21–25