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ORIGINAL ARTICLE

# New species and new record of *Alternaria* from onion leaf blight in Myanmar

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**Abstract**
Onion (*Allium cepa*) is an important globally cultivated crop and is known to be susceptible to purple blotch caused by *Alternaria porri*. The causal pathogens of blight symptoms from onion in Myanmar were isolated and identified. In addition to *Stemphylium vesicarium*, a large-spored *Alternaria* with unique morphology as well as a small-spored *Alternaria* were obtained. To identify the two *Alternaria* fungal pathogens, morphological characteristics and molecular phylogenies based on multigene sequence analysis of the internal transcribed spacer of ribosomal DNA (ITS) region, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), *Alternaria* major allergen (*ALT*), translation-elongation factor 1 (*EF1-α*), and RNA polymerase second largest subunit (*RPB2*) genes were used. This revealed the presence of a small-spored *Alternaria*, *A. burnsii*, and a new large-spored species here described as *A. cepae* sp. nov. The novel species is morphologically distinct from its closely related species of *A. montanica.* Pathogenicity assays revealed that *Stemphylium vesicarium*, *A. burnsii*, and *A. cepae* were the causal agents of the onion leaf blight of the current study, and that *A. cepae* exhibited the most virulence. However, *A. porri*, which has been reported as the most important onion pathogen worldwide, was absent during this investigation.

**Keywords** Ascomycota · Molecular phylogeny · Pathogenicity · Taxonomy · One new taxon

## Introduction

Onion (*Allium cepa* L., Alliaceae) is indigenous to central Asia and is widely cultivated globally. Its production is reduced by numerous diseases at various growth stages. Among these, purple blotch caused by *Alternaria porri* (Ellis) is one of the most destructive diseases, occurring on the foliage and reducing crop quality and yield (Gupta and Gupta 2013; Woudenberg et al. 2014). It typically appears as a brownish-purple lesion with concentric rings, leading to plant death when severe (Black et al. 2012). Another pathogen, *Stemphylium vesicarium* (Wall.), is common in warm and moist environments, causing damage on its own or in conjunction with *A. porri* (Aveling et al. 1993).

The genus *Alternaria*, for which there are about 589 legitimate species epithets, currently contains 366 accepted and recognizable species (Wijayawardene et al. 2020). These species, commonly found as plant pathogens, lead to substantial economic losses by causing leaf blight or leaf spot on various crops and as post-harvest pathogens (Andersen et al. 2001; Thomma 2003). Advanced analytical methods using molecular approaches have become essential for separating *Alternaria* species into sections and identifying them to species level. These analyses use multiple gene loci of the internal transcribed spacer regions 5.8S rDNA (ITS), 18S rDNA (SSU), 28S rDNA (LSU), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), RNA polymerase second largest subunit (*RPB2*), translation-elongation factor 1 (*EF1-α*), *Alternaria* major allergen gene (*ALT*),

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endopolygalacturonase (*endoPG*), and an anonymous gene region (*OPA10-2*) (Pryor and Gilbertson 2000; Hong et al. 2005; Lawrence et al. 2011, 2013; Woudenberg et al. 2013, 2014, 2015). *Stemphylium*. was proposed by Wallroth (1833) with *Stemphylium botryosum* as the type species. It is a monophyletic genus of filamentous ascomycetes comprising pathogens and saprobes with a wide range of host plants (Farr et al. 1989; Köhl et al. 2009; Crous et al. 2016; Woudenberg et al. 2017). There are approximately 200 reported names currently described as recognizable taxa of *Stemphylium* (Woudenberg et al. 2017).

In studies on onion diseases in India, *Alternaria* purple blotch and *Stemphylium* blight have been considered important foliage diseases in most cultivation regions (Gupta and Pathak 1988; Suheri and Price 2000; Mathur and Sharma 2006). In contrast, Myanmar has recorded few fungal diseases on cultivated crops. Thaung (1970) reported plant diseases on various hosts in Myanmar, and *A. porri* was identified as a pathogen causing onion purple blotch nationwide. However, to our knowledge, onion diseases have not been reported in Myanmar since then. Furthermore, yield losses caused by fungal diseases have not been well documented in Myanmar. During the 2018–2019 onion growing season, a disease outbreak occurred in Ywarthitgyi village in Naypyidaw, the capital of Myanmar. The typical symptoms were dark brown necrotic lesions, chlorotic foliage, and foliage dieback, distinct from purple blotch disease. As a consequence, 80% of the cultivated onion in that village was affected by the disease and production was shut down in 2019. The study was carried out to identify the causal agents of this onion disease based on morphological characters, molecular analyses, and pathogenicity tests.

## Materials and methods

### Sample collection and fungal isolation

In February 2019, leaves from six symptomatic plants were randomly sampled from three onion plantations in Ywarthitgyi village, Naypyidaw, Myanmar. Small leaf segments with disease lesions were placed in Petri dishes on moist filter paper and kept at 25℃ in the dark for 1 – 2 days to observe the causal fungal pathogens. Single spores emerging from the margins of the disease lesions were isolated using a sterile glass needle under a stereoscopic microscope and then transferred onto potato dextrose agar (PDA, Difco™, Detroit, MI, USA). Fifteen pure cultures representative of all plants were obtained (Table 1) and deposited in the Fungal Herbarium at Yangtze University, Jingzhou, China, and in the Agricultural Culture Collection of China (ACCC), Beijing, China.

### Morphological characterization

Two *Alternaria* strains, based on the colony and conidial characters, YZU 191023 representing large-spored and YZU 191042 representing small-spored, were selected for the further identification process. Colony characteristics were determined using PDA at 25℃ in the dark for 1 week (Deng et al. 2018). Morphology was then determined and characterized using a mycological color chart (Rayner 1970). The strains, grown on potato carrot agar (PCA) at 22℃ under an 8 h photoperiod for 1 week (Simmons 2007), were used to determine conidial characteristics. The conidia ($n = 50$) were mounted in lactophenol picric acid solution to be photographed for characterization, using a Nikon ECLIPSE Ni-U microscopic system (Nikon, Japan).

### DNA extraction, PCR amplification, and sequencing

Total genomic DNA extraction was carried out using fresh mycelia grown on PDA, according to Cenis (1992). PCR amplification and sequencing for *Alternaria* were performed to amplify genes of ITS region with primers ITS5/ITS4 (White et al. 1990), *EF1-α* with EF1-728F/EF1-986R (Carbone and Kohn 1999), *GAPDH* with GPD1/GPD2 (Berbee et al. 1999), *ALT* with Alt-for/Alt-rev (Hong et al. 2005), and *RPB2* with RPB2-6F/RPB2-7cR (Liu et al. 2019; Sung et al. 2007). Additionally, the *cmdA* gene using the primer pair CaldF1/CaldR1 (Lawrence et al. 2013), ITS and *GAPDH* genes, which were phylogenetically informative for species resolution within the *Pleospora* clade (Inderbitzin et al. 2009; Puig et al. 2015; Woudenberg et al. 2017), were used to identify *Stemphylium* species. PCR reactions were performed in a BIORAD T100 thermocycler (BIO-RAD, USA) with a total volume of 25 μL, comprising 12.5 μL 2 × Taq PCR Starmix (Genstar, Beijing, China), 1.25 μL of each primer, 2 μL template DNA, and 8 μL sterile distilled water. The resulting products were electrophoresed in 1% agarose gel and visualized under UV transillumination. Successfully amplified fragments were sequenced in both directions by BGI (Beijing Genomics Institute). The resulting sequences were viewed using BioEdit v7.0.9 (Hall 1999) and assembled in PHYDIT 3.2 (Chun 1995). Consensus sequences were deposited in GenBank (Table 1).

**Table 1** *Alternaria* strains used in this study and the GenBank accession numbers

| Section | Species | Strain | GenBank accession numbers | | | | |
|---|---|---|---|---|---|---|---|
| | | | ITS | *GAPDH* | *ALT* | *EF1-α* | *RPB2* |
| *Porri* | *A. allii* | CBS 121345 | KJ718104 | KJ717958 | KJ718624 | KJ718453 | KJ718278 |
| | | CBS 107.28 [T] | KJ718100 | KJ717954 | KJ718620 | KJ718449 | KJ718274 |
| | | CBS 116701[R] | KJ718103 | KJ717957 | KJ718623 | KJ718452 | KJ718277 |
| | *A. carthami* | CBS 116440 | KJ718132 | KJ717982 | KJ718650 | KJ718480 | KJ718306 |
| | | CBS 117091[R] | KJ718133 | KJ717983 | KJ718651 | KJ718481 | KJ718307 |
| | *A. carthamicola* | CBS 117092 [T] | KJ718134 | KJ717984 | KJ718652 | KJ718482 | KJ718308 |
| | *A. catananches* | CBS 137456 [T] | KJ718139 | KJ717989 | KJ718657 | KJ718487 | KJ718313 |
| | *A. centaureae* | CBS 116446 [T] | KJ718140 | KJ717990 | KJ718658 | KJ718488 | KJ718314 |
| | ***A. cepae* sp. nov** | **YZU 191024, ACCC39718** | **MN656134** | **MN656149** | **MN656139** | **MN656144** | **MN656152** |
| | | **YZU 191023, ACCC39717** | **MN656133** | **MN656148** | **MN656138** | **MN656143** | **MN656151** |
| | | **YZU 191025, ACCC39719** | **MN656135** | **MN656150** | **MN656140** | **MN656145** | **MN656153** |
| | *A. cichorii* | CBS 102.33 [T] | KJ718141 | KJ717991 | KJ718659 | KJ718489 | KJ718315 |
| | *A. cirsinoxia* | CBS 113261 [T] | KJ718143 | KJ717993 | KJ718661 | KJ718491 | KJ718317 |
| | *A. echinaceae* | CBS 116117 [T] | KJ718170 | KJ718015 | KJ718684 | KJ718518 | KJ718343 |
| | *A. ipomoeae* | CBS 219.79 [T] | KJ718175 | KJ718020 | KJ718689 | KJ718523 | KJ718348 |
| | *A. jesenskae* | CBS 133855 [T] | KJ718177 | KJ718022 | KJ718691 | KJ718525 | KJ718350 |
| | *A. linariae* | CBS 105.41 [T] | KJ718180 | KJ718024 | KJ718692 | KJ718528 | KJ718353 |
| | | CBS 109156 | KJ718183 | JQ646347 | GQ180101 | KJ718531 | KJ718356 |
| | *A. montanica* | CBS 121343 [T] | KJ718194 | KJ718033 | KJ718703 | KJ718541 | KJ718367 |
| | *A. passiflorae* | CBS 117102[R] | KJ718212 | KJ718047 | KJ718720 | KJ718558 | KJ718385 |
| | *A. porri* | CBS 116699 [T] | KJ718218 | KJ718053 | KJ718727 | KJ718564 | KJ718391 |
| | | CBS 116698[R] | DQ323700 | KC584132 | KJ718726 | KC584679 | KC584679 |
| | *A. ranunculi* | CBS 116330 [T] | KJ718225 | KJ718058 | KJ718732 | KJ718571 | KJ718398 |
| | *A. scorzonerae* | CBS 116703[R] | KJ718192 | KJ718031 | KJ718700 | KJ718539 | KJ718365 |
| | | CBS 478.83 [T] | KJ718191 | JQ646334 | KJ718699 | KJ718538 | KJ718364 |
| | *A. silybi* | CBS 134092 [T] | KJ718233 | KJ718063 | KJ718740 | KJ718579 | KJ718407 |
| | *A. solani-nigri* | CBS 121347 | KJ718248 | KJ718076 | KJ718754 | KJ718594 | KJ718423 |
| | | CBS 117101[R] | KJ718247 | KJ718075 | KJ718753 | KJ718593 | KJ718422 |
| | | CBS 109155 | KJ718242 | JQ646360 | JQ646444 | KJ718588 | KJ718417 |
| | *A. thunbergiae* | CBS 116331 [T] | KJ718257 | KJ718084 | KJ718764 | KJ718603 | KJ718432 |
| | | CBS 120986 | KJ718258 | KJ718085 | KJ718765 | KJ718604 | KJ718433 |

**Table 1** (continued)

| Section | Species | Strain | GenBank accession numbers | | | | |
|---|---|---|---|---|---|---|---|
| | | | ITS | *GAPDH* | *ALT* | *EF1-α* | *RPB2* |
| *Alternaria* | *A. alternata* | CBS 877.95 | KP124321 | KP124176 | KP123871 | KP125097 | KP124789 |
| | | CBS 916.96 T | AF347031 | AY278808 | AY563301 | KC584634 | KC584375 |
| | | CBS 918.96 | AF347032 | AY278809 | AY563302 | KC584693 | KC584435 |
| | | CBS 121336 | KJ862254 | KJ862255 | KJ862259 | KP125141 | KP124833 |
| | *A. arborescens* | CBS 119544 | KP124408 | JQ646321 | KP123955 | KP125186 | KP124878 |
| | | CBS 102605 T | AF347033 | AY278810 | AY563303 | KC584636 | KC584377 |
| | | CBS 101.13 | KP124392 | KP124244 | KP123940 | KP125170 | KP124862 |
| | | CBS 119545 | KP124409 | KP124260 | KP123956 | KP125187 | KP124879 |
| | *A. betae-kenyensis* | CBS 118810 T | KP124419 | KP124419 | KP123966 | KP125197 | KP124888 |
| | *A. burnsii* | CBS 879.95 | KP124422 | KP124272 | KP123969 | KP125200 | KP124891 |
| | | CBS 107.38 T | KP124420 | JQ646305 | KP123967 | KP125198 | KP124889 |
| | | CBS 108.27 | KC584236 | KC584162 | KP123850 | KC584727 | KC584468 |
| | | **YZU 191003** | **MN656136** | **MN718662** | **MN656141** | **MN656146** | **MN656154** |
| | | **YZU 191042, ACCC39720** | **MN656137** | **MN718663** | **MN656142** | **MN656147** | **MN656155** |
| | *A. eichhorniae* | CBS 489.92 T | KC146356 | KP124276 | KP124276 | KP125204 | KP124895 |
| | *A. gaisen* | CBS 118488R | KP124427 | KP124278 | KP123975 | KP125206 | KP124897 |
| | | CBS 632.93 R | KC584197 | KC584116 | KP123974 | KC584658 | KC584399 |
| | *A. gossypina* | CBS 104.32 T | KP124430 | JQ646312 | JQ646395 | KP125209 | KP124900 |
| | *A. iridiaustralis* | CBS 118486 T | KP124435 | KP124284 | KP123981 | KP125214 | KP124905 |
| | *A. jacinthicola* | CBS 133751 T | KP124438 | KP124287 | KP123984 | KP125217 | KP124908 |
| | *A. longipes* | CBS 121333R | KP124444 | KP124293 | KP123990 | KP125223 | KP124914 |
| | *A. tomato* | CBS 114.35 | KP124446 | KP124295 | KP123992 | KP125225 | KP124916 |
| | *A. tomato* | CBS 103.30 | KP124445 | KP124294 | KP123991 | KP125224 | KP124915 |

Strains generated in the present study are marked in bold. Type strains are marked "T." Representative strains are marked "R"

## Phylogenetic analyses

Newly generated gene sequences were preliminarily subjected to BLASTn search in NCBI (https://www.blast.ncbi.nlm.nih.gov/). Subsequently, related sequences and reference sequences (Woudenberg et al. 2014, 2015) were retrieved from the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). Phylogeny based on ITS, *GAPDH*, *ALT*, *EF1-α*, and *RPB2* gene sequences of the concatenated dataset was aligned using MEGA 6.0 (Tamura et al. 2013). In addition, the sequences of three different loci (ITS, *GAPDH*, *cmdA*) were concatenated and subjected to phylogenetic analysis for *Stemphylium* strains, YZU 191366 and YZU 191367. Phylogenetic analyses of each alignment were performed using maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI) methods. The best-fit model was GTR + I + G, as recommended by MRMODELTEST 2.3 (Nylander 2004). ML analyses were performed in RAxML 7.0.3 (Stamatakis et al. 2008) using the GTR + I + G model, with 1000 bootstrap replicates. MP analyses were conducted in PAUP 4.0b10 (Swofford 2003) using heuristic searches involving random sequence additions, with the tree bisection-reconnection branch-swapping algorithm. Other supportive tree scores, namely tree length, consistency index, retention index, and the rescaled consistency index, were also calculated (Table 2). Gaps within the alignments were treated as missing data. BI analyses were done in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), implemented for 1,000,000 generations of Markov chain Monte Carlo (MCMC) searches, to determine the posterior probability, branch length, and substitution parameters using the

**Table 2** Summary of sequenced gene loci, number of characters, and tree statistics used in individual MP analyses

| Locus | No. of MPTs | No. of constant characters | Parsimony-informative character | Parsimony-uninformative character | No. of MPTs | MPT length | CI | RI |
|---|---|---|---|---|---|---|---|---|
| ITS | 499 | 475 | 23 | 1 | 2 | 25 | 1 | 1 |
| *GAPDH* | 576 | 514 | 47 | 15 | 8 | 75 | 0.893 | 0.98 |
| *ALT* | 425 | 330 | 71 | 24 | 32 | 130 | 0.862 | 0.975 |
| *EF1-α* | 340 | 278 | 54 | 8 | 2 | 68 | 0.971 | 0.996 |
| *RPB2* | 525 | 458 | 46 | 21 | 39 | 81 | 0.889 | 0.983 |

*MPTs*, most parsimonious trees; *CI*, consistency index; *RI*, retention index

previously mentioned best model. We discarded the first 25% of the samples as burn-in and calculated a majority-rule consensus tree. The yielded trees were visualized in FigTree 1.3.1 (Rambaut and Drummond 2010).

### Pathogenicity tests

Pathogenicity tests were conducted on local onion obtained from Ywarthitgyi village by using a spore suspension inoculation (20 μL drop with the concentration of $1 \times 10^5$ conidia $mL^{-1}$) technique in parallel with the colonized agar plug (2 mm in diameter from 5-day-old PDA cultures) technique. For the spore suspension assay, the strains were cultured on PCA for 7 days and then flooded with sterilized distilled water. The conidia were then dislodged into the water by rubbing the colony surface with a sterile glass rod. The number of conidia was counted using a hemocytometer and adjusted to the final concentrations. Sterilized distilled water and uncolonized agar plugs were used as negative controls. The tests were repeated three times. All inoculated plants were covered with a clean polythene bag to maintain the moisture content and were kept in the greenhouse at 25℃. Disease development was observed daily. We calculated disease severity (the disease index) on a scale of 0–5: 0, no disease symptoms; 1, lesion diameter < 10 mm; 2, lesion diameter 10–20 mm; 3, lesion diameter 20–30 mm; 4, lesion diameter 30–40 mm; and 5, complete drying or leaves split from the center. The disease index was determined as $DI = (0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5)/5\,N \times 100$, where $n_{0-5}$ represent the number of leaves with each scale (0–5), and $N$ represents the total number of leaves. Re-isolation and re-identification were attempted to fulfill Koch's postulates.

## Results

### Phylogenetic analysis

Phylogeny of the combined ITS, *GAPDH*, *ALT*, *EF1-α*, and *RPB2* gene sequences was constructed to determine a more accurate placement of newly collected *Alternaria* strains. The phylogenetic tree information was presented in Table 1. The full-length alignment of the combined dataset was stored in TreeBASE (Study no. 26265). The tree topology generated by ML was identical to that generated using MP and BI analyses and was therefore used as the basal tree (Fig. 1). All large-spored strains recovered in this study formed a distinct clade with high support values of 1.0 (BI), 100% (ML), and 99% (MP) that did not include any reference strains, as a sister clade to *A. montanica* and *A. scorzonerae*, suggesting that this is a new species. The small-spored strains formed a well-supported clade (BI, 1.0; MP, 100; ML, 100) that contained reference sequences of *A. burnsii* and *A. tomato*. However, morphological differences between *A. burnsii* and *A. tomato* suggested that the small-spored strains recovered during the current study are *A. burnsii* (see "Taxonomy" section below). In PCR amplification of *Stemphylium*, the ITS region, *GAPDH*, and *cmdA* gene resulted in sequences of 538 nt, 525 nt, and 667 nt, respectively. Sequences were deposited in GenBank with the accession number MW052760–MW052764 (Supplementary Table S1). Phylogenetic analyses of the combined dataset confirmed that two representative strains grouped together with *S. vesicarium* reference strains, CBS 322.49 and CBS 133905, with high support values of 1.0 (PP), 100% (ML), and 99% (BS) (Supplementary Fig. S1).

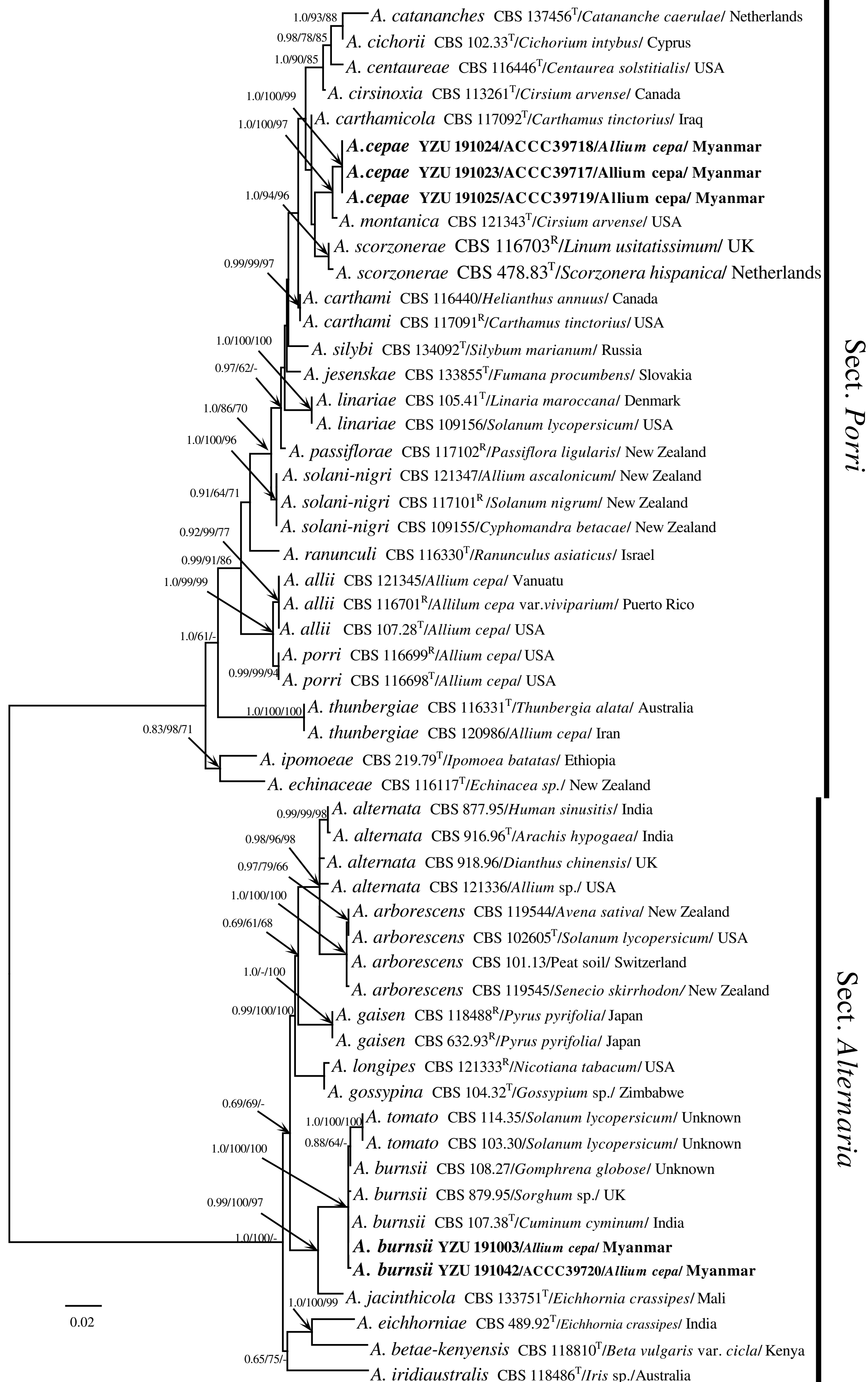
### Taxonomy

***Alternaria cepae*** **A. A. Htun & J. X. Deng, sp. nov.**

MycoBank No: MB 835551

**Etymology.** The specific epithet refers to the species of the host plant, *Allium cepa*.

**Descriptions.** *Colonies* on PDA in the dark at 25℃ (Fig. 2B), surface smooth, pale luteous to bay at the margin, colony reverse chestnut, scarlet, and rust pigmentation, 53–54 mm in diam., and *colonies* on PCA under fluorescent light/dark cycle of 8/16 h at 22℃ (Fig. 2C), surface ochreous, cinnamon, amber pigmentation, sulfur-yellow in reverse, 59−61 mm diam. *Conidiophore* macronematous, solitary, single conidiogenous locus arising directly from the aerial hyphae, 26–75 (–124) × 5–10 μm, normally



A. catananches CBS 137456T/Catananche caerulae/ Netherlands
A. cichorii CBS 102.33T/Cichorium intybus/ Cyprus
A. centaureae CBS 116446T/Centaurea solstitialis/ USA
A. cirsinoxia CBS 113261T/Cirsium arvense/ Canada
A. carthamicola CBS 117092T/Carthamus tinctorius/ Iraq
A.cepae YZU 191024/ACCC39718/Allium cepa/ Myanmar
A.cepae YZU 191023/ACCC39717/Allium cepa/ Myanmar
A.cepae YZU 191025/ACCC39719/Allium cepa/ Myanmar
A. montanica CBS 121343T/Cirsium arvense/ USA
A. scorzonerae CBS 116703R/Linum usitatissimum/ UK
A. scorzonerae CBS 478.83T/Scorzonera hispanica/ Netherlands
A. carthami CBS 116440/Helianthus annuus/ Canada
A. carthami CBS 117091R/Carthamus tinctorius/ USA
A. silybi CBS 134092T/Silybum marianum/ Russia
A. jesenskae CBS 133855T/Fumana procumbens/ Slovakia
A. linariae CBS 105.41T/Linaria maroccana/ Denmark
A. linariae CBS 109156/Solanum lycopersicum/ USA
A. passiflorae CBS 117102R/Passiflora ligularis/ New Zealand
A. solani-nigri CBS 121347/Allium ascalonicum/ New Zealand
A. solani-nigri CBS 117101R /Solanum nigrum/ New Zealand
A. solani-nigri CBS 109155/Cyphomandra betacae/ New Zealand
A. ranunculi CBS 116330T/Ranunculus asiaticus/ Israel
A. allii CBS 121345/Allium cepa/ Vanuatu
A. allii CBS 116701R/Allilum cepa var.viviparium/ Puerto Rico
A. allii CBS 107.28T/Allium cepa/ USA
A. porri CBS 116699R/Allium cepa/ USA
A. porri CBS 116698T/Allium cepa/ USA
A. thunbergiae CBS 116331T/Thunbergia alata/ Australia
A. thunbergiae CBS 120986/Allium cepa/ Iran
A. ipomoeae CBS 219.79T/Ipomoea batatas/ Ethiopia
A. echinaceae CBS 116117T/Echinacea sp./ New Zealand
A. alternata CBS 877.95/Human sinusitis/ India
A. alternata CBS 916.96T/Arachis hypogaea/ India
A. alternata CBS 918.96/Dianthus chinensis/ UK
A. alternata CBS 121336/Allium sp./ USA
A. arborescens CBS 119544/Avena sativa/ New Zealand
A. arborescens CBS 102605T/Solanum lycopersicum/ USA
A. arborescens CBS 101.13/Peat soil/ Switzerland
A. arborescens CBS 119545/Senecio skirrhodon/ New Zealand
A. gaisen CBS 118488R/Pyrus pyrifolia/ Japan
A. gaisen CBS 632.93R/Pyrus pyrifolia/ Japan
A. longipes CBS 121333R/Nicotiana tabacum/ USA
A. gossypina CBS 104.32T/Gossypium sp./ Zimbabwe
A. tomato CBS 114.35/Solanum lycopersicum/ Unknown
A. tomato CBS 103.30/Solanum lycopersicum/ Unknown
A. burnsii CBS 108.27/Gomphrena globose/ Unknown
A. burnsii CBS 879.95/Sorghum sp./ UK
A. burnsii CBS 107.38T/Cuminum cyminum/ India
A. burnsii YZU 191003/Allium cepa/ Myanmar
A. burnsii YZU 191042/ACCC39720/Allium cepa/ Myanmar
A. jacinthicola CBS 133751T/Eichhornia crassipes/ Mali
A. eichhorniae CBS 489.92T/Eichhornia crassipes/ India
A. betae-kenyensis CBS 118810T/Beta vulgaris var. cicla/ Kenya
A. iridiaustralis CBS 118486T/Iris sp./Australia
1.0/93/88
0.98/78/85
1.0/90/85
1.0/100/99
1.0/100/97
1.0/94/96
0.99/99/97
1.0/100/100
0.97/62/-
1.0/86/70
1.0/100/96
0.91/64/71
0.92/99/77
0.99/91/86
1.0/99/99
1.0/61/-
0.99/99/94
1.0/100/100
0.83/98/71
0.99/99/98
0.98/96/98
0.97/79/66
1.0/100/100
0.69/61/68
1.0/-/100
0.99/100/100
0.69/69/-
1.0/100/100
0.88/64/-
1.0/100/100
0.99/100/97
1.0/100/-
1.0/100/99
0.65/75/-
0.02
Sect. Porri
Sect. Alternaria

◂**Fig. 1** ML phylogenetic tree combined from ITS, *GAPDH*, *ALT*, *EF1-α*, and *RPB2* gene sequences of *Alternaria* species from *Allium cepa* and the related taxa. Bayesian posterior probabilities (PP) > 0.70, maximum likelihood (ML) > 70%, and parsimony bootstrap values (BS) > 70% are indicated above/below the branches (PP/ML/BS). Taxon names, strain numbers, host, and geographic origins are provided. The scale bar represented the number of nucleotide substitutions. Strains from the present study are shown in bold. Ex-type strains (T) and representative strains (R) are noted in superscript

with 3−5 septate, brown, smooth-walled. *Conidia* on PCA (Fig. 2D, E), solitary, narrow obclavate, internal cell formation retains distosepta throughout conidial enlargement, 53–85 (–90) × 12–30 µm, 5–7 transverse septa but rarely with a longitudinal euseptum, some conidia having a short-to-medium beak (8–30 µm) around 4.5–10 µm wide, occasionally up to 38 µm long, and similar *conidia* on host, 43–75 (–82) × 16–24 (–26) µm at 22℃, normally with short blunter beaks 5–24 (–40) µm long, and 49–79 × 15–23 (–26) µm at 25℃, with 10–26(–46) µm beaks.

**Holotype.** MYANMAR. NAYPYIDAW: Ywarthitgyi village (194,751), 19°52′ 27.372″N, 96°11′34.332″E, 115 m, isolated from leaf blight of onion, single spore isolation, colonies grown on PDA and PCA for 7 days, 12 Feb 2019, A. A. Htun. (Holotype YZU-H-0035). Ex-type culture: YZU 191023 = ACCC39717.

**Additional specimen examined.** MYANMAR. NAYPYIDAW: Ywarthitgyi village, 12 Feb 2019, A. A. Htun (YZU 191024 = ACCC39718, YZU191025 = ACCC39719).

**Habitat.** Leaf blight on *Allium cepa*.

**Distribution.** Naypyitaw (Central Myanmar).

**Notes:** Phylogenetically, the species is the closest to *A. montanica* in section *Porri* based on the ITS, *GAPDH*, *ALT*, *EF1-α*, and *RPB2* gene sequences. In conidial morphology, it differs significantly from *A. montanica*, which produces conidia with a long, narrow, and tapered apical beak that becomes a short, broad secondary conidiophore near the conidial apex (Table 3).

***Alternaria burnsii*** Uppal, Patel & Kamat, Indian J.Agric.Sci.8:61 (1938).

**Descriptions.** *Colonies* on PDA for 7 days in darkness (Fig. 3A), surface buff to honey, cottony to vinaceous buff, united margin, 72−73 mm in diameter. *Colonies* on PCA (Fig. 3B) incubated for 7 days under fluorescent light/dark cycle of 8/16 h at 22℃, surface pale-smoky gray, vinaceous buff, grayish sepia, fuscous black in reverse, 59−63 mm diam. *Conidia* narrow ovoid to long ovoid or long ellipsoid, 20−50 × 8−15 µm, with beaks 3−30 µm, rarely forming longitudinal septa and 4−7 transverse septa, normally 5−9 catenated conidia in a chain (Fig. 3C, D).

**Notes.** *Alternaria burnsii* was first described in India from *Cuminum cyminum* (Uppal et al. 1938). In the present study, the conidia of this species were similar to the morphological description by Simmons (2007) (Table 3). It is phylogenetically near to *A. tomato*; however, this species differs from *A. burnsii* by having ellipsoid to long-ovoid conidia (39–65 × 13−22 µm), with a single beak

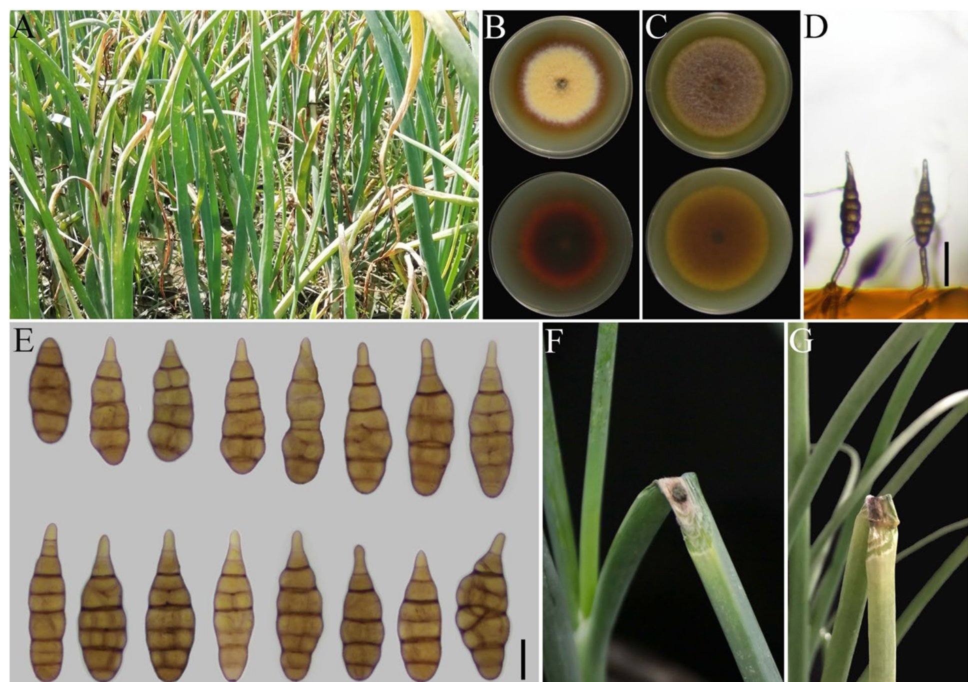



**Fig. 2** Morphological characteristics of *Alternaria cepae* and its causal symptoms on *Allium cepa*. Symptoms in the field (**A**); colony on PDA (**B**) and on PCA (**C**) for 7 days; sporulation patterns (**D**) and conidia (**E**) on PCA; pathogenicity test symptoms on living leaves inoculated with mycelium plug method (**F**) and conidial suspension (**G**) (4 days after inoculation). Scale bars: D = 50 µm, E = 25 µm

**Table 3** Morphological comparison of *A. cepae*, *A. burnsii*, and the related *Alternaria* spp.

| Species | Conidial shape | Conidial body | | Beak (µm) | | Catenation | Medium |
|---|---|---|---|---|---|---|---|
| | | Size (µm) | Septation | | | | |
| *A. allii* | Long narrow ovoid | 80–120 × 18–21 | 8–11 | Multiple up to 6 | 175–190 | Solitary | V8A[a] |
| *A. carthamicola* | Smooth-walled, ovoid | (39–)58–64(–82) × (13–)15–16(–17) | 6−7 | Filamentous 1−2 (−3) beaks | (40–)158–186(–219) | Solitary rarely in chains of two conidia | SNA[b] |
| *A. cepae* sp. nov | Obclavate | 53–85(–90) × 12–30 | 5–7 | Short-to-medium blunt-taper | 8–30(–38) | Singly | PCA[c] |
| | Obclavate | 43–75(–82) × 16–24(–26) (22℃)<br>49–79 × 15–23(–26) (25℃) | – | Blunt-taper | 5–24(–40) (22℃)<br>10–26(–46) (25℃) | Singly | Host[c] |
| *A. cepulicola* | Long obclavate | 90–150 × 18–40 | 10–14 | Blunt-taper | – | Singly or short chain (2–3 units) | Host[a] |
| | Obclavate to muriform | 58.8–184.8 × 21–46.2 | 6–15 | – | – | Singly or short chain (2–3 units) | Host[d] |
| *A. montanica* | Subcylindric, narrow ellipsoid, long ovoid, or obclavate | 80–105 × 14–22 | 7–10 | Long narrow-taper, occasionally with a branch | Juvenile 20–40 and mature up to 100–165 | Solitary or short chain (2–4 units) | V8A[a] |
| *A. porri* | Ovoid, nearly cylindrical | 70–105 × 19–24 | 8–12 | Long narrow or 1 to 2 branched | 95–160 | Solitary | V8A[a] |
| *A. scorzonerae* | Long narrow ellipsoid, subcylindrical, or obclavate | 85–110 × 14–22 | 9–13 | Narrow-taper beak or 2–3 beaks | Solitary one 140–250 | Solitary | V8A[a] |
| *A. burnsii* | Ovoid or ellipsoid | 30–50 × 9–13 | 5–8 | Beakless or a short beak | – | Solitary or short chain | Host[a] |
| | | 30–40 × 8–14 | 3–7 | – | – | – | PCA or V8A[a] |
| | Narrow ovoid or ellipsoid | 20–50 × 8–15 | 4–7 | Beakless or a short beak | 3–30 | 5−9 units per chain | PCA[c] |
| *A. tomato* | Ellipsoid to long ovoid | 39–65 × 13−22 | 6–9 | Narrow apical beak to almost filiform | 60–105 | Singly | Host[a] |

[a]Referenced from Simmons (2007)

[b]Referenced from Woudenberg et al. (2014)

[c]Determined in the present study

[d]Referenced from Rao (1963)

60−105 µm, and no evidence of catenation (Table 3). The host range of *A. burnsii* is reported as Apiaceae: *Cuminum cyminum* (Uppal et al. 1938), *Bunium persicum* (Mondal et al. 2002), *Apium graveolens* (Zhang 2003; Zhuang 2005), and Cucurbitaceae: *Cucurbita maxima* (Paul et al. 2015). In the present study, *A. burnsii* was isolated from Liliaceae: *Allium cepa*.

## Pathogenicity tests

The new species of *A. cepae* and *A. burnsii* were pathogenic to inoculated plants, regardless of the inoculation technique. A small necrotic spot was first observed on the inoculated leaves 1 day after inoculation (dpi) for the new species. The spot expanded aggressively and transformed into a water-soaked lesion with concentric rings at the edge, similar to the symptoms observed in the field (Fig. 2A, F, G). All of the inoculated leaves eventually collapsed at 3–5 dpi. For *A. burnsii* strains, water-soaked lesions appeared 3 dpi and gradually converted into sunken lesions with a creamy margin of 5 dpi (Fig. 3E, F). Using both inoculation methods, the disease incidence for the new species was 100%, with a severity of 80%; for *A. burnsii*, it was 40%, with a severity of 25%. The disease symptom of *S. vesicarium* on the inoculated leaves initially comprised a small, brown, and water-soaked lesion. Finally, the center of the lesion turned from brown to black with the concentric ring at the margin (Supplementary Fig. S2), which was very similar to that of *A. cepae*.

# Discussion

*Alternaria*, an omnipresent fungus consisting of hundreds of species, leads to pre- and post-harvest crop losses and has been recorded as a critical fungal pathogen because of its global dissemination on various hosts (Lawrence et al. 2016; Meena et al. 2017). *Alternaria* profoundly attacks *Allium* species at two growth stages, when the leaves mature and before the bulb develops (Stavely and Slana

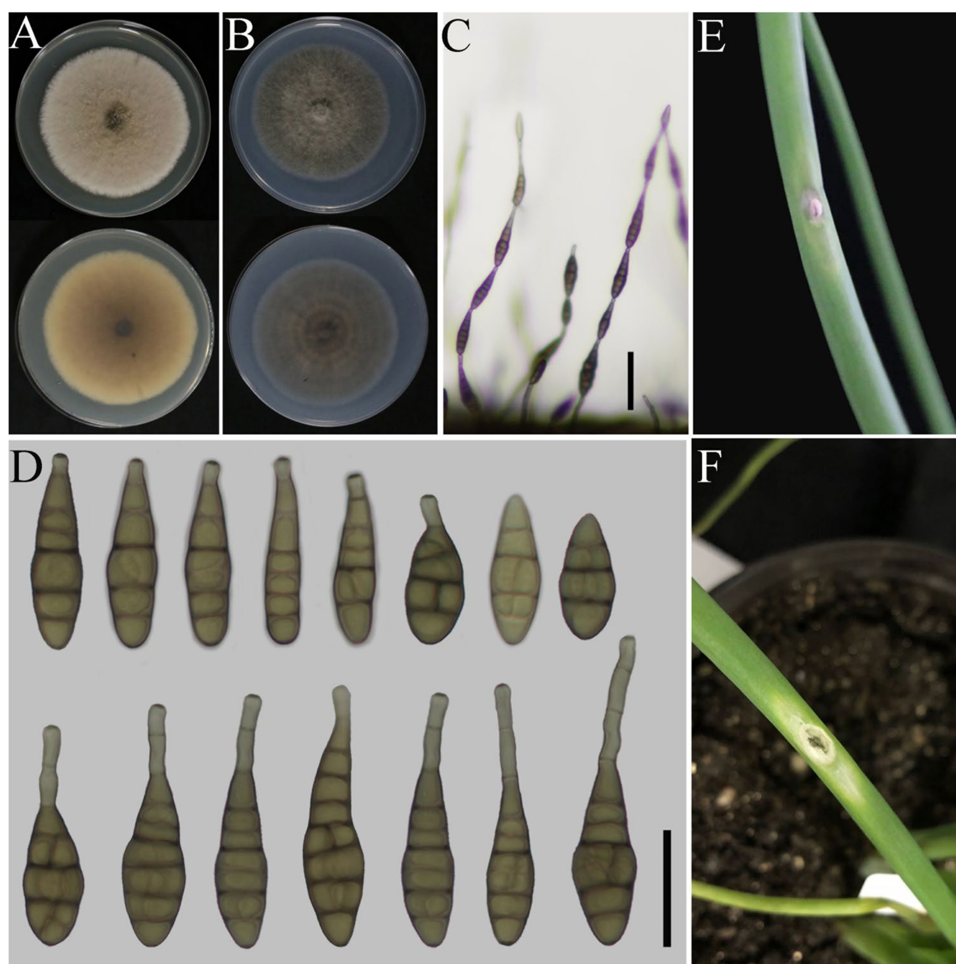



**Fig. 3** Morphological characteristics of *Alternaria burnsii* and its pathogenicity on *Allium cepa*. Colony on PDA (**A**) and on PCA (**B**) for 7 days; sporulation patterns (**C**) and conidia (**D**) on PCA; pathogenicity tests on living leaves inoculated with mycelium plug method (**E**) and conidial suspension (**F**) (7 days after inoculation). Scale bars: C = 50 μm, D = 25 μm

1971). There is a long history of studies on *Alternaria* species on *Allium* cultivars, specifically *A. allii*, *A. palandui*, *A. porri* (purple blotch), *A. cepulicola*, *A. ascaloniae*, *A. iranica*, *A. prasonis*, *A. vanuatuensis*, *A. alternata*, and *A. tenuissima* (Simmons 2007; Vélez-Rodríguez and Rivera-Vargas 2007).

Woudenberg et al. (2013, 2014, 2015), via multilocus phylogenetic analysis, indicated the misidentification of some of these *Alternaria* species in the past based mainly on morphological and host data. Among the *Alternaria* species infecting onions, *A. ascaloniae* has been synonymized as *A. solani-nigri*, *A. iranica* as *A. thunbergiae*, *A. vanuatuensis* as *A. allii*, and *A. tenuissima* and *A. palandui* as *A. alternata*. Gene sequence information for *A. cepulicola* is not available from GenBank, consequently, its phylogenetic position and possible synonymy with some of these species remain uncertain. In addition, *A. cepulicola* were obviously different from *A. cepae* by producing large conidia in a short chain (Table 3). In the current study, multigene phylogeny was employed alongside morphological characteristics to identify two *Alternaria* species that had not previously been associated with onions globally.

A new *Alternaria* species, named as *A. cepae* in the current study, was found to be associated with leaf blight in onion plantations in Naypyidaw, Myanmar. The taxonomy of *Alternaria* strains from this onion leaf blight was examined based on morphological characters and multilocus DNA sequence data. The combined gene phylogeny revealed that the strains formed a distinct lineage with well-supported bootstrap values. The other *Alternaria* species recovered from onion in this study, *A. burnsii*, was distinguished from its closest phylogenetic relative, *A. tomato*. Although these species were reciprocally monophyletic in the seven gene phylogeny of Woudenberg et al. (2015), support for the *A. burnsii* clade in that phylogeny was relatively low (0.88 Bayesian posterior probability, 68% maximum likelihood bootstrap support). Al-Nadabi et al. (2018) also failed to distinguish between *A. burnsii* and *A. tomato* using multigene phylogeny of the same five genes used in this study and resorted to referring to these species as the *A. burnsii*–*A. tomato* species complex.

Woudenberg et al. (2015) described that in most cases, *Alternaria* species cannot be fully resolved using single-gene phylogenies and reiterate the conclusion of Al-Nadabi et al. (2018) that additional gene regions might be needed to differentiate between *A. burnsii* and *A. tomato*.

Furthermore, pathogenic *Stemphylium vesicarium* was isolated from the diseased tissue (Supplementary Figs. S1–S2). This species has been recorded as an onion pathogen in Myanmar (https://www.ippc.int/static/media/files/pestreport/2016/12/01/Pests_of_Onion_in_Myanmar.pdf). Although most previous studies report significant destruction of onion crops when *S. vesicarium* co-occurs with *A. porri* (Aveling et al. 1993, Suheri and Price 2001, Mathur and Sharma 2006), *A. porri* was not obtained in the current study, even though it is a recognized onion fungal pathogen in Myanmar (Thaung 1970). Rather, *A. cepae*, *A. burnsii*, and *S. vesicarium* were recorded as the causal pathogens of onion leaf blight, leading to plant death. This study shed new light on the pathogenic fungi of onion, *Alternaria* and *Stemphylium*, based on their morphology, molecular data, and pathogenicity. Furthermore, *A. cepae* was described as a new taxon in *Alternaria*. To the best of our knowledge, this is the first report of onion leaf blight caused by *A. burnsii* in Myanmar, as well as the first report of the onion being a host for *A. burnsii*.

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**Author contribution** All authors contributed to the study conception and design. The samples collection were carried out by Aye Aye Htun. Deng Jian Xin supported scientific guidance during laboratory and field studies. The initial fungal isolation was performed by Aye Aye Htun, who led the entire research work with Liu Hai Feng and He Lin. Xia Zhen Zhou and Sein Lai Lai Aung contributed to data analysis. The manuscript was written by Aye Aye Htun, and all authors provided critical feedback and helped shape the research, analysis, and manuscript. Deng Jian Xin supervised the final version as well. All authors read and approved the final manuscript.

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**Data availability** All data generated or analyzed during this study are included in this published article (and its supplementary files). All sequences data generated in this study are available in NCBI GenBank.

## Declarations

**Conflict of interest** The authors declare no competing interests.

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