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New Species of Large-Spored Alternaria in Section Porri Associated with Compositae Plants in China

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Keywords: Alternaria; compositae; morphology; multi-locus sequence analyses; taxonomy

Abstract: Alternaria is a ubiquitous fungal genus including saprobic, endophytic, and pathogenic species associated with a wide variety of substrates. It has been separated into 29 sections and seven monotypic lineages based on molecular and morphological data. Alternaria sect. Porri is the largest section, containing the majority of large-spored Alternaria species, most of which are important plant pathogens. Since 2015, of the investigations for large-spored Alternaria species in China, 13 species were found associated with Compositae plants based on morphological comparisons and phylogenetic analyses. There were eight known species and five new species (A. anhuiensis sp. nov., A. coreopsidis sp. nov., A. nanningensis sp. nov., A. neimengguensis sp. nov., and A. sulphureus sp. nov.) distributed in the four sections of Helianthinficientes, Porri, Sonchi, and Teretispora, and one monotypic lineage (A. argyranthemi). The multi-locus sequence analyses encompassing the internal transcribed spacer region of rDNA (ITS), glyceraldehydes-3-phosphate dehydrogenase (GAPDH), Alternaria major allergen gene (Alt a 1), translation elongation factor 1-alpha (TEF1), and RNA polymerase second largest subunit (RPB2), revealed that the new species fell into sect. Porri. Morphologically, the new species were illustrated and compared with other relevant large-spored Alternaria species in the study. Furthermore, A. calendulae, A. lecanthemi, and A. tagetica were firstly detected in Brachyactis ciliate, Carthamus tinctorius, and Calendula officinalis in China, respectively.

Keywords: Alternaria; porri; compositae; morphology; multi-locus sequence analyses; taxonomy

1. Introduction

Alternaria is a cosmopolitan and widely distributed fungal genus described originally by Nees (1816), which is characterized by the dark-coloured phaeodictyospores in chains and a beak of tapering apical cells [1]. It is also associated with nearly every environmental substrate including animal, plant, agricultural product, soil, and the atmosphere. Species of Alternaria are known as serious plant pathogens, causing enormous losses on many crops [1,2]. The taxonomy is mainly based on sporulation patterns and their conidial shape, size, and seption [2,3]. Around 280 species are summarised and recognised on the basis of morphology [2], comprising two groups, large-spored (60–100 µm long conidial body) and small-spored (below 60 µm conidial body) [4–6].

Since the 20th century, molecular approaches, especially multi-locus phylogenetic analyses, have been used to identify Alternaria species [7–10]. Over ten gene regions are used in the classification, such as the internal transcribed spacer region of rDNA (ITS), large subunit ribosomal DNA (LSU), mitochondrial small subunit (mtSSU), glyceraldehydes-3-phosphate dehydrogenase (GAPDH), Alternaria major allergen gene (Alt a 1), translation elongation factor 1-alpha (TEF1), RNA polymerase second largest subunit (RPB2), and plasma membrane ATPase [1,4,7,9,11–18]. Alternaria has been separated into 29 sections and
seven monotypic lineages [19–21]. The introduction of a molecular phylogenetic approach has helped to clarify their taxonomy, combining many allied genera into one large genus of *Alternaria* complex [1].

Due to the effects of *Alternaria* on humans and their surroundings, the identification is particularly important to agriculture, medicine, and science. The Compositae plants serve as food plant, oil seed, seed plant, ornamental, and sources of medicine and insecticide worldwide [22], of which nearly 3000 species almost 240 genera have been found in China [23]. Most *Alternaria* are commonly plant pathogens leading to substantial economic losses caused by *Alternaria* leaf spots and defoliation [18,24–26]. Large-spored *Alternaria* species encompassing 148 species are almost phytopathogenic demonstrated [2].

During the investigation of large-spored *Alternaria* in China, five new species were encountered from diseased leave samples of composite plants. The objectives of this study were to identify them on the basis of the cultural and conidial morphology incorporate with multi-loci phylogeny (ITS, GAPDH, Alt a 1, TEF1, and RPB2). The present multi-locus analysis supplemented with cultural and morphological data forms an example for *Alternaria* species recognition. The five new species described in this study add species diversity to large-spored *Alternaria* and provide theoretical and practical basis for the further identification and disease management.

2. Materials and Methods

2.1. Sample Collection and Fungal Isolation

Symptomatic samples of composite plants (14) have been randomly collected from different provinces in China since 2015. For fungal isolation, the samples were put into sterile plastic bags and taken to the laboratory. Small leaf segments (2 mm) with disease lesions were placed into petri dishes with moist filter papers and incubated at 25 °C in dark for conidial sporulation. Single spore of large-spored *Alternaria* was picked by a sterilized glass needle under the stereoscopic microscope and transferred to potato dextrose agar (PDA: Difco, Montreal, Canada). Over ten similar spores were randomly picked from a sample for sub-culturing to obtain the pure cultures, and two to three strains were selected for deposition when exhibiting similar cultural morphology on PDA. A total of 81 strains were kept in test-tube slants and deposited at 4 °C. Living ex-type strains were preserved in the Fungi Herbarium of Yangtze University (YZU), in Jingzhou, Hubei, China.

2.2. Morphological Observations

To determine cultural characteristics including growth rate, color and texture of colonies [27], mycelial plugs (6 mm in diameter) were taken from the edge of colonies grown on PDA. Then, the plugs were put on fresh PDA plates (90 mm) at 25 °C for 7 days in darkness. To observe the conidial morphology (conidial sporulation patterns, shape, size, etc.), mycelia were grown on potato carrot agar (PCA) and V8 juice agar (V8A) inoculated at 22 °C with a light period of 8 h light/16 h dark [2]. After 7 days, conidia and sporulation patterns were observed. Conidiophores and conidia were mounted with lactophenol picric acid solution and photographed with a Nikon ECLIPSE Ni-U microscope (Nikon, Japan). Randomly selected conidia \((n = 50)\) were separately measured for each characterization.

2.3. DNA Extraction and PCR Amplification

Genomic DNA extraction was performed using fresh mycelia collected from colonies grown on PDA [28]. Polymerase chain reaction (PCR) amplifications of the internal transcribed spacer region of rDNA (ITS), glyceraldehydes-3-phosphate dehydrogenase (GAPDH), *Alternaria* major allergen gene (Alt a 1), translation elongation factor 1-alpha (TEF1), and RNA polymerase second largest subunit (RPB2) gene regions were amplified with the primer pairs ITS5/ITS4 [29], EF1-728F/EF1-986R [30], gpd1/gpd2 [31], Alt-for/Alt-rev [12], and RPB2-5F2/RPB2-7cR [32,33], respectively. A 25 µL of the PCR reaction mixture comprising 21 µL of 1.1 × Taq PCR Star Mix (TSINGKE, Beijing, China), 2 µL template DNA and 1 µL of each primer was applied and performed in a BIORAD T100 thermocycler [1].
Successfully amplified PCR products were purified and sequenced by TSINGKE company (Beijing, China).

2.4. Phylogenetic Analyses

The resulted sequences were examined by BioEdit v.7.0.9 [34] and assembled with PHYDIT 3.2 [35]. All newly generated sequences were deposited in GenBank (Table 1). Relevant sequences [4] were retrieved from NCBI database based on the results of BLAST searches (Table 1). The concatenated sequence dataset of multiple loci was aligned using MEGA v.6.0 [36]. Phylogenetic analyses of each alignment were performed using maximum likelihood (ML) and Bayesian inference (BI) methods. ML analysis was conducted using RAxML v.7.2.8 [37]. Bootstrapping with 1000 replicates was performed using the model of nucleotide substitution obtained by MrModeltest. For the BI analysis, it was performed using parameters including 1,000,000 Markov chain Monte Carlo (MCMC) algorithm with Bayesian posterior probabilities [38]. MrModel test v.2.3 used the best-fit model (GTR+I+G) according to the Akaike Information Criterion (AIC). Two MCMC chains were run from random trees for $10^6$ generations, and the trees were sampled every 100th generation. After discarding the first 25% of the samples, the 50% majority rule consensus tree and posterior probability values were calculated. Finally, the resulting trees were edited in FigTree v.1.3.1 [39]. Branch support of the groupings (>60%/0.6 for ML bootstrap value-BS/posterior probability-PP) were indicated in the phylogram. *Alternaria gypsophilae CBS 107.41* in sect. *Gypsophilae* was used as an outgroup.

| Section | Species | Strain | Locality | Substrate | ITS | GAPDH | Alt a 1 | TEF1 | RPB2 |
|---------|---------|--------|----------|-----------|-----|-------|--------|------|------|
| Porri   | A. acalyphicola | CBS 541.94 T | Seychelles | *Acalypha indica* | KJ718097 | KJ717952 | KJ718617 | KJ718446 | KJ718271 |
| Porri   | A. agerati | CBS 117221 R | USA | *Ageratum houstonianum* | KJ718098 | KJ717953 | KJ718618 | KJ718447 | KJ718272 |
| Porri   | A. agripastis | CBS 577.94 T | Canada | *Euphorbia esula*, stem lesion | KJ718099 | JQ646356 | KJ718619 | KJ718448 | KJ718273 |
| Porri   | A. allii | CBS 116701 R | USA | *Allium cepa* var. *viviparum* | KJ718103 | KJ717957 | KJ718623 | KJ718452 | KJ718277 |
| Porri   | A. alternarioides | CBS 105.51 T | UK | *Anagallis arvensis*, leaf spot | KJ718105 | KJ717959 | KJ718625 | KJ718454 | KJ718279 |
| Porri   | A. anagallioides | CBS 117129 R | New Zealand | *Coreopsis basalis*, leaf spot | KJ718109 | KJ717962 | KJ718629 | KJ718457 | KJ718283 |
| Porri   | A. anubiusis sp. nov. | ZYU 171206 T | China | *Coreopsis basalis*, leaf spot | MK264916 | MK303949 | MK303953 | MK303958 | MK303960 |
| Porri   | A. aragakii | PPRI 123576 | South Africa | *Anoda cristate*, leaf spot | KJ718110 | KJ717963 | KJ718630 | KJ718458 | KJ718284 |
| Porri   | A. argyroxiphii | CBS 117222 T | USA | *Argyroxiphium edulis*, leaf spot | KJ718111 | KJ717964 | KJ718631 | KJ718459 | KJ718285 |
| Porri   | A. azadirachtae | CBS 116444 T | Australia | *Azadirachta indica*, leaf spot | KJ718112 | JQ646350 | KJ718632 | KJ718460 | KJ718286 |
| Porri   | A. bataticola | CBS 531.63 T | Japan | *Ipomoea batatas* | KJ718115 | KJ717967 | KJ718635 | KJ718463 | KJ718289 |
| Porri   | A. blumeae | CBS 113764 T | Thailand | *Blumea aurita* | KJ718117 | JQ646349 | JQ646333 | KJ718465 | KJ718291 |
| Porri   | A. calendulae | CBS 224.76 T | Germany | *Calendula officinalis*, leaf spot | KJ718127 | KJ717977 | KJ718648 | KJ718475 | KJ718301 |
| Porri   | A. calendulae | CBS 101498 | New Zealand | *Calendula officinalis*, leaf spot | KJ718128 | KJ717978 | KJ718645 | KJ718476 | KJ718302 |
| Porri   | A. carthami | CBS 116439 T | New Zealand | *Rosa sp.*, leaf spot | KJ718129 | KJ717979 | KJ718646 | KJ718477 | KJ718303 |
| Porri   | A. carthamicola | CBS 116650 R | Japan | *Calendula officinalis*, leaf spot | KJ718130 | KJ717980 | KJ718647 | KJ718478 | KJ718304 |
| Porri   | A. carthamini | CBS 117091 R | USA | *Carthamus tinctorius*, leaf spot | KJ718134 | KJ717984 | KJ718652 | KJ718308 | KJ718308 |
| Section | Species        | Strain     | Locality       | Substrate                                      | ITS       | GAPDH    | Alt a 1 | TEF1      | RPB2      |
|---------|----------------|------------|----------------|------------------------------------------------|-----------|----------|---------|-----------|-----------|
| Porri   | A. cassiae     | CBS 116119 T | Malaysia       | Sauropus androgynus                            | KJ718136  | KJ717986 | KJ71865  | KJ718484  | KJ718310  |
| Porri   | A. catananches | CBS 137456 T | Netherlands    | Catananche caerulea                            | KJ718139  | KJ717989 | KJ718657 | KJ718487  | KJ718313  |
| Porri   | A. centaureae  | CBS 116446 T | USA            | Centaurea solstitialis, leaf spot              | KJ718140  | KJ717990 | KJ718658 | KJ718488  | KJ718314  |
| Porri   | A. cichorii    | CBS 102.33 T | Cyprus         | Citrullus vulgaris, fruit                       | KJ718141  | KJ717991 | KJ718659 | KJ718489  | KJ718315  |
| Porri   | A. cirsinoxia  | CBS 113261 T | Canada         | Datura stramonium, leaf spot                   | KJ718143  | KJ717993 | KJ718661 | KJ718491  | KJ718317  |
| Porri   | A. citrullicola | CBS 103.32 T | Cyprus         | Datura stramonium, leaf spot                   | KJ718144  | KJ717994 | KJ718665 | KJ718495  | KJ718320  |
| Porri   | A. coreopsidis | YZU 161159 | China          | Coreopsis basalisis, leaf                      | KJ718145  | KJ717997 | KJ718668 | KJ718501  | KJ718326  |
| Porri   | A. crassa      | CBS 110.38 T | Cyprus         | Luffa acutangula                               | KJ718147  | KJ717997 | KJ718665 | KJ718495  | KJ718320  |
| Porri   | A. cucumerina  | CBS 116114 T | USA            | Luffa acutangula                               | KJ718149  | KJ717997 | KJ718665 | KJ718495  | KJ718320  |
| Porri   | A. cyamopsidis | CBS 117219 R | USA            | Cucumis melo, leaf spot                         | KJ718151  | KJ718000 | KJ718668 | KJ718501  | KJ718326  |
| Porri   | A. dauci       | CBS 112590 R | USA            | Cucumis melo, leaf spot                         | KJ718152  | GQ180072 | GQ180088 | KJ718500  | KJ718325  |
| Porri   | A. deserticola | CBS 116114 T | USA            | Cucumis melo, leaf spot                         | KJ718153  | KJ718000 | KJ718668 | KJ718501  | KJ718326  |
| Porri   | A. echinacea   | YZU 161160 T | China          | Coreopsis basalisis, leaf                      | KJ718154  | KJ718001 | KJ718669 | KJ718502  | KJ718327  |
| Porri   | A. grandis     | CBS 116695 R | New Zealand    | Coreopsis basalisis, leaf                      | KJ718155  | KJ718001 | KJ718669 | KJ718502  | KJ718327  |
| Porri   | A. ipomoeae    | CBS 219.79 T | Ethiopia       | Coreopsis basalisis, leaf                      | KJ718156  | KJ718001 | KJ718669 | KJ718502  | KJ718327  |
| Porri   | A. jesenskae   | CBS 113855 T | New Zealand    | Coreopsis basalisis, leaf                      | KJ718157  | KJ718001 | KJ718669 | KJ718502  | KJ718327  |
| Porri   | A. linariae    | CBS 105.41 T | USA            | Coreopsis basalisis, leaf                      | KJ718158  | KJ718001 | KJ718669 | KJ718502  | KJ718327  |
| Porri   | A. passiflora  | CBS 630.93 T | USA            | Coreopsis basalisis, leaf                      | KJ718159  | KJ718001 | KJ718669 | KJ718502  | KJ718327  |
| Porri   | A. pseudorostrata | CBS 116333 T | New Zealand    | Coreopsis basalisis, leaf                      | KJ718210  | KJ718210 | KJ718718 | KJ718556  | KJ718383  |
| Porri   | A. pipionipisi | CBS 116115 T | India          | Coreopsis basalisis, leaf                      | KJ718211  | KJ718211 | KJ718718 | KJ718557  | KJ718384  |
| Porri   | A. porri       | CBS 116699 T | USA            | Coreopsis basalisis, leaf                      | KJ718212  | KJ718212 | KJ718718 | KJ718557  | KJ718384  |
| Porri   | A. proteenta   | CBS 116437 T | New Zealand    | Coreopsis basalisis, leaf                      | KJ718220  | KJ718220 | KJ718718 | KJ718557  | KJ718384  |
| Porri   | A. scorzonerae | CBS 117366 T | USA            | Scorzonera hispatica, leaf                     | KJ718229  | KJ718229 | KJ718736 | KJ718575  | KJ718402  |
| Porri   | A. sennae      | CBS 477.81 T | India          | Scorzonera hispatica, leaf                     | KJ718230  | KJ718230 | KJ718736 | KJ718575  | KJ718402  |

**Table 1.** Cont.
Table 1. Cont.

| Section | Species           | Strain    | Locality     | Substrate                  | ITS         | GAPDH       | Alt a 1      | TEF1          | RPB2         |
|---------|------------------|-----------|--------------|----------------------------|-------------|-------------|--------------|---------------|--------------|
| Porri   | A. sesami        | CBS 115264 R | India        | Sesamum indicum, seedling  | JF780939    | KJ718061    | KJ718738     | KJ718577      | KJ718405     |
| Porri   | A. sidae         | CBS 117730 T | Kiribati     | Sida fallax, leaf spot     | KJ718232    | KJ718062    | KJ718739     | KJ718578      | KJ718406     |
| Porri   | A. silybi        | CBS 134092 T | Russia       | Silybum marianum, leaf     | KJ718233    | KJ718063    | KJ718740     | KJ718579      | KJ718407     |
| Porri   | A. solani        | CBS 134093 T | Russia       | Silybum marianum, leaf     | KJ718234    | KJ718064    | KJ718741     | KJ718580      | KJ718408     |
| Porri   | A. solani-niger  | CBS 109157 R | USA          | Solanum tuberosum, leaf    | KJ718238    | KJ718065    | KJ718742     | KJ718581      | KJ718409     |
| Porri   | A. steviae       | CBS 117362 T | Japan        | S. rebaudiana, leaf spot   | KJ718247    | KJ718075    | KJ718753     | KJ718593      | KJ718422     |
| Porri   | A. tagetica      | CBS 117217 R | USA          | Tagetes sp., leaf spot     | KJ718252    | KJ718079    | KJ718758     | KJ718598      | KJ718427     |
| Porri   | A. tagetica      | CBS 297.79 T | UK           | Tagetes sp., seed          | KJ718253    | KJ718080    | KJ718759     | KJ718599      | KJ718428     |
| Porri   | A. tagetica      | CBS 298.79 T | UK           | Tagetes sp., seed          | KJ718254    | KJ718081    | KJ718760     | KJ718600      | KJ718429     |
| Porri   | A. tagetica      | CBS 480.81 R | USA          | Tagetes erecta, leaf spot  | KJ718255    | KJ718082    | KJ718762     | KJ718601      | KJ718430     |
| Porri   | A. thunbergiae   | CBS 116331 T | Australia    | Thunbergia alata, leaf spot| KJ718257    | KJ718084    | KJ718764     | KJ718603      | KJ718432     |
| Porri   | A. tillandsiae   | CBS 116116 T | New Zealand  | Tillandsia usneoides, leaf | KJ718260    | KJ718087    | KJ718767     | KJ718606      | KJ718435     |
| Porri   | A. tropica       | CBS 631.93 T | USA          | Passiflora edulis, fruit   | KJ718261    | KJ718088    | KJ718768     | KJ718607      | KJ718436     |
| Porri   | A. venezuelensis | CBS 116121 T | Venezuela    | Phacelia vulgaris, leaf     | KJ718263    | KJ718263    | KJ718770     | KJ718609      | KJ718438     |
| Porri   | A. zinniae       | CBS 117223 R | New Zealand  | Zinnia elegans, leaf spot  | KJ718270    | KJ718096    | KJ718777     | KJ718616      | KJ718445     |
| Porri   | A. zinniae       | CBS 118.44 T | Hungary      | Callistephus chinensis, seed| KJ718264    | KJ718643    | KJ718771     | KJ718610      | KJ718439     |
| Porri   | A. zinniae       | CBS 117.59 T | Italy        | Zinnia elegans              | KJ718266    | KJ718092    | KJ718773     | KJ718612      | KJ718441     |
| Porri   | A. zinniae       | CBS 299.79 T | UK           | Zinnia sp., seed           | KJ718268    | KJ718094    | KJ718775     | KJ718614      | KJ718443     |
| Gypsophilae | A. gypsophilae  | CBS 107.41 T | Netherlands  | Gypsophila elegans, seed   | KJ718270    | KJ718096    | KJ718777     | KJ718616      | KJ718445     |

Note: The bold indicate the newly generated sequences. T, ex-type strain; R, representative strain.

3. Results

In the present study, large-spored Alternaria species associated with Compositae leaf spot in China since a survey from 2015 are summarized based on the phylogenetic analysis of GAPDH and RPB2 gene fragments (Figure S1 and Table S1). A total of 13 species including the present five new taxa revealed in four sections of Helianthiinficientes (A. helianthiinficiens), Porri (A. calendulae, A. tagetica and A. zinniae), Sonchi (A. cinerariae and A. sonchi), and Teretispora (A. leucanthei), and one monotypic lineage (A. argyranthemi) (Figure S1). Meanwhile, a comprehensive description of the five new species in sect. Porri are described as A. anhuiensis sp. nov., A. coreopsis sp. nov., A. nanningensis sp. nov., A. neimengguensis sp. nov., and A. sulphureus sp. nov.
3.1. Phylogenetic Analysis

The multi-gene phylogeny was constructed to determine the accurate positions of the new Alternaria based on five sequence loci (ITS + GAPDH + Alt a 1 + TEF1 + RPB2) (Table 1). The analysis comprised sequences of the ITS (504 characters), GAPDH (526 characters), Alt a 1 (457 characters), TEF1 (342 characters), and RPB2 (672 characters) gene regions with a total length of 2501 characters. The tree topologies (Figure 1) computed from the ML and BI analyses, were similar to each other, resulting in identical species-clades and the ML topology was presented as basal tree. The present strains fell into five separate branches in sect. Porri of Alternaria. Strain YZU 171206 was sister to A. alternariacida supported with a PP value of 1.0, which close to A. silybi with low BS and PP values surpport. Strains YZU 161159 and YZU 161160 formed an independent clade (BS/PP = 100%/1.0). Strain YZU 171523 fell into an individual branch close to A. obtecta and A. tillandsiae well-supported by 97%/0.99 (BS/PP). Strain YZU 171784 was clustered with A. cirsinoxia, A. centaureae, A. cichorii, and A. cantannaches supported by values of 79%/1.0 (BS/PP). Strain YZU 191448 was out group of strain YZU 171206, A. silybi and A. alternariacida with BS and PP values below 60% and 0.6. The results indicated that the five branches represent five new species from three different hosts (Coreopsis basalis, Cosmos sulphureus, and Lactuca seriola).

3.2. Taxonomy

**Alternaria anhuiensis** H. Luo and J.X. Deng, sp. nov. (Figure 2).
MycoBank No: 844033.
**Etymology:** Named after the collecting locality, Anhui Province.
**Typification:** China, Anhui Province, Hefei City, from leaf spot of Coreopsis basalis. June, 2017, J.X Deng, ex-type culture YZU 171206.
**Description:** Colonies on PDA circular, buff in the centre, flocculent with brown halo at the edge; reverse crimson pigment at centers, light yellow at margins, 59–60 mm in diam, at 25 °C for 7 days. On V8A, conidiophores arising from substrate or lateral of aerial hyphae with geniculate conidiogenous loci at or near apex, straight or curved, smooth-walled, septate, pale to dark brown, (40–) 60–145 (–203) × (4.5–) 5–7.5 (–8) µm; conidia solitary, long-narrow ovoid or ellipsoid body, apex rounded, base narrow, smooth-walled, single to double beak, dark brown, 61–100 (–111.5) × (11.5–) 13–19.5 µm, 6–11 transverse septa, 0–1 (–2) longitudinal septa; beak long-narrowed filiform, 1-beak, (32–) 58–133 (–150.5) × 2.5–4 (–4.5) µm; 2-beak, (22–) 60.5–90.5 (–116.5) × 2.5–3.5 µm. On PCA, conidiophores straight or curved, smooth-walled, septate, (42.5–) 50–140 × 4.5–6.5 (–9) µm; conidia solitary, long-narrow ovoid or ellipsoid body, single to double beak, triple or quadruple beaks not common, black brown, (55–) 66–105 × 11–16 µm, 5–10 (–11) transverse septa, 0–1 longitudinal septum; beak long-narrowed filiform, 1-beak, 95–217 (–236) × 2.5–4 (–5.5) µm; 2-beak, 60–140 × 2.5–3.5 µm; 4-beak (n = 1), 82 × 3 µm.

**Notes:** Phylogenetic analysis of the species based on a combined dataset of ITS, GAPDH, Alt a 1, TEF1, and RPB2 gene fragments falls in an individual clade close to A. alternariacida and A. silybi in sect. Porri (Figure 1). Morphologically, its primary conidiophores can generate geniculate conidiogenous loci at or near apex which differed from those two species (Figure 2, Table 2). It can be easily distinguished from A. alternariacida by producing more transverse septa and shorter beaks. Moreover, its conidia are solitary while A. alternariacida forms solitary or in unbranched chains of 2 (–3) conidia.

**Alternaria coreopsidis** H. Luo and J.X. Deng, sp. nov. (Figure 3).
MycoBank No: 844034.
**Etymology:** Named after the host genus name, Coreopsis.
**Typification:** China, Shaanxi Province, Xian City, from leaf spot of Coreopsis basalis. June, 2016, J.X Deng, ex-type culture YZU 161160.
Figure 1. Maximum likelihood (ML) phylogram of new five Alternaria species from the Compositae family based on a combined dataset of ITS, GAPDH, Alt a 1, TEF1, and RPB2 gene sequences. The RAxML bootstrap support values >60% (ML) and Bayesian posterior probabilities >0.6 (PP) are given at the nodes (ML/PP). The present strains are in bold.

Description: Colonies on PDA circular, buff halo in the centre, villiform with white at the edge; reverse dark brown at centers, vinaceous buff pigment at margins, 47–48 mm in diam, at 25 °C for 7 days. On V8A, conidiophores arising from substrate or lateral of aerial hyphae, solitary, simple, straight to slightly curved, septate, pale to dark brown, apical conidiogenous locus, pale brown, (34–) 50–86 (–115.5) × 5–7 (–9) µm; conidia solitary or in unbranched chains of 2 conidia, long-narrow ovoid or ellipsoid body, smooth-walled, single beak, yellow or brown, (48.5–) 55–80 (–85) × (9–) 10–15 µm, 6–9 transverse septa,
0–1 longitudinal septa; beak filamentous, 1-beak, (20–) 30–140 (–206) × (2–) 2.5–4 µm; normally, false beak swollen at the apex, around 8–10.5 (–14) × 4.5–5 (–6) µm. On PCA, conidiophores straight or curved, smooth-walled, septate, (24–) 50–90 (–135) × 5–7.5 (–9) µm; conidia long-narrow ovoid or ellipsoid body, apex rounded, single beak, pale brown, (40–) 45–70 × 9–13 µm, (5–) 6–8 (–9) transverse septa, 0–1 longitudinal septa; beak filamentous, 1-beak, (0–) 15–100 (–175) × (0–) 2–4 µm; swollen apex of false beak commonly 10–13 (–16.5) × 5–6 (–6.5) µm.

Figure 2. Morphology of Alternaria anhuiensis sp. nov. (A, B) Natural symptoms of Coreopsis basalis; (C, D) Colony phenotypes (on PDA for 7 days at 25 °C); (E, F) Sporulation patterns (on V8A at 22 °C); (G, H) Conidiophores (on V8A at 22 °C); (I) Conidia (on V8A at 22 °C); (J) Conidia (on PCA at 22 °C). Bars: (E–J) = 25 µm.

Figure 3. Morphology of Alternaria coreopsidis sp. nov. (A, B) Natural symptoms of Coreopsis basalis; (C, D) Colony phenotypes (on PDA for 7 days at 25 °C); (E) Sporulation patterns (on V8A at 22 °C); (F, G) Conidiophores (on V8A at 22 °C); (H) Conidia (on V8A at 22 °C); (I) Conidia (on PCA at 22 °C). Bars: (E–I) = 25 µm.
Table 2. Morphological comparisons of the five new *Alternaria* species and their closely related species.

| Species Strain          | Conidia Shape                      | Size (µm) | Transverse septa | Beak (µm) | Sporulation Pattern                  | Medium     | Reference       |
|-------------------------|------------------------------------|-----------|-----------------|-----------|--------------------------------------|------------|-----------------|
| *A. alternariacida* CBS 105.51 | Smooth-walled, narrowly ovoid; smooth-walled | (85–) 99–111 | (3–) 5–6 (–) | (47–) 129–257 | Solitary or in unbranched chains of 2 (–) conidia | SNA        | [4]             |
| *A. anhuiensis* sp. nov. YZU 171206 | Long-narrow ovoid or ellipsoid; smooth-walled | 61–100 (–111.5) × (11.5–) 13–19.5 | 6–11 | (22–) 58–133 | Solitary | V8A | This study |
| *A. catananches* CBS 137456 | Narrowly ovoid, ornamented in lower half of the conidium | (26–) 37–43 (–57) × (7–) 8–9 (–11) | (2–) 4 (–) | (77–) 126–160 | Solitary | SNA | [4]             |
| *A. centaureae* CBS 116446 | Long-narrow-ellipsoid or long-ovoid; smooth-walled | 75–100 × 15–24 | 7–10 | 140–190 | Solitary | V8A | [2]             |
| *A. chinensis* CBS 117218 | Narrow-ovoid or narrow-ellipsoid; smooth-walled | 60–80 × 14–18 | 7–12 | 120–240 | Terminal clumps of 4–5 conidia | V8A        | [2]             |
| *A. cirrata* CBS 113261 | Long-narrow-ellipsoid or ellipsoid; smooth-walled | 70–90 × 12–22 | 7–9 | 80–165 | Solitary or tufts of 2–7 conidia | V8A        | [2]             |
| *A. coreopidis* sp. nov. YZU 161160 | Long-narrow ovoid or ellipsoid; smooth-walled | (48.5–) 55–80 (–85) × (9–) 10–15 | 6–9 | (20–) 30–140 | Solitary or 2-conidium chains | V8A        | This study |
| *A. nanningensis* sp. nov. A. neimenggensis sp. nov. YZU 171513 | Ovoid or ellipsoid; smooth-walled | (40.5–) 47–79 (–87) × 9–13.5 (–13) | 6–10 (–11) | 10–30 (1–) | Solitary | V8A | This study |
| *A. obtecta* CBS 134278 | Long-narrow-ellipsoid or ellipsoid; smooth or punctulate-walled | 65–95 × 18–22 | 7–10 | 55–150 | Solitary | PCA | [2]             |
| *A. porri* CBS 116698 | Long-ovoid, sometimes broad or nearly cylindrical; smooth or punctulate-walled | 70–105 × 19–24 | 8–12 | 95–160 (2–) 2–6.5 | Solitary | V8A | [2]             |
| *A. silphi* CBS 134093 | Long-ellipsoid, subcylindrical or long-ovoid; smooth or punctulate-walled | 50–80 × 15–20 (–22) | (5–) 7–10 | 70–130 (–190) × 3 | Solitary | V4A | [40]           |
| *A. stenae* CBS 117362 | Long-ovoid, subellipsoid, or obovoid; smooth or punctulate-walled | 55–95 × 18–30 | 7–10 | 60–120 | Solitary or tiny distal clumps | V8A        | [2]             |
| *A. sulphureus* sp. nov. YZU 191448 | Ovoid, ellipsoid, or obovoid; smooth-walled | (64–) 74–116 (12.5–) 14–20 (–28.5) | (5–) 7–11 | (25.5–) 34–151 (–159.5) × 2.5–4.5 | Solitary | V8A | This study |
| *A. tillandsiae* CBS 116116 | Long-ovoid, ellipsoid, long-ovoid; smooth or a minor punctulate-walled | 70–102 × 16–19 | 8–11 | 75–120 | Solitary | V8A | [2]             |

Materials examined: China, Shaanxi Province, Xian City, from leaf spot of *Coreopsis basalis*. June 2016, J.X Deng, living culture YZU 161159.

Notes: Phylogenetically, the species falls into an independent lineage outside of a clade comprising type species of *A. porri* of sect. *Porri* (Figure 1). It can be delimited based on either of GAPDH and RPB2 gene sequences (Figure S1). The species is characterized by producing conidia with false beak swollen at the apex up to 8–13 (–16.5) × 4.5–6.5 µm (Figure 3; Table 2).

*Alternaria nanningensis* H. Luo and J.X. Deng, sp. nov. (Figure 4).
Figure 4. Morphology of *Alternaria nanningensis* sp. nov. (A,B) Natural symptoms of *Cosmos sulphureus*; (C,D) Colony phenotypes (on PDA for 7 days at 25 °C); (E,F) Sporulation patterns (on V8A at 22 °C); (G) Conidiophores (on V8A at 22 °C); (H) Conidia (on V8A at 22 °C); (I) Conidia (on PCA at 22 °C). Bars: (E–J) = 25 µm.

**MycoBank No**: 844035.

**Etymology**: Named after the collecting locality, Nanning City.

**Typification**: China, Guangxi Province, Nanning City, from leaf spot of *Cosmos sulphureus*. July 2017, J.X. Deng, ex-type culture YZU 171523.

**Description**: Colonies on PDA irregular, pistac, entire; reverse dark olive green, slightly protuberant with white at margins, 56–57 mm in diam, at 25 °C for 7 days. On V8A, conidiophores arising from substrate or lateral of aerial hyphae with geniculate conidiogenous loci at apex, straight or curved, smooth-walled, septate, pale brown, 38–59 (–64) × 4–5 (–6) µm; conidia solitary, ovoid or ellipsoid body, base narrow, smooth-walled, single beak, pale to yellow brown, (40.5–) 47–79 (–87) × 9–13.5 (–15) µm, 6–10 (–11) transverse septa, 0–1 longitudinal septa; beak long-narrowed filiform, 1-beak, 10–30 × (1–) 1.5–2 (–3) µm. On PCA, conidiophores straight or curved, smooth-walled, septate; 32–70 (–86) × 4–5.5 µm; conidia solitary, ovoid or ellipsoid body, single beak, pale to yellow brown, (49–) 55–77 (–82) × 10.5–13.5 (–15) µm, 5–9 (–10) transverse septa, 0–1 longitudinal septum; beak long-narrowed filiform, 1-beak, 13–26 (–44) × 1.5–2 (–2.5) µm.

**Notes**: The species is phylogenetically recognized as a distinct species in sect. *Porri* based on ITS, GAPDH, Alt a 1, TEF1, and RPB2 which displays a close relationship with *A. obtecta*, *A. tillandsiae*, and *A. steviae* (Figure 1). Compared with them, it is quite different by producing smaller conidia with short beaks (Figure 4; Table 2). Furthermore, its conidia are smooth-walled while some conidia of *A. obtecta* and *A. steviae* are minutely punctulate. *Alternaria nanningensis* forms simple conidiophores (solitary). But many conidiophores of *A. steviae* produce geniculate extensions and additional conidia, yielding tiny distal clumps of sporulation.

**Alternaria neimengguensis** H. Luo and J.X. Deng, sp. nov. (Figure 5).

**MycoBank No**: 844036.

**Etymology**: Named after the collecting locality, Inner Mongolia Autonomous Region.

**Typification**: China, Inner Mongolia Autonomous Region, Inner Mongolia Agricultural University, IMAU, from leaf spot of *Lactuca seriola*. September 2017, J.X. Deng, ex-type culture YZU 171784.
**Figure 5.** Morphology of *Alternaria neimengguensis* sp. nov. (A) Natural symptoms of *Lactuca seriola*; (B,C) Colony phenotypes (on PDA for 7 days at 25 °C); (D,E) Sporulation patterns (on V8A at 22 °C); (F) Conidiophores (on V8A at 22 °C); (G) Conidia (on V8A at 22 °C); (H) Conidia (on PCA at 22 °C). Bars: (D–H) = 25 µm.

**Description:** Colonies on PDA circular, pale brown en masse, flocculent, reverse dark olive green at centers, pale brown at margins, 51–54 mm in diam, at 25 °C for 7 days. On V8A, conidiophores arising from substrate or lateral of aerial hyphae, straight or curved, smooth-walled, septate, brown, 26–45 (–51) × 5–7 (–8) µm; conidia solitary, ovoid or ellipsoid body, apex rounded, base wide, smooth-walled, single to double beak, brown, (70–) 77–130 (–143.5) × (13–) 15–20 (–23) µm, 6–11 (–12) transverse septa, 0–1 (–2) longitudinal septa; beak long-narrowed filiform, 1-beak, (24.5–) 35–65 (–76) × (1.5–) 2–3 (–4) µm; 2-beak, (33–) 45–65 (–92) × (2–) 2.5–3 (–3.5) µm. On PCA, conidiophores straight or curved, smooth-walled, septate; 35–70 (–75) × 5–6.5 (–7.5) µm; conidia solitary, ovoid or ellipsoid body, apex rounded, single to double beak, pale to yellow brown, (59–) 66–104 (–120.5) × 13–18 (–20) µm, (5–) 6–10 (–11) transverse septa, 0–1 (–2) longitudinal septa; beak long-narrowed filiform, 1-beak, (13–) 31.5–60 (–93) × 1.5–3 µm; 2-beak, (12–) 26–53 (–80) × 1.5–2.5 (–3) µm.

**Notes:** In the phylogeny, the species is sister to *A. cirsinoxia*, *A. centaureae*, *A. cichorii*, and *A. catananches* (Figure 1). The conidiophores are distinct to *A. cirsinoxia* whose are 2–3 arm branches near a conidiophore tip and progressively geniculate, yielding tufts of several conidia. They are different from *A. cichorii* whose are frequently branch or proliferate in a geniculate manner near the apex, yielding terminal clumps of 4–5 conidia. In conidial morphology, it is obviously different from those four species by producing larger conidia (Table 2).

*Alternaria sulphureus* L. Zhao and J.X. Deng, sp. nov. (Figure 6).
Figure 6. Morphology of *Alternaria sulphureus* sp. nov. (A,B) Natural symptoms of *Cosmos sulphureus*; (C,D) Colony phenotypes (on PDA for 7 days at 25 °C); (E,F) Sporulation patterns (on PCA at 22 °C); (G) Conidiophores (on PCA at 22 °C); (H) Conidia (on V8A at 22 °C); (I) Conidia (on PCA at 22 °C). Bars: (E–I) = 25 μm.

**Mycobank No:** 844037.

**Etymology:** Named after the host species name, *Cosmos sulphureus*.

**Typification:** China, Shanxi Province, from leaf spot of *Cosmos sulphureus*. September 2019, J.X Deng, ex-type culture YZU 191448.

**Description:** Colonies on PDA circular, light brown in the centre, buff texture velutinous at the edge, reverse black brown at centers, 62–63 mm in diam, at 25 °C for 7 days. On V8A, conidiophores arising from substrate, solitary, simple, straight to slightly curved, septate, apical conidiogenous locus, pale brown; (50–) 63–100 (–108) × 6–8 (–9) μm; conidia solitary, sometimes in chains of two conidia, ovoid, ellipsoid or obovoid body, smooth-walled, pale to yellow, (64–) 74–116 × (12.5–) 14–20 (–25.5) μm, (5–) 7–11 transverse septa, 0–1 (–2) longitudinal septa; beak long-narrowed filiform, 1-beak, (25.5–) 34–151 (–159.5) × 2.5–4.5 (–5.5) μm; 2-beak (*n* = 1), 129 × 4 μm. On PCA, conidiophores straight or curved, smooth-walled, septate, (34.5–) 40.5–56 (–85) × 5–7.5 μm; conidia ovoid, ellipsoid, or obovoid body, apex rounded, single to double beak, triple beaks not common, pale brown, 80–110 × 16–24 μm, 6–10 transverse septa, 0–1 longitudinal septum; beak long-narrowed filiform, 1-beak, (73–) 110–195 × 3–5 μm; 2-beak, (74–) 96–170 × 3–4 μm; 3-beak (*n* = 1), 109.5 × 3.5 μm.

**Notes:** This species is phylogenetically related to *A. silybi*, *A. alternariacida* and *A. anhuiensis* sp. nov. in sect. *Porri* (Figure 1). It could be distinguished from *A. silybi* and *A. alternariacida* by forming larger conidia (Figure 6; Table 2) and is quite different from *A. alternariacida* by producing multiple and shorter beaks.
4. Discussion

Thirteen large-spored *Alternaria* species associated with Compositae leaf spot in China were assigned to four sections and one monotypic lineage in this study. Among these species, five new species (*A. anhuiensis* sp. nov., *A. coreopsidis* sp. nov., *A. nanningensis* sp. nov., *A. neimengguensis* sp. nov., and *A. sulphureus* sp. nov.) were clearly recognized in section *Porri*. The section is speciose assessing encompassing 117 large-spored *Alternaria* [5]. In 2014, the section is reduced 82 morphospecies in to 63 phylogenetic species [2]. They are commonly pathogenic and could induce typical black necrotic lesions surrounded by chlorotic areas. There are some important famous plant pathogens, such as *A. porri* on *Allium* plants (Liliaceae), *A. solani* for potato (Solanaeaceae), *A. sesami* for sesame (Pedaliaceae) and *A. dauci* for carrot (Umbelliferae) [2]. Twenty-one species are comprised in sect. *Porri* associated with the Compositae family [4]. This study provides new data supplements for the *Alternaria* taxonomy of sect. *Porri*.

Morphologically, large-spored *Alternaria* species in sect. *Porri* are characterised by broadly ovoid, obclavate, ellipsoid, subcylindrical or obovoid, medium to large conidia containing multiple transverse and longitudinal septa, solitary or in short chains with a simple or branched, long to filamentous beak [4]. Among these characteristics, sporulation patterns, conidial body, transverse septa, and beak type provide useful information for the preliminary separation into sections [2]. Morphology is quite important for new fungal species identification, which can be defined based on unique morphological characters when the molecular data is not well-supported [41]. Morphological comparisons of the present new species and their relevant species in sect. *Porri* were conducted (Table 2). For the sporulation patterns, the conidia of *A. anhuiensis*, *A. nanningensis*, *A. neimengguensis*, and *A. sulphureus* are solitary produced except *A. coreopsidis*, which similar to *A. alternariacida*, *A. cichorii*, *A. cirsinoxia*, and *A. steviae* forming chain of 2 (~3) units [2,4]. In conidial morphology, *A. anhuiensis*, *A. nanningensis*, *A. neimengguensis*, and *A. sulphureus* are distinguishable from their closely related species based on the size of conidial bodies (Table 2) and also the wall ornamentations [2,4]. On the other hand, *A. anhuiensis*, *A. neimengguensis*, and *A. sulphureus* are readily be distinguished by producing multiple beaks. By the way, there are no significant differences on conidial morphology of PCA and V8A medium for all species.

In addition, morphological variation and fundamental pleomorphism complicate the *Alternaria* species recognition, and host plants reflect some evidences for the identification [3]. With the discovery of *Alternaria* species, it has been found from various plants of Compositae [1,4,21,42,43]. *Alternaria calendulae* has been reported from *Calendula officinalis* in Czech Republic [2], Germany [4], Japan [4], and Korea [44]. It also is found on *C. officinalis* in China and firstly on *Brachyactis ciliata* in the study. *Alternaria leucanthemi* has previously been found on *Chrysanthemum maximum* from Netherlands [1] and *Helianthus annuus* from China [45]. It is firstly isolated from *Carthamus tinctorius* in this study. In addition, *A. tagetica* is commonly associated with *Tagetes* plants (*Tagetes erecta* and *Tagetes patula*) [3,4,46–48], which firstly encountered from *Calendula officinalis* in this study. Interestingly, the five new species are isolated from three different composite hosts (*Coreopsis basalis*, *Cosmos sulphureus*, and *Lactuca seriola*) and *A. cinerariae* are found on five different composite plants in China (Figure S1; Table S1). The results suggest that an *Alternaria* species may associated with several host plants.

5. Conclusions

The present data indeed revealed a diversity of large-spored *Alternaria* associated with Compositae plants in China. A total of 13 large-spored *Alternaria* species were obtained and circumscribed as eight known species and five new species belonging to the four sections of *Helianthinficientes*, *Porri*, *Sonchi*, and *Teretispora*, and one monotypic lineage (*A. argyranthemi*) based on the morphological characteristics and molecular properties of multiple DNA sequences (ITS, GAPDH, Alt a 1, TEF1, and RPB2). *Alternaria calendulae*, *A. leucantheri*, and *A. tagetica* were firstly isolated from *Brachyactis ciliata*, *Carthamus tinctorius*, and *Calendula officinalis* in China, respectively. Since large-spored *Alternaria* species are
almost demonstrated phytopathogens, further study on the pathogenicity is needed to verify in the future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jof8060607/s1, Figure S1: Phylogenetic tree of large-spored Alternaria from the Compositae family in China using a maximum likelihood (ML) analysis based on combined GAPDH and RPB2 gene sequences. The RAxML bootstrap support values > 60% (ML) and Bayesian posterior probabilities >0.6 (PP) are given at the nodes (ML/PP); Table S1: The other Alternaria species associated with the Compositae plants from China analyzed by phylogeny.

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