**ABSTRACT**

A new species of *Alternaria* causing leaf spots on the rubber tree (*Hevea brasiliensis*) in Yunnan, China, was isolated, examined, and illustrated. Morphologically, it belongs to the section *Porri* of *Alternaria*, which produces relatively large conidia and a simple or branched, filamentous long beak. It is, however, characterized by conidiophores gradually enlarging near the apex into a clavate conidiogenous cell and long ellipsoid to obclavate, smooth-walled conidia with a long filamentous beak. Molecular phylogenetic analyses based on ITS rDNA, GAPDH, and TEF1-alpha sequences demonstrate that the phytopathogen falls in the clade of the section *Porri*, being most closely related to *A. sidae*, *A. sennae*, *A. deserticola*, *A. cyamopsidis*, *A. rostellata*, *A. nitrimali*, *A. thunbergiae*, *A. solani*, and *A. tomatophila*. *A. porri* causes purple blotch of onion [6], *A. bataticola* causes leaf petiole and stem blight of sweet potato [7], *A. solani* is the causative agent of early blight of potato [8], and *A. tomatophila* is known for causing early blight of tomato [9].

Earlier Martin [10] reported *Alternaria* sp. infection on *Hevea brasiliensis* in Mexico. In 2006, another *Alternaria* leaf blight with similar symptom on rubber trees caused by *A. alternata* was identified by Roy et al. [11] in India. Our research group reported that *A. heveae* and *A. alternata* were able to incite black leaf spot of rubber tree in China [12,13]. The disease symptoms initially appear as minute spots on leaves. The spots are circular with black center surrounded by a yellow halo, and lesions slightly sunken.

During a survey on rubber tree diseases, a fungus isolated from rubber tree leaf spot lesions was morphologically identified to be an *Alternaria* species in the section *Porri* but which did not fit any known species of this genus and further confirmed molecularly by grouping within the section of *Porri*. Herein, we propose this new species with morphological description, illustration, and molecular phylogeny.

**1. Introduction**

The *Alternaria* is saprobic and ubiquitous, and some species are well-known as major plant pathogens. They can also cause many human health disorders, while many can play an important role in decay and decomposition [1]. *Alternaria* was divided into 24 sections on the basis of morphological and multi-loci molecular phylogeny (18S, ITS and 28S rDNA, GAPDH, RPB2, and TEF1-alpha) [2–5]. The section *Porri* is the largest *Alternaria* section with species characterized by medium to large conidia with a simple or branched, filamentous long beak [2,3]. This section includes some important phytopathogens, such as *A. porri*, *A. bataticola*, *A. solani*, and *A. tomatophila*. *A. porri* causes purple blotch of onion [6], *A. bataticola* causes leaf petiole and stem blight of sweet potato [7], *A. solani* is the causative agent of early blight of potato [8], and *A. tomatophila* is known for causing early blight of tomato [9].

**2. Materials and methods**

**2.1. Isolation and cultures**

Fungus-infected leaves of the rubber tree were collected in May 2014 from Hula village, Ruili city, Yunnan Province, China. Segmented leaf lesions between symptomatic and healthy tissue were surface sterilized with 70% ethanol for 2 min and then air-dried, plated on potato dextrose agar (PDA; 20 g white potato boiled and filtered, 20 g dextrose, 15 g agar, and 1 L distilled water), and incubated at 28 °C with natural day/night cycles of light for 5–7 days. Single spores were isolated by following the method of Ho and Ko [14] and cultured and transferred onto a new PDA plate to establish pure cultures. Isolates were maintained at the Herbarium of Department of Plant Pathology (YITC 5109 and YITC...
5114), Yunnan institute of Tropic Crops. Ex-type was stored in the Agricultural Culture Collection of China, Institute of Plant Protection, Chinese Academy of Agricultural Sciences (ACCC 39327) and also in the China General Microbial Culture Collection, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (CGMCC3.1890). The type was deposited in the Herbarium Mycologicum Academiae Sinicæ, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS247784).

2.2. Pathogenicity assay
Pathogenicity assays using a field rubber tree inoculation method were conducted as previously described by Cai et al. [13]. Fungal conidia were harvested by flooding 7-day-old cultures with sterile water from single conidial cultures, centrifuging, and adjusting the concentration to $1 \times 10^4$ conidia/ml, and spraying the spore suspension to the copper-color leaves (approximately 5–7 cm in length) of rubber tree using manual pressure sprayer. Rubber plants sprayed with sterile distilled water were used as controls. After inoculation, the plants were covered with plastic bags for 48 h. The plastic bags were removed after 2 days postinoculation and monitored daily for symptom development.

2.3. Morphological observations
The isolate YITC 5109 was used for morphological descriptions. Colonial characteristics (color, size, and texture) were assessed after 7 days of growth on the PDA plates and V-8 juice agar (V-8; 100 mL V-8 juice, 0.2 g CaCO₃, 20 g agar, and 900 mL distilled water) at 25°C under a fluorescent light/dark cycle of 8/16 h. Conidial morphology was examined according to the method of Simmons [15,16]. Isolates were inoculated on potato carrot agar (PCA; 20 g white potato boiled and filtered, 20 g carrot, and 20 g agar in 1 L distilled water) and V-8 juice agar. The plates were stored at 22°C for 7 days in a chamber without humidity control (a gradually drying atmosphere in unsealed plates). A total of 100 conidia were randomly chosen and measured by using an OLYMPUS CH30RF200 light microscope (Olympus, Tokyo, Japan) with a Moticam 5000 digital camera (Motic Deutschland GmbH, Wetzlar, Germany).

2.4. DNA extraction, amplification and sequencing
Isolates were grown on PDA for 5–7 days and then collected for DNA extraction. Genomic DNA was extracted following a previously described method [17] with some modifications. The nuclear ribosomal internal transcribed spacer of ribosomal DNA (ITS rDNA) region was amplified with the universal primers ITS1 and ITS4 [18], the glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) with the primers gpd1 and gpd2 [19], the TEF1-alpha gene (TEFI) with the primers EFL-728F and EF1-986R [20]. The PCR mixtures included 9.5 μL of ddH₂O, 2 μL of each primer (10 μM), 1 μL of genomic DNA, 12.5 μL of 2 × EsTaq Master Mix containing 0.5 units of EsTaq DNA Polymerase, 3 mM of MgCl₂ and 200 μM of each dNTP and 1× PCR buffer (Cwbio, Beijing, China). The PCR reactions were carried out in a Veriti 96-well thermocycler (Applied Biosystems, Carlsbad, CA). The cycling program was initiated at 94°C for 5 min, and then followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, and elongation at 72°C for 30 sec, and finalized with an extension at 72°C for 10 min. PCR products were sequenced by BGI (Beijing Genomics Institute, Beijing, China). The obtained sequences (ITS, GAPDH, and TEFI) were deposited in GenBank with the accession numbers shown in Table 1.

2.5. Phylogenetic analyses
The obtained sequences and relevant ones available in the GenBank database (Table 1) were aligned using CLUSTAL_X ver. 2.0.11 (EMBL-EBI, Cambridgeshire, UK), and adjusted manually [21]. The combined dataset of ITS, GAPDH, and TEFI gene sequences was analyzed using MEGA 6 software [22]. Maximum likelihood analysis was performed with 1000 bootstrap replicates and the General Time Reversible model of nucleotide substitution. The node reliability was assessed by no less than 70% of parsimony bootstrap support values A. gypsophilae (CBS107.41) were used as outgroups for the phylogeny analysis.

3. Results and discussion
3.1. Phylogenetic analysis
The combined dataset resulted in an alignment containing a total of 1434 characters, which comprised 583, 575, and 276 characters of ITS rDNA, GAPDH, and TEFI, respectively. Maximum likelihood analysis was used to generate the phylogenetic tree shown in Figure 1. The two isolates YITC 5109 and YITC 5114 of A. yunnanensis sp. nov. were identical in all these three genes, and formed a strongly supported clade in the phylogram, being most closely related to A. sidae, A. sennae, A. deserticola. These two species further clustered together with A. cyamopidis, A. rostellata, A. nitrimali, A. thunbergiae, and A. crassa.
| Name                     | Old name                  | Strain number | Host/Substrate                      | Locality               | GenBank accession numbers          |
|--------------------------|---------------------------|---------------|-------------------------------------|------------------------|------------------------------------|
| Alternaria acalyphicola  |                           | CBS 54194     | Acalypha indica                     | Seychelles             | KJ718097 KJ717952 KJ718446        |
| Alternaria agerati       |                           | CBS 117221    | Ageratum houstonianum               | USA, Illinois           | KJ718098 KJ717953 KJ718447        |
| Alternaria agrigestis    |                           | CBS 57794     | Euphorbia esula, stem lesion        | Canada, Saskatchewan    | KJ718099 JQ646356 KJ718448        |
| Alternaria allii         | Alternaria porti          | CBS 10728     | Allium cepa, leaf spot              | Puerto Rico             | KJ718100 KJ717954 KJ718449        |
| Alternaria alternarioidae| Alternaria vanuatuensis   | CBS 121945    | Allium cepa, leaf                   | Vanuatu                | KJ718104 KJ717958 KJ718453        |
| Alternaria solani        |                           | CBS 10551     | Solanum lycopersicum, fruit         | UK, England             | KJ718105 KJ717959 KJ718454        |
| Alternaria anagallidis   |                           | CBS 101004    | Anagallis arvensis, leaf spot       | New Zealand, Auckland   | KJ718107 KJ717960 KJ718455        |
| Alternaria anodae        |                           | PPRI 12376    | A. cepa (leaf spot)                 | South Africa, Gauteng   | KJ718108 KJ717961 KJ718456        |
| Alternaria aragakii      |                           | CBS 57793     | Passiflora edulis                   | USA, Hawaii             | KJ718110 KJ717964 KJ718459        |
| Alternaria argyroxiphii  |                           | CBS 117222    | Argyroxyphium sp.                   | USA, Hawaii             | KJ718112 JQ646350 KJ718460        |
| Alternaria azadirachiae  |                           | CBS 10551     | Ipomoea batatas, stem lesion        | South Africa, Gauteng   | KJ718113 KJ717965 KJ718461        |
| Alternaria batalicola    |                           | CBS 53163     | Ipomoea batatas                     | Australia, Queensland   | KJ718116 KJ717966 KJ718462        |
| Alternaria blumeae       | Alternaria brasilensis    | CBS 117215    | Phaeosolus vulgaris, leaf spot      | Australia, Queensland   | KJ718125 KJ717976 KJ718473        |
| Alternaria carthami      |                           | CBS 117564    | Brunera aurita                      | Australia, Queensland   | KJ718126 KJ717977 KJ718475        |
| Alternaria carthamica    |                           | CBS 116439    | Calendula officinalis, leaf spot    | Australia, Queensland   | KJ718129 KJ717979 KJ718477        |
| Alternaria cassiae       |                           | CBS 47881     | Senna obtusifolia, seedling         | Australia, Queensland   | KJ718131 KJ717981 KJ718479        |
| Alternaria caudata       | Alternaria caudata        | CBS 117092    | Sauropus androgynus                 | Australia, Queensland   | KJ718132 KJ717982 KJ718480        |
| Alternaria centaureae    |                           | CBS 116446    | Centarea solstitialis, leaf spot    | Australia, Queensland   | KJ718134 KJ717984 KJ718482        |
| Alternaria cercina       |                           | CBS 116119    | Cyamopsis tetragonoloba, leaf spot  | Australia, Queensland   | KJ718135 KJ717985 KJ718483        |
| Alternaria cerasinicii   |                           | CBS 116119    | Cyamopsis tetragonoloba, leaf spot  | Australia, Queensland   | KJ718136 KJ717986 KJ718484        |
| Alternaria conidiophora  | Alternaria cucumina       | CBS 10332     | Citrullus vulgaris, fruit            | Nepal                   | KJ718137 KJ717989 KJ718487        |
| Alternaria crassa        |                           | CBS 11033     | Datura stramonium, leaf spot        | Nepal                   | KJ718145 KJ717995 KJ718493        |
| Alternaria cucumerina    | Alternaria loofiae        | CBS 116114    | Luffa acutangula                    | Nepal                   | KJ718147 KJ717997 KJ718495        |
| Alternaria cyanopsis     |                           | CBS 36467     | Cyamopsis tetragonoloba, leaf spot  | USA, Maryland           | KJ718150 KJ718004 KJ718503        |
| Alternaria dauci         |                           | CBS 11138     | Cyamopsis tetragonoloba, leaf spot  | USA, Georgia            | KJ718157 KJ717998 KJ718505        |
| Alternaria dichardiae    |                           | CBS 117099    | Daucus carota, seed                 | Italy                   | KJ718158 KJ718005 KJ718506        |
| Alternaria deserticola   | Alternaria acalyphicola   | CBS 11974     | Dichondra repens, leaf spot         | Cyprus                  | KJ718144 KJ717994 KJ718492        |
| Alternaria echinacea     |                           | CBS 20074     | Dichondra repens, leaf spot         | Italy                   | KJ718167 KJ718012 KJ718515        |
| Alternaria echoinae      |                           | CBS 116117    | Echinacea sp., leaf lesion          | New Zealand, isborne    | KJ718170 KJ718015 KJ718518        |
| Alternaria echinacea     |                           | CBS 116118    | Echinacea sp., leaf lesion          | New Zealand, isborne    | KJ718171 KJ718016 KJ718519        |
| Name                          | Old name                          | Strain number | Host/Substrate | Locality            | ITS     | GAPDH     | TEF1     |
|-------------------------------|-----------------------------------|---------------|----------------|---------------------|---------|-----------|----------|
| *Alternaria grandis*          | CBS 109158; E.G.S. 44.106         | Solarium tuberosum, leaf spot | USA, Pennsylvania | KJ718239 | JQ646341 | EU130547 |
| *Alternaria euphorbiicola*    | CBS 188.36; E.G.S. 30.082         | Solarium tuberosum, leaf spot | USA, Pennsylvania | KJ718241 | KJ18070 | KJ18587  |
| *Alternaria gypsumophila*     | CBS 119140; E.G.S. 40.029         | Euphorbia pulcherrima | USA, Hawaii | KJ718172 | KJ18017 | KJ18520  |
| *Alternaria ipomoeae* sp. nov.| CBS 219.79                        | Ipomoea batata, stem | Ethiopia | KJ718175 | KJ18020 | KJ18523  |
| *Alternaria jeneskae*         | CBS 138855; CCM 8361              | Fumana procumbens, seed | Slovakia | KJ718177 | KJ18022 | KJ18525  |
| *Alternaria limicola*         | CBS 483.30; E.G.S. 35.090         | Citrus aurantiifolia, leaf spot | Mexico, Colima | KJ718178 | JQ646329 | KJ18526  |
| *Alternaria lineare*          | CBS 109164; E.G.S. 41.057         | Ipomoea batata, stem | USA, Indiana | KJ718184 | JQ646345 | KJ18531  |
| *Alternaria macrospora*       | CBS 117228; E.G.S. 50.190         | Ipomoea batata | New Zealand | KJ718186 | KJ18027 | KJ18533  |
| *Alternaria montanae*         | CBS 112154; E.G.S. 44.112         | Citrus arvense | USA, Montana | KJ718194 | KJ18033 | KJ18541  |
| *Alternaria multicornata*     | CBS 712868; ATCC 18515            | Richardia sagra, floral bract | USA, Georgia | KJ718195 | JQ646362 | EU130546 |
| *Alternaria novae-guineensis* | CBS 713688; ATCC 18517;           | Richardia sagra, floral bract | USA, Georgia | KJ718196 | KJ18034 | KJ18542  |
| *Alternaria nititinali*       | CBS 116163; E.G.S. 46.151         | Solarium viarum, leaf spot | Puerto Rico | KJ718201 | JQ646358 | KJ18547  |
| *Alternaria nigrisignata*     | CBS 116120; E.G.S. 47.198         | Citrus sp., dry leaf | Papua New Guinea | KJ718202 | KJ18039 | KJ18548  |
| *Alternaria obducta*          | CBS 117567; E.G.S. 42.063         | Euphorbia pulcherrima, seed | USA, California | KJ718203 | KJ18040 | KJ18549  |
| *Alternaria paradoxicola*     | CBS 116652; E.G.S. 46.113         | Linum usitatissimum, seed | Canada, Manitoba | KJ718206 | KJ18043 | KJ18552  |
| *Alternaria passiflora*       | CBS 630.93; E.G.S. 29.020         | Passiflora edulis | USA, Hawaii | KJ718210 | JQ646352 | KJ18556  |
| *Alternaria peripetioides*    | CBS 116292; E.G.S. 40.096         | Cajanus cajan, seed | New Zealand, uckland | KJ718211 | KJ18046 | KJ18557  |
| *Alternaria protracta*        | CBS 116932; E.G.S. 17.082         | Allium cepa, leaf | USA, Nebraska | KJ718217 | KJ18052 | KJ18563  |
| *Alternaria ranunculi*        | CBS 116699; E.G.S. 48.152         | Allium cepa, leaf | USA, New York | KJ718218 | KJ18053 | KJ18564  |
| *Alternaria ricini*           | CBS 21531                          | Rionus communis | Japan | KJ718226 | KJ18059 | KJ18572  |
| *Alternaria rostellata*       | CBS 117566; E.G.S. 42.061         | Euphorbia pulcherrima, leaf | USA, California | KJ718229 | JQ646332 | KJ18573  |
| *Alternaria scorzeneriae*     | CBS 103.346; Elliot No. 45-19C   | Linum usitatissimum | UK, Scotland | KJ718190 | JQ646336 | KJ18537  |
| *Alternaria sennae* sp. nov.* | CBS 116703; E.G.S. 36.110         | Linum usitatissimum, seed | UK, Derbyshire | KJ718192 | KJ18031 | KJ18539  |
| *Alternaria sesami*           | CBS 477.81; E.G.S. 34.030         | Senna corymbosa, leaf | India, Uttar Pradesh | KJ718230 | JQ646344 | EU130543 |
| *Alternaria sidae*            | CBS 117730; E.G.S. 12.129         | Sesamum indicum | India | KJ718231 | JQ646343 | KJ18576  |
| *Alternaria silbyi*           | CBS 134092; VKM F-1109            | Sesamum indicum, seedling | Russia, Vladivostok | KJ718233 | KJ18063 | KJ18579  |
| *Alternaria silybi*           | CBS 134093; VKM F-4117            | Sesamum indicum, leaf | Russia, Vladivostok | KJ718234 | KJ18064 | KJ18580  |

(continued)
| Name                  | Old name               | Strain number | Host/Substrate | Locality      | GenBank accession numbers |
|-----------------------|------------------------|---------------|----------------|---------------|--------------------------|
| Alternaria solani     | Alternaria danida      | CBS 1114; EGS 07.029 | Ageratum houstonianum, seed | Italy         | Y17070 KJ718068 KJ718584 |
| Alternaria solani-nigri | Alternaria viciae-fabae | CBS 116442; EGS 46.162 | Vicia faba | New Zealand | KJ718240 KJ718069 KJ718586 |
| Alternaria solani-nigri | Alternaria cyophomandum | CBS 109155; EGS 40.058 | Cyphomandra betacea, fruit | New Zealand, New Plymouth | KJ718242 JQ646360 KJ718588 |
| Alternaria solani-nigri | Alternaria herculiniae | CBS 116332; EGS 49.180 | Petroselinum crispum, plant | New Zealand, Taranaki | KJ718244 KJ718072 KJ718590 |
| Alternaria solani-nigri | Alternaria glyceriae   | CBS 116334; EGS 51.107 | Glyceria maxima, leaf spot | New Zealand, Waikato | KJ718245 KJ718073 KJ718591 |
| Alternaria steviae     | CBS 63138; IFO 31212   | Stevia rebaudiana, leaf spot | Japan, Kagawa | KJ718250 KJ718078 KJ718596 |
| Alternaria tagetiae    | CBS 27979; GST AM2     | Tagetes sp., seed | UK            | KJ718253 KJ718080 KJ718599 |
| Alternaria tagetiae    | CBS 47981; EGS 33.081  | Tagetes erecta, seed | UK, England   | KCS8421 KC84143 KC84692 |
| Alternaria tagetiae    | CBS 117217; EGS 44.045 | Tagetes sp., leaf spot | USA, Ohio     | KJ718256 KJ718083 KJ718602 |
| Alternaria thunbergiae | Alternaria iricana     | CBS 116331; EGS 41.073 | Thunbergia data, leaf spot | Australia, Queensland | KJ718257 KJ718084 KJ718603 |
| Alternaria thunbergiae | Alternaria iricana     | CBS 120986; EGS 51.075 | Allium cepa, leaf | Iran, Mandooba | KJ718258 KJ718085 KJ718604 |
| Alternaria thunbergiae | Alternaria iricana     | CBS 122597 | Thunbergia data | New Zealand, Auckland | KJ718259 KJ718086 KJ718605 |
| Alternaria tillandsiae | CBS 116116; EGS 43.074 | Tillandsia aeneoides | New Zealand | KJ718260 KJ718087 KJ718606 |
| Alternaria tropica     | CBS 63193; EGS 39.126  | Passiflora edulis, fruit | USA, Florida | KJ718261 KJ718088 KJ718607 |
| Alternaria venezuelensis | Alternaria venezuelensis | CBS 116212; EGS 48.085 | Passiflora edulis, fruit | USA, Florida | KJ718262 KJ718089 KJ718608 |
| Alternaria venezuelensis | Alternaria venezuelensis | CBS 116123; EGS 44.035 | Phaeolus vulgaris, leaf spot | Venezuela, Maracay | KJ718263 KJ718090 KJ718609 |

*ATCC: American Type Culture Collection, Manassas, VA, USA; BRIP: Queensland Plant Pathology Herbarium, Queensland, Australia; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, Netherlands; CCM: Czech Collection of Microorganisms, Brno, Czech Republic; CECT: Spanish Type Culture Collection, Valencia, Spain; CPC: Personal collection of P.W. Crous, Utrecht, Netherlands; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; DSM: German Collection of Microorganisms and Cell Cultures, Leibniz Institute, Braunschweig, Germany; E.G.S.: Personal collection of Dr. E.G. Simmons; Elliott: Personal collection of M.E. Elliott; GST: Personal collection of G.S. Taylor; ICMP: International Collection of Micro-organisms from Plants, Auckland, New Zealand; IFO: Institute for Fermentation Culture Collection, Osaka, Japan; IMI: Culture collection of CABI Europe UK Centre, Egham UK; LEV: Plant Health and Diagnostic Station, Levin, New Zealand; MUCL: (Agro)Industrial Fungi and Yeast Collection of the Belgian Co-ordinated Collections of Micro-organisms (BCCM), Louvain-la-Neuve, Belgium; Nattrass: Personal collection of R.M. Nattrass; PD: Plant Protection Service, Wageningen, Netherlands; PPRI: ARC-Plant Protection Research Institute, Roodeplaat, South Africa; QM: Quarter Master Culture Collection, Amherst, MA, USA; VKM: All-Russian Collection of Microorganisms, Moscow, Russia.
Figure 1. The Maximum-likelihood tree derived from combined ITS rDNA, GAPDH, and TEF1 gene sequences of Alternaria species. Bootstrap support values above 50% are shown at the nodes. Alternaria gypsophilae serve as outgroup. The strains YITC 5109 and YITC 5114 of a new species A. yunnanensis proposed in this study are emphasized in red.
3.2. Taxonomy

*Alternaria yunnanensis* Z.Y. Cai, X.Y. Liu, Y.X. Liu & Y.P. Shi, sp. nov. (Figure 2).

Etymology: In reference to the location of Yunnan Province from where the fungus was isolated.

Fungal Name: FN570554.

Colonies of *A. yunnanensis* on PDA agar develope well, attaining a diameter of 7.7–8.5 cm under a daily fluorescent light/dark cycle of 8/16 h at 25°C in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentric rings and a whitish aerial-mycelium margin, cottony, yellowish in 7 days, circular, with evident concentr
chains of two (Figure 2(C,E)), straight or slightly curved, long ellipsoid to obclavate, tapering to the beak which is equal in length to or up to fourfold as long as the body. The apical beaks are simple, pale, septate, up to 252 μm long and 2.5 μm wide (Figure 2(E)).

3.3. Holotype

China, isolated from rubber tree leaf, Hula village, Ruili city, Yunnan Province, China, May 2014. Z.Y. Cai (dried culture HMAS 247784), ex-holotype cultures (ACCC 39327 and CGMCC3.18901).

3.4. Pathogenicity assay

Four days after inoculation, typical black leaf spots observed, which resembles the field symptom with round, black, foliar spots, surrounded by yellow halos, and lesions sometimes slightly sink (Figure 2(D)). A fungus reisolated from the leaf lesions on inoculated plants has the same colonial and conidial morphology as the inocula *A. yunnanensis*, but not from asymptomatic leaves of control rubber plants, fulfilling Koch’s postulates. The result indicates that
the species is the causal agent of leaf spot diseases in rubber tree.

Based on phylogenetic analyses and morphological characteristics, the fungus isolated from the leaf spots of the rubber tree was identified. Both data indicate that the fungus is a novel species belonging to the genus *Alternaria*. With its long history of rubber cultivation, rubber trees have had ample opportunity to attract a wide variety of diseases. A number of significant new diseases have been discovered. At present, the *Alternaria* leaf disease is not very serious. However, identifying *Alternaria* species on rubber tree is important to control rubber tree disease in case the disease outbreaks. In China among the three known *Alternaria* disease of rubber tree, *A. alternata* is the most widely distributed, which can be found at every rubber planting region. *Alternaria heveae* and *A. yunnanensis* were only found at very few rubber plantations.

Along with the *A. yunnanensis*, 112 *Alternaria* strains including 63 species of the section *Porri* of *Alternaria* were analyzed. The phylogram (Figure 1) generated from the three-gene combined dataset (*ITS, GAPDH*, and *TEF1*) exhibits similar topology to previously reported one [3]. The phylogenetic analyses indicate that the new species, *A. yunnanensis*, belongs to the *Porri* species-group, all of which produce relatively large conidia with a simple or branched, filamentous long beak [2,3].

The *Porri* species-group includes 63 species [3]. Phylogenetic analysis showed that *A. yunnanensis* was most closely related to *A. sidae, A sennae, A. deserticola*, and relevant to *A. cyamopsidis, A. rostellata, A. thunbergiae, A. nitrimali*, and *A. crassa* in the section *Porri*.

Based on conidial morphology, *A. yunnanensis* is most similar to *A. sennae*. Conidia of *A. yunnanensis* have 5–10 transverse septa and often evidently constricted at the septa, whereas those of *A. sennae* have 7–8 transverse septa, slightly constricted near some transverse septa. Besides the conidia of *A. sennae* (55–62 × 10–12 µm) are narrower than that of *A. yunnanensis* (41–97 × 13–29 µm) [3]. The culture
Table 2. Morphological characteristics of the present isolates and relevant species.

| Species MB or FN No. | Size (µm) | Shape | Septation | Ornamentation | Conidia |
|----------------------|-----------|-------|-----------|---------------|---------|
| A. yunnanensis        | 41–97 × 13–29 | Straight or curved, long ellipsoid | 5–10 transverse, 1–8 longitudinal or some-times cylindrical | Smooth | A. c. with a punctate surface
|                      |           |       |           |               | Conidia bear a scattering of granules
| A. sidae             | 70–110 × 18–27 | Narrowly ovoid, ellipsoid, or obclavate | 7–10 transverse, 2–5 longitudinal or oblique septa | Smooth to moderately punctulate | A. c. a filamentous beak, occasionally
|                      |           |       |           |               | long filamentous up to fourfold as long
| A. cyamopsidis       | 55–62 × 10–12 | Broadly ovoid, ellipsoid, or obclavate | 7–10 transverse, 2–5 longitudinal or oblique septa | Smooth | A. c., usually shorter, A. c.
|                      |           |       |           |               |
| A. thunbergiae       | 60–90 × 17–25 | Broadly ovoid, ellipsoid or oblate | 7–9 transverse, 2–5 longitudinal septa | Smooth to moderately punctulate | A. c.
|                      |           |       |           |               | One or inconspicuously punctulate
| A. nitrimali         | 50–80 × 20–30 | Broadly ovoid, ellipsoid or oblate | 8–10 transverse, 2–5 longitudinal septa | Smooth | A. c.
|                      |           |       |           |               |
| A. deserticola       | 96–112 × 22–32 | Ovoid or ellipsoid | 7–10 transverse, 2–5 longitudinal septa | Smooth to moderately punctulate | A. c.
|                      |           |       |           |               | One or inconspicuously punctulate
| A. heveae            | 70–87 × 14–17.5 | Ovoid or ellipsoid | 7–10 transverse, 2–5 longitudinal septa | Smooth to moderately punctulate | A. c.

The conidia of A. yunnanensis are obviously smaller than that of A. thunbergiae (96–112 × 27–32 µm). Additionally, the apical beaks of A. yunnanensis are simple and long filamentous up to fourfold as long as the body, which differs from A. rostellata with a narrow beak, ca 30–55 × 2 µm or usually shorter, A. sennae with a single beak which occasionally branches once. A detailed comparison between A. yunnanensis and members of the Porri species-group is provided in Table 2.

Compared with other Alternaria pathogens on rubber tree, including A. alternata and A. heveae, the conidia of A. yunnanensis are quite different, which are obviously larger than that of A. alternata (22.5–67.5 × 10–15 µm) and A. heveae (22.75–49.5 × 8–20 µm) [12,13]. In GenBank, there are only ITS sequences of A. heveae and A. alternata (KF953884 and KM111289) of these known Alternaria pathogens of rubber tree. We compared our sequences with them, and they were different (data not shown). Thus, we confirm the novelty of A. yunnanensis.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by the Founds for Technical Innovation Talents Training of Yunnan [2016HB016] and the Sci-Tech Innovation System Construction for Tropical Crops Grant of Yunnan Province [No. RF2017-6].

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