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RESEARCH ARTICLE

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Alternaria yunnanensis sp. nov., a New *Alternaria* Species Causing Foliage Spot of Rubber Tree in China

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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. deseriticola*, *A. cyamopsidis, A. rostellata, A. nitrimali, A. crassa*, and *A. thunbergiae.*

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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].

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.

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

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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 Sinicae, 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 1×10^4 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 $CaCO_3$, 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 (*TEF1*) with the primers EF1-728F and EF1-986R [20]. The PCR mixtures included 9.5 µL of ddH_2O, 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 $MgCl_2$ and 200 µM of each dNTP and 1× PCR buffer (Cwbio, Beijing, China). The PCR reactions were carried out in a Veriti 96-well thermo cycler (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 *TEF1*) 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 *TEF1* 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 *TEF1*, 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. cyamopsidis, A. rostellata, A. nitrimali, A. thunbergiae*, and *A. crassa*.

Table 1. Isolates used in this study and their GenBank accession numbers.

Name	Old name	Strain number[a]	Host/Substrate	Locality	GenBank accession numbers		
					ITS	*GAPDH*	*TEF1*
Alternaria acalyphicola		CBS 541.94; E.G.S. 38.100	*Acalypha indica*	Seychelles	KJ718097	KJ717952	KJ718446
Alternaria agerati		CBS 117221; E.G.S. 30.001	*Ageratum houstonianum*	USA, Illinois	KJ718098	KJ717953	KJ718447
Alternaria agripestis		CBS 577.94; E.G.S. 41.034	*Euphorbia esula*, stem lesion	Canada, Saskatchewan	KJ718099	JQ646356	KJ718448
Alternaria allii	*Alternaria porri*	CBS 107.28; E.G.S. 48.084	*Allium cepa*, leaf spot	Puerto Rico	KJ718100	KJ717954	KJ718449
	Alternaria vanuatuensis	CBS 121345; E.G.S. 45.018	*Allium cepa*, leaf	Vanuatu	KJ718104	KJ717958	KJ718453
Alternaria alternariacida sp. nov.	*Alternaria solani*	CBS 105.51; ATCC 11078;	*Solanum lycopersicum*, fruit	UK, England	KJ718105	KJ717959	KJ718454
Alternaria anagallidis		CBS 101004	*Anagallis arvensis*, leaf spot	New Zealand, Auckland	KJ718107	KJ717960	KJ718455
		CBS 117128; E.G.S. 42.074	*Anagallis arvensis*, leaf spot	New Zealand, Auckland	KJ718108	KJ717961	KJ718456
Alternaria anodae		PPRI 12376	*Anoda cristata*, leaf	South Africa, Gauteng	KJ718110	KJ717963	KJ718458
Alternaria aragakii		CBS 594.93; E.G.S. 29.016	*Passiflora edulis*	USA, Hawaii	KJ718111	KJ717964	KJ718459
Alternaria argyroxiphii		CBS 117222; E.G.S. 35.122	*Argyroxiphium* sp.	USA, Hawaii	KJ718112	JQ646350	KJ718460
		PPRI 11848	*Ipomoea batatas*, stem lesion	South Africa, Gauteng	KJ718113	KJ717965	KJ718461
		PPRI 11971	*Ipomoea batatas*, leaf	South Africa, pumalanga	KJ718114	KJ717966	KJ718462
Alternaria azadirachtae		CBS 116444; E.G.S. 46.195;	*Azadirachta indica*, leaf spot	Australia, Queensland	KJ718115	KJ717967	KJ718463
		CBS 116445; E.G.S. 46.196;	*Azadirachta indica*, leaf spot	Australia, Queensland	KJ718116	KJ717968	KJ718464
A. yunnanensis sp. nov.		YITC 5109	*Hevea brasiliensis*, leaf spot	China,	MH044725	MH044727	MH044726
		YITC 5114	*Hevea brasiliensis*, leaf spot	China,	MH044728	MH044730	MH044729
Alternaria bataticola		CBS 531.63; IFO 6187	*Ipomoea batatas*	Japan	KJ718117	JQ646349	KJ718465
		CBS 117096; E.G.S. 42.158;	*Ipomoea batatas*, leaf spot	Australia, Queensland	KJ718120	KJ717971	KJ718468
Alternaria blumeae	*Alternaria brasiliensis*	CBS 117215; E.G.S. 39.116	*Phaseolus vulgaris*, leaf spot	Brazil, Esperito Santo	KJ718125	KJ717976	KJ718473
		CBS 117364; E.G.S. 40.149	*Blumea aurita*	Thailand, Yala Province	KJ718126	AY562405	KJ718474
Alternaria calendulae		CBS 224.76; ATCC 38903;	*Calendula officinalis*, leaf spot	Germany	KJ718127	KJ717977	KJ718475
		CBS 116439; E.G.S. 42.197	*Rosa sp.*, leaf spot	New Zealand, Auckland	KJ718129	KJ717979	KJ718477
Alternaria carthami		CBS 635.80	*Carthamus tinctorius*, leaf	Italy, Perugia	KJ718131	KJ717981	KJ718479
	Alternaria heliophytonis	CBS 116440; E.G.S. 3.143;	*Helianthus annuus*, leaf	Canada, Saskatchewan	KJ718132	KJ717982	KJ718480
Alternaria carthamicola	*Alternaria carthami*	CBS 117092; E.G.S. 37.057	*Carthamus tinctorius*	Iraq	KJ718134	KJ717984	KJ718482
Alternaria cassiae		CBS 478.81; E.G.S. 33.147	*Senna obtusifolia*, seedling	USA, Mississippi	KJ718135	KJ717985	KJ718483
	Alternaria sauropodis	CBS 116119; E.G.S. 47.112	*Sauropus androgynus*	Malaysia, Sarawak	KJ718136	KJ717986	KJ718484
	Alternaria hibiscinficiens	CBS 117369; E.G.S. 50.166	*Hibiscus sabdariffa*, leaf	Fiji	KJ718138	KJ717988	KJ718486
Alternaria catananches sp. nov.		CBS 137456; PD 013/05703936	*Catananche caerulea*	Netherlands	KJ718139	KJ717989	KJ718487
Alternaria centaureae		CBS 116446; E.G.S. 47.119	*Centaurea solstitialis*, leaf spot	USA, California	KJ718140	KJ717990	KJ718488
Alternaria cichorii		CBS 102.33; E.G.S. 07.017	*Cichorium intybus*, leaf spot	Cyprus	KJ718141	J717991	KJ718489
Alternaria cirsinoxia		CBS 113261; E.G.S. 41.136	*Cirsium arvense*, stem lesion	Canada, askatchewan	KJ718143	KJ717993	KJ718491
Alternaria citrullicola sp. nov.	*Alternaria cucumerina*	CBS 103.32; VKM F-1881	*Citrullus vulgaris*, fruit	Cyprus	KJ718144	KJ717994	KJ718492
Alternaria conidiophora sp. nov.		CBS 137457	–	Netherlands	KJ718145	KJ717995	KJ718493
Alternaria crassa		CBS 110.38	*Datura stramonium*, leaf spot	Cyprus	KJ718147	KJ717997	KJ718495
Alternaria cucumerina	*Alternaria loofahae*	CBS 116114; E.G.S. 35.123	*Luffa acutangula*	USA, Hawaii	KJ718153	KJ718000	KJ718501
Alternaria cyamopsidis		CBS 364.67; E.G.S. 17.065;	*Cyamopsis tetragonoloba*, leaf spot	USA, Maryland	KJ718156	KJ718003	KJ718504
		CBS 117219; E.G.S. 13.120;	*Cyamopsis tetragonoloba*, leaf spot	USA, Georgia	KJ718157	KJ718004	KJ718505
Alternaria dauci		CBS 111.38	*Daucus carota*, seed	Italy	KJ718158	KJ718005	KJ718506
	Alternaria cichorii	CBS 101592	*Daucus carota*, seed	Netherlands	KJ718162	KJ718009	KJ718510
		CBS 117099; E.G.S. 47.131	*Daucus carota*, seed	USA, California	KJ718164	KJ718011	KJ718512
Alternaria deserticola sp. nov.	*Alternaria acalyphicola*	CBS 110799	desert soil	Namibia	KJ718249	KJ718077	KJ718595
Alternaria dichondrae		CBS 199.74; E.G.S. 38.007	*Dichondra repens*, leaf spot	Italy	KJ718166	JQ646357	KJ718514
		CBS 200.74; E.G.S. 38.008	*Dichondra repens*, leaf spot	Italy	KJ718167	KJ718012	KJ718515
Alternaria echinaceae		CBS 116117; E.G.S. 46.081	*Echinacea sp.*, leaf lesion	New Zealand, isborne	KJ718170	KJ718015	KJ718518
		CBS 116118; E.G.S. 46.082	*Echinacea sp.*, leaf lesion	New Zealand, isborne	KJ718171	KJ718016	KJ718519

(*continued*)

Table 1. Continued.

Name	Old name	Strain number[a]	Host/Substrate	Locality	GenBank accession numbers		
					ITS	*GAPDH*	*TEF1*
Alternaria grandis		CBS 109158; E.G.S. 44.106	*Solanum tuberosum*, leaf spot	USA, Pennsylvania	KJ718239	JQ646341	EU130547
		CBS 116695; E.G.S. 44.108	*Solanum tuberosum*, leaf spot	USA, Pennsylvania	KJ718241	KJ718070	KJ718587
Alternaria euphorbiicola		CBS 198.86; E.G.S. 38.082	*Euphorbia pulcherrima*	USA, Florida	KJ718172	KJ718017	KJ718520
		CBS 119410; E.G.S. 41.029	*Euphorbia pulcherrima*	USA, Hawaii	KJ718173	KJ718018	KJ718521
Alternaria gypsophilae		CBS 107.41; E.G.S. 07.025;	*Gypsophila elegans*, seed	Netherlands	KC584199	KC584118	KC584660
Alternaria ipomoeae sp. nov.	*Alternaria cucumerina*	CBS 219.79	*Ipomoea batatas*, stem	Ethiopia	KJ718175	KJ718020	KJ718523
		PPRI 8988	*Ipomoea batatas*, stem	South Africa, Gauteng	KJ718176	KJ718021	KJ718524
Alternaria jesenskae		CBS 133855; CCM 8361	*Fumana procumbens*, seed	Slovakia	KJ718177	KJ718022	KJ718525
Alternaria limicola		CBS 483.90; E.G.S. 39.070	*Citrus aurantiifolia*, leaf spot	Mexico, Colima	KJ718178	JQ646329	KJ718526
		CBS 117360; E.G.S. 43.009	*Citrus* sp.	Mexico, Jalisco	KJ718179	KJ718023	KJ718527
Alternaria linariae	*Alternaria tomatophila*	CBS 109156; E.G.S. 42.156	*Solanum lycopersicum*, leaf spot	USA, Indiana	KJ718183	JQ646347	KJ718531
	Alternaria subcylindrica	CBS 109161; E.G.S. 45.113	*Solanum lycopersicum* var. *cerasiforme*, leaf spot	USA, Louisiana	KJ718184	JQ646345	KJ718532
	Alternaria cretica	CBS 109164; E.G.S. 46.188	*Solanum lycopersicum*, leaf spot	Greece, Crete	KJ718185	JQ646342	EU130545
	Alternaria cucumericola	CBS 116438; E.G.S. 41.057	*Cucumis sativus*, leaf spot	New Zealand	KJ718186	KJ718027	KJ718533
Alternaria macrospora		CBS 117228; E.G.S. 50.190	*Gossypium barbadense*	USA, Arizona	KC584204	KC584124	KC584668
Alternaria montanica		CBS 121343; E.G.S. 44.112	*Cirsium arvense*	USA, Montana	KJ718194	KJ718033	KJ718541
Alternaria multirostrata		CBS 712.68; ATCC 18515	*Richardia scabra*, floral bract	USA, Georgia	KJ718195	JQ646362	EU130546
		CBS 713.68; ATCC 18517;	*Richardia scabra*, floral bract	USA, Georgia	KJ718196	KJ718034	KJ718542
Alternaria neoipomoeae sp. nov.		PPRI 11845	*Ipomoea batatas*, stem	South Africa, Gauteng	KJ718198	KJ718036	KJ718544
		PPRI 11847	*Ipomoea batatas*	South Africa, Mpumal	KJ718199	KJ718037	KJ718545
Alternaria nitrimali		CBS 109163; E.G.S. 46.151	*Solanum viarum*, leaf spot	Puerto Rico	KJ718201	JQ646358	KJ718547
Alternaria novae-guineensis		CBS 116120; E.G.S. 47.198	*Citrus sp.*, dry leaf	Papua New Guinea	KJ718202	KJ718039	KJ718548
		PPRI 12171	*Galinsoga parviflora*, leaf	South Africa, Gauteng	KJ718203	KJ718040	KJ718549
Alternaria obtecta		CBS 117367; E.G.S. 42.063	*Euphorbia pulcherrima*, leaf	USA, California	KJ718204	KJ718041	KJ718550
		CBS 134278; E.G.S. 42.064	*Euphorbia pulcherrima*	USA, California	KJ718205	KJ718042	KJ718551
Alternaria paralinicola sp. nov.	*Alternaria linicola*	CBS 116652; E.G.S. 47.157;	*Linum usitatissimum*, seed	Canada, Manitoba	KJ718206	KJ718043	KJ718552
Alternaria passiflorae	*Alternaria hawaiiensis*	CBS 630.93; E.G.S. 29.020;	*Passiflora edulis*	USA, Hawaii	KJ718210	JQ646352	KJ718556
	Alternaria gaurae	CBS 116333; E.G.S. 50.121	*Gaura lindheimeri*, leaf	New Zealand, uckland	KJ718211	KJ718046	KJ718557
Alternaria pipionipisi		CBS 116115; E.G.S. 40.096	*Cajanus cajan*, seed	India	KJ718214	KJ718049	KJ718560
Alternaria porri	*Alternaria allii*	CBS 116649; E.G.S. 17.082	*Allium cepa*, leaf	USA, Nebraska	KJ718217	KJ718052	KJ718563
		CBS 116699; E.G.S. 48.152	*Allium cepa*, leaf spot	USA, New York	KJ718218	KJ718053	KJ718564
Alternaria protenta	*Alternaria hordeiseminis*	CBS 116437; E.G.S. 32.076	*Hordeum vulgare*, seed	New Zealand	KJ718220	KJ718055	KJ718566
	Alternaria solani	CBS 116651; E.G.S. 45.020	*Solanum tuberosum*, tuber	USA, California	KC584217	KC584139	KC584688
Alternaria pseudorostrata		CBS 119411; E.G.S. 42.060	*Euphorbia pulcherrima*	USA, California	JN383483	AY562406	KC584680
Alternaria ranunculi		CBS 116330; E.G.S. 38.039	*Ranunculus asiaticus*, seed	Israel	KJ718225	KJ718058	KJ718571
Alternaria ricini		CBS 215.31	*Ricinus communis*	Japan	KJ718226	KJ718059	KJ718572
		CBS 353.86	*Ricinus communis*	Italy, Sardinia	KJ718227	JQ646331	KJ718573
Alternaria rostellata		CBS 117366; E.G.S. 42.061	*Euphorbia pulcherrima*, leaf	USA, California	KJ718229	JQ646332	KJ718575
Alternaria scorzonerae	*Alternaria linicola*	CBS 103.46; Elliot No. 45-190C	*Linum usitatissimum*	UK, Scotland	KJ718190	JQ646363	KJ718537
		CBS 478.83; E.G.S. 38.011	*Scorzonera hispanica*, leaf spot	Netherlands, Reusel	KJ718191	JQ646334	KJ718538
	Alternaria linicola	CBS 116703; E.G.S. 36.110	*Linum usitatissimum*, seed	UK, Derbyshire	KJ718192	KJ718031	KJ718539
Alternaria sennae sp. nov.	*Alternaria cassiae*	CBS 477.81; E.G.S. 34.030	*Senna corymbosa*, leaf	India, Uttar Pradesh	KJ718230	JQ646344	EU130543
Alternaria sesami		CBS 240.73	*Sesamum indicum*	Egypt	KJ718231	JQ646343	KJ718576
		CBS 115264;	*Sesamum indicum*, seedling	India	JF780939	KJ718061	KJ718577
Alternaria sidae		CBS 117730; E.G.S. 12.129	*Sida fallax*, leaf spot	Kiribati, Phoenix islands	KJ718232	KJ718062	KJ718578
Alternaria silybi		CBS 134092; VKM F-4109	*Silybum marianum*, leaf	Russia, Vladivistok	KJ718233	KJ718063	KJ718579
		CBS 134093; VKM F-4117	*Silybum marianum*, leaf	Russia, Vladivistok	KJ718234	KJ718064	KJ718580

(*continued*)

Table 1. Continued.

Name	Old name	Strain number[a]	Host/Substrate	Locality	GenBank accession numbers		
					ITS	*GAPDH*	*TEF1*
Alternaria solani	*Alternaria danida*	CBS 111.44; E.G.S. 07.029	*Ageratum houstonianum*, seed	Italy	Y17070	KJ718068	KJ718584
	Alternaria viciae-fabae	CBS 116442; E.G.S. 46.162	*Vicia faba*	New Zealand	KJ718240	KJ718069	KJ718586
Alternaria solani-nigri	*Alternaria cyphomandrae*	CBS 109155; E.G.S. 40.058	*Cyphomandra betacea*, fruit	New Zealand, New Plymouth	KJ718242	JQ646360	KJ718588
	Alternaria herbiculinae	CBS 116332; E.G.S. 49.180	*Petroselinum crispum*, plant	New Zealand, Taranaki	KJ718244	KJ718072	KJ718590
	Alternaria glyceriae	CBS 116334; E.G.S. 51.107	*Glyceria maxima*, leaf spot	New Zealand, Waikato	KJ718245	KJ718073	KJ718591
Alternaria steviae		CBS 631.88; IFO 31212	*Stevia rebaudiana*, leaf spot	Japan, Kagawa	KJ718250	KJ718078	KJ718596
		CBS 117362; E.G.S. 37.019	*Stevia rebaudiana*, leaf spot	Japan, Kagawa	KJ718252	KJ718079	KJ718598
Alternaria tagetica		CBS 297.79; GST AM2	*Tagetes* sp., seed	UK	KJ718253	KJ718080	KJ718599
		CBS 479.81; E.G.S. 33.081	*Tagetes erecta*, seed	UK, England	KC584221	KC584143	KC584692
		CBS 117217; E.G.S. 44.045	*Tagetes* sp., leaf spot	USA, Ohio	KJ718256	KJ718083	KJ718602
Alternaria thunbergiae		CBS 116331; E.G.S. 41.073	*Thunbergia alata*, leaf spot	Australia, Queensland	KJ718257	KJ718084	KJ718603
	Alternaria iranica	CBS 120986; E.G.S. 51.075	*Allium cepa*, leaf	Iran, Miandoab	KJ718258	KJ718085	KJ718604
		CBS 122597	*Thunbergia alata*	New Zealand, Auckland	KJ718259	KJ718086	KJ718605
Alternaria tillandsiae		CBS 116116; E.G.S. 43.074	*Tillandsia usneoides*	New Zealand	KJ718260	KJ718087	KJ718606
Alternaria tropica		CBS 631.93; E.G.S. 39.126	*Passiflora edulis*, fruit	USA, Florida	KJ718261	KJ718088	KJ718607
		CBS 117216; E.G.S. 39.125	*Passiflora edulis*, fruit	USA, Florida	KJ718262	KJ718089	KJ718608
Alternaria venezuelensis		CBS 116121; E.G.S. 48.065	*Phaseolus vulgaris*, leaf spot	Venezuela, Maracay	KJ718263	KJ718090	KJ718609
Alternaria zinniae		CBS 117223; E.G.S. 44.035	*Zinnia elegans*, leaf spot	New Zealand, Auckland	KJ718270	KJ718096	KJ718616

[a] 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.

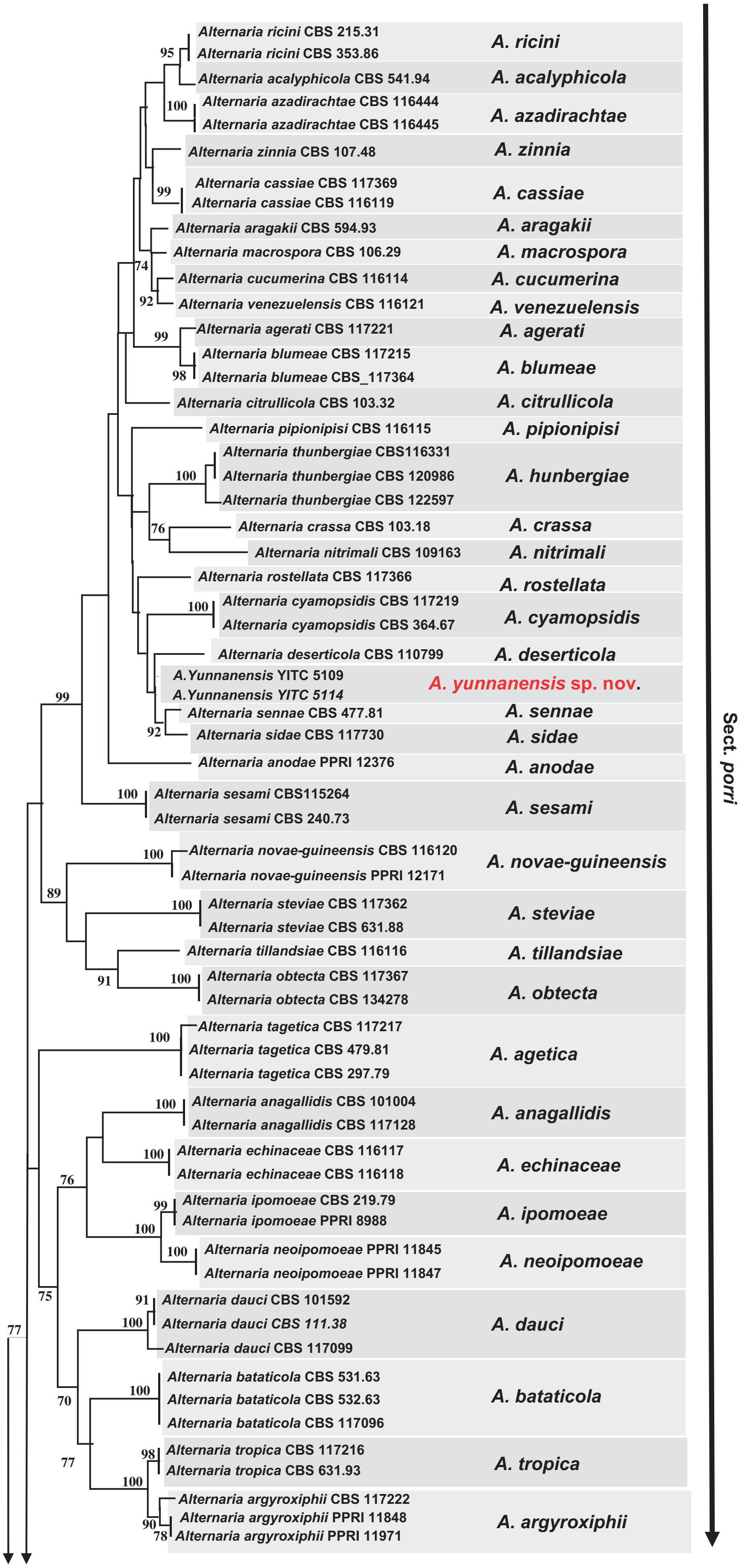

Figure 1. The Maximum-likelihood tree derived from combined *ITS rDNA*, *GAPDH*, and *TEF1* gene sequences of *Alternaria* species. Bootstrap support values above 50% are shown at the nodes. *Alternaria gypsophilae* serve as outgroup. The strains YITC 5109 and YITC 5114 of a new species *A. yunnanensis* proposed in this study are emphasized in red.

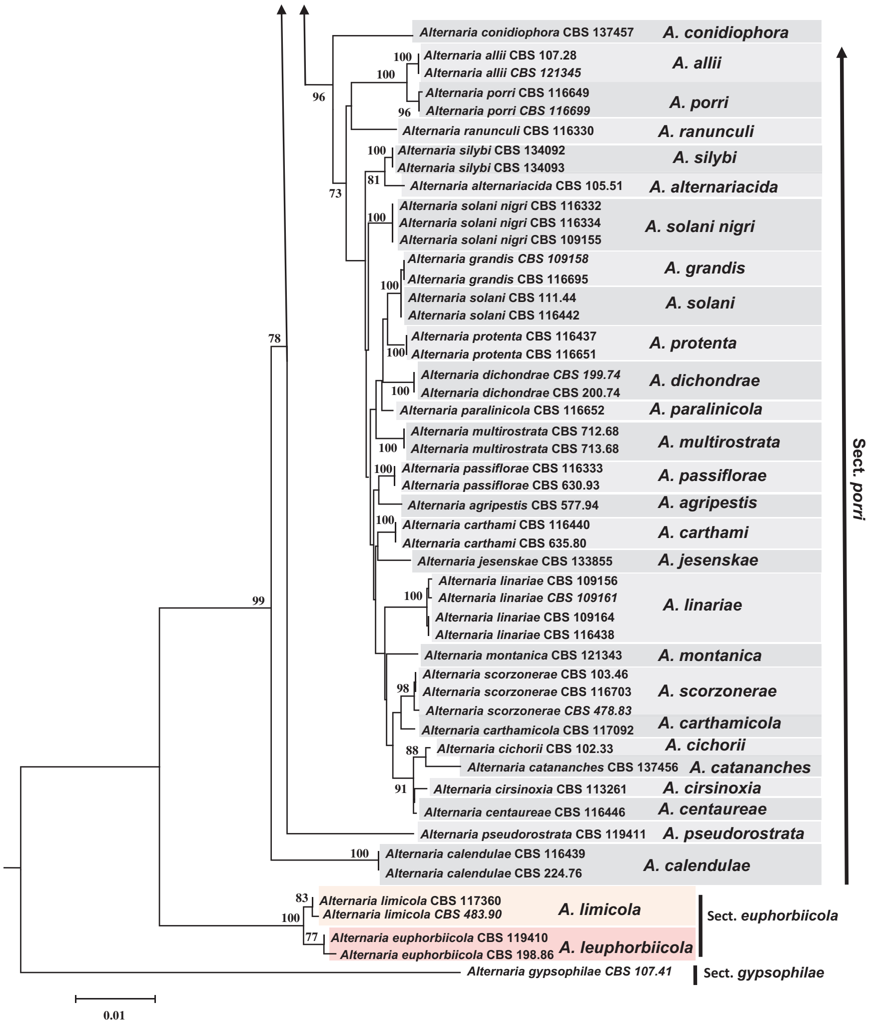

Figure 1. Continued.

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 to light gray, secreting an orangish pigment into the medium (Figure 2(A)). Mycelia produced on V-8 agar are light to dark gray (Figure 2(B)). Conidiophores are simple or sparingly branched, septate, pale brown to mid brown, smooth, arising beneath the agar surface or directly from hyphae, ca. 40–104 × 5–7µm, and gradually enlarge near the apex into a clavate conidiogenous cell that produces conidia (Figure 2(F)). Conidia are abundant on V-8 agar, and few on PDA and PCA, smooth, dilute dull brown, 41–97 × 13–29 µm, with 5–10 transverse septa and 1–6 longitudinal or oblique septa, often constricted at the septa, usually solitary or rarely in 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

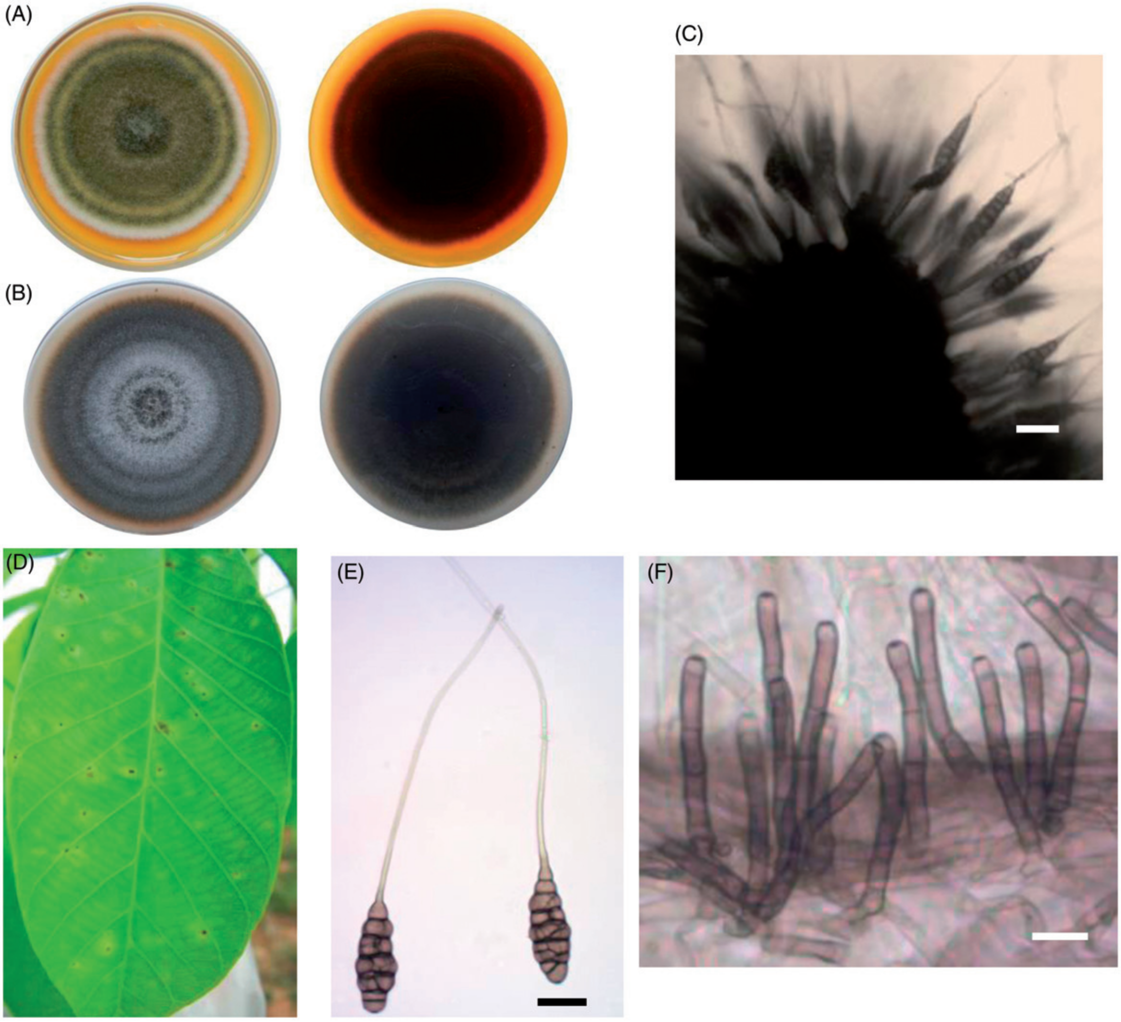

Figure 2. *Alternaria yunnanensis* (ACCC 39327: ex-holotype). (A) Obverse (left) and reverse (right) of colony on PDA at 25 °C for 7 days; (B) Obverse (left) and reverse (right) of colony on V-8 juice agar at 25 °C for 7 days; (C) features of the sporulation apparatus; (D) Symptoms on *Hevea brasiliensis* leaves; (E) Conidia produced on V-8 juice agar at 22 °C for 7 days; (F) Conidiophores produced on V-8 juice agar at 22 °C for 7 days. Scale bars: 50 µm for (C), 40 µm for (E), 10 µm for (F).

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.	Conidia				Beak
	Shape	Size (μm)	Septation	Ornamentation	
A. yunnanensis FN 570554	Straight or curved, long ellipsoid to obclavate	41–97 × 13–29	5–10 transverse, 1–6 longitudinal or oblique septa	Smooth	A filamentous beak, up to 252 μm long and 2.5 μm wide
A. sidae MB 489129	Variant of a long-elliptical or sometimes cylindric	70–110 × 18–27	7–10 transverse septa and 1–2 longisepta	Conidia with a pustulate surface	A filamentous beak reach 95–110 × 1.5 μm
A. sennae MB 809000	Narrowly ovoid	55–62 × 10–12	7–8 transverse distosepta and 2–3 longitudinal septa	Smooth	A filamentous beak, occasionally branches once, ca.99–163 × 2 μm
A. cyamopsidis MB 292412	Broadly ovoid, ellipsoid, or sometimes obovoid	60–90 × 17–25	7–10 transverse distosepta with 1–3 longisepta	Conidia bear a scattering of granules	A filamentous beak up to 100–170 × 1.5–2.0 μm
A. rostellata MB 414600	Ellipsoid to broadly ovoid	50–80 × 20–30	7–9 transverse septa and 1–3 longitudinal septa	Smooth to moderately punctate-rough	A narrow beak, ca 30–55 × 2 μm or usually shorter
A. thunbergiae MB 504986	Broadly ovoid, ellipsoid, or obclavate	96–112 × 27–32	8–12 transverse septa and 1–2 longitudinal septa	Either smooth or, closely punctulate	tapered beak up to 225–320 × 2 μm
A. nitrimali MB 467495	Ovoid or ellipsoid,	70–87 × 14–17.5	7–10 transverse distosepta and only 1 longiseptum	Smooth or inconspicuously punctulate	A long filamentous beak 115–186 × 2 μm

of *A. deserticola* (CBS 110799) is sterile. However, Conidia of *A. yunnanensis* are abundant on V-8 agar. Forthmore *A. yunnanensis* differs from *A. deserticola* of its closest phylogenetic neighbour based on alleles of all three loci (positions derived from respective alignments of the separate loci: *ITS* positions 389(C), 390(C), and 504(T); *GAPDH* positions 111(G), 172(C), 332(C), 482(T), and 500(C); *TEF1* positions 264(C) and 310(A) [3]. Conidium outer walls of *A. yunnanensis* are smooth, while those of relevant species (*A. sidae, A. cyamopsidis, A. rostellata, A. thunbergiae* and *A. nitrimali*) are punctulate to strongly punctate ornamentation. 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 occasinally 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.

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