

Cryptic diversity found in Didymellaceae from Australian native legumes

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Abstract

Ascochyta koolunga (Didymellaceae, Pleosporales) was first described in 2009 (as *Phoma koolunga*) and identified as the causal agent of Ascochyta blight of *Pisum sativum* (field pea) in South Australia. Since then *A. koolunga* has not been reported anywhere else in the world, and its origins and occurrence on other legume (Fabaceae) species remains unknown. Blight and leaf spot diseases of Australian native, pasture and naturalised legumes were studied to investigate a possible native origin of *A. koolunga*.

Ascochyta koolunga was not detected on native, naturalised or pasture legumes that had leaf spot symptoms, in any of the studied regions in southern Australia, and only one isolate was recovered from *P. sativum*. However, we isolated five novel species in the Didymellaceae from leaf spots of Australian native legumes from commercial field pea regions throughout southern Australia. The novel species were classified on the basis of morphology and phylogenetic analyses of the internal transcribed spacer region and part of the RNA polymerase II subunit B gene region. Three of these species, *Nothophoma garlbiwalawarda* **sp. nov.**, *Nothophoma naiawu* **sp. nov.** and *Nothophoma ngayawang* **sp. nov.**, were isolated from *Senna artemisioides*. The other species described here are *Epicoccum djirangnandiri* **sp. nov.** from *Swainsona galegifolia* and *Neodidymelliopsis tinkyukuku* **sp. nov.** from *Hardenbergia violaceae*. In addition, we report three new host-pathogen associations in Australia, namely *Didymella pinodes* on *S. artemisioides* and *Vicia cracca*, and *D. lethalis* on *Lathyrus tingitanus*. This is also the first report of *Didymella prosopidis* in Australia.

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Keywords

Alternative host, multilocus phylogeny, pathogen reservoir

Introduction

The Didymellaceae was established to accommodate *Ascochyta*, *Didymella*, and other allied *Phoma*-like genera (de Gruyter et al. 2009). To date, more than 5,400 species from 31 genera have been recorded, including recently established genera such as *Dimorphoma* and *Macroascochyta* (Hou et al. 2020). Species of Didymellaceae are cosmopolitan and occupy a broad range of environments. Many species are plant pathogens that cause leaf and stem lesions, often with a broad host range (Aveskamp et al. 2009; Aveskamp et al. 2010; Chen et al. 2015b). Multilocus phylogenetics and a polyphasic approach to classify species have helped to revise taxa and refine systematic relationships in the Didymellaceae (Aveskamp et al. 2009, de Gruyter et al. 2009; Aveskamp et al. 2010; Chen et al. 2015a, de Gruyter 2012; Hou et al. 2020).

In Australia, reports of taxa in the Didymellaceae mostly refer to plant pathogenic species, particularly on crop and pasture legumes (Fabaceae). In Australia, the disease Ascochyta blight of *Pisum sativum* (field pea) is typically caused by three fungal species, *Ascochyta koolunga*, *Didymella pinodella*, and *D. pinodes*. A fourth species, *Ascochyta pisi*, is very rarely isolated. One species in particular, *A. koolunga*, is an important part of the Ascochyta blight disease complex of field pea in South Australia (Davidson et al. 2009a). First described in 2009, *A. koolunga* (syn. *Phoma koolunga*) had spread across southern Australia and had been detected in Victoria and Western Australia by 2015 (Davidson et al. 2011; Tran et al. 2015a).

Molecular techniques are now routinely used to understand the genetic diversity and population structure of Didymellaceae (Aveskamp et al. 2010; Salam et al. 2011, de Gruyter 2012; Chen et al. 2015a, Hou et al. 2020). To date, there has not been a systematic inventory of leaf spot pathogens associated with Australian native legume species despite international reports from a diversity of countries on Ascochyta blight since 2009 (Le May et al. 2009; Mathew et al. 2010; Panicker and Ramraj 2010; Skoglund et al. 2011; Soylu and Dervis 2011; Gaurilcikiene and Viciene 2013; Liu et al. 2013; Ahmed et al. 2015; Liu et al. 2016). *Ascochyta koolunga* is only known to occur in Australia, which suggests an Australasian origin, with perhaps an association with native legume species. The aim of this study was to determine the species of Didymellaceae associated with leaf spot diseases, and to investigate possible native sources of *A. koolunga*. To this end we collected legume specimens from both cultivated and neighbouring natural ecosystems. In particular, we collected specimens from Australian native, pasture and naturalised legumes in the field pea growing regions of eastern and southern Australia.

Materials and methods

Sample collection and culturing

Samples of leaf tissue displaying leaf spot disease symptoms on legumes were obtained from 22 field pea trial sites, from the immediate surrounds of experimental and commercial crops and roadsides around crops in field pea growing regions of southern Australia. In total, 124 samples (stems with multiple leaves and more rarely seed pods and flowers) were collected during four separate 4–5 day (d) periods in August, September and October 2017. In addition to trial sites, local agronomists were contacted to obtain approval to allow access to growers' properties in Eyre Peninsula (South Australia) and Horsham (Victoria).

The national parks, or conservation areas, nearest to the field pea sampling sites were identified prior to field trips and permits were obtained to enable collections of samples from native plants that exhibited leaf disease symptoms within these neighbouring natural ecosystems. Leaf disease samples were also collected from two botanic gardens, Adelaide Botanic Garden, Adelaide, South Australia and the Australian Botanic Garden, Mount Annan, New South Wales. Plants with leaf spots were photographed in the field with a Samsung galaxy S5 or S8 mobile phone camera and the GPS locations recorded. Representative leaf samples were placed in plastic bags, labelled and stored at 4 °C.

Within 5 d of collection, leaf specimens were surface disinfected by spraying with 70% v/v ethanol and blotted dry with fresh, non-sterilised tissue paper. Excised leaf pieces were placed on plates of potato dextrose agar (PDA) (Oxoid) acidified by supplementation with 1 ml of 85% v/v lactic acid per litre (APDA) to minimise bacterial contamination. Incubation was under a 12 hour (h) black and fluorescent light /12 h dark cycle at 22 °C for 7–10 d, when fungal colonies were examined microscopically for pycnidia and conidia. Representative isolates were subcultured onto PDA using hyphal tips and deposited in the culture collection of the Queensland Plant Pathology Herbarium (BRIP).

DNA extraction, PCR and sequencing

Genomic DNA was extracted from 7 d old mycelium grown on PDA from the subculture isolates using the FastDNA Kit (Q-biogene Inc. Irvine, California, USA) according to the manufacturer's instructions. A section of DNA from the internal transcribed spacer (ITS) region was amplified with the primers ITS1 and ITS4 (White et al. 1990), and the partial region of the RNA polymerase II subunit B (*rpb2*) gene was amplified with the primers RPB2-5F2 (Sung et al. 2007) and RPB2-7cR (Liu et al. 1999). The PCR conditions were as described by White et al. (1990) for ITS and O'Donnell et al. (2007) for *rpb2*. All PCRs were undertaken

in 25 µl reaction volumes containing the final concentrations; 1 unit of PCR 5X buffer (Promega Corporation, Madison, Wisconsin, USA), 1.6 mM of 25 mM MgCl₂ (Sigma-Aldrich Corporation, Louis, Missouri, USA), 0.025 U/µl of GoTaq™ (Promega), 0.6 mM of primer 1 and primer 2 and 1.6 mM of each dNTP (Promega). The PCR amplicons were purified using ExoSAP-IT (USB Corporation) following the manufacturer's instructions. The purified amplicons were sent to the Ramaciotti Centre for Gene Function Analysis (University of New South Wales, Kensington, NSW), where DNA sequences were determined using an ABI PRISM 3700 DNA Analyser (Applied Biosystems Inc).

Phylogenetic analysis

Forward and reverse sequences were assembled using Geneious v. 11.1.5 (Biomatters Ltd) and deposited in GenBank (Table 1, in bold). The sequences were aligned with selected reference sequences of Didymellaceae (Table 1) using the multiple alignment MAFFT algorithm (Katoh et al. 2009) in Geneious. *Neoscochyta desmazieri* strain CBS 267.69 was included as the outgroup. The sequences of each locus were aligned separately and manually adjusted where necessary.

Maximum likelihood (ML) analysis was run using the RAxML v. 7.2.8 (Stamatakis and Alachiotis 2010) plug-in in Geneious v. 11.1.5 starting from a random tree topology. The nucleotide substitution model used was general time-reversible (GTR) with a gamma-distributed rate variation. The Bayesian analysis was performed using the MrBayes v.3.2.1 (Ronquist and Huelsenbeck 2003) plug-in in Geneious v. 11.1.5. To remove the need for a priori model testing, the Markov chain Monte Carlo (MCMC) analysis was set to sample across the entire GTR model space with a gamma-distributed rate variation across the nucleotide sites. Ten million random trees were generated using the MCMC procedure with four chains. The sample frequency was set at 2000 and the temperature of the heated chain was 0.1. "Burn-in" was set at 25%, after which the log-likelihood values were stationary.

Morphology

Fungal isolates were cultured on four media types; PDA, oatmeal agar (OA), malt extract agar (MEA) (Boerema et al. 2004; Chen et al. 2015a), and carnation leaf agar (CLA). The colonies were measured at 7 d, and morphology examined after 12–14 d incubation in the same light and temperature conditions described above. Images of the colonies were captured by an Epson Perfection V700 scanner at a 300 dpi resolution. Colony colour was determined on surface and reverse using the colour charts of Rayner (1970). Isolates were characterised microscopically from the PDA plates. Lactic acid (100 % v/v) was used as the mounting fluid. Specimens were examined using a Leica DM5500B compound microscope with a Leica DFC 500 camera fitted to capture images under Nomarski differential interference contrast illumination. Micromorphological measurements and descriptions of pycnidia,

pycnidial wall cells and conidia were taken from up to 20 samples, and septation and colour recorded. Images of pycnidia were taken from CLA plates using a Leica M165C stereo microscope and Lecia DFC 500 camera. The NaOH spot test on MEA culture plates helped distinguish taxa (Boerema et al. 2004).

Results

From 124 samples of legumes collected at 22 locations, 194 isolates were obtained of which 54 isolates were identified as Didymellaceae by ITS sequences. Of these, 36 isolates were further sequenced (*rpb2* locus). Duplicate isolates were excluded where they were from the same host species, which left 18 isolates for multilocus sequence analysis and inclusion in the phylogenetic analysis.

Phylogeny

A multilocus sequence analysis based on the ITS region and partial region of the *rpb2* gene was used to infer the relationship of the 18 isolates and recognised species in Didymellaceae (Table 1). The resulting concatenated aligned dataset comprised 124

Table 1. Didymellaceae isolates examined in this study. Novel taxa and newly generated sequences are indicated in **bold**.

Species	Strain ¹	Host	Locality ²	GenBank accessions ³	
				ITS	<i>rpb2</i>
<i>Ascochyta astragalina</i>	CBS 113797	<i>Lathyrus vernus</i>	Sweden	KT389482	MT018257
<i>Ascochyta benningiorum</i>	CBS 144957 ^T	Soil	The Netherlands	MN823581	MN824606
<i>Ascochyta coronillae-meri</i>	MFLUCC 13-0820 ^T	<i>Hippocrepis emerus</i>	Italy	MH069661	MH069679
<i>Ascochyta fabae</i>	CBS 524.77	<i>Phaseolus vulgaris</i>	Belgium	GU237880	MT018241
<i>Ascochyta herbicola</i>	CBS 629.97	Water	USA, Montana, Missoula	GU237898	KP330421
<i>Ascochyta koolunga</i>	DAR 78535 ^T	<i>Pisum sativum</i>	Australia, SA, Minnipa	EU338416	EU874849
	BRIP 70265	<i>Pisum sativum</i>	Australia, SA, Riverton	MN567671	MN604922
	BRIP 69590	<i>Pisum sativum</i>	Australia, SA, Mundulla	MN567672	MN604923
<i>Ascochyta lentis</i>	CBS 370.84	<i>Lens culinaris</i>	Unknown	KT389474	MT018246
<i>Ascochyta medicaginicola</i>	CBS 112.53 ^T	<i>Medicago sativa</i>	USA	GU237749	MT018251
<i>Ascochyta nigripynidia</i>	CBS 116.96 ^T	<i>Vicia cracca</i>	Russia	GU237756	MT018253
<i>Ascochyta phacae</i>	CBS 184.55 ^T	<i>Phaca alpine</i>	Switzerland	KT389475	MT018255
<i>Ascochyta pilosella</i>	CBS 583.97 ^T	<i>Clintonia uniflora</i>	Canada	MN973590	MT018258
<i>Ascochyta pisi</i>	CBS 122785	<i>Pisum sativum</i>	The Netherlands	GU237763	MT018244
<i>Ascochyta rabiei</i>	CBS 237.37 ^T	<i>Cicer arietinum</i>	Bulgaria	KT389479	MT018256
<i>Ascochyta rosae</i>	MFLUCC 15-0063 ^T	<i>Rubus ulmifolius</i>	Italy	KY496751	KY514409
<i>Ascochyta syringae</i>	CBS 545.72 ^T	<i>Syringa vulgaris</i>	The Netherlands	KT389483	MT018245
<i>Ascochyta versabilis</i>	CBS 876.97	<i>Silene</i> sp.	The Netherlands, Wageningen	GU237909	KT389561
<i>Ascochyta viciae</i>	CBS 451.68	<i>Vicia sepium</i>	The Netherlands, Baarn, Praamgracht	KT389484	KT389562
<i>Ascochyta viciae-pannonicae</i>	CBS 254.92	<i>Vicia pannonica</i>	Czechoslovakia	KT389485	MT018250
<i>Ascochyta viciae-villosae</i>	CBS 255.92	<i>Vicia villosa</i>	Czechoslovakia	MN973584	MT018249
<i>Didymella americana</i>	CBS 185.85	<i>Zea mays</i>	USA, Georgia	FJ426972	KT389594
<i>Didymella anserina</i>	CBS 253.80		Germany	KT389498	KT389595
<i>Didymella arachidicola</i>	CBS 333.75 ^T	<i>Arachis hypogaea</i>	South Africa, Cape Province	GU237833	KT389598
<i>Didymella aurea</i>	CBS 269.93 ^T	<i>Medicago polymorpha</i>	New Zealand, Auckland	GU237818	KT389599
<i>Didymella chlamydospora</i>	YW23-14 ^T	Soil	South Korea	MK836111	LC480708

Species	Strain ¹	Host	Locality ²	GenBank accessions ³	
				ITS	<i>rpb2</i>
<i>Didymella coffeae-arabicae</i>	CBS 123380 ^T	<i>Coffea Arabica</i>	Ethiopia	FJ426993	KT389603
<i>Didymella combreti</i>	CBS 137982 ^T	<i>Combretum mossambiciensis</i>	Zambia	MN973525	MT018139
<i>Didymella curtisii</i>	CBS 251.92	<i>Nerine</i> sp.	The Netherlands	FJ427038	MT018131
<i>Didymella degraffiae</i>	CBS 144956 ^T	Soil	The Netherlands	MN823444	MN824470
<i>Didymella eucalyptica</i>	CBS 377.91	<i>Eucalyptus</i> sp.	Australia, WA	GU237846	KT389605
<i>Didymella gardeniae</i>	CBS 626.68 ^T	<i>Gardenia jasminoides</i>	India	FJ427003	KT389606
<i>Didymella glomerata</i>	CBS 528.66	<i>Chrysanthemum</i> sp.	The Netherlands	FJ427013	GU371781
<i>Didymella guttulata</i>	CBS 127976 ^T	Soil	Zimbabwe	MN973524	MT018138
<i>Didymella heteroderiae</i>	CBS 109.92 ^T	Undefined food material	The Netherlands	FJ426983	KT389601
<i>Didymella keratinophila</i>	UTHSC DI16-200 ^T	<i>Homo sapiens</i>	USA	LT592901	LT593039
<i>Didymella lethalis</i>	CBS 103.25			GU237729	KT389607
	BRIP 69584	<i>Lathyrus tingitanus</i>	Australia, SA, Brownhill Creek	MN567674	MN604925
<i>Didymella magnoliae</i>	MFLUCC 18-1560 ^T	<i>Magnolia grandiflora</i>	China	MK347814	MK434852
<i>Didymella maydis</i>	CBS 588.96 ^T	<i>Zea mays</i>	USA, Wisconsin, Hancock	FJ427086	GU371782
<i>Didymella mitis</i>	CBS 443.72 ^T	Soil	South Africa	MN973523	MT018137
<i>Didymella musae</i>	CBS 463.69	<i>Mangifera indica</i>	India	FJ427026	MT018148
<i>Didymella nigricans</i>	CBS 444.81	<i>Acer palmatum</i>	Japan	KY742075	KY742158
<i>Didymella pinodela</i>	CBS 318.90	<i>Pisum sativum</i>	The Netherlands	FJ427051	MN983533
	BRIP 69589	<i>Pisum sativum</i>	Australia, VIC, Rainbow	MN567675	MN604926
<i>Didymella pinodes</i>	CBS 525.77 ^T	<i>Pisum sativum</i>	Belgium	GU237883	KT389614
	BRIP 69581	<i>Senna artemisioides</i>	Australia, SA, Blanchetown	MN567676	MN604927
	BRIP 69593	<i>Senna artemisioides</i>	Australia, SA, Blyth	MN567677	MN604928
	BRIP 69596	<i>Senna artemisioides</i>	Australia, SA, Wudinna	MN567678	MN604929
	BRIP 69578	<i>Vicia cracca</i>	Australia, NSW, Cowra	MN567679	MN604930
<i>Didymella pomorum</i>	CBS 539.66	<i>Polygonum tataricum</i>	The Netherlands	FJ427056	KT389618
<i>Didymella prolaticolla</i>	CBS 126182 ^T	Soil	Namibia	MN973533	MT018157
<i>Didymella prosopidis</i>	CBS 136414 ^T	<i>Prosopis</i> sp.	South Africa	FJ777180	MT018149
	BRIP 69579	<i>Gastrolobium celsianum</i>	Australia, SA, Adelaide	MN5676780	MN604931
<i>Didymella protuberans</i>	CBS 381.96 ^T	<i>Lycium halifolium</i>	The Netherlands	GU237853	KT389620
<i>Didymella sancta</i>	CBS 281.83 ^T	<i>Ailanthus altissima</i>	South Africa	FJ427063	KT389623
<i>Didymella sinensis</i>	CGMCC 3.18348 ^T	<i>Cerasus pseudocerasus</i>	China	KY742085	MT018127
<i>Didymella subglobispora</i>	CBS 364.91 ^T	<i>Ananas sativus</i>		MN973531	MT018153
<i>Didymella subglomerata</i>	CBS 110.92	<i>Triticum</i> sp.	USA, North Dakota	FJ427080	KT389626
<i>Epicoccum brahmansense</i>	CBS 990.95 ^T	Soil	Papua New Guinea	MN973513	MT018119
<i>Epicoccum brasiliense</i>	CBS 120105 ^T	<i>Amaranthus</i> sp.	Brazil	GU237760	KT389627
<i>Epicoccum camelliae</i>	CGMCC 3.18343 ^T	<i>Camellia sinensis</i>	China	KY742091	KY742170
<i>Epicoccum catenisorum</i>	CBS 181.80 ^T	<i>Oryza sativa</i>	Guinea-Bissau	FJ427069	LT623253
<i>Epicoccum dendrobii</i>	CGMCC 3.18359 ^T	<i>Dendrobium fimbriatum</i>	China	KY742093	MT018084
<i>Epicoccum dickmanii</i>	CBS 124671 ^T	<i>Acropora Formosa</i>	Australia	MN973509	MT018113
<i>Epicoccum djarangnandiri</i> sp. nov.	BRIP 69585 ^T	<i>Swainsona galegifolia</i>	Australia, NSW, Mount Annan	MN567673	MN604924
<i>Epicoccum draconis</i>	CBS 186.83	<i>Dracaena</i> sp.	Rwanda	GU237795	KT389628
<i>Epicoccum duchesneae</i>	CGMCC 3.18345 ^T	<i>Duchesnea indica</i>	China	KY742095	MT018115
<i>Epicoccum henningsii</i>	CBS 104.80	<i>Acacia mearnsii</i>	Kenya	GU237731	KT389629
<i>Epicoccum hordei</i>	CGMCC 3.18360 ^T	<i>Hordeum vulgare</i>	Australia	KY742097	MT018102
<i>Epicoccum huancayense</i>	CBS 105.80 ^T	<i>Solanum</i> sp.	Peru	GU237732	KT389630
<i>Epicoccum italicum</i>	CGMCC 3.18361 ^T	<i>Acca sellowiana</i>	Italy	KY742099	KY742172
<i>Epicoccum keratinophilum</i>	UTHSC DI16-271 ^T	<i>Homo sapiens</i>	USA	LT592930	LT593068
<i>Epicoccum laticollum</i>	CGMCC 3.18346 ^T	<i>Sorghum bicolor</i>	China	KY742101	KY742174
<i>Epicoccum longiostiolatum</i>	CBS 886.95 ^T	<i>Stellaria</i> sp.	Papua New Guinea	FJ427074	MT018108
<i>Epicoccum mackenziei</i>	MFLUCC 16-0335 ^T	<i>Ononis spinose</i>	Italy	KX698039	KX698035
<i>Epicoccum mezzettii</i>	CBS 173.38 ^T	<i>Populus pulp</i>	Italy	MN973496	MT018095
<i>Epicoccum nigrum</i>	CBS 173.73 ^T	<i>Dactylis glomerata</i>	USA	FJ426996	KT389632
<i>Epicoccum oviporum</i>	CBS 180.80 ^T	<i>Zea mays</i>	South Africa	FJ427068	LT623252
<i>Epicoccum phragmospora</i>	CGMCC 3.19339 ^T	<i>Saccharum officinarum</i>	China	MN215619	MN255460
<i>Epicoccum pimprinum</i>	CBS 246.60 ^T	Soil	India	FJ427049	MT018100
<i>Epicoccum plurivorum</i>	CBS 558.81 ^T	<i>Setaria</i> sp.	New Zealand	GU237888	KT389634

Species	Strain ¹	Host	Locality ²	GenBank accessions ³	
				ITS	<i>rpb2</i>
<i>Epicoccum pneumoniae</i>	UTHSC DI16-257 ^T	<i>Homo sapiens</i>	USA	LT592927	LT593065
<i>Epicoccum poaeicola</i>	MFLUCC 15-0448 ^T	Poaceae	Thailand	KX965727	KX898365
<i>Epicoccum poae</i>	CGMCC 3.18363 ^T	<i>Poa annua</i>	USA	KY742113	KY742182
<i>Epicoccum polychromum</i>	CBS 141502 ^T	<i>Paspalum dilanatum</i>	France	MN973506	MT018109
<i>Epicoccum proteae</i>	CBS 114179 ^T	<i>Protea compacta</i> x <i>Protea neriifolia</i>	South Africa, Somerset West	JQ044433	LT623251
<i>Epicoccum pseudokeratinophilum</i>	MFLUCC 18-1593 ^T	<i>Prunus avium</i>	China	MH827002	MH853659
<i>Epicoccum purpurascens</i>	CBS 128906	Soil	USA	MN973488	MT018083
<i>Epicoccum sorghinum</i>	CBS 179.80	<i>Sorghum bicolor</i>	Puerto Rico	FJ427067	KT389635
<i>Epicoccum tobaicum</i>	CBS 384.36 ^T	Soil	Indonesia	MN973493	MT018092
<i>Epicoccum variabile</i>	CBS 119733 ^T	<i>Coffea Arabica</i>	Brazil	MN973501	MT018103
<i>Epicoccum viticis</i>	CGMCC 3.18344 ^T	<i>Vitex negundo</i>	China	KY742118	KY742186
<i>Neoscochyta desmazieri</i> (outgroup)	CBS 297.69 ^T	<i>Lolium perenne</i>	Germany, Hohenlieth	KT389508	KT389644
<i>Neodidymelliopsis achlydis</i>	CBS 256.77 ^T	<i>Achlys triphylla</i>	Canada, British Columbia, Vancouver Island	KT389531	MT018293
<i>Neodidymelliopsis cannabis</i>	CBS 234.37	<i>Cannabis sativa</i>	Unknown	GU237804	KP330403
<i>Neodidymelliopsis farokhinejadii</i>	CBS 142853	<i>Conocarpus erectus</i>	Iran	KY449009	KY464922
<i>Neodidymelliopsis longicolla</i>	CBS 382.96 ^T	Soil	Israel, En Avdat, Negev desert	KT389532	MT018298
<i>Neodidymelliopsis moricola</i>	MFLUCC 17-1063	<i>Morus alba</i>	Russia	KY684939	KY684943
<i>Neodidymelliopsis negundinis</i>	JZB380011	<i>Acer negundo</i>	Russia	MG564165	MG564166
<i>Neodidymelliopsis polemonii</i>	CBS 109181 ^T	<i>Polemonium caeruleum</i>	The Netherlands	GU237746	KP330427
<i>Neodidymelliopsis ranunculi</i>	CBS 286.72	<i>Citrus limonium</i>	Italy	MN973612	MT018294
<i>Neodidymelliopsis tillae</i>	CBS 519.95 ^T	<i>Tilia</i> sp.	Italy	MN973610	MT018287
<i>Neodidymelliopsis tinkyukuku</i> sp. nov.	BRIP 69592 ^T	<i>Hardenbergia violacea</i>	Australia, SA, Clare	MN5676781	MN604932
<i>Neodidymelliopsis xanthina</i>	CBS 383.68 ^T	<i>Delphinium</i> sp.	The Netherlands, Baarn	GU237855	KP330431
<i>Nothophoma acaciae</i>	CBS 143404 ^T	<i>Acacia melanoxylon</i>	Australia	MG386056	MG386144
<i>Nothophoma anigozanthi</i>	CBS 381.91 ^T	<i>Anigozanthus maugeisii</i>	The Netherlands	GU237852	KT389655
<i>Nothophoma arachidis-hypogaeae</i>	CBS 125.93	<i>Anachis hypogaea</i>	India, Madras	GU237771	KT389656
<i>Nothophoma brennandiae</i>	CBS 145912 ^T	Soil	The Netherlands	MN823579	MN824604
<i>Nothophoma garbiwalawardae</i> sp. nov.	BRIP 69580	<i>Senna artemisioides</i>	Australia, SA, Adelaide	MN5676782	MN604933
	BRIP 69586	<i>Senna artemisioides</i>	Australia, SA, Berri	MN5676783	MN604934
<i>Nothophoma garbiwalawardae</i> sp. nov.	BRIP 69587	<i>Senna artemisioides</i>	Australia, SA, Berri	MN5676784	MN604935
	BRIP 69594	<i>Senna artemisioides</i>	Australia, SA, Kimba	MN5676785	MN604936
	BRIP 69595 ^T	<i>Senna artemisioides</i>	Australia, SA, Wudinna	MN5676786	MN604937
<i>Nothophoma eucalyptigena</i>	CBS 142535 ^T	<i>Eucalyptus</i> sp.	Australia	KY979771	KY979852
<i>Nothophoma gossypicola</i>	CBS 377.67	<i>Gossypium</i> sp.	USA, Texas	GU237845	KT389658
<i>Nothophoma infossa</i>	CBS 123395 ^T	<i>Fraxinus pennsylvanica</i>	Argentina, Buenos Aires Province, La Plata	FJ427025	KT389659
<i>Nothophoma infusata</i>	CBS 121931 ^T	<i>Acacia longifolia</i>	New Zealand	MN973559	MN973559
<i>Nothophoma macrospora</i>	UTHSC DI16-199 ^T	<i>Homo sapiens</i>	USA, Arizona	LN880536	LT593073
<i>Nothophoma naiauw</i> sp. nov.	BRIP 69583 ^T	<i>Senna artemisioides</i>	Australia, SA, Blanchetown	MN5676787	MN604938
	BRIP 69582 ^T	<i>Senna artemisioides</i>	Australia, SA, Blanchetown	MN5676788	MN604939
<i>Nothophoma nullicana</i>	CPC 32330 ^T	<i>Acacia falciformis</i>	Australia	NR_156665	MG386143
<i>Nothophoma pruni</i>	MFLUCC 18-1600	<i>Prunus avium</i>	China	MH827005	MH853662
<i>Nothophoma quercina</i>	CBS 633.92	<i>Microsphaera alphitoides</i> from <i>Quercus</i> sp.	Ukraine	GU237900	KT389657
<i>Nothophoma variabilis</i>	UTHSC DI16-285 ^T	<i>Homo sapiens</i>	USA	LT592939	LT593078

¹ BRIP, Queensland Plant Pathology Herbarium, Brisbane, QLD, Australia; CBS, Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CGMCC, China General Microbiological Culture Collection, Beijing, China; MFLUCC, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; UTHSC, Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, Texas, USA.

² NSW, New South Wales; SA, South Australia; VIC, Victoria; WA, Western Australia.

³ ITS, internal transcribed spacer region; *rpb2*, RNA polymerase II second subunit.

^T ex-type strain.

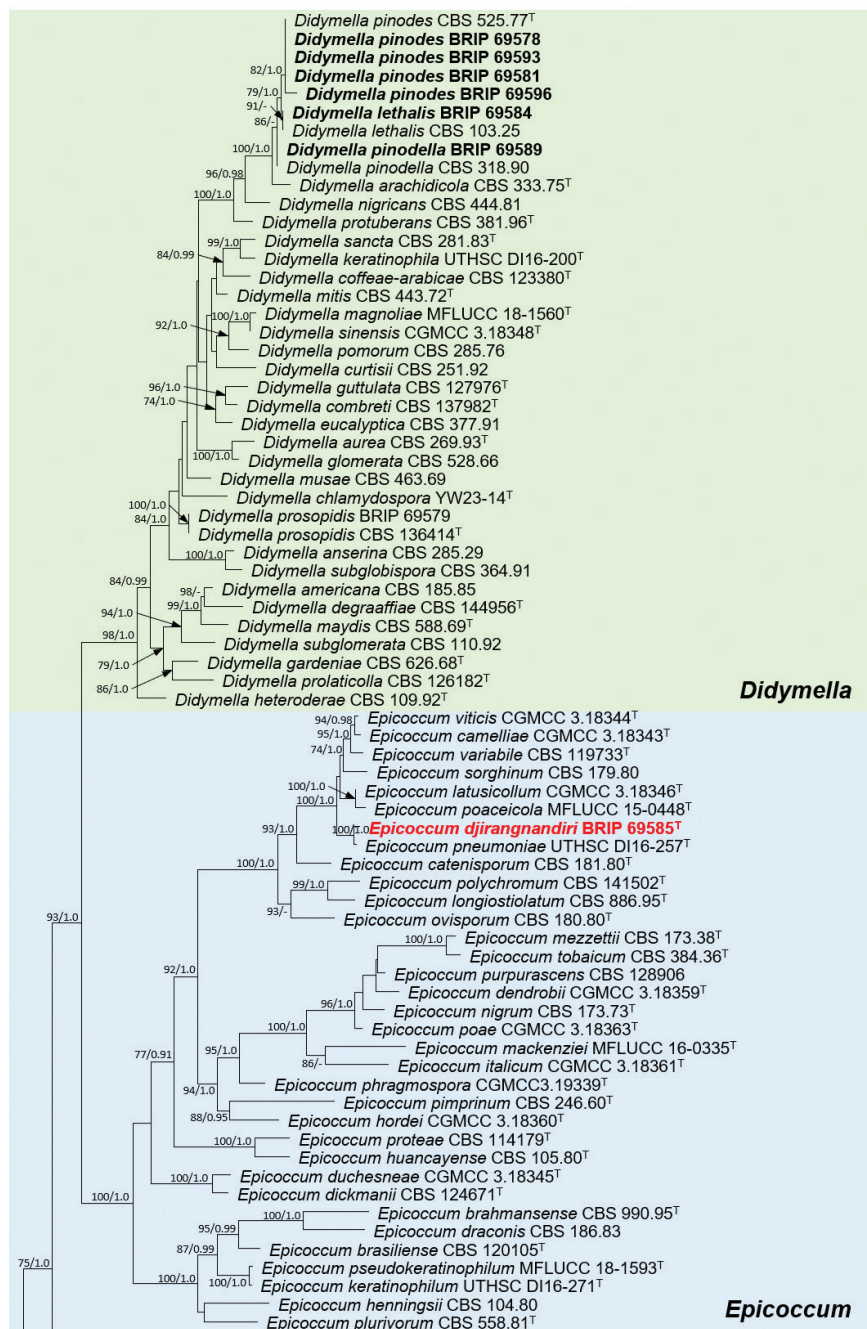


Figure 1. Phylogenetic tree based on maximum likelihood analysis of the combined multilocus (*rpb2* and ITS) alignment. RAxML bootstrap values (bs) greater than 70 % and Bayesian posterior probabilities (pp) greater than 0.95 are given at the nodes (bs/pp). Genera are delimited in coloured boxes, with the genus name indicated to the right. Isolates identified in this study are in **bold**, and novel taxa are in **red bold**. Ex-type isolates are marked with ^T. The outgroup is *Neoscochyta desmazieri* (CBS 297.69).

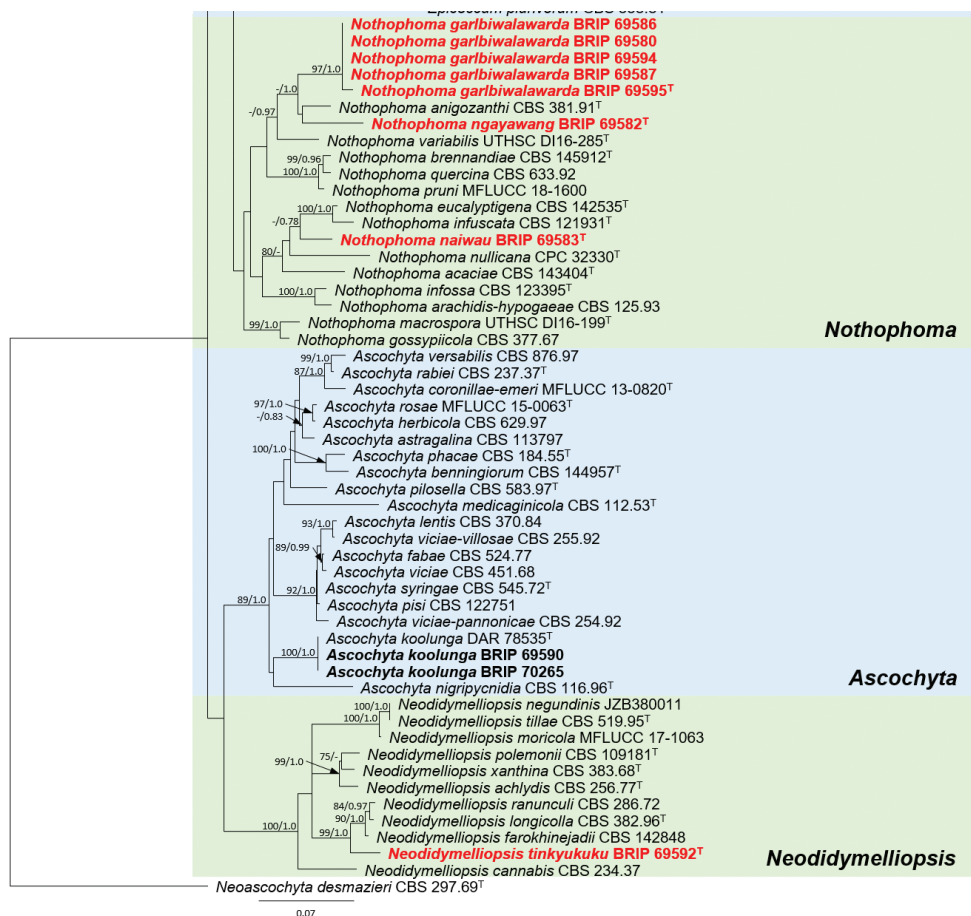


Figure 1. Continued.

ingroup isolates from 111 taxa, and consisted of 1,090 characters (493 for ITS, and 596 for *rpb2*, including alignment gaps). The ML tree based on the combined dataset is presented, with bootstrap support values (BS) greater than 70% and Bayesian posterior probabilities (PP) greater than 0.95 indicating four well-supported clades, and limited support for *Nothophoma* (Fig. 1). The ITS phylogeny, using either ML or Bayesian analysis, provided poor resolution at the genus and species level (data not shown). The phylogenetic tree based on the concatenated alignment of ITS and *rpb2* indicates the placement of the 18 isolates (Fig. 1), five of which represent novel species (Figs 2–6).

We identified three new host-pathogen associations, and one new record for Australia *Didymella pinodes* (strains BRIP 69581, 69593, and 69596) was isolated from native *S. artemisioides* from three locations in South Australia separated by over 400 km. *Didymella pinodes* (strain BRIP 69578) was also isolated from naturalised *Vicia cracca* (tufted vetch) in New South Wales from an area which did not cultivate *P. sativum*.

Didymella lethalis (strain BRIP 69584) was isolated from the naturalised *Lathyrus tingitanus* (tangier pea) from a recreational walking area within an urban environment. *Didymella prosopidis* (strain BRIP 69579) was isolated from *Gastrolobium celsianum* from the botanic gardens in the capital city of South Australia, Adelaide.

Taxonomy

Multilocus sequence analysis and morphological comparisons classified nine fungal isolates from legumes in southern Australia into five novel species from three Didymellaceae genera. The novel species are described and illustrated in Figs 2–6. Nomenclatural novelties are registered in MycoBank.

The species epithets were derived from Indigenous Australian Peoples' language groups to provide a uniquely Australian theme. Permission to use words from the local language of the area in which the fungi were collected was granted by elders or community representatives.

***Epicoccum djirangnandiri* E.C. Keirnan, M.H. Laurence, R.G. Shivas & Y.P. Tan, sp. nov.**

MycoBank No: 833689

Fig. 2

Type. AUSTRALIA, New South Wales, Mount Annan, *Swainsona galegifolia*, 19 Jan. 2017, E.C. Keirnan (holotype BRIP 69585, includes culture ex-type).

Description. Colonies on OA, 76–80 mm diam. after 7 d, covered in dense aerial mycelium, variable shades of grey, pale cinnamon towards centre; reverse dark vinaceous; on MEA, 70–72 mm after 7 d, margin entire, covered in low dense aerial mycelium, pale mouse grey with lighter patches; reverse olivaceous with radiating spokes; on PDA, 73–80 mm after 7 d, margin entire, mycelia felty, mouse grey becoming vinaceous buff towards centre; reverse fuscous black. *NaOH spot test*: negative. *Conidiomata* on CLA, pycnidial, globose 100–200 µm diam., pale brown becoming black, solitary, glabrous, non-papillate; pycnidial wall composed of textura globulosa, pale brown, cells 5–15 µm diam. *Conidiogenous* cells phialidic, cylindral, thin-walled, hyaline, rounded ends. *Conidia* aseptate, 5–7 × 2–3 µm.

Etymology. From the language of the Indigenous Australian Dharawal people, meaning leaf spot. The Dharawal people are from the western Sydney region in New South Wales, which includes Mount Annan, where the holotype was collected.

Notes. *Epicoccum djirangnandiri* is phylogenetically close to *E. pneumoniae* ex-type strain UTHSC DI16-257 (Fig. 1) and is distinguished in *rpb2* sequences with 99% identity. Morphological comparisons could not be made as *E. pneumoniae* was sterile in culture (Valenzuela-Lopez et al. 2018). *Epicoccum djirangnandiri* is only known from one specimen on *Swainsona galegifolia*.

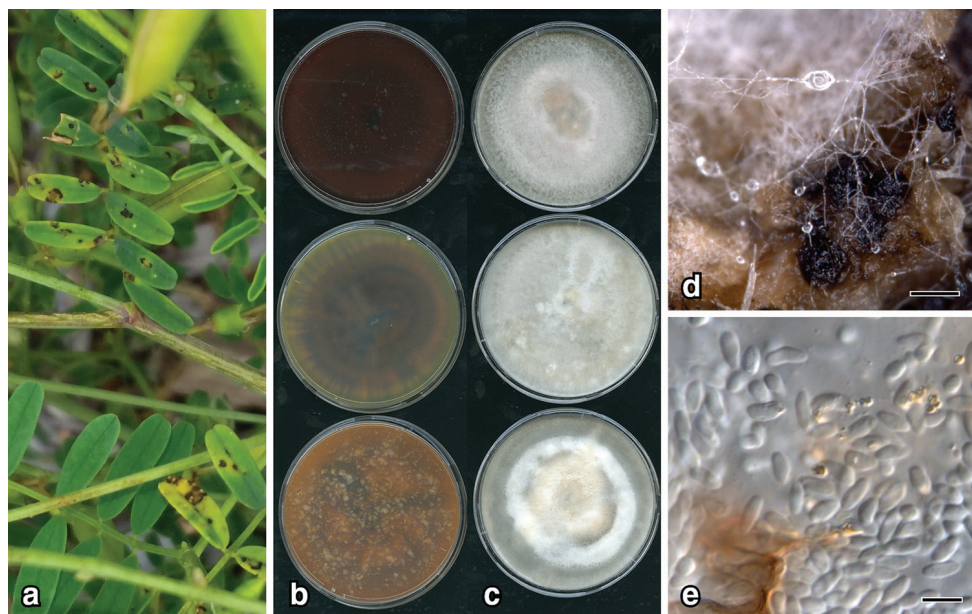


Figure 2. *Epicoccum djirangnandiri*: **a** leaf lesions on *Swainsona galegifolia* **b** 14-d old colonies on PDA, MEA, OA (left, top to bottom) and lower surface (right) **c** upper surface **d** pycnidia on CLA **e** conidia. Scale bars: 200 μ m (**d**); 7 μ m (**e**).

***Neodidymelliopsis tinkyukuku* E.C. Keirnan, M.H. Laurence, R.G. Shivas & Y.P. Tan, sp. nov.**

Mycobank No: 833692

Fig. 3

Type. AUSTRALIA, South Australia, Clare, *Hardenbergia violacea*, 17 Sep. 2017, *E.C. Keirnan* (holotype BRIP 69592, includes culture ex-type).

Description. *Colonies* on OA, 26–28 mm diam. after 7 d, dense low aerial mycelium, buff with numerous grey patches, darker with abundant pycnidia at centre; reverse buff to rosy buff with darker concentric rings towards centre; on MEA, 28–30 mm after 7 d, margin entire, dense low aerial mycelium, vinaceous buff paler at margin; reverse rosy buff to buff at margin with abundant scattered pycnidia; on PDA, 35–38 mm after 7 d, margin entire, dense low aerial mycelium, pale mouse grey lighter at margin; reverse cinnamon with concentric dark rings, darker at centre. *NaOH spot test*: light yellow. *Conidiomata* on CLA pycnidial, globose to ampulliform, 250–350 μ m diam., brown becoming black, solitary, abundant in centre of colony, zonate, glabrous, non-papillate; ostiole c. 25 μ m diam.; pycnidial wall composed of textura angularis, pale brown, cells 5–8 μ m diam. *Conidiogenous cells* phialidic, cylindrical, thin-walled, hyaline. *Conidia* occasionally septate, 6–9 $\times$ 2–3 μ m, cylindrical, hyaline, thin-walled.

Etymology. From the language of the Indigenous Australian Kurna people, meaning leaf disease. The Kurna people are from the Adelaide plains region, which includes Clare, the locality where the holotype was collected.

Notes. *Neodidymelliopsis tinkyukuku* (strain BRIP 69592) is sister to a clade that includes *N. farokhinejadii* (strain CBS 142853), *N. longicolla* (ex-type strain CBS 382.96) and *N. ranunculi* (strain CBS 286.72) (Fig. 1). *Neodidymelliopsis* conidial dimensions are distinct from *N. farokhinejadii* ($4.6\text{--}7.5 \times 2.4\text{--}3.9 \mu\text{m}$), *N. longicolla* ($12\text{--}15 \times 4\text{--}7 \mu\text{m}$), and *N. ranunculi* ($3\text{--}5 \times 7.5\text{--}10 \mu\text{m}$). *Neodidymelliopsis tinkyukuku* can be easily distinguished from these three species by DNA sequences of the *rpb2* locus.

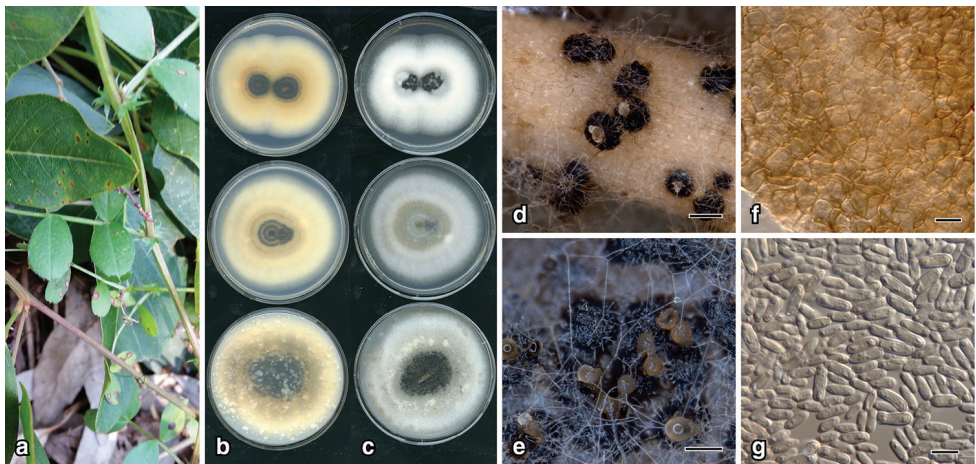


Figure 3. *Neodidymelliopsis tinkyukuku*: **a** leaf lesions on *Hardenbergia violacea* **b** 12-d old colonies top to bottom on PDA, MEA, OA (left, top to bottom) and lower surface (right) **c** upper surface **d** pycnidia on CLA **e** pycnidia **f** pycnidial wall **g** conidia. Scale bars: 300 μm (**d**, **e**); 10 μm (**f**); 7 μm (**g**).

***Nothophoma garlbiwalawarda* E.C. Keirnan, M.H. Laurence, R.G. Shivas & Y.P. Tan, sp. nov.**

MycoBank No: 833693

Fig. 4

Type. AUSTRALIA, South Australia, Wudinna, *Senna artemisioides*, 19 Aug. 2017, E.C. Keirnan (holotype BRIP 69595, includes culture ex-type).

Description. *Colonies* on OA, 27–30 mm diam. after 7 d, flat with scant aerial mycelia with a few zonate rings, vinaceous to dark vinaceous; vinaceous to dark vinaceous; on MEA, 23–25 mm after 7 d, margin entire, flat, scant aerial mycelium towards centre, amber with abundant pycnidia; reverse amber darker towards centre; on PDA, 28–30 mm after 7 d, margin irregular, flat with aerial mycelia tufted in centre, dark with abundant pycnidia in concentric rings, buff at margin; reverse dark becoming buff at margin. *NaOH spot test*: reddish. *Conidiomata* pycnidial, globose to

subglobose, 130–320 µm diam., pale brown, scattered, abundant, zonate, glabrous, non-papillate; ostiole c. 25 µm diam.; pycnidial wall composed of textura angularis, pale to medium brown, cells 5–12 µm diam. *Conidiogenous* cells phialidic, cylindrical, thin-walled, hyaline 5–12 × 2–4 µm long, narrower at the apex. *Conidia* aseptate, 5–7.0 × 2.0–3.0 µm, parallel to narrowly ellipsoidal, hyaline, wall c. 0.5 µm.

Etymology. From the native language of the Indigenous Australian Barngarla people, meaning leaf-fun-guy. The Barngarla people are from the Eyre Peninsula region, which includes Wudinna, the locality where the holotype was collected.

Additional material examined. AUSTRALIA, South Australia, Adelaide, *Senna artemisioides*, 26 Oct. 2016, E.C. Keirnan (BRIP 69580); Berri, *Senna artemisioides*, 01 Jul. 2017, E.C. Keirnan (BRIP 69586); *ibid*, 01 Jul. 2017, E.C. Keirnan (BRIP 69587); Kimba, *Senna artemisioides*, 17 Sep. 2017, E.C. Keirnan (BRIP 69594).

Notes. *Nothophoma garlbiwalawarda* is phylogenetically closest to *No. anigozanthi* and two novel species (see below for notes) (Fig. 2). *Nothophoma garlbiwalawarda* is distinguished from *No. anigozanthi* by its larger conidia (cf. 3.5–5 × 1.5–2.5 µm), *rpb2* sequence (93% identity), and its reaction to *NaOH* spot test on MEA (dull green then black).

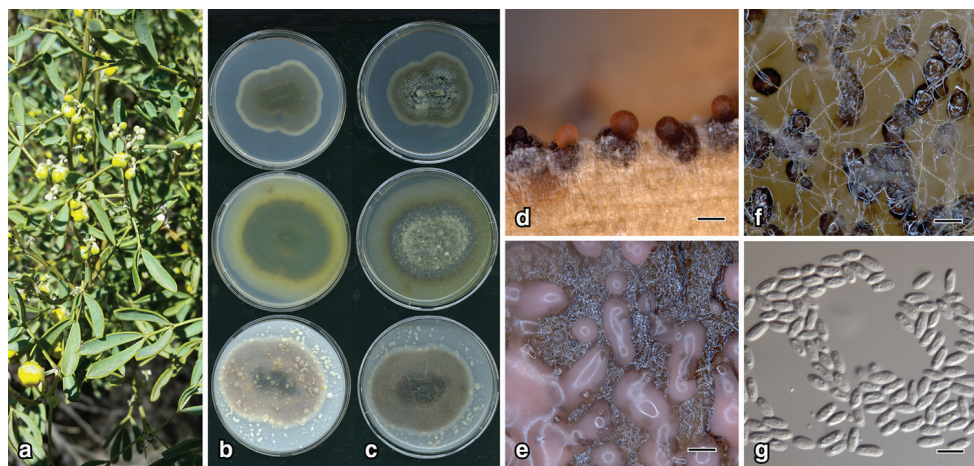


Figure 4. *Nothophoma garlbiwalawarda*: **a** pin-prick leaf spots on *Senna artemisioides* from Wudinna SA **b** 12-d old colonies top to bottom on PDA, MEA, OA (left, top to bottom) and lower surface (right) **c** upper surface **d** pycnidia on CLA **e** pycnidia and pycnidial ooze on OA **f** pycnidia on PDA **g** conidia. Scale bars: 300 µm (**d**, **e**, **f**); 7 µm (**g**).

Nothophoma naiawu E.C. Keirnan, M.H. Laurence, R.G. Shivas & Y.P. Tan, sp. nov.
Mycobank No: 833694

Fig. 5

Type. AUSTRALIA, South Australia, Blanchetown, from *Senna artemisioides*, 22 Oct. 2016, E.C. Keirnan, holotype BRIP 69583 (includes culture ex-type).

Description. *Colonies* on OA, 21–25 mm diam. after 7 d, flat with scant aerial mycelia, rosy vinaceous, dark at centre; reverse rosy buff, dark at centre, with a few dark radiating fissures; on MEA, 27–30 mm after 7 d, margin entire, flat, with sparse aerial mycelium towards centre rosy vinaceous; reverse peach, darker at centre; on PDA, 27–30 mm after 7 d, margin entire, flat felty, rosy buff; reverse peach, dark at centre. *NaOH spot test*: slightly yellow. *Conidiomata* pycnidial, globose to subglobose, 200–300 µm diam., pale brown becoming black, semi-immersed, confluent on MEA, glabrous, non-papillate; ostiole c. 25 µm diam.; pycnidial wall composed of textura globulosa, pale brown, cells 5–8 µm diam.. *Conidiogenous* cells phialidic, cylindrical, very thin-walled, hyaline. *Conidia* aseptate or 1-septate, 8–12 × 4–6 µm, cylindrical to narrow ellipsoidal, pale yellow.

Etymology. A variation of the Indigenous Australian Ngayawang people's language group, who lived in the Murray River region of South Australia, which includes Blanchetown, the locality where this specimen was collected.

Notes. *Nothophoma naiawu* is phylogenetically close to *No. eucalyptigena* and *No. infuscata* (Fig. 2). *Nothophoma naiawu* is easily distinguished from *No. eucalyptigena* and *No. infuscata* by the ITS region (98 % identity to both) and the *rpb2* locus (95%, and 94% identity, respectively). *Nothophoma infuscata* produce a pale red discolouration in response to *NaOH spot test* on MEA media, which is distinct from the slightly yellow response by *No. naiawu*.

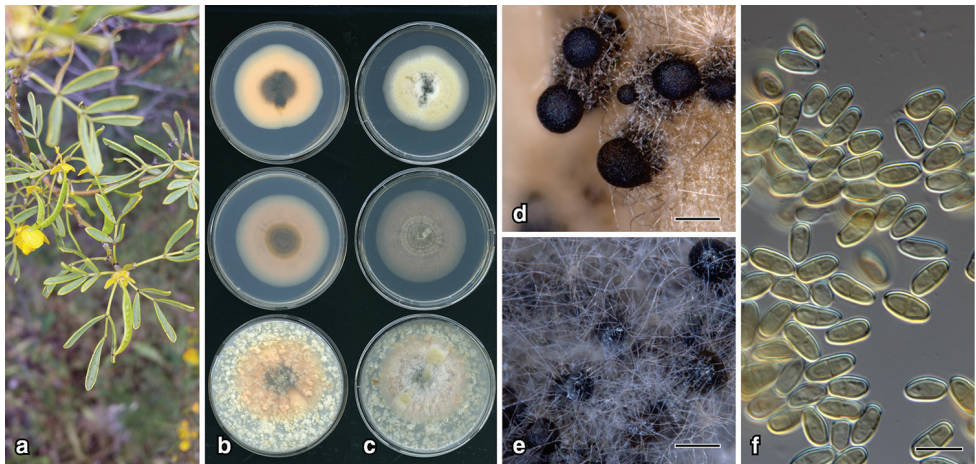


Figure 5. *Nothophoma naiawu*: **a** pin-prick leaf spots on *Senna artemisioides* **b** 14-d old colonies top to bottom on PDA, MEA, OA (left, top to bottom) and lower surface (right) **c** upper surface **d** pycnidia on CLA **e** pycnidia **f** conidia. Scale bars: 300 µm (**d**, **e**); 10 µm (**f**).

Nothophoma ngayawang E.C. Keirnan, M.H. Laurence, R.G. Shivas & Y.P. Tan, sp. nov.

Mycobank No: 833695

Fig. 6

Type. AUSTRALIA, South Australia, Blanchetown, *Senna artemisioides*, 22 Oct. 2016, E.C. Keirnan, holotype BRIP 69582 (includes culture ex-type).

Description. *Colonies* on OA, 18–20 mm diam. after 7 d, covered by scant tufted aerial mycelia at centre becoming abundant and floccose towards margin, rosy buff becoming darker towards centre; reverse salmon with centre and margins pale isabelline; on MEA, 15–20 mm after 7 d, margin irregular, felty buff becoming white towards the margin; reverse pale rosy buff, darker at centre becoming paler near margin; on PDA, 18–21 mm after 7 d, margin regular, aerial mycelia tufted in centre becoming floccose toward the margin, white to pale rosy buff; reverse pale rosy buff with few scattered vinaceous spots. *NaOH spot test*: slightly yellow. *Conidiomata* pycnidial, globose to subglobose, 200–300 μm diam., pale brown becoming black, solitary, abundant in centre of colony, glabrous, non-papillate; ostiole c. 25 μm diam.; pycnidial wall composed of textura globulosa, pale brown, cells 5–8 μm diam. *Conidiogenous* cells phialidic, cylindrical, thin-walled, hyaline. *Conidia* aseptate, $2.5\text{--}4.0 \times 1.0\text{--}2.0$ μm , cylindrical to narrow ellipsoidal, hyaline, thin-walled.

Etymology. Named after the Indigenous Australian Ngayawang people's language group, who existed in the Murray River region of South Australia, which includes Blanchetown, the locality where this specimen was collected.

Notes. *Nothophoma ngayawang* is phylogenetically close to *No. anigozanthi* ex-type strain CBS 381.91 (Fig. 2). *Nothophoma ngayawang* is distinguished from *No. variabilis* by the ITS region (98 % identity) and the *rpb2* locus (93% identity). The *NaOH spot test* of *No. variabilis* was negative on MEA, which is distinguished from the slightly yellow reaction of *No. ngayawang*.

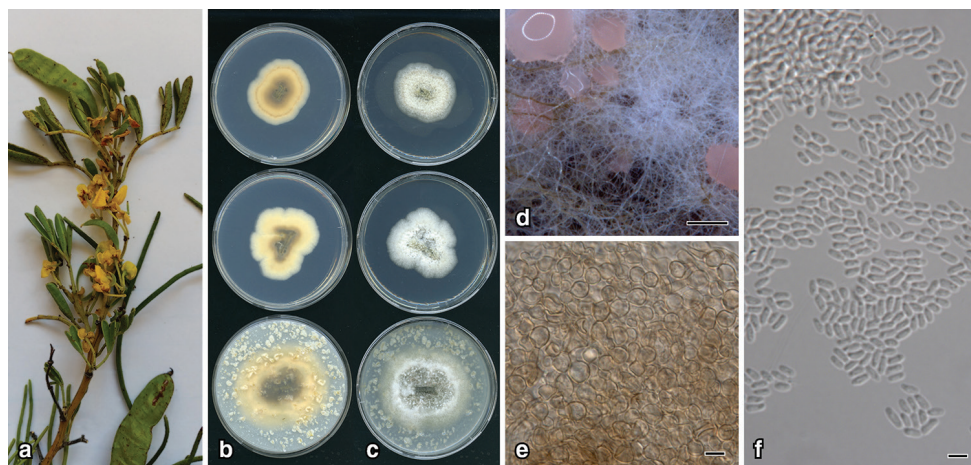


Figure 6. *Nothophoma ngayawang*: **a** leaf and pod lesions on *Senna artemisioides* **b** 14-d old colonies, top to bottom on PDA, MEA, OA (left, top to bottom) and lower surface (right) **c** upper surface **d** pycnidia **e** pycnidial wall **f** conidia. Scale bars: 250 μm (**d**); 8 μm (**e**); 3 μm (**f**).

Discussion

Our investigations did not identify *A. koolunga* from native Australian legumes. In fact, the incidence was low in that only one isolate (BRIP 69590) was collected from

P. sativum in South Australia. It is difficult to make an association between the low incidence of *A. koolunga* on *P. sativum* and the absence of *A. koolunga* on other legumes. While the current evidence suggests that *A. koolunga* is unlikely to have originated from Australian native legumes, additional field surveys may be required to investigate the possible source of *A. koolunga*.

Our investigations instead uncovered five novel Didymellaceae species not yet known to science. *Epicoccum djirangnandiri* on *S. galegifolia* was collected from the botanic garden in New South Wales, where the host is endemic. *Neodidymelliopsis tinkyukuku* on *H. violacea* was collected from a public garden in South Australia. Growing in the same garden is *V. sativa* from which *D. pinodes* (strain BRIP 69578), a known Ascochyta blight pathogen, was isolated. *Hardenbergia violacea* has a wide distribution in southern and eastern Australia. These three native Australian legume species were found in a cultivated environment rather than in a natural environment. Further studies are warranted to understand how widespread these fungal species may be in cultivated or natural environments, and if they are host specific.

Leaf spots were commonly seen on the native legume *S. artemisioides* throughout the regions sampled in South Australia. Three novel *Nothophoma* species were isolated from *S. artemisioides*. *Nothophoma garlbiwalawarda* was collected from five locations across South Australia, separated by over 400 km, in field pea and non-field pea growing regions. *Nothophoma naiawu* and *No. ngayawang* were collected from the South Australian Murray River region on the roadside of a main highway. The leaf spot symptoms for the three *Nothophoma* species were similar (small pin-prick lesions), with some larger spots on the seed pods caused by *No. ngayawang*.

Our investigations also identified new host-pathogen associations, namely *D. pinodes* on *S. artemisioides* and *V. cracca*, and *D. lethalis* on *L. tingitanus*. These hosts could be a reservoir of Ascochyta blight inoculum if found growing adjacent to field pea crops. The discovery of an alternative host has implications for disease epidemiology and management. The symptoms of *D. pinodes* on *S. artemisioides* are indistinguishable from the pin-prick leaf spot symptoms caused by the three *Nothophoma* species described in this study. *Didymella pinodes* was isolated from five locations. Four of these locations also yielded a novel *Nothophoma* species. *Didymella prosopidis* was isolated from the Australian native *G. celsianum*, a species first described as associated with stem disease of *Prosopis* sp. (also a member of the Fabaceae family) in South Africa (Crous et al. 2013). This is the first report of *D. prosopidis* outside of South Africa.

At the outset, our study sought to identify if any *A. koolunga* could be isolated from Australian native legumes causing leaf spot disease. This study uncovered five novel isolates in the Didymellaceae from Australian native legumes, and identified three new legume host-pathogen associations for Australia. *Ascochyta koolunga* was not isolated from hosts other than field pea, which might be an artefact of the low incidence of the fungus during the collection period. Further investigations using a longitudinal systematic survey are needed to identify any native hosts of *A. koolunga*.

and to further investigate the diversity and prevalence of Didymellaceae species on Australian native, pasture and naturalised legumes, to classify novel isolates and to identify new Australian hosts for known species.

Acknowledgements

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References

- Ahmed H, Chang K-F, Hwang S-F, Fu H, Zhou Q, Strelkov S, Conner R, Gossen B (2015) Morphological characterization of fungi associated with the ascochyta blight complex and pathogenic variability of *Mycosphaerella pinodes* on field pea crops in central Alberta. *The Crop Journal* 3: 10–18. <https://doi.org/10.1016/j.cj.2014.08.007>
- Ali SM, Dennis J (1992) Host range and physiologic specialisation of *Macrophomina phaseolina* isolated from field peas in South Australia. *Journal of Experimental Agriculture* 32: 1121–1125. <https://doi.org/10.1071/EA9921121>
- Ariyawansa HA, Hyde KD, Jayasiri SC (2015) Fungal diversity notes 111–252—taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* 75: 27–274. <https://doi.org/10.1007/s13225-015-0346-5>
- Aveskamp MM, Verkley GJM, de Gruyter J, Murace MA, Perello A, Woudenberg JHC, Groenewald JZ, Crous PW (2009) DNA phylogeny reveals polyphyly of *Phoma* section *Peyronellaea* and multiple taxonomic novelties. *Mycologia* 101: 363–382. <https://doi.org/10.3852/08-199>
- Aveskamp MM, de Gruyter J, Woudenberg JH, Verkley GJ, Crous PW (2010) Highlights of the *Didymellaceae*: A polyphasic approach to characterise *Phoma* and related pleosporalean genera. *Studies in Mycology* 65: 1–60. <https://doi.org/10.3114/sim.2010.65.01>
- Boerema GH, De Gruyter J, Noordeloos ME, Hamers MCE (2004) *Phoma* identification manual differentiation of specific and intra-specific taxa in culture. CABI Publishing, Cambridge, MA, USA, Wallingford, OX, UK, <https://doi.org/10.1079/9780851997438.0000>
- Chen Q, Jiang JR, Zhang GZ, Crous PW (2015a) Resolving the *Phoma* enigma. *Studies in Mycology* 82: 137–217. <https://doi.org/10.1016/j.simyco.2015.10.003>
- Chen Q, Zhang KE, Zhang G, Cai L (2015b) A polyphasic approach to characterise two novel species of *Phoma* (*Didymellaceae*) from China. *Phytotaxa* 197: 267–281. <https://doi.org/10.11646/phytotaxa.197.4.4>

- Chen Q, Hou LW, Duan WJ, Crous PW, Cai L (2017) *Didymellaceae* revisited. *Studies in Mycology* 87: 105–159. <https://doi.org/10.1016/j.simyco.2017.06.002>
- Chilvers MI, Rogers JD, Dugan FM, Stewart JE, Chen W, Peever TL (2009) *Didymella pisi* sp. nov., the teleomorph of *Ascochyta pisi*. *Mycological Research* 113: 391–400. <https://doi.org/10.1016/j.mycres.2008.11.017>
- Crous PW, Wingfield MJ, Guarro J, Cheewangkoon R, van der Bank M, Swart WJ, Stchigel AM, Cano-Lira JF, Roux J, Madrid H, Damm U, Wood AR, Shuttleworth LA, Hodges CS, Munster M, de Jesús Yáñez-Morales M, Zúñiga-Estrada L, Cruywagen EM, de Hoog GS, Silvera C, Najafzadeh J, Davison EM, Davison PJ, Barrett MD, Barrett RL, Manamgoda DS, Minnis AM, Kleczewski NM, Flory SL, Castlebury LA, Clay K, Hyde KD, Maússe-Sitoe SN, Chen S, Lechat C, Hairaud M, Lesage-Meessen L, Pawłowska J, Wilk M, Sliwińska-Wyrzychowska A, Mętrak M, Wrzosek M, Pavlic-Zupanc D, Maleme HM, Slippers B, Mac Cormack WP, Archuby DI, Grünwald NJ, Tellería MT, Dueñas M, Martín MP, Marincowitz S, de Beer ZW, Perez CA, Gené J, Marin-Felix Y, Groenewald JZ (2013b) Fungal Planet description sheets: 154–213. *Persoonia* 31: 188–296. <https://doi.org/10.3767/003158513X675925>
- Crous PW, Groenewald JZ (2016) They seldom occur alone. *Fungal Biology* 120: 1392–1415. <https://doi.org/10.1016/j.funbio.2016.05.009>
- Das K, Lee S-Y, Jung H-Y (2020) Molecular and morphological characterization of two novel species collected from Soil in Korea. *Mycobiology* 48:1, 9–19. <https://doi.org/10.1080/12298093.2019.1695717>
- Davidson JA, Hartley D, Priest M, Krysinska-Kaczmarek M, Herdina, McKay A, Scott ES (2009) A new species of *Phoma* causes ascochyta blight symptoms on field peas (*Pisum sativum*) in South Australia. *Mycologia* 101: 120–128. <https://doi.org/10.3852/07-199>
- Davidson JA, Krysinska-Kaczmarek M, Wilmshurst CJ, McKay A, Herdina, Scott ES (2011) Distribution and survival of ascochyta blight pathogens in field-pea-cropping soils of Australia. *Plant Disease* 95: 1217–1223. <https://doi.org/10.1094/PDIS-01-11-0077>
- Dear S, Staden R (1992) A standard file format for data from DNA sequencing instruments. *DNA Sequence*. 3: 107–110. <https://doi.org/10.3109/10425179209034003>
- de Gruyter J, Aveskamp MM, Woudenberg JH, Verkley GJ, Groenewald JZ, Crous PW (2009) Molecular phylogeny of *Phoma* and allied anamorph genera: towards a reclassification of the *Phoma* complex. *Mycological Research* 113: 508–519. <https://doi.org/10.1016/j.mycres.2009.01.002>
- de Gruyter J (2012) Revised taxonomy of *Phoma* and allied genera. PhD Dissertation, Wageningen University, Wageningen, NL, 181 pp.
- Gaurilcikiene I, Viciene RC (2013) The susceptibility of pea (*Pisum sativum* L.) to ascochyta blight under Lithuanian conditions. *Zemdirbyste (Agriculture)* 100: 283–288. <https://doi.org/10.13080/z-a.2013.100.036>
- Hibbett D, Abarenkov K, Koljalg U, Opik M, Chai B, Cole JR, Wang Q, Crous PW, Robert VA, Helgason T, Herr J, Kirk P, Lueschow S, O'Donnell K, Nilsson H, Oono R, Schoch CL, Smyth C, Walker D, Porras-Alfaro A, Taylor JW, Geiser DM (2016) Sequence-based classification and identification of Fungi. *Mycologia* 108: 1049–1068.

- Hou LW, Groenewald JZ, Pfenning LH, Yarden O, Crous PW, Cai L (2020) The phoma-like dilemma. *Studies in Mycology* 96: 309–396. <https://doi.org/10.1016/j.simyco.2020.05.001>
- Katoh K, Asimenos G, Toh H (2009) Multiple alignment of DNA sequences with MAFFT. In: Posada D (Ed) *Bioinformatics for DNA Sequence Analysis*. Humana Press, New York, NY 10013, USA, 39–64. https://doi.org/10.1007/978-1-59745-251-9_3
- Le May C, Potage G, Andrivon D, Tivoli B, Outreman Y (2009) Plant disease complex: Antagonism and synergism between pathogens of the *Ascochyta* blight complex on pea. *Journal of Phytopathology* 157: 715–721. <https://doi.org/10.1111/j.1439-0434.2009.01546.x>
- Liu J, Cao T, Feng J, Chang K-F, Hwang S-F, Strelkov SE (2013) Characterization of the fungi associated with ascochyta blight of field pea in Alberta, Canada. *Crop Protection* 54: 55–64. <https://doi.org/10.1016/j.cropro.2013.07.016>
- Liu N, Xu S, Yao X, Zhang G, Mao W, Hu Q, Feng Z, Gong Y (2016) Studies on the Control of *Ascochyta* Blight in Field Peas (*Pisum sativum* L.) Caused by *Ascochyta pinodes* in Zhejiang Province, China. *Frontiers in Microbiology* 7: 481–453. <https://doi.org/10.3389/fmicb.2016.00481>
- Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* 16: 1799–1808. <https://doi.org/10.1093/oxfordjournals.molbev.a026092>
- Mathew FM, Goswami RS, Markell SG, Osborne L, Tande C, Ruden B (2010) First report of *Ascochyta* blight of field pea caused by *Ascochyta pisi* in South Dakota. *Plant Disease* 94: 789. <https://doi.org/10.1094/PDIS-94-6-0789A>
- O'Donnell K, Sarver BAJ, Brandt M, Chang DC, Noble-Wang J, Park BJ, Sutton DA, Benjamin, L, Lindsley M, Padhye A, Geuser DM, Ward TJ (2007) Phylogenetic diversity and microsphere array-based genotyping of human pathogenic fusaria, including isolates from the multistate contact lens - Associated US Keratitis outbreaks of 2005 and 2006. *Journal of Clinical Microbiology* 45: 2235–2248. <https://doi.org/10.1128/JCM.00533-07>
- Panicker S, Ramraj B (2010) Studies on the epidemiology and control of *Ascochyta* blight of peas (*Pisum sativum* L) caused by *Ascochyta pinodes*. *Archives of Phytopathology and Plant Protection* 43: 51–58. <https://doi.org/10.1080/03235400701652417>
- Quaedvlieg W, Binder M, Groenewald JZ, Summerell BA, Carnegie AJ, Burgess TI, Crous PW (2014) Introducing the consolidated species concept to resolve species in the *Teratosphaeriaceae*. *Persoonia* 33: 1–40. <https://doi.org/10.3767/003158514X681981>
- Ramaciotti Centre for Genomics (2019) Guide to Sanger Sequencing at RAMAC. https://www.ramaciotti.unsw.edu.au/sites/default/files/2019-04/RAMAC_Sanger_Sequencing_Service_Guide_2019_v1.0.pdf
- Rayner RW (1970) A mycological colour chart. Commonwealth Mycological Institute, Kew.
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Salam MU, Davidson JA, Thomas GJ, Ford R, Jones RAC, Lindbeck KD, MacLeod WJ, Kimber RBE, Galloway J, Mantri N (2011) Advances in winter pulse pathology research in Australia. *Australasian Plant Pathology* 40: 549–567. <https://doi.org/10.1007/s13313-011-0085-3>

- Skoglund LG, Harveson RM, Chen W, Dugan F, Schwartz HF, Markell SG, Porter L, Burrows ML, Goswami R (2011) Ascochyta Blight of Peas. Plant Health Progress, 1–9. <https://doi.org/10.1094/PHP-2011-0330-01-RS>
- Snyder WC, Hansen HN (1947) Advantages of natural media and environments in the culture of fungi. Phytopathology 37: 420–421.
- Soylu S, Dervis S (2011) Determination of prevalence and incidence of fungal disease agents of pea (*Pisum sativum* L.) plants growing in Amik plain of Turkey. Research on Crops 12: 588–592.
- Stamatakis A, Alachiotis N (2010) Time and memory efficient likelihood-based tree searches on phylogenomic alignments with missing data. Bioinformatics 26: i132–i139. <https://doi.org/10.1093/bioinformatics/btq205>
- Sung GH, Sung JM, Hywel-Jones NL (2007) A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): identification of localized incongruence using a combinational bootstrap approach. Molecular Phylogenetics and Evolution 44: 1204–1223. <https://doi.org/10.1016/j.ympev.2007.03.011>
- Thambugala KM, Daranagama DA, Phillips AJL (2017) Microfungi on Tamarix. Fungal Diversity 82: 239–306. <https://doi.org/10.1007/s13225-016-0371-z>
- Tran HS, You MP, Khan TN, Barbetti MJ (2015) Pea black spot disease complex on field pea: dissecting the roles of the different pathogens in causing epicotyl and root disease. European Journal of Plant Pathology 144: 595–605. <https://doi.org/10.1007/s10658-015-0798-1>
- Valenzuela-Lopez N, Cano-Lira JF, Guarro J, Sutton DA, Wiederhold N, Crous PW, Stchigel AM (2018) Coelomycetous *Dothideomycetes* with emphasis on the families *Cucurbitariaceae* and *Didymellaceae*. Studies in Mycology 90: 1–69. <https://doi.org/10.1016/j.simyco.2017.11.003>
- White TJ, Bruns T, Lee S (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJe (Eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, USA, 315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Wijayawardene NN, Hyde KD, Wanasinghe DN (2016) Taxonomy and phylogeny of dematiaceous coelomycetes. Fungal Diversity 77: 1–316. <https://doi.org/10.1007/s13225-016-0360-2>
- Woudenberg JH, De Gruyter J, Crous PW, Zwiers LH (2012) Analysis of the mating-type loci of co-occurring and phylogenetically related species of *Ascochyta* and *Phoma*. Molecular Plant Pathology 13: 350–362. <https://doi.org/10.1111/j.1364-3703.2011.00751.x>

Notes on *Trochila* (Ascomycota, Leotiomyces), with new species and combinations

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Abstract

Studies of *Trochila* (Leotiomyces, Helotiales, Cenangiaceae) are scarce. Here, we describe two new species based on molecular phylogenetic data and morphology. *Trochila bostonensis* was collected at the Boston Harbor Islands National Recreation Area, Massachusetts. It was found on the stem of *Asclepias syriaca*, representing the first report of any *Trochila* species from a plant host in the family Apocynaceae. *Trochila urediniophila* is associated with the uredinia of the rust fungus *Cerotelium fici*. It was discovered during a survey for rust hyperparasites conducted at the Arthur Fungarium, in a single sample from 1912 collected in Trinidad. Macro- and micromorphological descriptions, illustrations, and molecular phylogenetic analyses are presented. The two new species are placed in *Trochila* with high support in both our six-locus (SSU, ITS, LSU, *rpb1*, *rpb2*, *tef1*) and two-locus (ITS, LSU) phylogenetic reconstructions. In addition, two species are combined in *Trochila*: *Trochila colensoi* (formerly placed in *Pseudopeziza*) and *T. xishuangbanna* (originally described as the only species in *Calycellinopsis*). This study reveals new host plant families, a new ecological strategy, and a new country record for the genus *Trochila*. Finally, our work emphasizes the importance of specimens deposited in biological collections such as fungaria.

Keywords

4 new taxa, biological collections, Boston Harbor Islands, fungarium specimens, fungicolous fungi, South America, taxonomy, Trinidad

Introduction

The genus *Trochila* Fr. (Ascomycota, Leotiomyces) was erected by Fries (1849) to accommodate four species previously placed in *Phacidium* Fr., *Sphaeria* Haller, and *Xyloma* Pers. *Trochila craterium* (DC) Fr. was the first species listed by Fries, based on *Sphaeria craterium* DC., which was later selected by Clements and Shear (1931) as the type species of *Trochila*. The other three species included by Fries (1849) were: *T. ilicis* (Fr.) Fr. [= *Sphaeria ilicis* Fr.], *T. laurocerasi* (Desm.) Fr. [= *Phacidium laurocerasi* Desm.], and *T. taxi* (Fr.) Fr. [= *Xyloma taxi* Fr.]. Only the genus and one species (*T. laurocerasi*) were briefly described by Fries (1849). However, the type species, *T. craterium*, was well described macromorphologically by Lamarck and de Candolle (1805). The description can be translated loosely from French as “a fungus growing on the lower surface of ivy leaves, initially forming a flat white disc, then turning blackish and concave opening by a split along radial lines, the disc usually surrounded by a whitish membrane” (Lamarck and de Candolle 1805). Later, the generic concept was expanded to include other types of apothecial opening. Rehm (1896) remarked that the covering layer of the apothecia could also open completely like a lid depending on host characters such as cuticle thickness. After the inclusion of this new character describing the genus, *Stegia ilicis* (Chevall.) Gillet was transferred as *Trochila ilicina* (Nees ex Fr.) Courtec (Crouan and Crouan 1867; Rehm 1896).

In our current circumscription of the genus *Trochila*, apothecia are sunken in the host tissues and hymenia are exposed either by splitting along radial lines or by splitting into a number of lobes that roll outward exposing the hymenium. The excipulum is composed of dark, globose-angular cells; asci contain eight ellipsoid, hyaline ascospores with oil guttules (except *T. substrictica* Rehm and *T. tetraspora* E. Müll. & Gamundí, which both have asci containing four ascospores); and paraphyses possess yellowish guttules (Dennis 1978; Baral and Marson 2005). Thirty-three names have been applied in the genus (Index Fungorum 2021). Jaklitsch et al. (2016) suggest that only ca. 10 names should be accepted.

Fries (1849) included *Trochila* in “Patellariacei” (= Patellariaceae). Later, it was transferred to Dermateaceae, Helotiales (Fuckel 1869; Karsten 1869; Saccardo 1884; Lambotte 1888). *Trochila* remained in this family (Korf 1973; Dennis 1978) into the molecular era (Lumbsch and Huhndorf 2010). Jaklitsch et al. (2016) placed *Trochila* in the resurrected family Cenangiaceae based on morphological and molecular data. Later, the relationships among genera in this family were supported in another, 5–15-locus phylogeny of Leotiomyces (Johnston et al. 2019).

Most species of *Trochila* have been described from their sexual morph. The asexual morph has the characteristics of the form-genus *Cryptocline* Petr. (Morgan-Jones 1973; Kiffer and Morelet 2000; Hyde et al. 2011). Two species of *Trochila* have been linked to their asexual morphs: *T. craterium* to *C. paradoxa* (De Not.) Arx and *T. laurocerasi* to *C. phacidiella* (Grove) Arx (von Arx 1957). The paucity of culture and molecular data of both *Cryptocline* and *Trochila* species has hindered the linkage of sexual and asexual morphs for most species. *Trochila viburnicola* Crous & Denman was the first species

of the genus to be described based on the combination of morphology and molecular data, but only its asexual morph is known (Crous et al. 2018). The species was named referring to its host, *Viburnum* sp. (Dipsacales, Adoxaceae). In addition to *T. viburnicola*, two other species have been reported on this host genus, but only from their sexual morph, *T. ramulorum* Feltgen and *T. tini* (Duby) Quél. [currently *Pyrenopeziza tini* (Duby) Nannf.]. Due to the lack of sequences or cultures of these two species, a comparison with *T. viburnicola* is impossible (Feltgen 1903; Crous et al. 2018).

Most *Trochila* members have a restricted record of geographical distribution and ecological strategy. *Trochila* records typically originate from the Northern Hemisphere limited to temperate regions in Europe and North America (Ziolo et al. 2005; Stoykov and Assyov 2009; Crous et al. 2018; Stoykov 2019; Global Biodiversity Information Facility 2020). Nonetheless, a number of putative *Trochila* reports are known from the Southern hemisphere (Spegazzini 1888, 1910, 1921; Rehm 1909; Gamundí et al. 1978). In addition, species of *Trochila* are typically recorded as saprotrophs on dead leaves and branches of both herbaceous plants and trees. However, a few species have been found infecting living plant tissues. *Trochila ilicina* is reported as both a weak parasite and a saprotroph because of its presence on living, decaying, and fallen leaves of *Ilex aquifolium* (Aquifoliales, Aquifoliaceae) (Ziolo et al. 2005), *T. laurocerasi* as a parasite of living leaves of *Prunus laurocerasus* (Rosales, Rosaceae) (Gregor 1936), and *T. symploci* as a pathogen of living leaves of *Symplocos japonica* (Ericales, Symplocaceae) (Hennings 1900; Stevenson 1926).

Here, we describe two new species, *T. bostonensis* and *T. urediniophila*, collected at the Boston Harbor Islands National Recreation Area, Massachusetts and at Port of Spain, Trinidad, respectively. We also make two new combinations in *Trochila* based on morphological studies and phylogenetic analyses. We reveal two new host plant families (Apocynaceae and Asparagaceae) and a new ecological strategy (fungicolous symbiont) for the genus. Finally, we provide a comparative table of characters, based on literature review, for all currently accepted species of *Trochila* (*sensu* Index Fungorum 2021).

Material and methods

Collected samples

Samples were collected in the field and from fungaria. One collection of *Trochila* was discovered during the Boston Harbor Islands (BHI) National Recreation Area fungal ATBI (Haelewaters et al. 2018a). In this project, above-ground, ephemeral fruiting bodies of non-lichenized fungi were collected. In the field, specimens were placed in plastic containers or brown paper bags. BHI-F collection numbers were assigned. Date, specific locality when applicable, GPS coordinates, substrate, and habitat notes were recorded. Specimens were dried using a Presto Dehydro food dehydrator (National Presto Industries, Eau Claire, Wisconsin) set at 35 °C for 7–9 hours. Collections were packaged, labeled, and deposited at FH. A second *Trochila* collection came to our attention during

a survey for hyperparasites of rust fungi at PUR. The specimen was found on the uredinia of the rust fungus *Cerotelium fici* on the underside of *Ficus maxima* leaves. Fungarium acronyms follow Thiers (continuously updated).

Morphological studies

Methods to study the morphological characteristics of the *Trochila* specimens followed the process given in Baral (1992). Macro- and micromorphological features were examined on both fresh and dried apothecia for the specimen collected at the BHI and on dried apothecia for the specimen found at PUR. Apothecia from the BHI specimen were observed under an EZ4 stereomicroscope (Leica, Wetzlar, Germany) and studied under a B1 compound microscope (Motic, Barcelona, Spain). Apothecia from the PUR specimen were examined on an SZ2-ILTS dissecting microscope (Olympus, Center Valley, Pennsylvania) and studied using a BH2-RFCA compound microscope (Olympus). Sections of apothecia were cut free-hand and mounted in water or pre-treated in 5% KOH. Sections were also mounted in Melzer's reagent with and without KOH-pretreatment to determine dextrinoid or amyloid reactions. At least 10 measurements were made for each structure at 400–1000× magnification. Measurements for each character are given as $(a-)b-c(-d)$, with $b-c$ indicating the 95% confidence interval and a and d representing the smallest and large single measurement, respectively. Macro- and microphotographs were taken with a USB Moticam 2500 camera (Motic) (BHI specimen) or an Olympus SC30 camera (PUR specimen). Measurements were made using the following software suites: Motic Images Plus 2.0 and cellSens Standard 1.18 Imaging Software (Olympus). Color coding refers to Kelly (1965). Abbreviations were adopted from Baral (1992) and Baral and Marson (2005) as follows:

*	living state;	LBs	lipid bodies;
†	dead state;	MLZ	Melzer's reagent;
IKI	Lugol's solution;	OCI	oil content index;
KOH	potassium hydroxide;	VBs	refractive vacuolar bodies.

DNA isolation, PCR amplifications, sequencing

Genomic DNA was isolated from 1–3 apothecia per specimen using the E.Z.N.A. HP Fungal DNA Kit (Omega Bio-Tek, Norcross, Georgia), QIAamp DNA Micro Kit (Qiagen, Valencia, California), following the manufacturer's instructions, and the Extract-N-Amp Plant PCR Kit (Sigma-Aldrich, St. Louis, Missouri), following Haelewaters et al. (2018a). We amplified the following loci: nuclear small and large ribosomal subunits (SSU and LSU), internal transcribed spacer region of the ribosomal DNA (ITS), RNA polymerase II second largest subunit (*rpb2*), and translation elongation factor 1- α (*tef1*). Primer combinations were as follows: NS1/NS2 and NS1/NS4 for SSU (White et al. 1990); LR0R/LR5 for LSU (Vilgalys and Hester 1990; Hopple 1994); ITS1F/ITS4, ITS9mun/ITS4A, and ITS5/ITS2 for ITS (White et al.

1990; Gardes and Bruns 1993; Egger 1995); RPB2-5F2/fRPB2-7cR for *rpb2* (Liu et al. 1999; Sung et al. 2007); and EF1-983F/EF1-1567R and EF1-983F/EF1-2218R for *tefl* (Rehner and Buckley 2005). All 25- μ l PCR reactions were conducted on a Mastercycler ep gradient Thermal Cycler (Eppendorf model #5341, Hauppauge, New York) and consisted of 12.5 μ l of 2 $\times$ MyTaq Mix (Bioline, Swedesboro, New Jersey), 1 μ l of each 10 μ M primer, and 10.5 μ l of 1/10 diluted DNA extract. Amplifications of rDNA and *rpb2* loci were run under the following conditions: initial denaturation at 95 °C for 5 min (94 °C for LSU); followed by 40 cycles of denaturation at 95 °C for 30 sec (94 °C for LSU), annealing at 45 °C (ITS) / 50 °C (LSU) / 55 °C (SSU, *rpb2*) for 45 sec, and elongation at 72 °C for 45 sec (1 min for LSU); and final extension at 72 °C for 7 min (1 min for SSU). Amplification of *tefl* was done with a touchdown PCR as follows: initial denaturation at 95 °C for 10 min; followed by 30 cycles of 95 °C for 1 min, 62 °C for 1 min (decreasing 1 °C every 3 cycles), 72 °C for 90 sec; then 30 cycles of 95 °C for 30 sec, 55 °C for 30 sec, and 72 °C for 1 min; and final extension at 72 °C for 7 min (Don et al. 1991; Haelewaters et al. 2018b). PCR products were visualized by gel electrophoresis. Purification of successful PCR products and subsequent sequencing in both directions were outsourced to Genewiz (South Plainfield, New Jersey). Raw sequence reads were assembled and edited using Sequencher version 5.2.3 (Gene Codes Co., Ann Arbor, Michigan).

Sequence alignment and phylogenetic analysis

Edited sequences were blasted against the NCBI GenBank nucleotide database (<http://ncbi.nlm.nih.gov/blast/Blast.cgi>) to search for closest relatives. For phylogenetic placement of our isolates, we downloaded SSU, ITS, LSU, *rpb1*, *rpb2*, and *tefl* sequences of *Trochila* from GenBank. We also downloaded sequence data of selected clades of Helotiales, mainly from Pärtel et al. (2017) but also other sources (details in Table 1), as a basis for our six-locus phylogenetic analysis. We selected representative taxa of Cenangiaceae, Cordieritidaceae, Rutstroemiaceae, and Sclerotiniaceae, with taxa in the family Chlorociboriaceae serving as outgroups (Johnston et al. 2019). Alignment of DNA sequences was done for each locus separately using MUSCLE version 3.7 (Edgar 2004), available on the Cipres Science Gateway 3.3 (Miller et al. 2010). The aligned sequences for each locus were concatenated in MEGA7 (Kumar et al. 2016). Maximum likelihood (ML) inference was performed using IQ-TREE from the command line (Nguyen et al. 2015) under partitioned models (Chernomor et al. 2016). Nucleotide substitution models were selected under Akaike's information criterion corrected for small sample size (AICc) with the help of the built-in program ModelFinder (Kalyaanamoorthy et al. 2017). Ultrafast bootstrap analysis was implemented with 1000 replicates (Hoang et al. 2017).

For the purpose of species delimitation, we constructed a second dataset of ITS–LSU consisting of isolates of *Trochila* and closely related taxa in the family Cenangiaceae. We included *Trochila* spp., *Calycellinopsis xishuangbanna*, and *Pseudopeziza colensoi*, with *Cenangiopsis* spp. serving as outgroup. In this analysis, we included *T. ilicina*, for

Table 1. Sequences used in phylogenetic analyses. Accession numbers in boldface indicate sequences that were generated during the course of this study.

Isolate	Species	Family	SSU	ITS	LSU	<i>rpb1</i>	<i>rpb2</i>	<i>tefi</i>	Reference
KL391	<i>Arveghiniella australis</i>	Cordieritiaceae	KX090893		KX090841	KX090787		KX090690	Parrel et al. (2017)
AD283531 [†]	<i>Annabellia australensis</i>	Cordieritiaceae		MK328475	MK328476				Fryar et al. (2019)
AFTOL-ID 59	<i>Boryotinia fückeliana</i>	Sclerotiniaceae	AY544695		AY544651	DQ471116	DQ247786	DQ471045	Spatafora et al. (2006)
HMAS:187063	<i>Calyndrinopsis xizhuangbanua</i>	Cenangiaceae	GU936124		KR094163	MH729338	MH729345		W.Y. Zhuang et al. (unpubl.)
KL375	<i>Cenangiopis alpestris</i>	Cenangiaceae			KX090837	KX090784	KX090736		Parrel et al. (2017)
KL378	<i>Cenangiopis alpestris</i>	Cenangiaceae	KX090891	LT158470	KX090839	KX090786	KX090738		Parrel et al. (2017)
KL157	<i>Cenangiopis alpestris</i>	Cenangiaceae	KX090858	LT158421	KX090806	KX090709		KX090663	Parrel et al. (2017)
KL174	<i>Cenangiopis quercicola</i>	Cenangiaceae	KX090862	LT158425	KX090811	KX090713			Parrel et al. (2017)
KL377	<i>Cenangiopis</i> sp.	Cenangiaceae	KX090890	KX090900	KX090838	KX090737			Parrel et al. (2017)
KL276	" <i>Cenangium</i> " <i>actium</i>	<i>Piccomphale</i> clade	KX090879	LT158445	KX090828	KX090727		KX090680	Parrel et al. (2017)
KL243	" <i>Cenangium</i> " <i>actium</i>	<i>Piccomphale</i> clade	KX090873	LT158439	KX090822	KX090767	KX090720	KX090674	Parrel et al. (2017)
KL390	<i>Cenangium ferrugineosum</i>	Cenangiaceae	KX090892	LT158471	KX090840	KX090739			Parrel et al. (2017)
KL167	<i>Chlorenchocelia tortia</i>	Cenangiaceae		LT158424	KX090810	KX090759			Parrel et al. (2017)
KP606	<i>Chlorenchocelia versiformis</i>	Cenangiaceae	KX090894			KX090788	KX090740	KX090692	Parrel et al. (2017)
KL21	<i>Chlorenchocelia versiformis</i>	Cenangiaceae	KX090846	LT158427	KX090795				Parrel et al. (2017)
KL152	<i>Chlorociboria aeruginascens</i>	Chlorociboriaceae		LT158419		KX090752	KX090706	KX090657	Parrel et al. (2017)
KL247	<i>Chlorociboria aeruginella</i>	Chlorociboriaceae				KX090769	KX090722	KX090676	Parrel et al. (2017)
KL238	<i>Chlorociboria glauca</i>	Chlorociboriaceae	KX090875	LT158438	KX090821	KX090766		KX090673	Parrel et al. (2017)
KL212	<i>Ciboria nitidifusa</i>	Sclerotiniaceae	KX090863	LT158429	KX090812				Parrel et al. (2017)
KL254	<i>Crumenulopsis sororia</i>	Cenangiaceae		LT158442	KX090826		KX090725		Parrel et al. (2017)
KL317	<i>Diplacarpa bloxamii</i>	Cordieritiaceae	KX090885		KX090834	KX090778	KX090745	KX090688	Parrel et al. (2017)
SK80	<i>Diplacarpia nanula</i>	Cordieritiaceae	KX090896	KP984782	KX090790				Parrel et al. (2017)
TU:109263	<i>Dumontinia tuberosa</i>	Sclerotiniaceae	KX090897	LT158412	KX090843	KX090792			Etayo et al. (2015), Parrel et al. (2017)
KL111	<i>Enocelia fimbriata</i>	Cenangiaceae	KX090852		KX090800		KX090703	KX090697	Parrel et al. (2017)
KL108	<i>Enocelia fufrinacea</i>	Cenangiaceae	KX090851		KX090799		KX090702	KX090654	Parrel et al. (2017)
KL107	<i>Enocelia fufrinacea</i>	Cenangiaceae	KX090850	LT158416	KX090798	KX090749	KX090701	KX090653	Parrel et al. (2017)
KL106	<i>Enocelia fufrinacea</i>	Cenangiaceae	KX090849	LT158415		KX090748		KX090652	Parrel et al. (2017)
KL92	<i>Enocelia fufrinacea</i>	Cenangiaceae	KX090847	LT158482	KX090796			KX090651	Parrel et al. (2017)
KL164	<i>Enocelia heteromera</i>	Cenangiaceae	KX090861		KX090809	KX090758	KX090712	KX090662	Parrel et al. (2017)
KL304	<i>Enocelia heteromera</i>	Cenangiaceae	KX138404			KX138400			Parrel et al. (2017)
KL244	Helotiales sp.	Cenangiaceae	KX090874	LT158440	KX090823	KX090768	KX090721	KX090675	Parrel et al. (2017)
KL20	<i>Heyderia abietis</i>	Cenangiaceae	KX090845	LT158426		KX090747	KX090699	KX090650	Parrel et al. (2017)
HMAS:71954	<i>Heyderia abietis</i>	Cenangiaceae	AY789295	AY789297					Wang et al. (2005)
KL216	<i>Heyderia pusilla</i>	Cenangiaceae	KX090865	LT158430	AY789296	KX090762	KX090715	KX090665	Parrel et al. (2017)
KL299	<i>Ionomidotis frondosa</i>	Cordieritiaceae	KX090882			KX090775		KX090685	Parrel et al. (2017)
KL231	<i>Ionomidotis filamentigens</i>	Cordieritiaceae	KX090870		KX090819	KX090765	KX090719	KX090671	Parrel et al. (2017)

Isolate	Species	Family	SSU	ITS	LSU	<i>rpb1</i>	<i>rpb2</i>	<i>ref1</i>	Reference
KL239	<i>Ionanidopsis filiostringens</i>	Cordieritiaceae	KX138403		KX138407	KX138399	KX138401		Parrel et al. (2017)
KL154	<i>Ionanidopsis irregularis</i>	Cordieritiaceae	KX090856		KX090804	KX090754		KX090658	Parrel et al. (2017)
KL301	<i>Ionanidopsis olivaceus</i>	Cordieritiaceae	KX090883		KX090833	KX090776	KX090732	KX090686	Parrel et al. (2017)
CBS:811.85	<i>Lambertella subrepenspora</i>	Rustroemiaceae	KF545416	AB956097	MH873604				Zhao et al. (2016), Parrel et al. (2017), Vu et al. (2019)
LL95	<i>Limonietta terricola</i>	Cordieritiaceae	KX090895		KX090842	KX090789	KX090741	KX090693	Parrel et al. (2017)
AFTOL-ID 169	<i>Monilinia laxa</i>	Sclerotiniaceae	AY544670		AY544670	FJ238425	DQ470889	DQ471057	Spatafora et al. (2006)
KL374	<i>Piccomphale bulgaroides</i>	Piccomphale clade	KX090889	LT158469	KX090836	KX090783			Parrel et al. (2017)
KL98	<i>Piccomphale bulgaroides</i>	Piccomphale clade	KX090848	LT158483	KX090797		KX090700		Parrel et al. (2017)
PDD:112240	<i>Pseudopeziza olenoi</i>	Cenangiaceae		MH921874	MH985297	MH986706	MH986705		P.R. Johnston and D. Park (unpubl.)
KL267	<i>Pycnopieziza sejanetii</i>	Sclerotiniaceae	KX090878	LT158443	KX090827	KX090772	KX090726	KX090679	Parrel et al. (2017)
AFTOL-ID 907	<i>Rhodocline laticis</i>	Cenangiaceae	DQ471002		DQ470954	DQ471146	DQ470904	DQ471073	Spatafora et al. (2006)
KL292	<i>Rustroemia firma</i>	Rustroemiaceae	KX090881	LT158450	KX090832	KX090774	KX090731	KX090684	Parrel et al. (2017)
KL291	<i>Rustroemia firma</i>	Rustroemiaceae		LT158449	KX090831		KX090730	KX090683	Parrel et al. (2017)
KL290	<i>Rustroemia firma</i>	Rustroemiaceae			KX090830		KX090729	KX090682	Parrel et al. (2017)
KL222	<i>Rustroemia firma</i>	Rustroemiaceae	KX138402		KX138406			KX138397	Parrel et al. (2017)
KL310	<i>Rustroemia johnstonii</i>	Rustroemiaceae	KX090884	LT158454		KX090777	KX090733	KX090687	Parrel et al. (2017)
KL234	<i>Rustroemia juniperi</i>	Rustroemiaceae	KX090871		KX090820			KX090672	Parrel et al. (2017)
KL217	<i>Rustroemia lateritrescens</i>	Rustroemiaceae		LT158431	KX090814	KX090763	KX090716	KX090666	Parrel et al. (2017)
KL160	<i>Rustroemia tiliacea</i>	Rustroemiaceae	KX090860	LT158423	KX090808	KX090757	KX090711	KX090661	Parrel et al. (2017)
KL393	<i>Rustroemiaceae</i> sp.	Rustroemiaceae	KX138405	LT158472	KX138408		KX138398	KX090691	Parrel et al. (2017)
KL288	<i>Rustroemiaceae</i> sp.	Rustroemiaceae	KX090880	LT158446	KX090829	KX090773	KX090728	KX090681	Parrel et al. (2017)
CBS:273.74 ^T	<i>Sarastrochila longispora</i>	Cenangiaceae		KJ663836	KJ663877		KJ663918		Crous et al. (2014)
KL347	<i>Sclerochaeta fascicularis</i>	Sclerotiniaceae			KX090782				Parrel et al. (2017)
KL156	<i>Sclerochaeta fascicularis</i>	Sclerotiniaceae	KX090857		KX090805	KX090755	KX090708	KX090659	Parrel et al. (2017)
KL344	<i>Sclerochaeta pruinosula</i>	Sclerotiniaceae	KX090888			KX090781	KX090735		Parrel et al. (2017)
CBS:499.50	<i>Sclerotinia sclerotiorum</i>	Sclerotiniaceae	DQ471013		DQ470965		DQ470916		Spatafora et al. (2006)
NY:01231276	<i>Skyptea radiatilis</i>	Cordieritiaceae		KJ559538	KJ559560	KX090791	KX090742	KX090694	Sujia et al. (2015), Parrel et al. (2017)
TH90	<i>Thamnogella crombiei</i>	Cordieritiaceae	KJ559583	KJ559535	KJ559557		KX090743	KX090695	Parrel et al. (2017)
BH1-P974a ^T	<i>Trochila bostonensis</i>	Cenangiaceae	MT873949	MT873947	MT873952		MT861181	MT861183	This study
BH1-P974b ^T	<i>Trochila bostonensis</i>	Cenangiaceae	MT873950	MT873948	MT873948		MT861182	MT861184	This study
KL332	<i>Trochila craterium</i>	Cenangiaceae	KX090886			KX090779			Parrel et al. (2017)
KL336	<i>Trochila lauracensis</i>	Cenangiaceae	KX090887	LT158460	KX090835	KX090780	KX090734	KX090689	Parrel et al. (2017)
F18316 ^T	<i>Trochila undinophila</i>	Cenangiaceae		MT873946	MT873951				This study
CBS:144206 ^T	<i>Trochila viburnicola</i>	Cenangiaceae		MH107921	MH107967		MH108011	MH108031	Crous et al. (2018)
KL253	<i>Vellutaria rufopoliacea</i>	Cenangiaceae	KX090877		KX090825	KX090771	KX090724	KX090678	Parrel et al. (2017)

which only a single ITS sequence is available. The same methods as above were applied: alignment using MUSCLE (Edgar 2004), selection of nucleotide substitution models with the help of ModelFinder (Kalyaanamoorthy et al. 2017), ML using IQ-TREE (Nguyen et al. 2015; Chernomor et al. 2016; Hoang et al. 2017). Phylogenetic reconstructions with bootstrap values (BS) were visualized in FigTree version 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Results

Nucleotide alignment dataset and phylogenetic inferences

The concatenated six-locus dataset consisted of 11343 characters, of which 2655 were parsimony-informative. The percentage of parsimony-informative characters per locus was 9.3% for SSU, 48.1% for ITS, 21.4% for LSU, 48.9% for *rpb1*, 30.0% for *rpb2*, and 19.2% for *tef1*. A total of 71 isolates were included, of which *Chlorociboria aeruginascens* (Nyl.) Kanouse ex C.S. Ramamurthi, Korf & L.R. Batra, *C. aeruginella* (P. Karst.) Dennis, and *C. glauca* (Dennis) Baral & Pärtel (Helotiales, Chlorociboriaceae) served as outgroup taxa. The following models were selected by ModelFinder (AICc): TNe+R3 (SSU, $-\ln L = 23478.796$); GTR+F+I+G4 (ITS, $-\ln L = 18385.043$); TN+F+R4 (LSU, $-\ln L = 28398.591$); SYM+I+G4 (*rpb1*, $-\ln L = 41387.214$); GTR+F+R10 (*rpb2*, $-\ln L = 57025.083$); and GTR+F+R8 (*tef1*, $-\ln L = 35467.940$). Our ML analysis reveals five high to maximum-supported clades (Fig. 1): Cenangiaceae, Cordieritidaceae, Rutstroemiaceae, Sclerotiniaceae, and a clade with *Piceomphale bulgarioides* (P. Karst.) Svrček and “*Cenangium*” *acuum* Cooke & Peck (*Piceomphale* clade *sensu* Pärtel et al. 2017). As previously reported (e.g., Pärtel et al. 2017; Johnston et al. 2019), several genera in their current circumscription are polyphyletic: *Encoelia* (Fr.) P. Karst. in Cenangiaceae and Rutstroemiaceae, *Ionomidotis* E.J. Durand ex Thaxt. in Cordieritidaceae, *Rutstroemia* P. Karst. in Rutstroemiaceae, and *Trochila* in Cenangiaceae. *Trochila laurocerasi* is placed as a sister taxon to *Calycellinopsis xishuangbanna* W.Y. Zhuang and *Pseudopeziza colensoi* (Berk.) Massee. The other species of *Trochila*, including the type species *T. craterium* and the here described species, form a monophyletic clade (BS = 81).

The second two-locus dataset consisted of 2284 characters (ITS: 924, LSU: 1360), of which 2040 were parsimony-informative (ITS: 782, LSU: 1258). A total of 13 isolates were included, of which *Cenangiosis alpestris* (Baral & B. Perić) Baral, B. Perić & Pärtel, *C. quercicola* (Romell) Rehm, and *Cenangiosis* sp. served as outgroup taxa. The following models were selected by ModelFinder (AICc): GTR+F+I+G4 (ITS, $-\ln L = 5810.483$) and TIM+F+R2 (LSU, $-\ln L = 5595.374$). *Calycellinopsis xishuangbanna*, *Pseudopeziza colensoi*, and all *Trochila* species form a monophyletic clade with high support (BS = 96) (Fig. 2). Both new species of *Trochila* are distinct from previously described species. The undescribed *Trochila* species found on uredinia of *Cerotelium fici* is retrieved as sister to *T. viburnicola* (BS = 90).

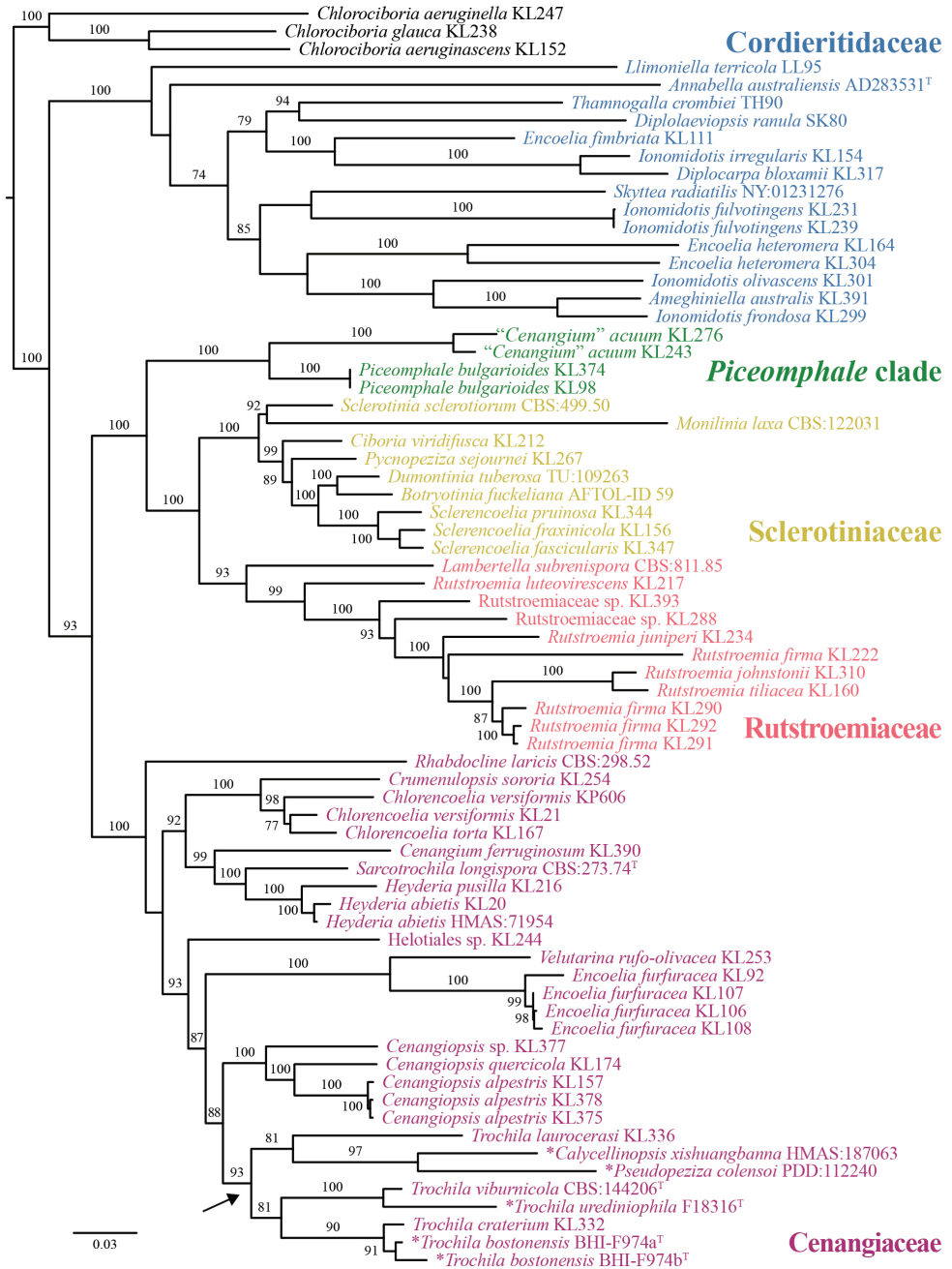


Figure 1. The best-scoring ML tree ($-\ln L = 87544.854$) of Cenangiaceae, Cordieritidaceae, Rutstroemiaceae, Sclerotiniaceae, and the *Piceomphale* clade, reconstructed from a concatenated six-locus dataset (SSU, ITS, LSU, *rpb1*, *rpb2*, and *tef1*). For each node, the ML bootstrap value (if ≥ 70) is presented above or in front of the branch leading to that node. The arrow denotes the genus *Trochila*. Species with an asterisk (*) are treated in the Taxonomy section.

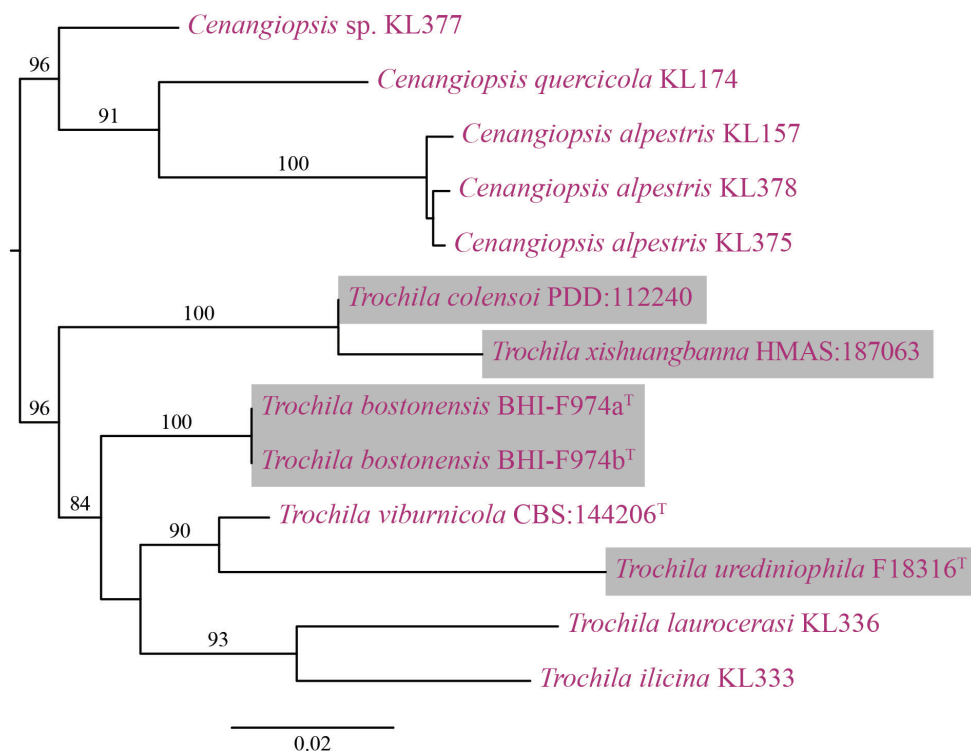


Figure 2. The best-scoring ML tree ($-\ln L = 5225.551$) of Cenangiaceae, reconstructed from a concatenated ITS–LSU dataset. For each node, the ML bootstrap value (if ≥ 70) is presented above the branch leading to that node. Species treated in the Taxonomy section are highlighted with gray shading.

Taxonomy

Leotiomyces O.E. Erikss. & Winka

Helotiales Nannf. ex Korf & Lizoň

Cenangiaceae Rehm

***Trochila bostonensis* Quijada & Haelew, sp. nov.**

Mycobank No: 836582

Fig. 3

Diagnosis. Differs from *Trochila craterium* and *T. laurocerasi* in its host (Apocynaceae), sizes of asci ($57\text{--}65.5 \times 5\text{--}6 \mu\text{m}$) and ascospores ($6.2\text{--}7.2 \times 2.6\text{--}2.8 \mu\text{m}$), and the inamyloidity of its ascus apex.

Type. Holotype: USA, Massachusetts, Boston Harbor Islands National Recreation Area, Plymouth County, Great Brewster Island, 42.3310722°N , 70.8977667°W , alt. 10 m a.s.l., 16 Oct 2017, leg. D. Haelewaters, J.K. Mitchell & L. Quijada, on hollow dead stem of *Asclepias syriaca* (Gentianales, Apocynaceae), FH:BHI-F0974. Ex-holotype sequences: isolates BHI-F0974a (1 apothecium, SSU: MT873949,

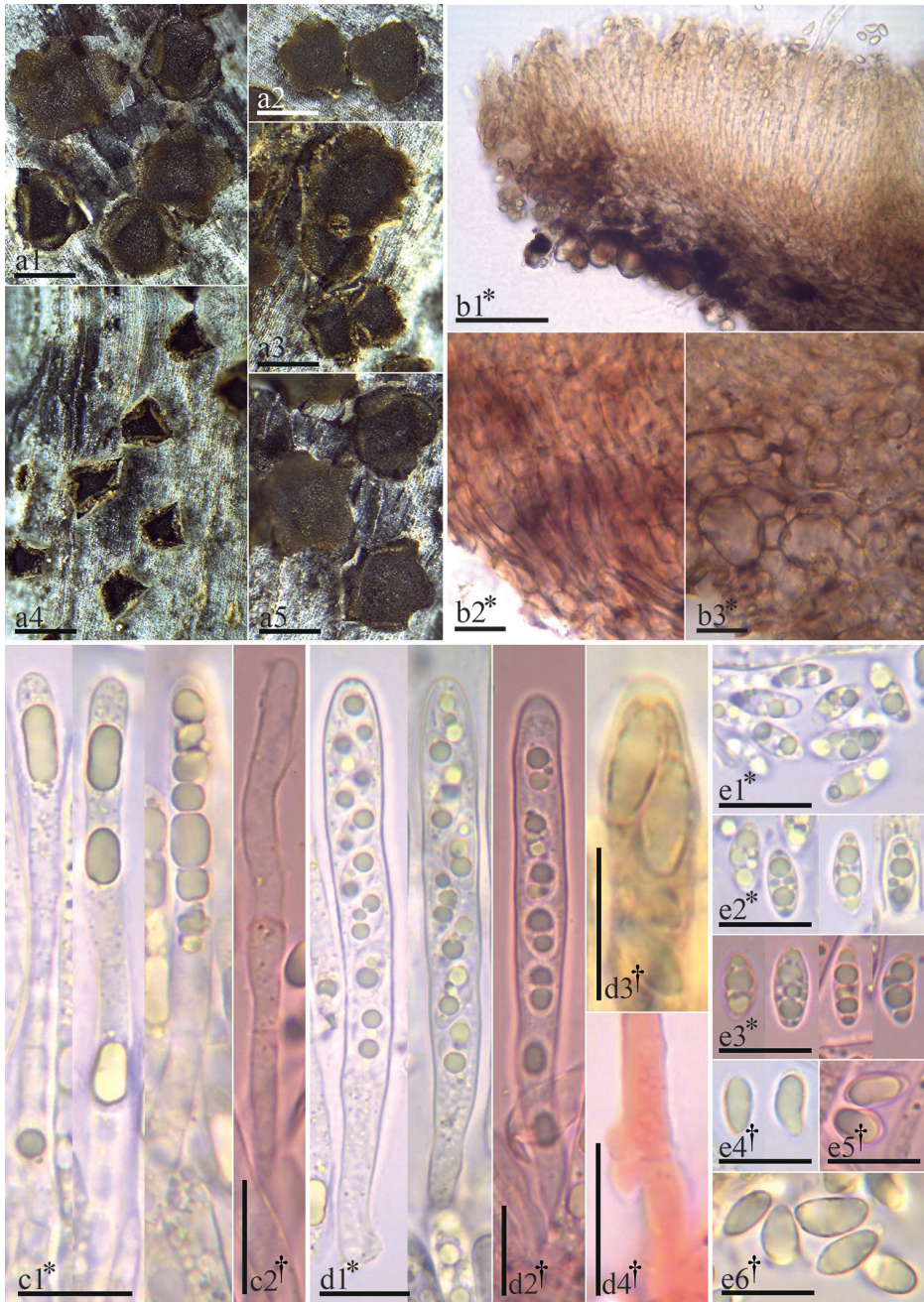


Figure 3. Morphological features of *Trochila bostonensis* (holotype collection FH:BHI-F0974) **a1–3, a5** fresh apothecia **a4** dried apothecia **b1** excipular tissues in median section **b2** cells at the base **b3** cells at the upper and lower flank **c1, c2** paraphyses **d1, d2** asci **d3** ascus pore with inamyloid reaction **d4** crozier at ascus base **e1–e6** ascospores. Mounted in: Congo Red (**c2, d2, d4, e3, e5**), H₂O (**b1–b3, c1, d1, e1, e2**), KOH (**e4**), MLZ (**d3, e6**). Scale bars: 500 μ m (**a1–a5**); 50 μ m (**b1**); 10 μ m (**b1, b2, c1, c2, d1–d4, e1–e6**).

ITS: MT873947, LSU: MT873952, *rpb2*: MT861181, *tefl*: MT861183) and BHI-F0974b (1 apothecium, SSU: MT873950, ITS: MT873948, LSU: MT873953, *rpb2*: MT861182, *tefl*: MT861184).

Etymology. *bostonensis* – referring to Boston, Massachusetts, the locality of the type collection.

Description. *Apothecia* erumpent singly or in groups of 2–3, protruding from the bark by lifting and rolling outward the host periderm, sessile on a broad base, closed and barely visible when dry, rehydrated 0.4–1.1 mm diam., 0.1–0.2 mm thick; mature flat to slightly cupulate, dark grayish red brown (47.D.gy.r.Br) to black (267.Black). Margin toothed and lighter than the disc, apothecia star-shaped, with 3–6 teeth of 0.1–0.3 mm in length, each tooth deep yellowish brown (75.deepyBr). *Asci* *(46.5–)55.5–66.5(–73) × (5.5–)6.0–6.5(–7.0) µm, †(50.5–)57–65.5(–66) × (4.5–)5.0–6.0 µm, 8-spored, cylindrical, pars sporifera *30–52 µm; apex rounded to subconical, inamyloid (IKI, KOH-pretreated or not), slightly thick-walled at apex, lateral walls thin; base slightly tapered and arising from croziers. *Ascospores* *(6.3–)6.7–7.7(–8.6) × 2.7–3.4 µm, †(5.8–)6.2–7.2 × 2.6–2.8 µm, ellipsoid-cuneate, inequilateral, ends rounded or subacute, aseptate, hyaline, smooth, thick-walled, oligoguttulate, containing 2–5 grayish yellow (90.gy.Y) oil drops (LBs), 1–2.4 µm diam., OCI = (45–)60–75(–90)%. *Paraphyses* slightly to medium clavate, terminal cell *(17.5–)18–23(–29.5) × 3–4 µm, secondary cells *(8–)9–10(–11) × 2.5–3 µm, lower cells *(7.5–)8.5–10.5(–11.5) × 2.5–3 µm, unbranched, thin-walled, smooth, with one or several cylindric to globose refractive drops (VBs, not present after KOH-pretreated), *3.5–14 × 2–3.5 µm. *Medullary excipulum* 17.5–54 µm thick, grey yellowish brown (80.gy.yBr), upper part of *textura porrecta*, lower part dense *textura intricata*, cells with tiny globose deep yellow (85.deepyY) refractive drops (VBs). *Ectal excipulum* of thin-walled *textura globulosa-angularis* at base and lower flanks, dark yellowish brown (78.d.yBr) to dark brown (59.d.Br), (40–)55–78 µm thick, cells *(7.0–)9.5–13(–15.5) × (3.0–)5.0–8.5(–10) µm; at upper flanks and margin of *textura prismatica*, 30–40 µm thick, cells *(5.5–)6.5–7.5(–8.5) × 2.5–3.5 µm, entirely without drops and slightly gelatinized, cells slightly thick-walled with irregular patches of dark brown exudates in areas of mutual contact, cortical cells in flanks covered by amorphous refractive deep yellow (88.d.Y) granular exudates, at margin some cells protruding like short hairs (*6.5–14 × 2.5–3.5 µm). *Asexual state* unknown.

Notes. *Trochila bostonensis* is the only species of the genus found on a member of Apocynaceae (Table 2). It was growing in the outer layer of a dead stem of *Asclepias syriaca*, which had fallen on the ground. The host was close to the shore in a shrubby thicket of *Rhus*. There are two similar species. *Trochila laurocerasi* has wider asci (6.0–8.0 µm vs. 4.5–6.0 µm) and larger ascospores (6.3–10 × 2.5–4.6 µm vs. 5.8–7.2 × 2.6–2.8 µm) compared to *T. bostonensis*. Ascus and ascospore length are similar in *T. bostonensis* and *T. craterium*, although ascospores are slightly larger in *T. craterium*. The two species mostly differ in the width of their asci (7–12 µm in *T. craterium* vs. 4.5–6.0 µm in *T. bostonensis*). We used the measurements in dead state to compare *T. bostonensis* with other species in the genus (see Table 2).

Table 2. Comparative table of currently accepted species of *Trochila* (except *T. viburnicola*). For each species, the following characters are presented: host plant, host family, measurements of asci and ascospores (dead state). The asterisk (*) indicates a fungal host.

Species	Host Plant	Host Family	Asci (μm)		Ascospores (μm)		Reference
			Length	Width	Length	Width	
<i>T. andromedae</i>	<i>Andromeda polifolia</i>	Ericaceae	80	12	15–18	4–5	Karsten (1871)
<i>T. astragali</i>	<i>Astragalus glycyphyllos</i>	Fabaceae	50–60	6–7	8	4	Rehm (1896)
<i>T. atrosanguinea</i>	<i>Carex rigida</i>	Cyperaceae	45–68	7–8	7–8	2–3	Rostrup (1885)
	<i>Carex vulgaris</i>	Cyperaceae					
<i>T. bostonensis</i>	<i>Asclepias syriaca</i>	Apocynaceae	(50.5)57–65.5(66)	(4.5)5–6	(5.8)6.2–7.2	2.6–2.8	This study
<i>T. chilensis</i>	<i>Lardizabala biternata</i>	Lardizabaleae	70–80	8–9	14–15	4	Spegazzini (1910)
<i>T. cinerea</i>	<i>Pyrola</i> sp.	Ericaceae	no data	no data	6–7	1.5	Patouillard (1886)
<i>T. colensoi</i>	<i>Cordyline</i> sp.	Asparagaceae	60–70	8–10	9–12.5	3.5–5	Dennis (1961)
<i>T. conioselinii</i>	<i>Conioselinum</i> sp.	Apiaceae	38–40	6–7	10–13	3	Rostrup (1886)
	<i>Gmelina</i> sp.	Apiaceae					
<i>T. craterium</i>	<i>Cassiope tetragona</i>	Araliaceae	50–60	8–12	6–8	4–5	Rehm (1896)
	<i>Hedera algeriensis</i>	Araliaceae	no data	7	6–8.2	3–4.5	Greenhalgh and Morgan-Jones (1964)
	<i>Hedera helix</i>	Araliaceae					
<i>T. epilobii</i>	<i>Epilobium angustifolium</i>	Onagraceae	75–95	17–20	15–17	8	Karsten (1871)
<i>T. exigua</i>	<i>Nardus stricta</i>	Poaceae	32	6	8–10	0.8	Rostrup (1888)
<i>T. fallens</i>	<i>Salix</i> sp.	Salicaceae	50–60	7–9	9–14	3.5–4.5	Karsten (1871)
<i>T. ilicina</i>	<i>Ilex aquifolia</i>	Aquifoliaceae	75–80	9–10	9–11	3.5–4.5	Rehm (1896)
	<i>Ilex aquifolium</i>	Aquifoliaceae	60–76	8.5–10	10–12.5	3.5–4.5	Greenhalgh and Morgan-Jones (1964)
	<i>Ilex colchica</i>	Aquifoliaceae					
	<i>Ilex platyphylla</i>	Aquifoliaceae	57.6–93.4	6.6–9.6	9.8–15.9	2.7–5.1	Ziolo et al. (2005)
<i>T. jaffuelii</i>	<i>Lapageria rosea</i>	Philesiaceae	50–70	25	13–14	6–7	Spegazzini (1921)
<i>T. juncicola</i>	<i>Juncus compressus</i>	Juncaceae	40–45	5–6	8–9	1–1.5	Rostrup (1886)
<i>T. laurocerasi</i>	<i>Laurocerasus officinalis</i>	Rosaceae	45–60	8–9	7–10	3.5–4	Rehm (1896)
	<i>Photinia serrulata</i>	Rosaceae					Greenhalgh and Morgan-Jones (1964)
	<i>Prunus laurocerasus</i>	Rosaceae	50–65	6–9	7.5–10	3–3.75	
	<i>Prunus lusitanica</i>	Rosaceae					
<i>T. leopoldina</i>	<i>Nectandra rigida</i>	Lauraceae	45–50	7	8–9	3	Rehm (1909)
<i>T. majalis</i>	<i>Fagus sylvatica</i>	Fagaceae	38–45	7–8	7–9	3–3.5	Kirschstein (1944)
<i>T. molluginea</i>	<i>Galium molluginis</i>	Rubiaceae	55–60	7	10–12	2.5	Mouton (1900)
<i>T. oleae</i>	<i>Olea europaea</i>	Oleaceae	no data	no data	no data	no data	Fries (1849)
<i>T. oxycoccus</i>	<i>Vaccinium oxycoccus</i>	Ericaceae	60–70	11–14	14–18	5	Karsten (1871)
<i>T. perexigua</i>	<i>Hippophae rhamnoides</i>	Elaeagnaceae	80	15	14	7	Spegazzini (1881)
<i>T. perseae</i>	<i>Persea lingue</i>	Lauraceae	50–60	10	9–10	3	Spegazzini (1910)
<i>T. plantaginea</i>	<i>Plantago major</i>	Plantaginaceae	42–50	12–16	18–25	4–4.5	Karsten (1871)
<i>T. prominula</i>	<i>Juniperus sabina</i>	Cupressaceae	65–70	10–12	18–20	6	Saccardo (1878)
<i>T. puccinioidea</i>	<i>Carex</i> sp.	Cyperaceae	no data	no data	no data	no data	De Notaris (1863)
<i>T. ramulorum</i>	<i>Viburnum opulus</i>	Viburnaceae	40–55	5.5–7	5–7	1.5–2	Felgen (1903)
<i>T. rhodiolae</i>	<i>Rhodiola</i> sp.	Crassulaceae	40	5–6	10	1–1.5	Rostrup (1891)
<i>T. staritziana</i>	<i>Ailanthus glandulosa</i>	Simaroubaceae	no data	no data	no data	no data	Kirschstein (1941)
	<i>Rhus glabra</i>	Anacardiaceae					
<i>T. substricta</i>	<i>Solidago virgaurea</i>	Asteraceae	60	9	12–14	6	Rehm (1884)
<i>T. symploci</i>	<i>Symplocos japonica</i>	Symplocaceae	65–85	5–7	8–11	4–5	Hennings (1900)
<i>T. tami</i>	<i>Tamus communis</i>	Dioscoreaceae	40–55	6–7	5–8	2.5–4	Grelet and de Crozals (1928)
<i>T. tetraspora</i>	<i>Nothofagus dombeii</i>	Nothofagaceae	58–72	7.7–9.6	12–15	3.4–4.8	Gamundi et al. (1978)
<i>T. urediniophila</i>	<i>Cerotelium fici</i> *	Phakopsoraceae	(86.4)102.4–111.2(121.8)	(9.1)10.5–11.6(13.1)	(7.6)9.0–9.7(10.9)	(5.1)6.3–7.1(8.1)	This study
<i>T. xishuangbanna</i>	no data	no data	55–60	3.5–4	8–11	1.2–1.7	Zhuang et al. (1990)
<i>T. winteri</i>	<i>Drymis Winteri</i>	Winteraceae	40–50	10–12	12–13	5	Spegazzini (1888)

***Trochila urediniophila* Gomez-Zap., Haelew. & Aime, sp. nov.**

Mycobank No: 836583

Fig. 4

Diagnosis. Differs from *Trochila ilicina* in ecological strategy (fungicolous symbiont); sizes of asci ($102.4\text{--}111.2 \times 10.5\text{--}11.6\ \mu\text{m}$), ascospores ($9.0\text{--}9.7 \times 6.3\text{--}7.1\ \mu\text{m}$), paraphyses ($3.2\text{--}3.6\ \mu\text{m}$ wide); and the inamyloidity of its ascus apex.

Type. Holotype: Reliquiae Farlowiana No. 723; Trinidad and Tobago, Port of Spain, Trinidad, Maraval Valley, ca. 10.5°N , 61.25°W , alt. $\pm 301\ \text{m a.s.l.}$, 1 Apr 1912, leg. R. Thaxter, on uredinia of *Cerotelium fici* [as *Phakopsora nishidana*] (Pucciniales, Phakopsoraceae) on the underside of *Ficus maxima* (Rosales, Moraceae) leaves, PUL F27668 (ex-PUR F18316). Ex-holotype sequences: isolate F18316 (3 apothecia, ITS: MT873946, LSU: MT873951).

Etymology. Referring to the intimate association of the fungus with the uredinia of *Cerotelium fici*.

Description. Apothecia protruding from uredinia of *Cerotelium fici*, gregarious in small groups or rarely solitary, discoid to irregular-ellipsoid when crowded, $0.4\text{--}1.0\ \text{mm}$ diam., sessile on a broad base, flat to slightly concave at maturity, dark grayish yellow brown (81.d.gy.yBr) to dark grayish brown (62.d.gy.Br), margin marked and lighter than hymenium, light grayish yellow brown (79.l.gr.yBr) to medium yellow brown (77.m.yBr), receptacle concolor with margin and surface slightly pruinose. Asci $\dagger(86.4\text{--})102.4\text{--}111.2\text{--}(121.8) \times (9.1\text{--})10.5\text{--}11.6\text{--}(13.1)\ \mu\text{m}$, 8-spored, cylindrical, $\dagger$ uniseriate; apex rounded to subconical, inamyloid (IKI, KOH-pretreated or not), base arising from croziers. Ascospores $\dagger(7.6\text{--})9.0\text{--}9.7\text{--}(10.9) \times (5.1\text{--})6.3\text{--}7.1\text{--}(8.1)\ \mu\text{m}$, ovoid to ellipsoid, aseptate, hyaline, smooth-walled, guttulate, containing $\dagger$ one to two pale yellow (89.p.Y) to yellow gray (93.y Gray) oil drops (LBs), $2\text{--}5\ \mu\text{m}$ diam., OCI = $(40\text{--})55.1\text{--}66.9\text{--}(81)\%$. Paraphyses cylindrical to slightly or medium clavate-spathulate, unbranched, smooth, septate, hyaline, $\dagger(2.3\text{--})3.2\text{--}3.6\text{--}(4.1)\ \mu\text{m}$ wide, apex up to $6.8\ \mu\text{m}$ wide. Medullary excipulum $\dagger 17.4\text{--}79.4\ \mu\text{m}$ thick, *textura intricata* strong brown (55.s.Br) to deep brown (56.deepBr). Ectal excipulum of *textura globulosa-angularis* at base and lower flanks, strong yellow brown (74.s.yBr) to dark brown (59.d.Br), $\dagger 32.8\text{--}93.5\ \mu\text{m}$ thick, cells $\dagger(7.3\text{--})9.0\text{--}10.8\text{--}(15.3) \times (6.0\text{--})7.5\text{--}8.7\text{--}(11.5)\ \mu\text{m}$; at upper flanks and margin cells vertically oriented of *textura prismatica*, $17\text{--}34\ \mu\text{m}$ thick, at margin and upper flank cells protruding like short hairs, hyaline, aseptate, cylindrical, $\dagger(9.5\text{--})16\text{--}20.6\text{--}(29.1) \times (3.0\text{--})3.9\text{--}4.5\text{--}(5.8)\ \mu\text{m}$. Asexual state unknown.

Notes. *Trochila urediniophila* is the first known fungicolous member of the genus. The specimen described here was discovered during a survey of hyperparasites of rust fungi at PUR. Apothecia of *T. urediniophila* were never observed in direct contact with the plant tissue; instead, they grew directly on the uredinia of *Cerotelium fici* on the underside of *Ficus maxima* leaves. *Trochila ilicina* is most similar to *T. urediniophila*, but *T. urediniophila* differs from *T. ilicina* in its distinctly wider ascospores, larger

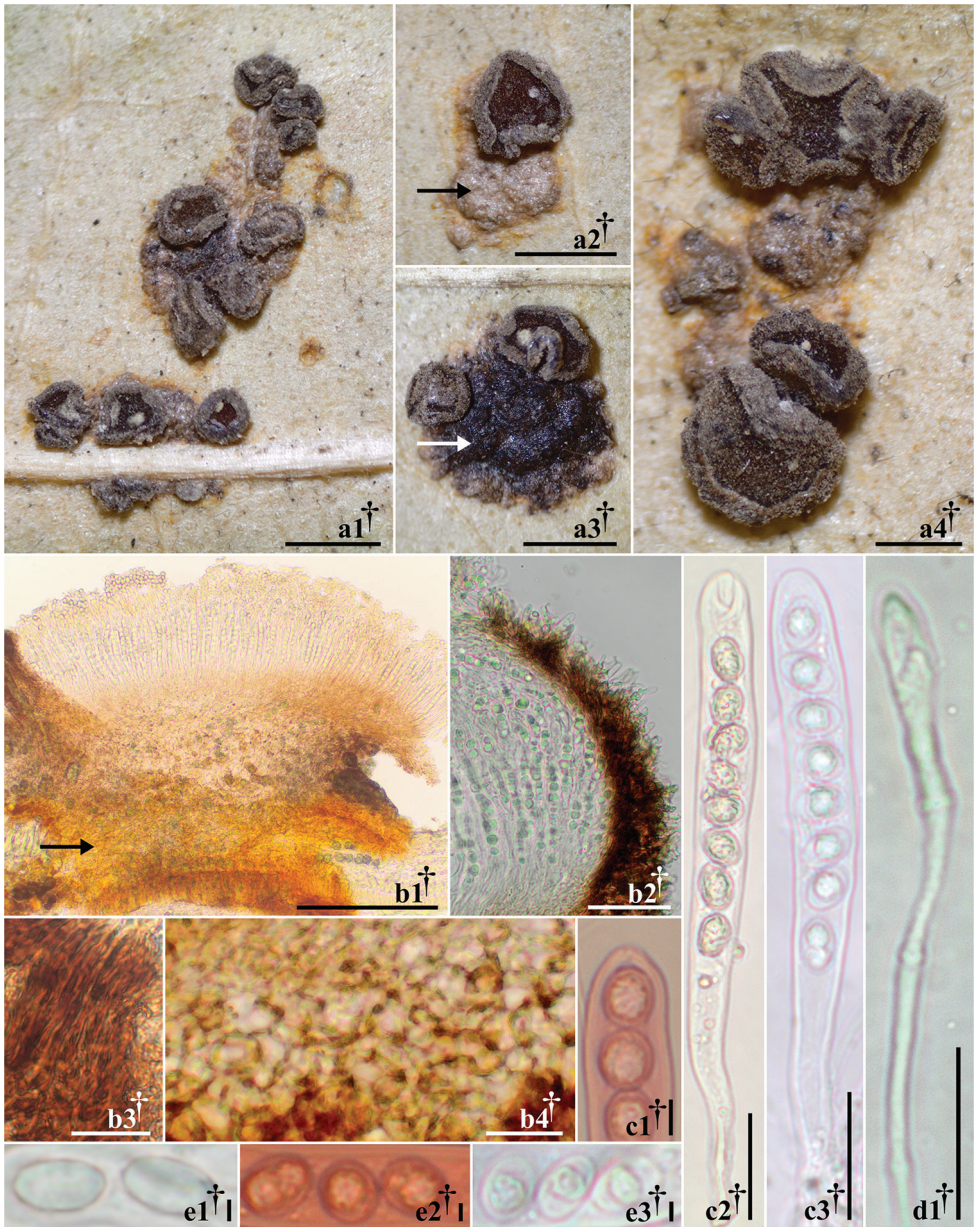


Figure 4. Morphological features of *Trochila urediniophila*, holotype collection (PUL F27668) **a1–a4** dried apothecia growing on uredinia of *Cerotelium fici* **a2, a3** substrate (uredinia) on which the apothecia grow (arrows) **b1** transverse section of apothecia; arrow pointing out the substrate **b2, b3** details of excipulum at margin and upper flanks **b4** cells at base **c1–c3** asci **d1** paraphyses **e1–e3** ascospores **e2, e3** oil drops (LBs) inside ascospores. Mounted in: Congo Red (**c1, e2**), H₂O (**b2, c3, d1, e1, e3**), KOH (**b1, b3, b4, c2**). Scale bars: 1 mm (**a1–a3**); 500 µm (**a4**); 200 µm (**b1**); 50 µm (**b2**); 20 µm (**b3, b4, c2, c3, d1**); 2 µm (**c1, e1–e3**).

asci, inamyloid ascus apex, and wider apex of the paraphyses. The uredinia of the host fungus, *C. fici*, become a solidified mass that changes in color from dark orange yellow (72.d.OY) without apothecia of *Trochila* to brownish black (65.brBlack) where apothecia are present.

A second duplicate of the Reliquiae Farlowiana No. 723 is also deposited at PUR (accession PUR F1098). However, no apothecia were present on this specimen, nor could additional specimens of *T. urediniophila* be found on any of the other specimens of *C. fici* housed at PUR. At least eight other duplicates are housed at BPI, CINC, CUP, F, ISC, MICH, and UC (MyCoPortal 2020). It is unknown whether any of them may host *T. urediniophila*.

New combinations

Trochila colensoi (Berk.) Quijada, comb. nov.

Mycobank No: 836591

≡ *Cenangium colensoi* Berk., Hooker, Bot. Antarct. Voy. Erebus Terror 1839–1843, II, Fl. Nov.-Zeal.: 201 (1855). [Basionym]

= *Pseudopeziza colensoi* (Berk.) Masee, J. Linn. Soc., Bot. 31: 468 (1896)

Notes. *Cenangium colensoi* is described from dead leaves of *Cordyline* sp. (Asparagales, Asparagaceae) in New Zealand (Hooker 1855). The host had been mistakenly reported as *Phormium* (Asparagales, Asphodelaceae) by Berkeley in Hooker (1855) and only recently corrected after re-study of the type collection (Landcare Research 2020). *Cenangium colensoi* was later combined in *Pseudopeziza* and described in more detail by Masee (1896). Both authors commented on the watery-grey disc and brownish receptacle of the apothecia. The apothecia develop among the rigid vascular bundles of the epidermis, first covered by the cuticle, then erumpent and opening by a narrow slit, becoming discoid when mature (Hooker 1855; Masee 1896). The habit of this fungus fits well with typical macromorphological features of the genus *Trochila* – a dark brown to black receptacle, which develops beneath the host tissues and eventually becomes erumpent to expose the hymenium by splitting along radial lines or by its splitting into lobes (von Höhnelt 1917; Greenhalgh and Morgan-Jones 1964; Dennis 1978; Baral and Marson 2005). Microscopically, *P. colensoi* was described with a parenchymatous excipulum (angular-globose or isodiametric cells), hyaline under the hymenium and dark brown at the cortex (Berkeley in Hooker 1855; Masee 1896), which is also in agreement with the excipular features of *Trochila* species. Finally, the hymenium of *P. colensoi* was described as composed of inamyloid, 8-spored asci with elliptical hyaline ascospores and slender paraphyses (*op. cit.*).

In 2018, P.R. Johnston collected two specimens (PDD:112240, PDD:112242, Landcare Research 2020) on leaves of *Cordyline australis* (Asparagaceae). The

morphology, ecology (host), and locality of these new collections agree with *P. colensoi*. The photographs of both specimens reveal features such as guttules in ascospores and paraphyses, protruding hyaline cells in the cortical layer of the upper flank and margin, and hyaline gelatinized hyphae covering the dark globose-angular cells of the ectal excipulum at the base and lower flanks. The latter excipular feature of the receptacle is reminiscent of Zhuang's (1990) description of *Calycellinopsis xishuangbanna*. An ITS sequence of this species was generated from the recent material (PDD:112240) and included in the Leotiomyces-wide ITS phylogeny of Johnston et al. (2019). Their results and those in this study (Figs 1, 2) show that *P. colensoi* is placed among species of *Trochila*.

***Trochila xishuangbanna* (W.Y. Zhuang) Quijada, comb. nov.**

Mycobank No: 836592

≡ *Calycellinopsis xishuangbanna* W.Y. Zhuang, Mycotaxon 38: 121 (1990). [Basionym]

Notes. The genus *Calycellinopsis* was proposed with a single species, *C. xishuangbanna*, which is a petiole-inhabiting fungus (Zhuang 1990). The genus was placed in Dermateaceae because of its isodiametric dark brownish excipular cells (Zhuang 1990). In 2002, a second collection of the same species was sampled (HMAS:187063), which was sequenced (Zhuang et al. 2010). Additional morphological details were provided, and the genus was placed in Helotiaceae (Zhuang et al. 2010). *Trochila* was treated in Dermateaceae until recently because of its excipular features (Fuckel 1869; Karsten 1869; Saccardo 1884; Lambotte 1888; Lumbsch and Huhndorf 2010). Collections of *Calycellinopsis* have a well-developed excipulum, with an outer layer of angular to isodiametric cells with brownish walls and cortical cells at flanks and margin with protruding hyaline cells. The medullary excipulum is subhyaline and composed of *textura angularis* to *textura intricata* (Zhuang 1990; Zhuang et al. 2010).

Species in *Trochila* usually have a poorly developed excipulum. For example, *T. bostonensis* and *T. craterium* produce only a thin layer of globose to angular dark excipular cells (von Höhnelt 1917; Greenhalgh and Morgan-Jones 1964; Baral and Marson 2005). However, other species, such as *T. laurocerasi* and *T. urediniophila*, have a well-developed excipulum (*op. cit.*). The excipulum of *Calycellinopsis* is very similar to those species of *Trochila* with a well-developed excipulum, composed of an outer layer of dark *textura globulosa-angularis* and an inner layer of hyaline medulla made of *textura angularis-porrecta-intricata*. At the flanks and margin of the excipulum, *Calycellinopsis* has protruding hyaline cells similar to *Trochila* species with a well-developed excipulum (Fig. 4). Although limited details about the living features can be obtained from the original description of *Calycellinopsis*, its hymenial features are consistent with *Trochila*. The ascospores of *Calycellinopsis* are described with several guttules, a feature that is also observed in species of *Trochila*.

Discussion

Taxonomy of *Trochila*

This study represents the first attempt to investigate the systematics of *Trochila* using both morphological features and DNA sequences. We have added four species to *Trochila*, bringing the total number of species described in the genus to 37. Most *Trochila* species have been delimited based on the size of asci and ascospores, but we find that amyloidity of ascus apex, excipular features, details of the paraphyses, and presence vs. absence of guttules are also diagnostic (Table 2). For this study, we also applied a two-dataset approach for phylogenetic analyses (e.g., Aime and Phillips-Mora 2005; Haelewaters et al. 2019). Our phylogenetic reconstruction of a six-locus dataset resolved *Trochila* as polyphyletic with respect to *C. xishuangbanna* and *P. colensoi* (Fig. 1). Because morphological data of these two taxa agree with *Trochila*, we recombined them in this genus. The second, two-locus dataset was used for species delimitation, which showed *T. bostonensis* and *T. urediniophila* as distinct from the other *Trochila* species. Our molecular phylogenetic results (Figs 1, 2) and morphological comparisons of *Trochila* species (Table 2) will facilitate future taxonomic studies in the genus.

Host associations

Thus far, members of *Trochila* have been reported from 31 families of both monocots and dicots (Table 2). In this study, we add two plant family hosts, Apocynaceae (for *T. bostonensis*) and Asparagaceae (for *T. colensoi*). In addition, we reveal a new ecological niche (for *T. urediniophila*) – a species that associates with uredinia of the rust species *Cerotelium fici*. This sample was collected in 1912 as a rust specimen and deposited in the Arthur Fungarium (PUR) at Purdue University. More than a century later, the exsiccatae sample was scanned for the presence of hyperparasites of rust fungi from South America. Apothecia of *T. urediniophila* were found exclusively on uredinia without any direct contact with the host plant. Due to the age and limited available material, ultrastructural examinations of the interaction between these two fungi could not be made. However, *T. urediniophila* is the first species in the genus that fruits exclusively from another fungus, hinting at more complex associations among *Trochila* species and other fungi on which they might act as mycoparasites.

Trochila in the Neotropics

South America is known to be one of the most biodiverse continents in the world (Dourojeanni 1990; Hawksworth 2001). However, its fungal communities are thought to be severely understudied (Mueller and Schmit 2007). Members of *Trochila* are no exception to this. Six species of *Trochila* have been described from South America. These are *T. chilensis* Speg., *T. jaffuelii* Speg., and *T. perseae* Speg. from Chile; *T. leopoldina* Rehm from Brazil; and *T. tetraspora*, and *T. winteri* Speg. from Argentina (Spegazzini 1888, 1910, 1921; Rehm 1909; Gamundí et al. 1978). Their type collections need to be

re-examined to determine if these species are in fact members of *Trochila*. One of our new species, *T. urediniophila*, was collected in Port of Spain, Trinidad. Little data are available regarding the Funga (*sensu* Kuhar et al. 2018) of Trinidad and Tobago (Baker and Dale 1951; Dennis 1954a, b). The most recent work on the fungal diversity from this country was published online (Jodhan and Minter 2006) derived from reference collections and data from scientific literature. Based on the available literature, no records of *Trochila* are known in Trinidad. As a result, *T. urediniophila* represents the first published report of the genus from Trinidad, and by extension from the Caribbean (Minter et al. 2001).

Trochila species are likely more broadly distributed than generally thought, and certainly not limited to the Northern Hemisphere. This is often the case for many fungi that are based on limited regional collecting and thus may not represent the full extent of their distributional ranges due to, for example, the lack of studies in sub-tropical and tropical ecosystems (Groombridge 1992; Hawksworth and Mueller 2005; Mueller and Schmit 2007; Aime and Brearley 2012; Cheek et al. 2020).

The importance of biological collections

Our work emphasizes the importance of specimens preserved in biological collections – such as fungaria and herbaria – for studies of biodiversity and applied biological sciences, and for climate change research (Hawksworth and Lücking 2017; Andrew et al. 2019; Lang et al. 2019; Ristaino 2020; Wijayawardene et al. 2020). Because of the well-preserved specimens deposited at PUR, the genus *Trochila* is now known to be present in Trinidad and to form fungicolous associations. Another interesting example of the use of collections is *Trochila colensoi*. Known only from the type specimen for more than 100 years, additional specimens were only reported following the correction of the host substrate (as *Cordyline* rather than *Phormium*), which was based on re-examination of the type specimen preserved at K. Biological collections are not only important for morphological studies, but also as sources of genetic and genomic information (Bruns et al. 1990; Brock et al. 2009; Redchenko et al. 2012; Dentinger et al. 2016; this study). The single-oldest fungal specimen used for DNA extraction and sequencing was the type of *Hygrophorus cossus* (Sowerby) Fr. (Agaricales, Hygrophoraceae), collected in 1794 and deposited at K (Larsson and Jacobsson 2004). Our material of *T. urediniophila* gathered by Roland Thaxter in 1912 proves again that old samples can be used successfully for modern molecular phylogenetic analyses.

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References

- Aime MC, Brearley FQ (2012) Tropical fungal diversity: closing the gap between species estimates and species discovery. *Biodiversity and Conservation* 21: 2177–2180. <https://doi.org/10.1007/s10531-012-0338-7>
- Aime MC, Phillips-Mora W (2005) The causal agents of witches' broom and frosty pod rot of cacao (chocolate, *Theobroma cacao*) form a new lineage of Marasmiaceae. *Mycologia* 97: 1012–1022. <https://doi.org/10.3852/mycologia.97.5.1012>
- Andrew C, Diez J, James TY, Kauserud H (2019) Fungarium specimens: a largely untapped source in global change biology and beyond. *Philosophical Transactions of the Royal Society B* 374(1763): 20170392. <https://doi.org/10.1098/rstb.2017.0392>
- Baker RED, Dale WT (1951) Fungi of Trinidad and Tobago. *Mycological Papers* 33: 1–123.
- Baral H-O (1992) Vital versus herbarium taxonomy: morphological differences between living and dead cells of ascomycetes, and their taxonomic implications. *Mycotaxon* 44: 333–390.
- Baral H-O, Marson G (2005) In vivo veritas. Over 10000 images of fungi and plants (microscopical drawings, water colour plates, photo macro- & microphotographs), with materials on vital taxonomy and xerotolerance. Ed. 3. Privately distributed DVD-ROM.
- Brock PM, Döring H, Bidartondo MI (2009) How to know unknown fungi: The role of a herbarium. *New Phytologist* 181: 719–724. <https://doi.org/10.1111/j.1469-8137.2008.02703.x>
- Bruns TD, Fogel R, Taylor JW (1990) Amplification and sequencing of DNA from fungal herbarium specimens. *Mycologia* 82: 175–184. <https://doi.org/10.2307/3759846>
- Cheek M, Lughadha EN, Kirk P, Lindon H, Carretero J, Looney B, Douglas B, Haelewaters D, Gaya E, Llewellyn T, Ainsworth M, Gafforov Y, Hyde K, Crous P, Hughes M, Walker BE, Forzza RC, Meng WK, Niskanen T (2020) New scientific discoveries: Plants and fungi. *Plants, People, Planet* 2(5): 371–388. <https://doi.org/10.1002/ppp3.10148>
- Chernomor O, Von Haeseler A, Minh BQ (2016) Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology* 65: 997–1008. <https://doi.org/10.1093/sysbio/syw037>
- Clements FE, Shear CL (1931) *The genera of fungi*. H.W. Wilson Company, Bronx, New York, 496 pp.

- Crouan PL, Crouan MH (1867) Florule du Finistère. Contenant les descriptions de 360 espèces nouvelles de sporogames, de nombreuses observations et une synonymie des plantes cellulaires et vasculaires qui croissant spontanément dans ce département. Friedrich Klincksieck and J.B. et A. Lefournier, Paris & Brest, 262 pp. <https://doi.org/10.5962/bhl.title.11601>
- Crous PW, Quaedvlieg W, Hansen K, Hawksworth DL, Groenewald JZ (2014) *Phacidium* and *Ceuthospora* (Phaciaceae) are congeneric: taxonomic and nomenclatural implications. *IMA Fungus* 5: 173–193. <https://doi.org/10.5598/imafungus.2014.05.02.02>
- Crous PW, Schumacher RK, Wingfield MJ, Akulov A, Denman S, Roux J, Braun U, Burgess TI, Carnegie AJ, Váczy KZ, Guatimosim E, Schwartsburd PB, Barreto RW, Hernández-Restrepo M, Lombard L, Groenewald JZ (2018) New and Interesting Fungi. 1. *Fungal Systematics and Evolution* 1: 169–215. <https://doi.org/10.3114/fuse.2018.01.08>
- De Notaris G (1863) [printed 1864] Proposte di alcune rettificazioni al profilo dei Discomiceti. *Commentario della Società Crittogamologica Italiana* 1(5): 357–388.
- Dennis RWG (1954a) Operculate Discomycetes from Trinidad and Jamaica. *Kew Bulletin* 9(3): 417–421. <https://doi.org/10.2307/4108810>
- Dennis RWG (1954b) Some Inoperculate Discomycetes of Tropical America. *Kew Bulletin* 9(2): 289–348. <https://doi.org/10.2307/4114399>
- Dennis RWG (1961) Some Inoperculate Discomycetes from New Zealand. *Kew Bulletin* 15(2): 293–320. <https://doi.org/10.2307/4109373>
- Dennis RWG (1978) *British Ascomycetes*. J. Cramer, Vaduz, Liechtenstein, 585 pp.
- Dentinger BTM, Gaya E, O'Brien H, Suz LM, Lachlan R, Diaz-Valderrama JR, Koch RA, Aime MC (2016) Tales from the crypt: genome mining from fungarium specimens improves resolution of the mushroom tree of life. *Biological Journal of the Linnean Society* 117: 11–32. <https://doi.org/10.1111/bij.12553>
- Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS (1991) 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Research* 19(14): 4008–4008. <https://doi.org/10.1093/nar/19.14.4008>
- Dourojeanni MJ (1990) Entomology and biodiversity conservation in Latin America. *American Entomologist* 36: 88–93. <https://doi.org/10.1093/ae/36.2.88>
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Egger KN (1995) Molecular analysis of ectomycorrhizal fungal communities. *Canadian Journal of Botany* 73: S1415–S1422. <https://doi.org/10.1139/b95-405>
- Etayo J, Flakus A, Suija A, Kukwa M (2015) *Macroskytteia parmotrematis* gen. et sp. nov. (Helotiales, Leotiomycetes, Ascomycota), a new lichenicolous fungus from Bolivia. *Phytotaxa* 224: 247–257. <https://doi.org/10.11646/phytotaxa.224.3.3>
- Feltgen J (1903) Vorstudien zu einer Pilz-flora des Grossherzogthums Luxemburg. I. Theil – Ascomycetes. *Nachträge III. Recueil des mémoires et des travaux publiés par la Société de botanique du grand-duché de Luxembourg* 16: 3–328.
- Fries E (1849) *Summa Vegetabilium Scandinaviae. Sectio Posterior. Cl. XX. Fungi*. A. Bonnier, Stockholm & Leipzig, 261–572.
- Fryar SC, Haelewaters D, Catcheside DE (2019) *Annabella australiensis* gen. & sp. nov. (Helotiales, Cordieritidaceae) from South Australian mangroves. *Mycological Progress* 18: 973–981. <https://doi.org/10.1007/s11557-019-01499-x>

- Fuckel L (1869) *Symbolae mycologicae*. Beiträge zur Kenntniss der rheinischen Pilze. Mit VI lithographirten und colorirten Tafeln. Jahrbücher des Nassauischen Vereins für Naturkunde 23–24: 1–459. <https://doi.org/10.5962/bhl.title.47117>
- Gamundí, IJ, Arambarri, AM, Gaiotti A (1978) Micoflora de la hojarasca de *Nothofagus dombeyi*. Darwiniana 21: 81–114.
- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Global Biodiversity Information Facility (2020) *Trochila* Fr. GBIF Backbone Taxonomy. <https://www.gbif.org/species/2575690> [accessed 17 August 2020]
- Greenhalgh G, Morgan-Jones G (1964). Some species of *Trochila* and an undescribed discomycete on leaves of *Prunus laurocerasus*. Transactions of the British Mycological Society 47: 311–320. [https://doi.org/10.1016/S0007-1536\(64\)80002-4](https://doi.org/10.1016/S0007-1536(64)80002-4)
- Gregor MJF (1936) A disease of cherry laurel caused by *Trochila laurocerasi* (Desm.) Fr. Annals of Applied Biology 23: 700–704. <https://doi.org/10.1111/j.1744-7348.1936.tb06121.x>
- Grelet L-J, de Crozals A (1928) Discomycètes nouveaux (3ième série). Bulletin trimestriel de la Société mycologique de France 44: 336–340.
- Haelewaters D, Dirks AC, Kappler LA, Mitchell JK, Quijada L, Vandegrift R, Buyck B, Pfister DH (2018a) A preliminary checklist of fungi at the Boston Harbor islands. Northeastern Naturalist 25(sp9): 45–77. <https://doi.org/10.1656/045.025.s904>
- Haelewaters D, De Kesel A, Pfister DH (2018b) Integrative taxonomy reveals hidden species within a common fungal parasite of ladybirds. Scientific Reports 8: e15966. <https://doi.org/10.1038/s41598-018-34319-5>
- Haelewaters D, Pfliegler WP, Gorczak M, Pfister DH (2019) Birth of an order: comprehensive molecular phylogenetic study reveals that *Herpomyces* (Fungi, Laboulbeniomycetes) is not part of Laboulbeniales. Molecular Phylogenetics and Evolution 133: 286–301. <https://doi.org/10.1016/j.ympev.2019.01.007>
- Hammond PM (1992) Species inventory. In: Groombridge B (Ed.) Global biodiversity, status of the earth's living resources. Chapman & Hall, London, 17–39. https://doi.org/10.1007/978-94-011-2282-5_4
- Hawksworth DL (2001) The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycological Research 105: 1422–1432. <https://doi.org/10.1017/S0953756201004725>
- Hawksworth DL, Lücking R (2017) Fungal diversity revisited: 2.2 to 3.8 million species. In: Heitman J, Howlett B, Crous P, Stukenbrock E, James T, Gow N (Eds) The Fungal Kingdom. ASM Press, Washington, 79–95. <https://doi.org/10.1128/microbiolspec.FUNK-0052-2016>
- Hawksworth DL, Mueller GM (2005) Fungal communities: their diversity and distribution. In: Digthton J, White JF, Oudemans P (Eds) The fungal community: its organisation and role in the ecosystem. CRC Press, Boca Raton, 27–37. <https://doi.org/10.1201/9781420027891.ch2>
- Hennings P (1900) Fungi japonici. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 28: 273–280.
- Hoang DT, Chernomor O, Von Haeseler A, Minh BQ, Vinh LS (2017) UFBoot2: improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35: 518–522. <https://doi.org/10.1093/molbev/msx281>
- Höhnelt F von (1917) Über die Gattung *Trochila* Fries. Annales Mycologici 15: 330–334.

- Hooker JD (1855) The botany of the Antarctic Voyage of H.M. discovery ships Erebus and Terror, in the years 1839–1843. II. Flora Novae-Zelandiae. Part II. Flowerless plants. Lovell Reeve, London, 378 pp.
- Hopple JS (1994) Phylogenetic investigations in the genus *Coprinus* based on morphological and molecular characters. PhD Dissertation, Duke University, Durham.
- Hyde KD, McKenzie EHC, KoKo TW (2011) Towards incorporating anamorphic fungi in a natural classification – checklist and notes for 2010. *Mycosphere* 2: 1–88.
- Index Fungorum (2021) Index Fungorum. <http://www.indexfungorum.org/names/Names.asp> [accessed 22 June 2020]
- Jaklitsch W, Baral H-O, Lücking R, Lumbsch HT (2016) Syllabus of plant families. In: Frey W (Ed.) *Adolf Engler's Syllabus der Pflanzenfamilien*. Part 1/2 Ascomycota (13th edn). Borntraeger Science Publishers, Stuttgart, 322 pp.
- Jodhan D, Minter DW (2006) Fungi of Trinidad & Tobago. <http://www.cybertruffle.org.uk/trinfung> [accessed 14 November 2019]
- Johnston PR, Quijada L, Smith CA, Baral H-O, Hosoya T, Baschien C, Pärtel K, Zhuang K-Y, Haelewaters D, Park D, Carl S, López-Giráldez F, Wang Z, Townsend JP (2019) A multigene phylogeny toward a new phylogenetic classification of Leotiomyces. *IMA Fungus* 10: 1–22. <https://doi.org/10.1186/s43008-019-0002-x>
- Kalyanamoorthy K, Minh BQ, Wong TKE, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587–589. <https://doi.org/10.1038/nmeth.4285>
- Karsten PA (1869) *Monographia Pezizarum fennicarum*. Notiser ur Sällskapet pro Fauna et Flora fennica förhandlingar X: 99–206.
- Karsten PA (1871) *Mycologia Fennica I. Discomycetes*. Bidrag Kännedom Finland Natur Folk 19: 1–263.
- Kelly KL (1965) ISCC-NBS Colour-name charts illustrated with centroid colors. Inter-Society Colors Council. National Bureau of Standards, Circular 553 (Supplement). US Government Printing Office, Washington, 44 pp.
- Kiffer E, Morelet M (2000) The Deuteromycetes, mitosporic fungi: classification and generic key. Science Publishers, Enfield, 273 pp.
- Kirschstein W (1941) De plerisque novis ascomycetibus et paucis novis fungis imperfectis. *Hedwigia* 80: 119–137.
- Kirschstein W (1944) Über neue, seltene und kritische Kleinpilze. *Hedwigia* 81: 193–224.
- Korf RP (1973) Discomycetes and Tuberales. In: Ainsworth GC, Sparrow FK, Sussman AS (Eds) *The Fungi: An Advanced Treatise*. Vol. 4a. Academic Press, London, 249–319.
- Kuhar F, Furci G, Drechsler-Santos ER, Pfister DH (2018) Delimitation of Funga as a valid term for the diversity of fungal communities: the Fauna, Flora & Funga proposal (FF&F). *IMA Fungus* 9: 71–74. <https://doi.org/10.1007/BF03449441>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Lamarck JBPA, de Candolle AP (1805) *Flore française*, ii. Desray, Paris, 600 pp.
- Lambotte E (1888) *La flore mycologique de la Belgique*. Mémoires de la Société royale des sciences de Liège, sér. 2, 14: 1–350.

- Landcare Research (2020) Collection details. *Pseudopeziza colensoi* (Berk.) Massee (1896) [1895–97]. <https://nzfungi2.landcareresearch.co.nz/default.aspx?selected=NameDetails&TabNum=0&NameId=1CB1B798-36B9-11D5-9548-00D0592D548C> [accessed 16 August 2020]
- Lang PL, Willems FM, Scheepens JF, Burbano HA, Bossdorf O (2019) Using herbaria to study global environmental change. *New Phytologist* 221: 110–122. <https://doi.org/10.1111/nph.15401>
- Larsson E, Jacobsson S (2004) Controversy over *Hygrophorus cossus* settled using ITS sequence data from 200 year-old type material. *Mycological Research* 108: 781–786. <https://doi.org/10.1017/S0953756204000310>
- Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* 16: 1799–1808. <https://doi.org/10.1093/oxfordjournals.molbev.a026092>
- Lumbsch HT, Huhndorf SM (2010) Myconet Volume 14. Part One. Outline of Ascomycota – 2009. Part Two. Notes on Ascomycete Systematics. Nos. 4751–5113. *Fieldiana Life and Earth Sciences* 2010: 1–64. <https://doi.org/10.3158/1557.1>
- Massee GE (1896) Redescriptions of Berkeley's types of fungi. *Journal of the Linnean Society* 31: 463–524. <https://doi.org/10.1111/j.1095-8339.1896.tb00812.x>
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop*, 14 Nov. 2010, New Orleans, LA, 1–8. <https://doi.org/10.1109/GCE.2010.5676129>
- Minter DW, Rodríguez-Hernández M, Mena-Portales J (2001) *Fungi of the Caribbean. An annotated checklist*. PDMS Publishing, Isleworth, 950 pp.
- Morgan-Jones G (1973) *Genera coelomycetarum*. VII. *Cryptocline* Petrak. *Canadian Journal of Botany* 51: 309–325. <https://doi.org/10.1139/b73-039>
- Mouton V (1900) Quatrième notice sur des ascomycètes nouveaux ou peu connus. *Bulletin de la Société Royale de Botanique de Belgique* 39: 37–53.
- Mueller GM, Schmit JP (2007) Fungal biodiversity: what do we know? What can we predict? *Biodiversity and Conservation* 16: 1–5. <https://doi.org/10.1007/s10531-006-9117-7>
- MyCoPortal (2020) Mycology Collections data Portal. <http://mycoportal.org/portal/index.php> [accessed 7 December 2020]
- Nguyen L-T, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution* 32: 268–274. <https://doi.org/10.1093/molbev/msu300>
- Pärtel K, Baral H-O, Tamm H, Pöldmaa K (2017) Evidence for the polyphyly of *Encoelia* and *Encoelioideae* with reconsideration of respective families in *Leotiomyces*. *Fungal Diversity* 82: 183–219. <https://doi.org/10.1007/s13225-016-0370-0>
- Patouillard MN (1886) Quelques champignons de la Chine, récoltés par M. l'abbé Delavay dans la province du Yunnan. *Revue Mycologique* 8: 179–194.
- Redchenko O, Vondrák J, Košnar J (2012) The oldest sequenced fungal herbarium sample. *The Lichenologist* 44: 715–718. <https://doi.org/10.1017/S002428291200031X>
- Rehm H (1896) Abt. 3. Ascomyceten: hysteriaceen und discomyceten. In: Rabenhorst L (Ed.) *Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz*. Verlag von Eduard Kummer, Leipzig.
- Rehm H-J (1909) *Ascomycetes exs. Fasc. 45. Annales Mycologici* 7: 524–530.

- Rehner SA, Buckley E (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1- α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97: 84–98. <https://doi.org/10.3852/mycologia.97.1.84>
- Ristaino JB (2020) The importance of mycological and plant herbaria in tracking plant killers. *Frontiers in Ecology and Evolution* 7: e521. <https://doi.org/10.3389/fevo.2019.00521>
- Rostrup E (1885) Islands svampe. *Botanisk Tidsskrifte* 14: 218–229.
- Rostrup E (1886) Svampen fra Finmarken. *Botanisk Tidsskrifte* 15: 229–236.
- Rostrup E (1888) Oversigt over Grönlands svampe. *Meddelelser om Grønland* 3: 516–590.
- Rostrup E (1891) Tillaeg til “Grönlands svampe (1888)”. *Meddelelser om Grønland* 3: 591–643.
- Saccardo PA (1878) *Fungi Veneti novi vel critici*. Series IX. *Michelia* 4: 361–434.
- Saccardo PA (1884) *Conspectus generum discomycetum hucusque cognitorum*. *Botanisches Centralblatt* 18: 247–256.
- Spatafora JW, Sung GH, Johnson D, Hesse C, O’Rourke B, Serdani M, Spotts R, Lutzoni F, Hofstetter V, Miadlikowska J, Reeb V, Gueidan C, Fraker E, Lumbsch T, Lücking R, Schmitt I, Hosaka K, Aptroot A, Roux C, Miller AN, Geiser DM, Hafellner J, Hestmark G, Arnold AE, Büdel B, Rauhut A, Hewitt D, Untereiner WA, Cole MS, Scheidegger C, Schultz M, Sipman H, Schoch CL (2006) A five-gene phylogeny of Pezizomycotina. *Mycologia* 98: 1018–1028. <https://doi.org/10.1080/15572536.2006.11832630>
- Spegazzini C (1888) *Fungi Fuegiani*. *Boletín de la Academia Nacional de Ciencias en Córdoba* 11: 135–311. <https://doi.org/10.5962/bhl.title.4055>
- Spegazzini C (1910) Hongos chilenos. *Revista de la Facultad de Agronomía y Veterinaria*. 6: 130–132.
- Spegazzini C (1921) *Mycetes chilenses*. *Boletín de la Academia Nacional de Ciencias* 25: 1–124.
- Stevenson JA (1926) Foreign plant diseases. A manual of economic plant diseases which are new or not widely distributed in the United States. United States Department of Agriculture Federal Horticulture Board, Washington DC. <https://doi.org/10.5962/bhl.title.114939>
- Stoykov DY, Assyov B (2009) The genus *Trochila* in Bulgaria. *Mycotaxon* 109: 351–359. <https://doi.org/10.5248/109.351>
- Stoykov DY (2019) New records of *Trochila* (Cenangiaceae, Helotiales) from the Balkans. *Phytologia Balcanica* 25: 245–248.
- Suija A, Ertz D, Lawrey JD, Diederich P (2015) Multiple origin of the lichenicolous life habit in Helotiales, based on nuclear ribosomal sequences. *Fungal Diversity* 70: 55–72. <https://doi.org/10.1007/s13225-014-0287-4>
- Sung GH, Sung JM, Hywel-Jones NL, Spatafora JW (2007) A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): Identification of localized incongruence using a combinational bootstrap approach. *Molecular Phylogenetics and Evolution* 44: 1204–1223. <https://doi.org/10.1016/j.ympev.2007.03.011>
- Thiers B (2020) [continuously updated] Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanic Garden’s Virtual Herbarium. <http://sweetgum.nybg.org/ih/> [accessed 14 July 2020]
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246. <https://doi.org/10.1128/JB.172.8.4238-4246.1990>

- von Arx JA (1957) Revision der zu *Gloeosporium* gestellten Pilze. Verhandelingen der Koninklijke Nederlandse Akademie van Wetenschappen, Afd. Natuurkunde, Tweede Reeks 51: 1–153.
- Vu D, Groenewald M, de Vries M, Gehrman T, Stielow B, Eberhardt U, Al-Hatmi A, Groenewald JZ, Cardinali G, Houbraken J, Boekhout T, Crous PW, Robert V, Verkley GJM (2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. *Studies in Mycology* 92: 135–154. <https://doi.org/10.1016/j.simyco.2018.05.001>
- Wang Z, Binder M, Hibbett DS (2005) Life history and systematics of the aquatic discomycete *Mitrula* (Helotiales, Ascomycota) based on cultural, morphological, and molecular studies. *American Journal of Botany* 92: 1565–1574. <https://doi.org/10.3732/ajb.92.9.1565>
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand H, Sninsky JS, White TJ (Eds) *PCR protocols: A guide to methods and applications*. Academic Press, San Diego, 315–322. <https://doi.org/10.3181/00379727-150-39089>
- Wijayawardene NN, Hyde KD, Al-Ani LKT, Tedersoo L, Haelewaters D, Rajeshkumar KC, Zhao RL, Aptroot A, Leontyev DV, Saxena RK, Tokarev YS, Dai DQ, Letcher PM, Stephenson SL, Ertz D, Lumbsch HT, Kukwa M, Issi IV, Madrid H, Phillips AJL, Selbmann L, Pfliegler WP, Horváth E, Bensch K, Kirk PM, Kolaříková K, Raja HA, Radek R, Papp V, Dima B, Ma J, Malosso E, Takamatsu S, Rambold G, Gannibal PB, Triebel D, Gautam AK, Avasthi S, Suetrong S, Timdal E, Fryar SC, Delgado G, Réblová M, Doilom M, Dolatabadi S, Pawłowska J, Humber RA, Kodsueb R, Sánchez-Castro I, Goto BT, Silva DKA, de Souza FA, Oehl F, da Silva GA, Silva IR, Błaszczowski J, Jobim K, Maia LC, Barbosa FR, Fiuza PO, Divakar PK, Shenoy BD, Castañeda-Ruiz RF, Somrithipol S, Lateef AA, Karunarathna SC, Tibpromma S, Mortimer PE, Wanasinghe DN, Phookamsak R, Xu J, Wang Y, Tian F, Alvarado P, Li DW, Kušan I, Matočec N, Maharachchikumbura SSN, Papizadeh M, Heredia G, Wartchow F, Bakhshi M, Boehm E, Youssef N, Hustad VP, Lawrey JD, Santiago ALCMA, Bezerra JDP, Souza-Motta CM, Firmino AL, Tian Q, Houbraken J, Hongsanan S, Tanaka K, Dissanayake AJ, Monteiro JS, Grossart HP, Suija A, Weerakoon G, Etayo J, Tsurykau A, Vázquez V, Mungai P, Damm U, Li QR, Zhang H, Boonmee S, Lu YZ, Becerra AG, Kendrick B, Brearley FQ, Motiejūnaitė J, Sharma B, Khare R, Gaikwad S, Wijesundara DSA, Tang LZ, He MQ, Flakus A, Rodriguez-Flakus P, Zhurbenko MP, McKenzie EHC, Stadler M, Bhat DJ, Liu JK, Raza M, Jeewon R, Nassonova ES, Prieto M, Jayalal RGU, Erdoğdu M, Yurkov A, Schnittler M, Shchepin ON, Novozhilov YK, Silva-Filho AGS, Liu P, Cavender JC, Kang Y, Mohammad S, Zhang LF, Xu RF, Li YM, Dayarathne MC, Ekanayaka AH, Wen TC, Deng CY, Pereira OL, Navathe S, Hawksworth DL, Fan XL, Dissanayake LS, Kuhnert E, Grossart HP, Thines M (2020) Outline of Fungi and fungus-like taxa. *Mycosphere* 11: 1060–1456. <https://doi.org/10.5943/mycosphere/11/1/8>
- Zhao YJ, Hosaka K, Hosoya T (2016) Taxonomic re-evaluation of the genus *Lambertella* (Rutstroemiaceae, Helotiales) and allied stroma-forming fungi. *Mycological Progress* 15: 1215–1228. <https://doi.org/10.1007/s11557-016-1225-5>
- Zhuang WY (1990) *Calycellinopsis Xishuangbanna* gen. et sp. nov. (Dermateaceae), a petiole-inhabiting fungus from China. *Mycotaxon* 38: 121–124.

- Zhuang WY, Luo J, Zhao P (2010) The fungal genus *Calycellinopsis* belongs in Helotiaceae not Dermateaceae. Phytotaxa 3: 54–58. <https://doi.org/10.11646/phytotaxa.3.1.8>
- Ziolo E, Madej T, Blaszkowski J (2005) *Trochila ilicina* (Helotiales, Ascomycota), a fungus newly found in Poland. Acta Mycologica 40: 181–184. <https://doi.org/10.5586/am.2005.016>

Morphological and phylogenetic analyses reveal three new species of *Diaporthe* from Yunnan, China

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Abstract

Species of *Diaporthe* have often been reported as plant pathogens, endophytes or saprobes, commonly isolated from a wide range of plant hosts. Sixteen strains isolated from species of ten host genera in Yunnan Province, China, represented three new species of *Diaporthe*, *D. chrysalidocarpi*, *D. machili* and *D. pometiae* as well as five known species *D. arecae*, *D. hongkongensis*, *D. middletonii*, *D. osmanthi* and *D. pandanicola*. Morphological comparisons with known species and DNA-based phylogenies based on the analysis of a multigene (ITS, TUB, TEF, CAL and HIS) dataset support the establishment of the new species. This study reveals that a high species diversity of *Diaporthe* with wide host ranges occur in tropical rainforest in Yunnan Province, China.

Keywords

Diaporthaceae, Diaporthales, phylogeny, taxonomy, three taxa new to science

Introduction

The genus *Diaporthe* (Diaporthaceae Diaporthales) with asexual morphs previously known as *Phomopsis* spp. is based on the type species *Diaporthe eres* Nitschke (1870) from *Ulmus* sp. in Germany. Rossman et al. (2015) proposed to use the name *Diaporthe* over *Phomopsis* in the context of the one fungus – one name initiative, be-

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cause it was described first, is encountered commonly in literature and includes the majority of known species. The sexual morph of *Diaporthe* is characterised by immersed ascomata and an erumpent pseudostroma with elongated perithecial necks; asci are unitunicate, clavate to cylindrical; and ascospores are fusoid, ellipsoid to cylindrical, hyaline, biseriate to uniseriate in the ascus, sometimes with appendages (Udayanga et al. 2011; Senanayake et al. 2017, 2018). The asexual morph is characterised by ostiolate pycnidia with cylindrical phialides often producing three types of hyaline, aseptate conidia called α -conidia, β -conidia and γ -conidia (Udayanga et al. 2011; Gomes et al. 2013). The α -conidia and β -conidia are produced frequently, but the γ -conidia are rarely observed (Gomes et al. 2013; Guarnaccia and Crous 2017; Guo et al. 2020).

Currently, more than 1100 epithets of *Diaporthe* are listed in Index Fungorum (<http://www.indexfungorum.org/>; accessed 1 Nov. 2020), but only one-fifth of these taxa have been well-studied with ex-type cultures and supplementary DNA barcodes (Guo et al. 2020; Yang et al. 2020; Zapata et al. 2020). Species of *Diaporthe* are widely distributed and have a broad range of hosts including economically significant agricultural crops and ornamental plants such as species of *Camellia*, *Castanea*, *Citrus*, *Glycine*, *Helianthus*, *Juglans*, *Persea*, *Pyrus*, *Vaccinium*, *Vitis* and many more (van Rensburg et al. 2006; Santos and Phillips 2009; Crous et al. 2011a, b, 2016; Santos et al. 2011; Thompson et al. 2011; Grasso et al. 2012; Huang et al. 2013; Lombard et al. 2014; Gao et al. 2015, 2016, 2017; Udayanga et al. 2012, 2015; Guarnaccia et al. 2016; Dissanayake et al. 2017; Guarnaccia and Crous 2017; Fan et al. 2018; Senanayake et al. 2018; Guo et al. 2020). *Diaporthe* species have been reported as destructive plant pathogens, harmless endophytes or saprobes (Murali et al. 2006; Udayanga et al. 2012; Gomes et al. 2013; Ménard et al. 2014; Guarnaccia et al. 2016; Torres et al. 2016; Senanayake et al. 2018). However, the biology and lifestyle of some of these fungi remain unclear (Vilka and Volkova 2015).

In the past, methods of species identification of *Diaporthe* had previously been based only on host as well as morphological characters such as the size and shape of ascomata and conidiomata. Nowadays, molecular phylogenetic studies demonstrate that determining species boundaries only by morphological characters is not possible due to lack of host specificity and their variability under changing environmental conditions (Gomes et al. 2013). Phylogenetic analysis using a five-locus dataset (ITS-TUB-TEF-CAL-HIS) has been determined to be the optimal combination to identify species of *Diaporthe* species, as revealed by Santos et al. (2017). Many *Diaporthe* species are described based on a polyphasic approach together with morphological characterisation (Rehner and Uecker 1994; Udayanga et al. 2011; Gao et al. 2017; Guarnaccia and Crous 2017; Yang et al. 2018a, 2020; Crous et al. 2020; Dayarathne et al. 2020; Guo et al. 2020; Hyde et al. 2020; Li et al. 2020; Zapata et al. 2020).

The aim of this study was to explore the diversity of *Diaporthe* species from symptomatic leaves of plants in Yunnan Province. We present three novel species and five known species of *Diaporthe*, collected from species belonging to ten host genera, based on morphological characters and phylogenetic analysis.

Materials and methods

Isolation and morphological studies

Leaves of samples were collected in Yunnan Province, China. Isolations from surface sterilized leaf tissues were conducted following the protocol of Gao et al. (2014). Tissue fragments (5 × 5 mm) were taken from the margin of leaf lesions and surface-sterilized by immersing them in 75% ethanol solution for 1 min, 5% sodium hypochlorite solution for 30 s, and then rinsing in sterile distilled water for 1 min. The pieces were dried with sterilized paper towels and placed on potato dextrose agar (PDA) (Cai et al. 2009). PDA plates (90 mm) were incubated in an incubator at 25 °C for 2–4 days, and hyphae were picked out of the periphery of the colonies and inoculated onto new PDA plates.

Following 2–3 weeks of incubation, photographs of colonies were taken at 7 days and 15 days using a Powershot G7X mark II digital camera. Colour notations was done using the colour charts of Rayner (1970). Micromorphological characters were observed using an Olympus SZX10 stereomicroscope and Olympus BX53 microscope, both fitted with Olympus DP80 high definition colour digital cameras to document fungal structures. All fungal strains were stored in 10% sterilized glycerin at 4 °C for further studies. Voucher and type specimens were deposited in the Herbarium of Plant Pathology, Shandong Agricultural University (HSAUP). Living cultures were deposited in the Shandong Agricultural University Culture Collection (SAUCC). Taxonomic information of the new taxa was submitted to MycoBank (<http://www.mycobank.org>).

DNA extraction and amplification

Genomic DNA was extracted from fungal mycelium on PDA, using a modified cetyltrimethylammonium bromide (CTAB) protocol as described in Guo et al. (2000). The internal transcribed spacer regions with intervening 5.8S nrRNA gene (ITS), part of the beta-tubulin gene region (TUB), partial translation elongation factor 1- α (TEF), histone H3 (HIS) and calmodulin (CAL) genes were amplified and sequenced by using primers pairs ITS4/ITS5 (White et al. 1990), Bt2a/Bt2b (Glass and Donaldson 1995), EF1-728F/EF1-986R (Carbone and Kohn 1999), CAL-228F/CAL-737R (Carbone and Kohn 1999) and CYLH3F/H3-1b (Glass and Donaldson 1995; Crous et al. 2004), respectively.

PCR was performed using an Eppendorf Master Thermocycler (Hamburg, Germany). Amplification reactions were performed in a 25 μ L reaction volume, which contained 12.5 μ L Green Taq Mix (Vazyme, Nanjing, China), 1 μ L of each forward and reverse primer (10 μ M) (Biosune, Shanghai, China), and 1 μ L template genomic DNA in amplifier, and were adjusted with distilled deionized water to a total volume of 25 μ L.

PCR parameters were as follows: 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at a suitable temperature for 30 s, extension at 72 °C for 1 min and a final elongation step at 72 °C for 10 min. Annealing temperature for each gene were 55 °C for ITS, 60 °C for TUB, 52 °C for TEF, 54 °C for CAL

and 57 °C for HIS. The PCR products were visualised on 1% agarose electrophoresis gel. Sequencing was done bi-directionally, conducted by the Biosune Company Limited (Shanghai, China). Consensus sequences were obtained using MEGA 7.0 (Kumar et al. 2016). All sequences generated in this study were deposited in GenBank (Table 1).

Phylogenetic analyses

Novel sequences generated from the sixteen strains in this study, and all reference sequences of *Diaporthe* species downloaded from GenBank, were used for phylogenetic analyses. Alignments of the individual locus were determined using MAFFT v. 7.110 by default settings (Katoh et al. 2017) and manually corrected where necessary. To establish the identity of the isolates at species level, phylogenetic analyses were conducted first individually for each locus and then as combined analyses of five loci (ITS, TUB, TEF, CAL and HIS regions). Phylogenetic analyses were based on maximum likelihood (ML) and Bayesian inference (BI) for the multi-locus analyses. For BI, the best evolutionary model for each partition was determined using MrModeltest v. 2.3 (Nylander 2004) and incorporated into the analyses. ML and BI were run on the CIPRES Science Gateway portal (<https://www.phylo.org/>) (Miller et al. 2012) using RaxML-HPC2 on XSEDE (8.2.12) (Stamatakis 2014) and MrBayes on XSEDE (3.2.7a) (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; Ronquist et al. 2012), respectively. For ML analyses the default parameters were used and BI was carried out using the rapid bootstrapping algorithm with the automatic halt option. Bayesian analyses included five parallel runs of 5,000,000 generations, with the stop rule option and a sampling frequency of 500 generations. The burn-in fraction was set to 0.25 and posterior probabilities (PP) were determined from the remaining trees. The resulting trees were plotted using FigTree v. 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree>) and edited with Adobe Illustrator CS5.1. New sequences generated in this study were deposited at GenBank (<https://www.ncbi.nlm.nih.gov>; Table 1) and the alignments and trees were deposited in TreeBASE: S27479 (<http://treebase.org/treebase-web/home.html>).

Results

Phylogenetic analyses

Sixteen strains of *Diaporthe* isolated from plant hosts from Yunnan, China, were grown in culture and used for analyses of molecular sequence data. *Diaporthe* spp. were analysed by using multilocus data (ITS, TUB, TEF, CAL and HIS) from 115 isolates of *Diaporthe* spp. and *Diaporthella corylina* (CBS 121124) as the outgroup taxon. A total of 3005 characters including gaps were obtained in the phylogenetic analysis, viz. ITS: 1–656, TUB: 657–1329, TEF: 1330–1860, CAL: 1861–2444,

Table 1. Species and Genbank accession numbers of DNA sequences used in this study. New sequences in bold.

Species	Voucher	Host/Substrate	GeneBank accession number					Reference
			ITS	TUB	TEF	CAL	HIS	
<i>Diaporthe acuta</i>	PSCG 046	<i>Pyrus pyrifolia</i>	MK626958	MK691224	MK654803	MK691124	MK726162	Guo et al. 2020
	PSCG 047*	<i>Pyrus pyrifolia</i>	MK626957	MK691225	MK654802	MK691125	MK726161	Guo et al. 2020
<i>D. acutispora</i>	LC6160	<i>Camellia sauranqua</i>	KX986763	KX999194	KX999154	KX999273	KX999234	Gao et al. 2017
	LC6161	<i>Coffea sp.</i>	KX986764	KX999195	KX999155	KX999274	KX999235	Gao et al. 2017
<i>D. amanambophila</i>	MAFF 246900	<i>Ananathus tricolor</i>	LC459575	LC459579	LC459577	LC459583	LC459581	Rosman et al. 2015
	MAFF 246901	<i>Ananathus tricolor</i>	LC459576	LC459580	LC459578	LC459584	LC459582	Rosman et al. 2015
<i>D. angelicae</i>	CBS 111592*	<i>Henaleum sponylium</i>	KC343027	KC343095	KC343753	KC343269	KC343511	Gomes et al. 2013
<i>D. anhuensis</i>	CNUCC 201901*	<i>Cunninghamia lanceolata</i>	MN219718	MN227008	MN224668	MN224549	MN224556	Zhou and Hou 2019
	CNUCC 201902	<i>Cunninghamia lanceolata</i>	MN219727	MN227009	MN224669	MN224550	MN224557	Zhou and Hou 2019
<i>D. arcii</i>	DP0482	<i>Arctium sp.</i>	KJ590736	KJ610891	KJ590776	KJ612133	KJ659218	Udayanga et al. 2015
<i>D. arecae</i>	CBS 161.64*	<i>Areca catechu</i>	KC343032	KC344000	KC343758	KC343274	KC343516	Gomes et al. 2013
	CBS 535.75	<i>Citrus sp.</i>	KC343033	KC344001	KC343759	KC343275	KC343517	Gomes et al. 2013
<i>D. arengae</i>	SAUCC194.18	<i>Persea americana</i>	MT822546	MT85743	MT855860	MT855631	MT855515	This study
	CBS 114979*	<i>Arenca engleri</i>	KC343034	KC344002	KC343760	KC343276	KC343518	Gomes et al. 2013
<i>D. asenua</i>	MFLUCC 12-0299a*	On dead leaves	KT459414	KT459432	KT459448	KT459464	—	Dissanayake et al. 2017
<i>D. belharziæ</i>	BRIP 54792*	<i>Indigofera australis</i>	JX862529	KF170921	JX862535	—	—	Tan et al. 2013
	ZJUD 60	<i>Citrus sinensis</i>	KJ490595	KJ490416	KJ490474	—	KJ490537	Huang et al. 2017
<i>D. biconispora</i>	ZJUD 61	<i>Fortunella margarita</i>	KJ490596	KJ490417	KJ490475	—	KJ490538	Huang et al. 2017
	ZJUD 62	<i>Citrus grandis</i>	KJ490597	KJ490418	KJ490476	—	KJ490539	Huang et al. 2017
<i>D. brasiliensis</i>	CBS 133183*	<i>Aspidosperma tomentosus</i>	KC343042	KC344010	KC343768	KC343284	KC343526	Gomes et al. 2013
<i>D. cuatringensis</i>	URM 7486*	<i>Tachina thamoena</i>	KY085926	KY115600	KY115603	KY115597	KY115605	Grous et al. 2017
<i>D. camoreii</i>	JZB320143	<i>Urtica dioica</i>	MN535309	MN561316	MN984254	—	—	Hyde et al. 2020
<i>D. caryae</i>	NIBM-ABIJP	<i>Carica papaya</i>	MN335224	—	—	—	—	Rosman et al. 2015
	CFCC 52563	<i>Carya illinoensis</i>	MH121498	MH121581	MH121541	MH121422	MH121458	Yang et al. 2018
	CFCC 52564	<i>Carya illinoensis</i>	MH121499	MH121581	MH121541	MH121423	MH121459	Yang et al. 2018
	CFCC 52565	<i>Cercia chinensis</i>	MH121500	MH121582	MH121542	MH121424	MH121460	Yang et al. 2018
<i>D. cercidis</i>	CFCC 52566	<i>Chrysaliidoacarpus lutescens</i>	MT822561	MT855758	MT855587	MT855645	MT855530	This study
<i>D. chrysalidoacarp</i>	SAUCC194.33	<i>Chrysaliidoacarpus lutescens</i>	MT822563	MT855760	MT855586	MT855646	MT855532	This study
	MFLUCC 17-1023*	<i>Cichorium inulphus</i>	KY964220	KY964104	KY964176	KY964133	—	Dissanayake et al. 2017
<i>D. chichorii</i>	LC3083*	<i>Camellia sinensis</i>	KP267854	KP293434	KP267928	—	KP293508	Gao et al. 2016
<i>D. compacta</i>	CBS 136.25	<i>Cucumis sativus</i>	KC343031	KC343999	KC343757	KC343273	KC343515	Udayanga et al. 2014
<i>D. cucurbitae</i>	CBS 117499	<i>Aspalathus linearis</i>	KC343057	KC344025	KC343783	KC343299	KC343541	Udayanga et al. 2012
<i>D. cupacea</i>	CBS 109772	<i>Corylus avellana</i>	KC343059	KC344027	KC343785	KC343301	KC343543	Gomes et al. 2013
<i>D. decedens</i>	CBS 444.82	<i>Eugenia aromatica</i>	KC343098	KC344066	KC343824	KC343340	KC343582	Gomes et al. 2013
<i>D. eugeniae</i>	BRIP 54781*	<i>Fraxinus angustifolius</i>	JX862528	KF170920	JX862534	—	—	Tan et al. 2013
<i>D. fraxini-angustifoliae</i>	PSCG 051*	<i>Pyrus pyrifolia</i>	MK626858	MK691236	MK654806	MK691132	MK726163	Guo et al. 2020
<i>D. fulvicolor</i>	PSCG 057	<i>Pyrus pyrifolia</i>	MK626859	MK691233	MK654810	MK691131	MK726164	Guo et al. 2020
<i>D. garjiae</i>	CBS 180.91*	<i>Camnabis sativa</i>	KC343112	KC344080	KC343838	KC343354	KC343596	Gomes et al. 2013
<i>D. guangxensis</i>	JZBH 320094*	<i>Vitis vinifera</i>	MK335772	MK500168	MK523566	MK736727	—	Manawasinghe et al. 2019

Species	Voucher	Host/Substrate	GeneBank accession number					Reference
			ITS	TUB	TEF	CAL	HIS	
<i>D. gulyae</i>	MF-Ha 17-042*	<i>Helianthus annuus</i>	MK024252	MK033488	MK039420	–	–	Thompson et al. 2011
<i>D. hongkongensis</i>	CBS 115448*	<i>Didyma fefirigga</i>	KC343119	KC344087	KC343845	KC343361	KC343603	Gomes et al. 2013
	CGMCC.3.17.102	<i>Libocarpus glaber</i>	KF576275	KF576299	KF576250	KF576227	–	Gao et al. 2015
	LC 3478	<i>Camellia sinensis</i>	KP293484	KP293484	KP267978	–	KP293553	Gao et al. 2017
	SAUCC194.81	<i>Milletia reticulata</i>	MT822609	MT855806	MT855921	MT855688	MT855577	This study
<i>D. huangshanensis</i>	SAUCC194.87	<i>Camellia sinensis</i>	MT822615	MT855812	MT855927	MT855694	MT855583	This study
	CNUCC.201903	<i>Camellia oleifera</i>	MN219729	MN227010	MN24670	–	MN224558	Zhou and Hou 2019
	CNUCC.201904	<i>Camellia oleifera</i>	MN219730	MN227011	MN24671	–	MN224559	Zhou and Hou 2019
<i>D. infucanda</i>	CBS 133812*	<i>Schinus terebinthifolius</i>	KC343126	KC344094	KC343852	KC343368	KC343610	Gomes et al. 2013
<i>D. kribiensis</i>	MFUCC.17-2481*	<i>Bruguiera</i> sp.	MN047101	MN431495	MN433215	–	–	Dayaratne et al. 2020
<i>D. limonicola</i>	BRIP 54900*	<i>Litchi chinensis</i>	JX862533	KF170925	JX862539	–	–	Tan et al. 2013
<i>D. limonicola</i>	CPC 28200*	<i>Citrus limon</i>	MF418422	MF418501	MF418501	MF418256	MF418342	Guarnaccia and Crous 2017
<i>D. lusitanicae</i>	CBS 123212*	<i>Foeniculum vulgare</i>	KC343136	KC344104	KC343862	KC343578	KC343620	Phillips and Santos 2009
<i>D. machili</i>	SAUCC194.69	<i>Pometia pinnata</i>	MT822597	MT855794	MT855909	MT855677	MT855565	This study
	SAUCC194.111*	<i>Machilus pingii</i>	MT822639	MT855836	MT855951	MT855718	MT855606	This study
<i>D. malorum</i>	CAA752*	<i>Malus domestica</i>	KY435643	KY435671	KY435630	KY435651	KY435651	Santos et al. 2017
	CAA740	<i>Malus domestica</i>	KY435642	KY435670	KY435629	KY435660	KY435650	Santos et al. 2017
<i>D. manihotia</i>	CBS 505.76	<i>Manihot utilissima</i>	KC343138	KC344106	KC343864	KC343380	KC343622	Gomes et al. 2013
<i>D. mayteni</i>	CBS 133185*	<i>Maytenus ilicifolia</i>	KC343139	KC344107	KC343865	KC343381	KC343623	Gomes et al. 2013
<i>D. medicensis</i>	CPC 27873*	<i>Citrus limon</i>	MF418424	MF418584	MF418503	MF418258	MF418344	Guarnaccia and Crous 2017
<i>D. middletonii</i>	BRIP 54884e*	<i>Rapistrum rugosum</i>	KJ197286	KJ197266	KJ197248	–	–	Thompson et al. 2015
	SAUCC194.27	<i>Litchi chinensis</i>	MT822555	MT855752	MT855868	MT855639	MT855524	This study
	SAUCC194.45	<i>Libocarpus glaber</i>	MT822573	MT855770	MT855886	MT855654	MT855542	This study
	SAUCC194.46	<i>Libocarpus glaber</i>	MT822574	MT855771	MT855887	MT855655	MT855543	This study
	SAUCC194.48	<i>Libocarpus crabianus</i>	MT822576	MT855773	MT855889	MT855657	MT855545	This study
<i>D. millietiae</i>	GUCC9167*	<i>Milletia reticulata</i>	MK398674	MK502089	MK480609	MK502086	–	Long et al. 2019
<i>D. multigundata</i>	ZJUD 98*	<i>Citrus gundis</i>	KJ490633	KJ490454	KJ490512	–	KJ490575	Huang et al. 2015
<i>D. muigena</i>	CBS 129519*	<i>Musa</i> sp.	KC343143	KC344111	KC343869	KC343385	KC343627	Crous et al. 2011
<i>D. myracrodruonis</i>	URMT972	<i>Mynacrodruon urundeuva</i>	MK205289	MK205291	MK213408	MK205290	–	Silva et al. 2019
<i>D. neararii</i>	CBS 109490*	<i>Annohisia trifida</i>	KC343145	KC344113	KC343871	KC343387	KC343629	Gomes et al. 2013
<i>D. novem</i>	CBS 127270*	<i>Glycine max</i>	KC343156	KC344124	KC343882	KC343398	KC343640	Santos et al. 2011
<i>D. osmanthi</i>	GUCC9165*	<i>Osmanthus fragrans</i>	MK398675	MK502091	MK480610	MK502087	–	Long et al. 2019
<i>D. oze</i>	SAUCC194.21	<i>Litchi chinensis</i>	MT822549	MT855746	MT855862	MT855634	MT855518	This study
	CBS 133186*	<i>Maytenus ilicifolia</i>	KC343164	KC344132	KC343890	KC343406	KC343648	Gomes et al. 2013
	CBS 133187	<i>Maytenus ilicifolia</i>	KC343165	KC344133	KC343891	KC343407	KC343649	Gomes et al. 2013
<i>D. pandanicola</i>	MFUCC.17-0607	<i>Pandanus</i> sp.	MG646974	MG646930	–	–	–	Thipromma et al. 2018
	SAUCC194.82	<i>Milletia reticulata</i>	MT822610	MT855807	MT855922	MT855689	MT855578	This study
<i>D. paranensis</i>	CBS 133184*	<i>Maytenus ilicifolia</i>	KC343171	KC344139	KC343897	KC343413	KC343655	Gomes et al. 2013
<i>D. pascoei</i>	BRIP 54847*	<i>Persea americana</i>	JX862532	KF170924	JX862538	–	–	Tan et al. 2013
<i>D. perscae</i>	CBS 151.73	<i>Persea gratissima</i>	KC343173	KC344141	KC343899	KC343415	KC343657	Gomes et al. 2013
<i>D. peticola</i>	MFUCC.16-0105*	<i>Prunus persica</i>	KU557555	KU557579	KU557623	KU557603	–	Disanayake et al. 2017

Species	Voucher	Host/Substrate	GeneBank accession number					Reference
			ITS	TUB	TEF	CAL	HIS	
<i>D. podocarpi-macrophylli</i>	LC6155*	<i>Podocarpus macrophyllus</i>	KX986774	KX999207	KX999167	KX999278	KX999246	Gao et al. 2017
	LC6200	<i>Podocarpus macrophyllus</i>	KX986769	KX999201	KX999161	KX999276	KX999240	Gao et al. 2017
<i>D. pomatie</i>	SAUCC194.19	<i>Persia americana</i>	MT822547	MT85744	MT855861	MT855632	MT855516	This study
	SAUCC194.72*	<i>Pometia pinnata</i>	MT822600	MT855797	MT855913	MT855679	MT855568	This study
	SAUCC194.73	<i>Heliconia metallica</i>	MT822601	MT855798	MT855913	MT855680	MT855569	This study
<i>D. pseudomangiferae</i>	CBS 101339*	<i>Mangifera indica</i>	KC343181	KC344149	KC343907	KC343423	KC343665	Gomes et al. 2013
<i>D. pseudophoenicicola</i>	CBS 462.69*	<i>Phoenix dactylifera</i>	KC343184	KC343152	KC343910	KC343426	KC343668	Gomes et al. 2013
<i>D. pterocarpicola</i>	MFLUCC 10-0580a*	<i>Pterocarpus indicus</i>	JQ619887	JX275441	JX275403	JX197433	—	Udayanga et al. 2012
	MFLUCC 10-0580b	<i>Pterocarpus indicus</i>	JQ619888	JX275442	JX275404	JX197434	—	Udayanga et al. 2012
<i>D. pycnanthae</i>	CAA487*	<i>Pycnanthus coccinea</i>	KY435636	KY435667	KY435626	KY435657	KY435647	Santos et al. 2017
<i>D. nucumiae</i>	CPC 26646*	<i>Euclea nemosa</i>	MG600223	MG600223	MG600225	MG600219	MG600221	Marin-Felix et al. 2018
<i>D. monilicarporum</i>	CBS 133182*	<i>Spondias mombin</i>	KC343188	KC344156	KC343914	KC343430	KC343672	Gomes et al. 2013
<i>D. rosmaniae</i>	CAA 762*	<i>Vaccinium corymbosum</i>	MK792290	MK837914	MK828063	MK883822	MK871432	Hilario et al. 2020
<i>D. sacksonii</i>	BRIP 54669b*	<i>Helianthus annuus</i>	KJ197287	KJ197267	KJ197249	—	—	Thompson et al. 2015
<i>D. subnicola</i>	MFLU 18-0553*	<i>Xylocarpus</i> sp.	MN047098	—	MN077073	—	—	Thompson et al. 2015
	MFLU 17-2592	<i>Xylocarpus</i> sp.	MN047099	—	MN077074	—	—	Dayaratne et al. 2020
<i>D. schini</i>	CBS 133181*	<i>Schinus terebinthifolius</i>	KC343191	KC344159	KC343917	KC343433	KC343675	Gomes et al. 2013
<i>D. schoeni</i>	MFLU 15-2609	<i>Schoenus nigrifolius</i>	KY964229	KY964112	KY964185	KY964141	—	Dissanayake et al. 2017
<i>D. semiae</i>	CFCC 51636*	<i>Senna biapularis</i>	KY203724	KY228891	KY228885	KY228875	—	Yang et al. 2017
<i>D. senfinae</i>	BRIP 55665a*	<i>Helianthus annuus</i>	KJ197274	KJ197254	KJ197236	—	—	Thompson et al. 2015
<i>D. spinosa</i>	PSCG 383*	<i>Pyrus pyrifolia</i>	MK626849	MK691234	MK654811	MK691129	MK726156	Guo et al. 2020
<i>D. stewartii</i>	CBS 193.36*	<i>Cosmos bipinnatus</i>	FJ889448	JX275421	GQ250324	JX197415	—	Guo et al. 2010; Udayanga et al. 2012
<i>D. subordinaria</i>	CBS 101711	<i>Plantago lanceolata</i>	KC343213	KC344181	KC343939	KC343455	KC343697	Gomes et al. 2013
	CBS 464.90	<i>Plantago lanceolata</i>	KC343214	KC344182	KC343940	KC343456	KC343698	Gomes et al. 2013
<i>D. taicola</i>	PSCG485	<i>Prunus persica</i>	MK626869	MK691227	MK654812	MK691120	MK726173	Dissanayake et al. 2017
<i>D. tarconanthi</i>	CPC 37479	<i>Tarconanthus litardis</i>	MT223794	—	—	—	—	Crous et al. 2020
<i>D. tectonigena</i>	MFLUCC 12-0767*	<i>Tectonia grandis</i>	KU712429	KU743976	KU749371	KU749358	—	Dolom et al. 2016
<i>D. terebinthifolii</i>	CBS 133180*	<i>Schinus terebinthifolius</i>	KC343216	KC344184	KC343942	KC343458	KC343700	Gomes et al. 2013
<i>D. undulata</i>	LC6624*	Unknown host	KX986798	KX999230	KX999190	—	KX999269	Gao et al. 2017
	LC8110	Unknown host	KY491545	KY491565	KY491555	—	—	Gao et al. 2017
<i>D. uandreyi</i>	BRIP 57887a*	<i>Psidium guajana</i>	KR936126	KR936128	KR936129	—	—	Crous et al. 2015
<i>D. viniferae</i>	JZBH 320071	<i>Vitis vinifera</i>	MK341550	MK500112	MK500107	MK500119	—	Manawasinghe et al. 2019
	JZBH 320072	<i>Vitis vinifera</i>	MK341551	MK500113	MK500108	MK500120	—	Manawasinghe et al. 2019
<i>D. xishuangbanica</i>	LC6707*	<i>Camellia sinensis</i>	KX986783	KX999216	KX999175	—	KX999255	Gao et al. 2017
<i>Diaportheella corplina</i>	CBS 121124	<i>Corplia</i> sp.	KC343004	KC343972	KC343730	KC343246	KC343488	Gomes et al. 2013

Isolates marked with “*” are ex-type or ex-epitype strains.

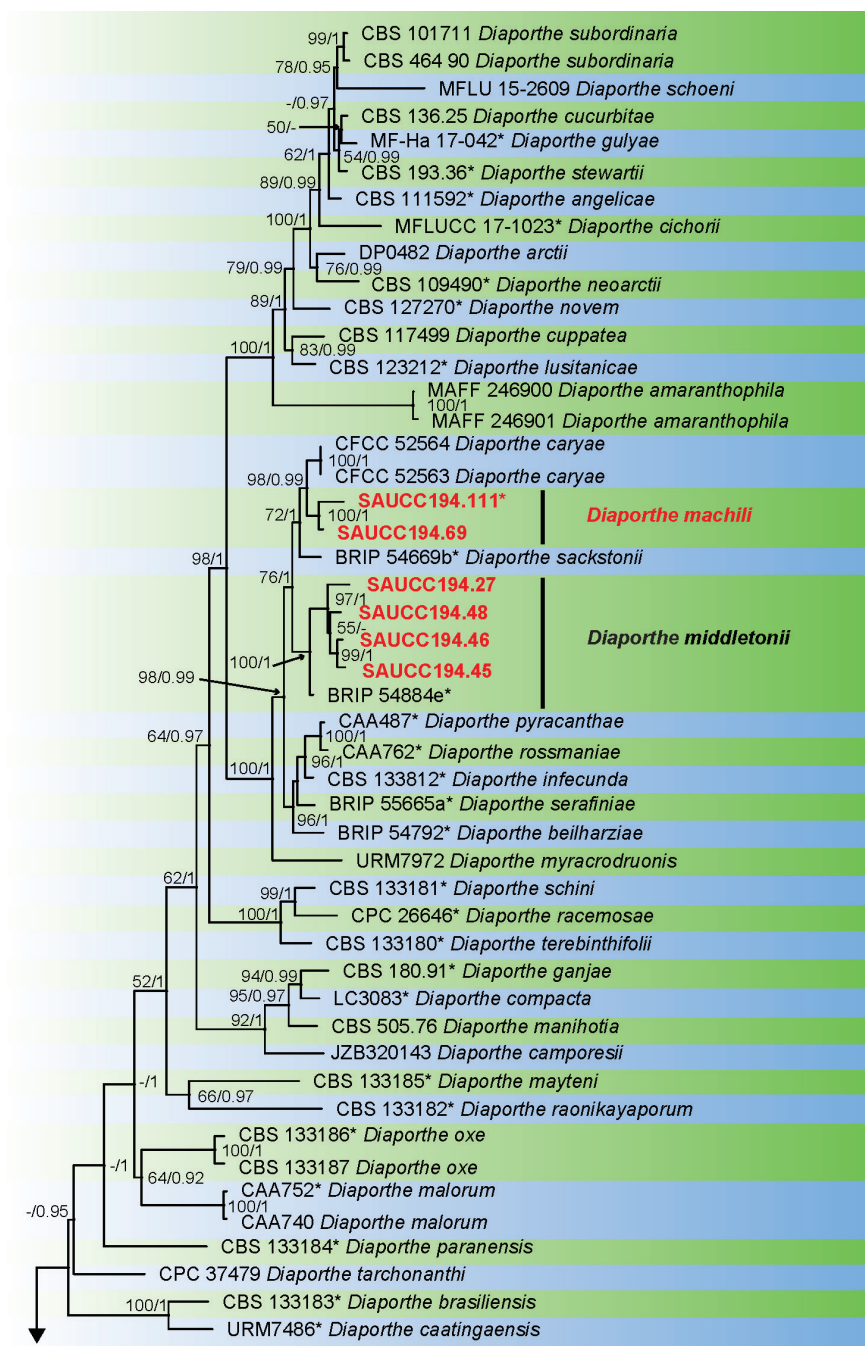


Figure 1. Phylogram of *Diaporthe* spp. based on combined sequence data of ITS, TUB, TEF, CAL and HIS genes. The ML and BI bootstrap support values above 50% and 0.90 BYPP are shown at the first and second position, respectively. Strains marked with "*" are ex-type or ex-epitype. Codes referring to strains from the current study are written in red. Some branches were shortened to fit them to the page as indicated by two diagonal lines with the number of times a branch was shortened indicated.

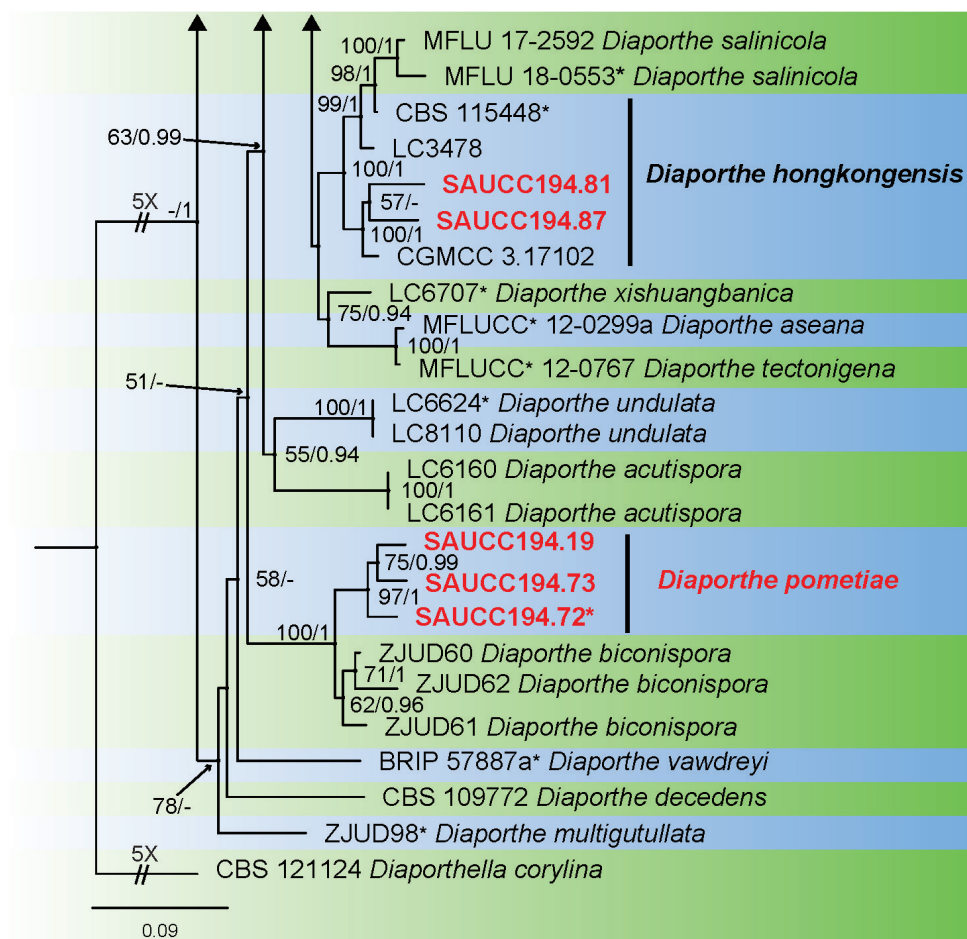


Figure 1. Continued.

HIS: 2445–3005. Of these characters, 1349 were constant, 453 were variable and parsimony-uninformative, and 1203 were parsimony-informative. For the BI and ML analyses, the substitution model GTR+I+G for ITS, TUB, TEF and HIS, HKY+I+G for and CAL were selected and incorporated into the analyses. The ML tree topology confirmed the tree topologies obtained from the BI analyses, and therefore, only the ML tree is presented (Fig. 1).

ML bootstrap support values ($\geq 50\%$) and Bayesian posterior probability (≥ 0.90) are shown as first and second position above nodes, respectively. Based on the five-locus phylogeny and morphology, nine isolates were assigned to five species, including *Diaporthe arecae* (1), *D. hongkongensis* (2), *D. middletonii* (4), *D. osmanthi* (1) and *D. pandanicola* (1), whereas seven isolates formed distinct well supported clades, which refer to novel species named *D. chrysalidocarpi* (2), *D. machili* (2) and *D. pometiae* (3), respectively.

Taxonomy

***Diaporthe arecae* (H.C. Srivast., Zakia & Govindar.) R.R. Gomes, Glienke & Crous, *Persoonia* 31: 16. (2013)**

Figure 2

Subramanella arecae H.C. Srivast., Zakia & Govindar., in Srivastava, Banu and Govindarajan (1962). Basionym.

Description. Asexual morph: Conidiomata pycnidial, several pycnidia grouped together, globose, black, erumpent, exuding creamy to yellowish conidial droplets from ostioles. Conidiophores hyaline, septate, branched, cylindrical, straight to sinuous, $25.0\text{--}32.0 \times 1.4\text{--}2.5 \mu\text{m}$. Conidiogenous cells $10.5\text{--}20.7 \times 1.4\text{--}2.0 \mu\text{m}$, phialidic, cylindrical, swollen at base, tapering towards apex, slightly curved. Alpha conidia hyaline, smooth, aseptate, ellipsoidal, guttulate, apex subobtuse, base subtruncate, $7.5\text{--}10.0 \times 1.8\text{--}3.0 \mu\text{m}$ (mean = $8.2 \times 2.4 \mu\text{m}$, $n = 20$). Beta conidia hyaline, aseptate, filiform, slightly curved, tapering towards base, $18.5\text{--}26.5 \times 1.0\text{--}1.8 \mu\text{m}$ (mean = $24.3 \times 1.4 \mu\text{m}$, $n = 20$). Gamma conidia not observed. Sexual morph not observed.

Culture characteristics. Cultures incubated on PDA at 25°C in darkness, growth rate $11.2\text{--}13.3 \text{ mm diam/day}$. Aerial mycelium white, cottony, feathery, abundant in center, sparse in margin, white on surface, reverse yellowish to tan.

Specimen examined. China, Yunnan Province: Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, on diseased leaves of *Persea americana* (Lauraceae). 19 April 2019, S.T. Huang, HSAUP194.18, living culture SAUCC194.18.

Notes. *Diaporthe arecae* (CBS 161.64) was originally described as *Subramanella arecae* on fruit of *Areca catechu* in India (Srivastava et al. 1962) and placed in *Diaporthe* by Gomes et al. (2013). The *Diaporthe* isolate from fruits of *Citrus* sp. (CBS 535.75) in Suriname was also placed in *D. arecae* by Gomes et al. (2013). In the present study, strain (SAUCC194.18) from symptomatic leaves of *Persea americana* was congruent with *D. arecae* based on morphology and DNA sequences data (Fig. 1). We therefore consider the isolated strain as *D. arecae*.

***Diaporthe chrysalidocarpi* S.T. Huang, J.W. Xia, W.X. Sun, & X.G. Zhang, sp. nov.**

MycoBank No: 837812

Figure 3

Etymology. Named after the host genus on which it was collected, *Chrysalidocarpus lutescens*.

Diagnosis. *Diaporthe chrysalidocarpi* can be distinguished from the phylogenetically most closely related species *D. spinosa* by longer beta conidia ($28.0\text{--}32.5 \times 1.2\text{--}1.6$ vs. $18.5\text{--}30.5 \times 1.0\text{--}1.5 \mu\text{m}$), and from other species *D. fulvicolor* by the types of conidia (*D. chrysalidocarpi* produces only beta conidia, while *D. fulvicolor* produces

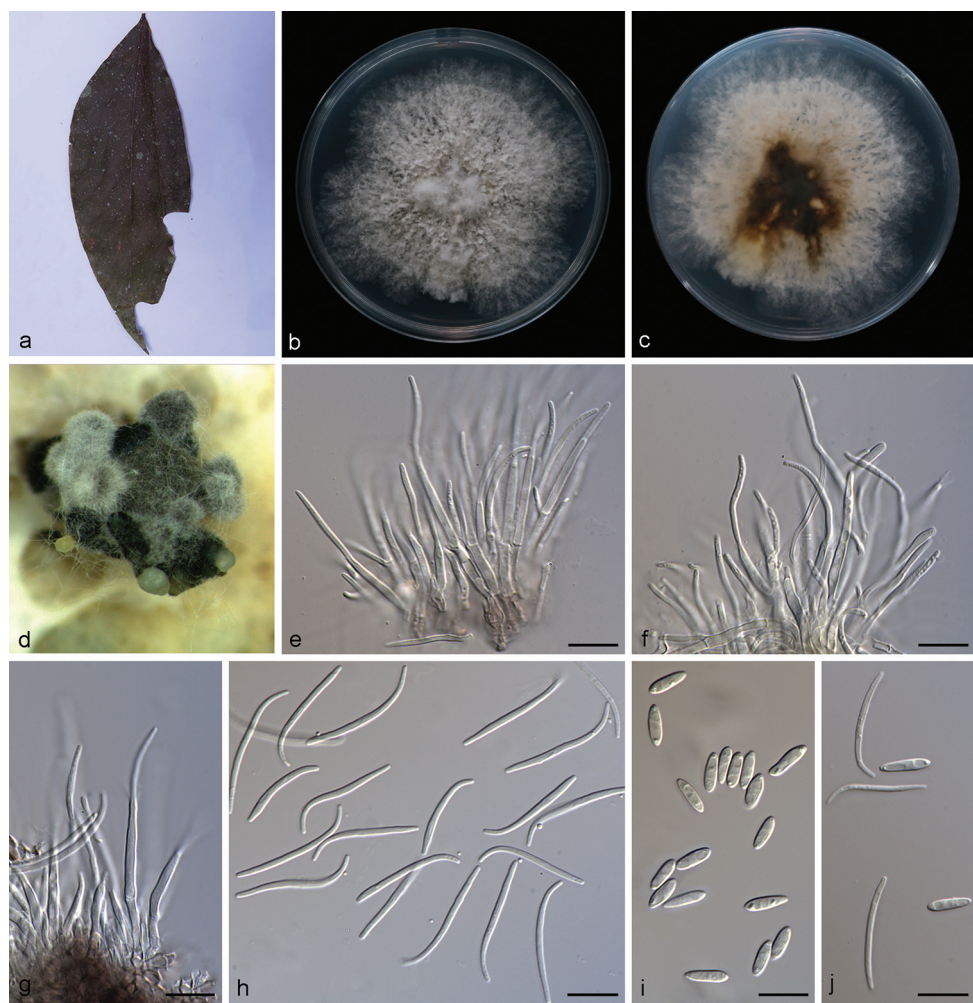


Figure 2. *Diaporthe arecae* (SAUCC194.18) **a** infected leaf of *Persea americana* **b, c** surface and reverse of a colony after 15 days on PDA **d** conidiomata **e–g** conidiophores and conidiogenous cells **h** beta conidia **i** alpha conidia **j** alpha conidia and beta conidia. Scale bars: 10 μ m (**e–j**).

only alpha conidia) and several loci (25/491 in the ITS region, 18/471 TUB, 4/298 TEF, 28/458 CAL and 13/441 HIS).

Type. China, Yunnan Province: Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, on diseased leaves of *Chrysalidocarpus lutescens* (Palmae). 19 April 2019, S.T. Huang, HSAUP194.35 holotype, ex-type living culture SAUCC194.35.

Description. Asexual morph: Leaf spots irregular, pale brown in center, brown to tan at margin. Conidiomata pycnidial, scattered or aggregated, black, erumpent, raising above surface of culture medium, subglobose, exuding white or yellowish creamy conidial droplets from central ostioles after 30 days in light at 25 °C; pycnidial wall

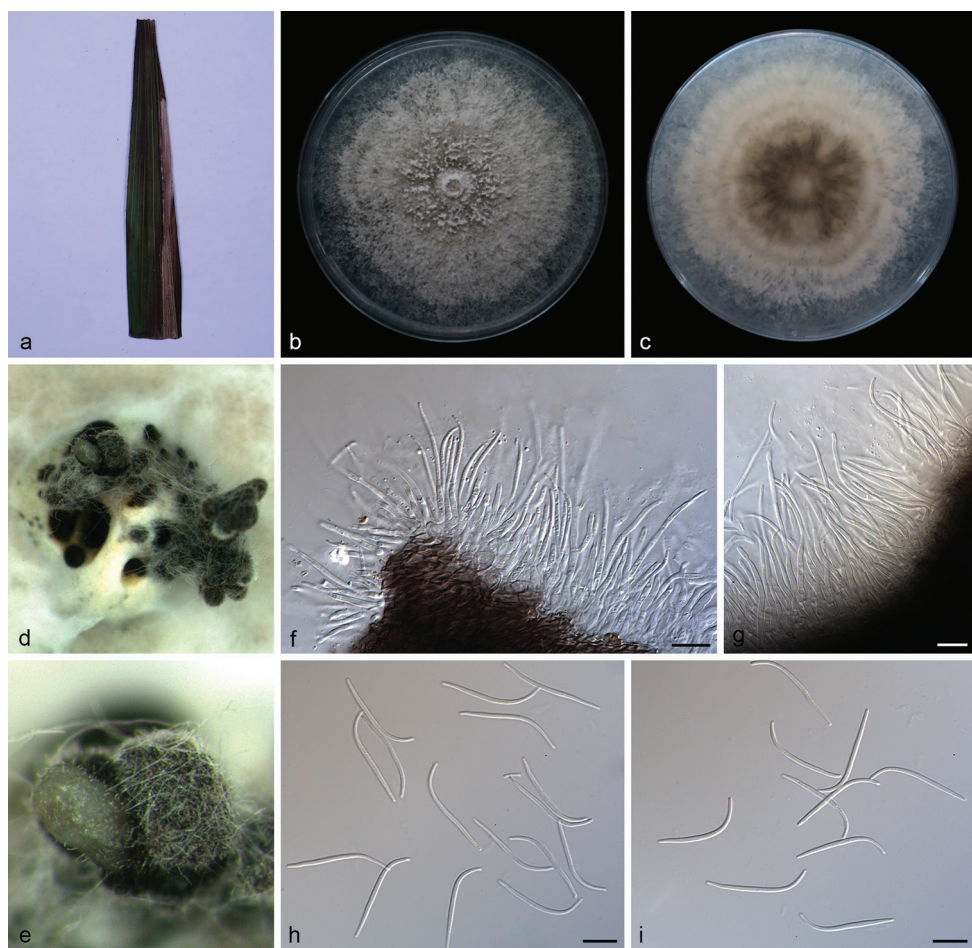


Figure 3. *Diaporthe chrysalidocarpi* (SAUCC194.35) **a** diseased leaf of *Chrysalidocarpus lutescens* **b, c** surface and reverse of a colony after 15 days on PDA **d, e** conidiomata **f, g** conidiophores and conidiogenous cells **h, i** beta conidia. Scale bars: 10 μm (**f–i**).

consists of black to dark brown, thin-walled cells. Conidiophores $27.5\text{--}35.0 \times 1.4\text{--}2.0 \mu\text{m}$, hyaline, slightly branched, swelling at base, subcylindrical, septate, smooth, straight or curved. Conidiogenous cells $10.5\text{--}23.0 \times 1.4\text{--}1.8 \mu\text{m}$, phialidic, cylindrical, terminal, straight to sinuous, tapering towards apex. Beta conidia $28.0\text{--}32.5 \times 1.2\text{--}1.6 \mu\text{m}$ (mean = $30.3 \times 1.3 \mu\text{m}$, $n = 20$), filiform, hyaline, straight or slightly curved, aseptate, base subtruncate, tapering towards the base. Alpha conidia and gamma conidia not observed. Sexual morph not observed.

Culture characteristics. Cultures incubated on PDA at 25°C in darkness, growth rate $13.3\text{--}15.2 \text{ mm diam/day}$, initially white, becoming greyish, reverse pale brown, with concentric rings of dense, sparse hyphae, irregular margin, fluffy aerial mycelium at center, pycnidia forming after 15 days.

Additional specimen examined. China, Yunnan Province: Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, on diseased leaves of *Chrysalidocarpus lutescens* (Palmae). 19 April 2019, S.T. Huang, HSAUP194.33 paratype; living culture SAUCC194.33.

Notes. Phylogenetic analysis of a combined five gene showed that *D. chrysalidocarpi* formed an independent clade (Fig. 1) and is phylogenetically distinct from *D. spinosa* and *D. fulvicolor*. This species can be distinguished from *D. spinosa* by 61 different nucleotides in the concatenated alignment (13/492 in the ITS region, 17/471 TUB, 4/298 TEF, 17/458 CAL and 10/441 HIS), and *D. fulvicolor* by 88 nucleotides (25/491 in the ITS region, 18/471 TUB, 4/298 TEF, 28/458 CAL and 13/441 HIS). Morphologically, *D. chrysalidocarpi* differs from *D. spinosa* in having longer beta conidia ($28.0\text{--}32.5 \times 1.2\text{--}1.6$ vs. $18.5\text{--}30.5 \times 1.0\text{--}1.5$ μm) (Guo et al. 2020). Furthermore, *Diaporthe chrysalidocarpi* produces only beta conidia, while *D. spinosa* produces alpha conidia and beta conidia and *D. fulvicolor* produces only alpha conidia (Guo et al. 2020). Therefore, we establish this fungus as a novel species.

***Diaporthe hongkongensis* R.R. Gomes, Glienke, Crous, *Persoonia* 31: 23. (2013)**

Figure 4

Description. Asexual morph: Conidiomata pycnidial, subglobose or globose, solitary, black, erumpent, coated with white hyphae, thick-walled, exuding creamy conidial droplets from central ostioles. Conidiophores hyaline, smooth, septate, unbranched, densely aggregated, cylindrical or clavate, straight to sinuous, swollen at base, tapering towards apex, $32.0\text{--}42.0 \times 2.0\text{--}2.9$ μm . Conidiogenous cells $20.0\text{--}24.2 \times 1.3\text{--}2.3$ μm , phialidic, cylindrical, terminal, slightly tapering towards apex. Alpha conidia, hyaline, smooth, aseptate, ellipsoidal or oval, 0–2 guttulate, apex subobtuse, base subtruncate, $5.5\text{--}7.0 \times 2.0\text{--}2.5$ μm (mean = 6.2×2.2 μm , $n = 20$). Beta conidia hyaline, aseptate, filiform, hamate, tapering towards both ends, mostly J-shaped, $21.5\text{--}27.0 \times 1.4\text{--}1.8$ μm (mean = 25.6×1.3 μm , $n = 20$). Gamma conidia not observed. Sexual morph not observed.

Culture characteristics. Cultures incubated on PDA at 25 °C in darkness, growth rate 19.0–21.5 mm diam/day, cottony, radial with abundant aerial mycelium, sparse at margin, with an obvious pale brown concentric ring of dense hyphae, white to grayish on surface with age, white to pale brown on the reverse side.

Specimens examined. China, Yunnan Province: Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, 19 April 2019, S.T. Huang. On diseased leaves of *Millettia reticulata* (Fabaceae) HSAUP194.81, living culture SAUCC194.81; on diseased leaves of *Camellia sinensis* (Theaceae) HSAUP194.87, living culture SAUCC194.87.

Notes. In the present study, two strains (SAUCC194.81 and SAUCC194.87) from symptomatic leaves of *Millettia reticulata* and *Camellia sinensis* were similar to *Diaporthe hongkongensis* (CGMCC 3.17102) (Gomes et al. 2013) and *D. salinicola* (MFLU 18-0553) (Dayarathne et al. 2020) based on DNA sequences data (Fig. 1). Morphologically, our strains were similar to *Diaporthe hongkongensis*, which was originally described with an asexual morph on fruits of *Dichroa febrifuga* in China,

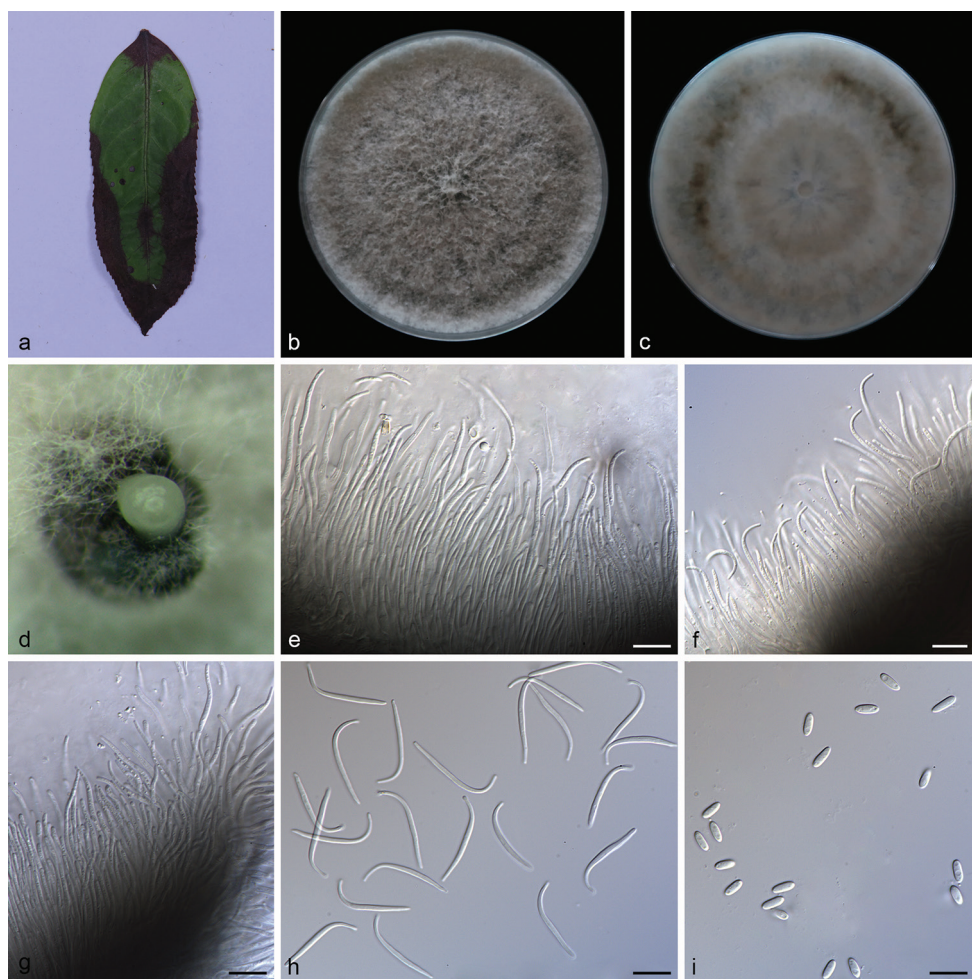


Figure 4. *Diaporthe hongkongensis* (SAUCC194.87) **a** diseased leaf of *Camellia sinensis* **b, c** surface and reverse of colony after 15 days on PDA **d** conidiomata **e–g** conidiophores and conidiogenous cells **h** beta conidia **i** alpha conidia. Scale bars: 10 μ m (**e–i**).

but the asexual morph of *D. salinicola* was undetermined. We therefore identify our strains as *D. hongkongensis*.

***Diaporthe machili* S.T. Huang, J.W. Xia, W.X. Sun, & X.G. Zhang, sp. nov.**

Mycobank No: 837814

Figure 5

Etymology. Named after the host genus on which it was collected, *Machilus pingii*.

Diagnosis. *Diaporthe machili* differs from *D. caryae* and *D. sackstonii* in the types of conidia (*D. machili* only produces beta conidia, while *D. caryae* produces alpha

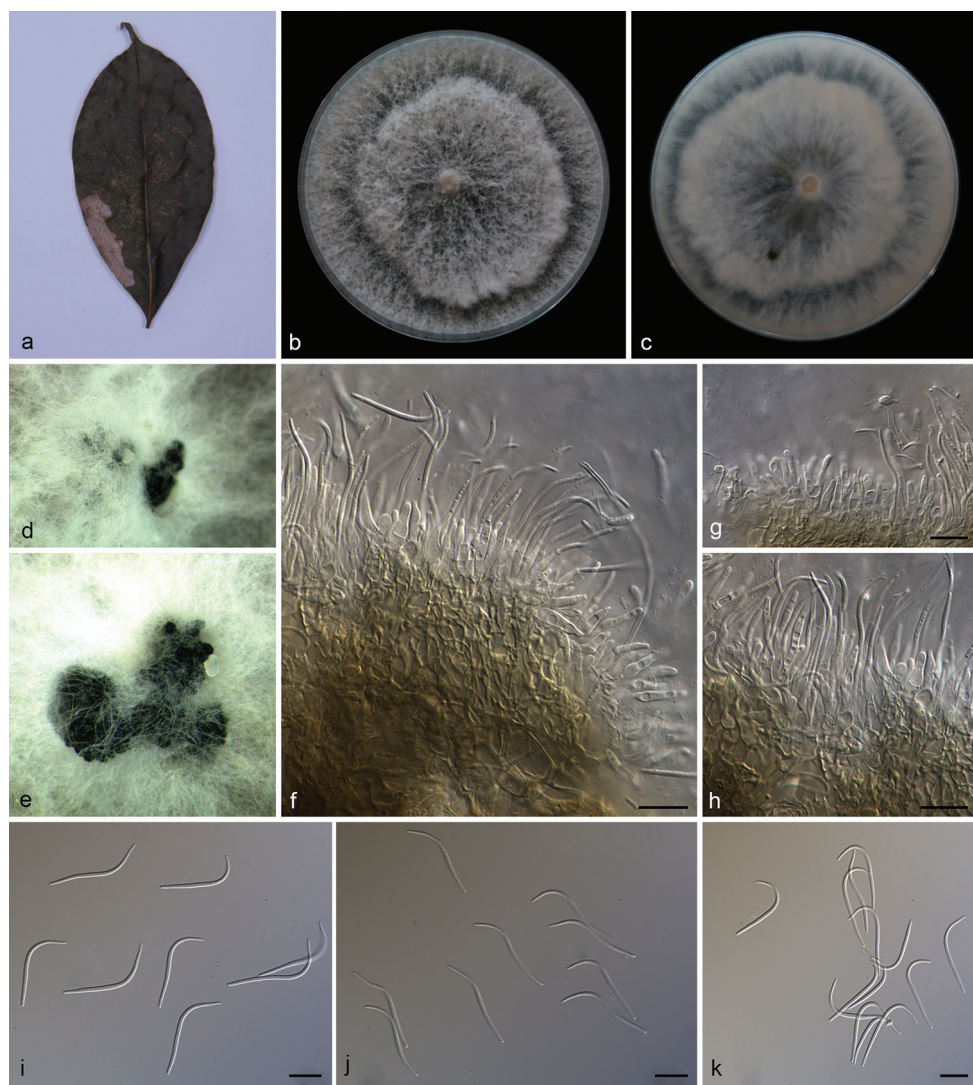


Figure 5. *Diaporthe machili* (SAUCC194.111) **a** infected leaf of *Machilus pingii* **b, c** surface and reverse of colony after 15 days on PDA **d, e** conidiomata **f–h** conidiophores and conidiogenous cells **i–k** beta conidia. Scale bars: 10 μ m (**f–k**).

conidia and beta conidia, and *D. sackstonii* only produces alpha conidia), and from *D. caryae* in longer beta conidia ($29.0\text{--}39.0 \times 1.3\text{--}1.5$ vs. $15.5\text{--}34.0 \times 1.1\text{--}1.4$ μ m).

Type. China, Yunnan Province: Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, on diseased leaves of *Machilus pingii* (Lauraceae). 19 April 2019, S.T. Huang, HSAUP194.111 holotype, ex-holotype living culture SAUCC194.111.

Description. Asexual morph: Conidiomata pycnidial, aggregated, black, erumpent, subglobose to globose, exuding creamy conidial droplets from central ostioles after 30

days in light at 25 °C. Conidiophores $7.0\text{--}11.4 \times 1.8\text{--}2.8 \mu\text{m}$, hyaline, unbranched, densely aggregated, mostly ampulliform, cylindrical, guttulate, septate, straight or slightly curved, swelling at base, tapering towards apex. Beta conidia $29.0\text{--}39.0 \times 1.3\text{--}1.5 \mu\text{m}$ (mean = $32.5 \times 1.4 \mu\text{m}$, $n = 20$), filiform, hyaline, aseptate, mostly curved, J-shaped, swelling in middle, tapering towards both ends. Alpha and gamma conidia not observed. Sexual morph not observed.

Culture characteristics. Cultures incubated on PDA at 25 °C in darkness, growth rate 16.3–17.5 mm diam/day, aerial mycelium abundant, white on surface, reverse white to pale yellow, with an obvious concentric zonation, pycnidia forming after 15 days.

Additional specimen examined. China, Yunnan Province: Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, on diseased leaves of *Pometia pinnata* (Sapindaceae). 19 April 2019, S.T. Huang, HSAUP194. 69 paratype; living culture SAUCC194. 69.

Notes. In the phylogenetic tree, *Diaporthe machili* forms an independent clade and is phylogenetically distinct from *D. caryae* and *D. sackstonii* (Fig. 1). *Diaporthe machili* can be distinguished from *D. caryae* in ITS, TUB, TEF, CAL and HIS loci by 67 nucleotide differences in concatenated alignment (5/459 in ITS, 10/416 in TUB, 15/334 in TEF, 7/454 in CAL and 30/455 in HIS), and from *D. sackstonii* in ITS, TUB and TEF loci by 58 nucleotide differences (12/559 in ITS, 23/486 in TUB and 23/348 in TEF). Moreover, *Diaporthe machili* differs from *D. caryae* in having longer beta conidia ($29.0\text{--}39.0 \times 1.3\text{--}1.5$ vs. $15.5\text{--}34.0 \times 1.1\text{--}1.4 \mu\text{m}$). *Diaporthe machili* only produces beta conidia, while *D. caryae* produces alpha conidia and beta conidia, and *D. sackstonii* only produces alpha conidia (Thompson et al. 2015; Yang et al. 2018b).

***Diaporthe middletonii* R.G. Shivas, L. Morin, S.M. Thomps. & Y.P. Tan, *Persoonia* 35: 45. (2015)**

Figure 6

Description. Asexual morph: Leaf spots discoid to irregular. Conidiomata pycnidial, scattered or aggregated in groups of 3–5 pycnidia, globose, black, erumpent, coated with white to greyish hyphae, thick-walled, exuding creamy translucent conidial droplets from central ostioles. Conidiophores hyaline, smooth, septate, unbranched, densely aggregated, cylindrical, straight to sinuous, tapering towards apex, $10.0\text{--}14.0 \times 1.3\text{--}2.3 \mu\text{m}$. Conidiogenous cells $5.0\text{--}9.5 \times 1.3\text{--}1.7 \mu\text{m}$, phialidic, cylindrical, terminal, slightly tapering towards apex. Alpha conidia hyaline, smooth, aseptate, biguttulate, ellipsoidal, oval, apex subobtuse, base subtruncate, $5.5\text{--}7.0 \times 2.5\text{--}3.2 \mu\text{m}$ (mean = $6.3 \times 2.8 \mu\text{m}$, $n = 20$). Beta conidia hyaline, aseptate, filiform, mostly curved by 90–180°, tapering towards both ends, $26.0\text{--}36.5 \times 1.0\text{--}1.6 \mu\text{m}$ (mean = $21.5 \times 1.2 \mu\text{m}$, $n = 20$). Gamma conidia not observed. Sexual morph not observed.

Culture characteristics. Cultures incubated on PDA at 25 °C in darkness, growth rate 22.5–24.0 mm diam/day, fluffy with abundant aerial mycelium, margin fimbriate, white on surface, white to pale yellow on reverse.

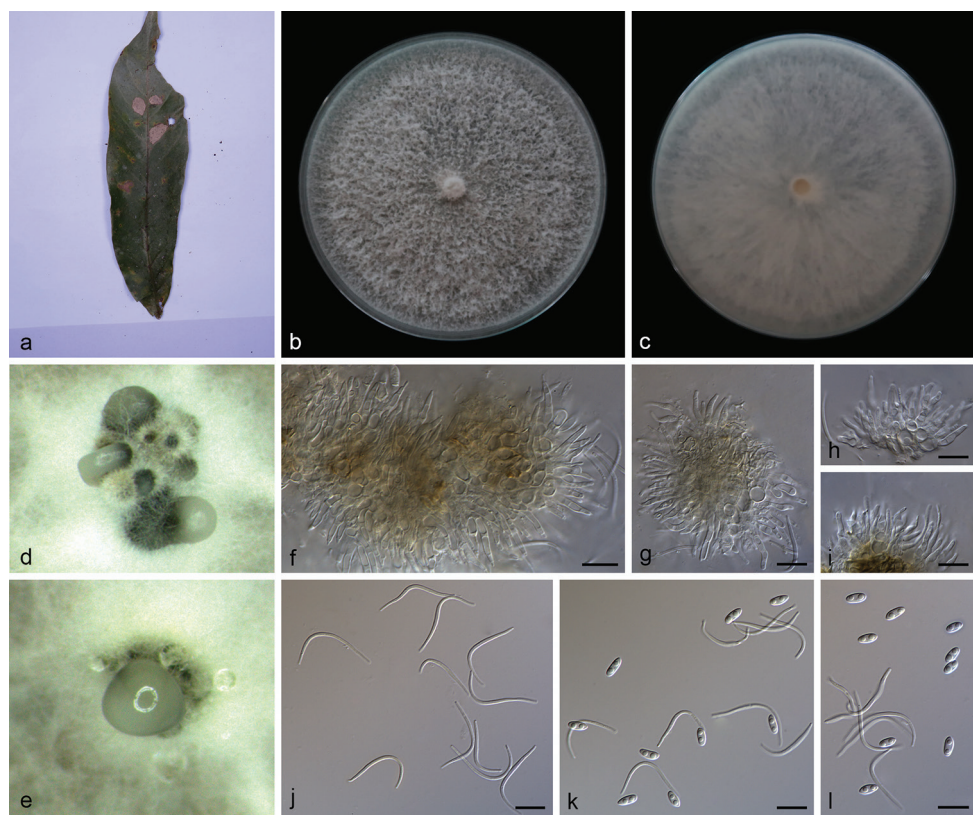


Figure 6. *Diaporthe middletonii* (SAUCC194.46) **a** infected leaf of *Lithocarpus glaber* **b, c** surface and reverse of colony after 15 days on PDA **d, e** conidiomata **f–i** conidiophores and conidiogenous cells **j** beta conidia **k, l** alpha conidia and beta conidia. Scale bars: 10 µm (**f–l**).

Specimens examined. China, Yunnan Province: Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, 19 April 2019, S.T. Huang. On diseased leaves of *Litchi chinensis* (Sapindaceae), HSAUP194.27, living culture SAUCC194.27; on diseased leaves of *Lithocarpus glaber* (Fagaceae), HSAUP194.45, living culture SAUCC194.45; on diseased leaves of *Lithocarpus glaber* (Fagaceae), 19 April 2019, S.T. Huang, HSAUP194.46, living culture SAUCC194.46; on diseased leaves of *Lithocarpus craibianus* (Fagaceae), HSAUP194.48, living culture SAUCC194.48.

Notes. *Diaporthe middletonii* was originally described from the stem of *Rapistrum rugosum* (BRIP 54884e) (Brassicaceae) and *Chrysanthemoides monilifera* subsp. *rotundata* (BRIP 57329) (Asteraceae) in Australia (Thompson et al. 2015). In the present study, four strains (SAUCC194.27, SAUCC194.45, SAUCC194.46 and SAUCC194.48) are closely related to *D. middletonii* in the combined phylogenetic tree (Fig. 1). The differences between nucleotides in the concatenated alignment (17/565 in ITS, 9/494 in TUB and 10/340 in TEF) were minor. Morphologically, our strains were similar to *D. middletonii* by slightly shorter and wider alpha conidia (5.0–7.0 ×

2.5–3.2 vs. 6.0–7.5 $\times$ 2.0–2.5 μm), and longer beta conidia (26.0–36.5 $\times$ 1.0–1.6 vs. 20.0–35.0 $\times$ 1.0–1.5 μm) (Thompson et al. 2015). We therefore identify our strains as *Diaporthe middletonii*.

***Diaporthe osmanthi* H. Long, K.D. Hyde, & Yong Wang bis, MycoKeys 57: 120. (2019)**

Figure 7

Description. Conidiomata pycnidial, globose, 5–10 pycnidia grouped together, dark brown to black, exuding creamy to yellowish conidial droplets from central ostioles. Conidiophores hyaline, smooth, densely aggregated, branched, cylindric-clavate, 20.5–32.0 $\times$ 1.8–2.4 μm . Conidiogenous cells phialidic, hyaline, terminal, cylindrical, straight, 14.0–20.5 $\times$ 1.5–2.0 μm , tapered towards apex. Alpha conidia hyaline, aseptate, fusiform, tapering towards both ends, guttulate, 7.3–9.3 $\times$ 1.8–2.3 μm (mean = 8.5 $\times$ 2.0 μm , n = 20). Beta conidia hyaline, aseptate, filiform, curved, 22.0–28.5 $\times$ 1.0–2.0 μm (mean = 27.2 $\times$ 1.3 μm , n = 20). Gamma conidia not observed. Sexual morph not observed.

Culture characteristics. Cultures incubated on PDA at 25 °C in darkness, growth rate 12.0–13.5 mm diam/day, cottony with abundant aerial mycelium, sparse at margin. With several concentric rings of dense hyphae, white on surface, white to pale brown on reverse.

Specimen examined. China, Yunnan Province: Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, 19 April 2019, S.T. Huang. On diseased leaves of *Litchi chinensis* (Sapindaceae) HSAUP194.21, living culture SAUCC194.21.

Notes. *Diaporthe osmanthi* was originally described from the leaves of *Osmanthus fragrans* (Oleaceae) in Guangxi province, China (Long et al. 2019). In the present study, phylogenetic analyses (Fig. 1) indicated that the strain SAUCC194.21 is closely related to *Diaporthe osmanthi* and *D. podocarpi-macrophylli* (Gao et al. 2017). Morphological comparison indicated that this strain was most similar to *D. osmanthi* by the size of alpha conidia and beta conidia. We therefore identify this strain as belonging to *D. osmanthi*.

***Diaporthe pandanicola* Tibpromma & K.D. Hyde, MycoKeys 33: 44 (2018)**

Figure 8

Description. Asexual morph: Conidiomata pycnidial, 3–5 pycnidia grouped together, superficial to embedded on PDA, erumpent, thin-walled, dark brown to black, globose or subglobose, exuding white creamy conidial mass from ostioles. Conidiophores hyaline, aseptate, cylindrical, smooth, straight to sinuous, unbranched, aggregated, 17.0–26.5 $\times$ 2.0–3.0 μm . Conidiogenous cells phialidic, cylindrical, terminal, 10.0–20.0 $\times$ 1.5–1.8 μm . Alpha conidia hyaline, smooth, aseptate, ellipsoidal, eguttulate, apex subobtuse, base subtruncate, 6.5–9.0 $\times$ 1.8–2.5 μm (mean = 7.5 $\times$ 2.0 μm , n = 20). Beta conidia hyaline, aseptate, filiform, curved, tapering towards apex, base truncate,

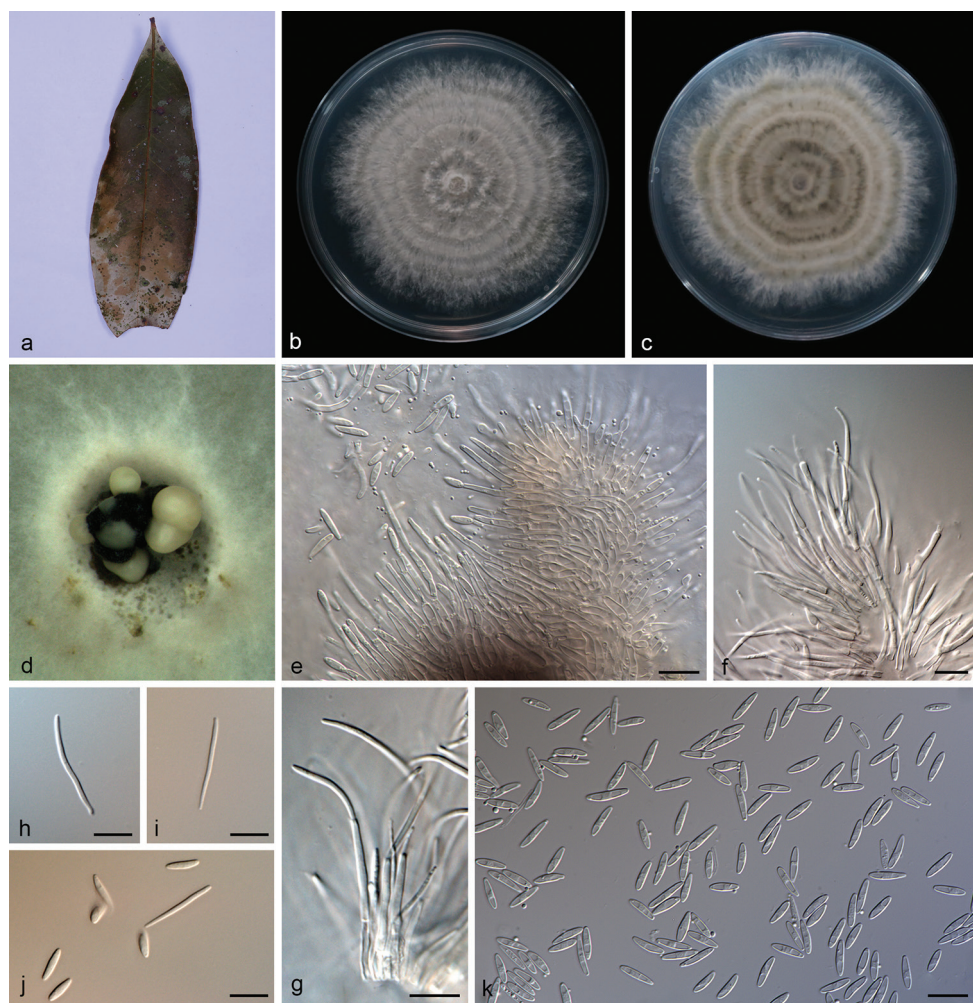


Figure 7. *Diaporthe osmanthi* (SAUCC194.21) **a** infected leaf of *Litchi chinensis* **b, c** surface and reverse of colony after 15 days on PDA **d** conidiomata **e–g** conidiophores and conidiogenous cells **h, i** beta conidia **j, k** alpha conidia. Scale bars: 10 µm (**e–k**).

26.0–32.8 × 1.0–1.6 µm (mean = 29.0 × 1.3 µm, n = 20). Gamma conidia infrequent, aseptate, smooth, straight, hyaline, 12.5–14.5 × 1.3–1.8 µm (mean = 13.5 × 1.6 µm, n = 6). Sexual morph not observed.

Culture characteristics. Cultures incubated on PDA at 25 °C in darkness, growth rate 12.8–15.0 mm diam/day, flat, cottony in centre, with aerial mycelium sparse toward margin, white on surface, white to pale yellow on reverse.

Specimen examined. China, Yunnan Province: Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, on diseased leaves of *Millettia reticulata* (Fabaceae). 19 April 2019, S.T. Huang, HSAUP194.82, living culture SAUCC194.82.

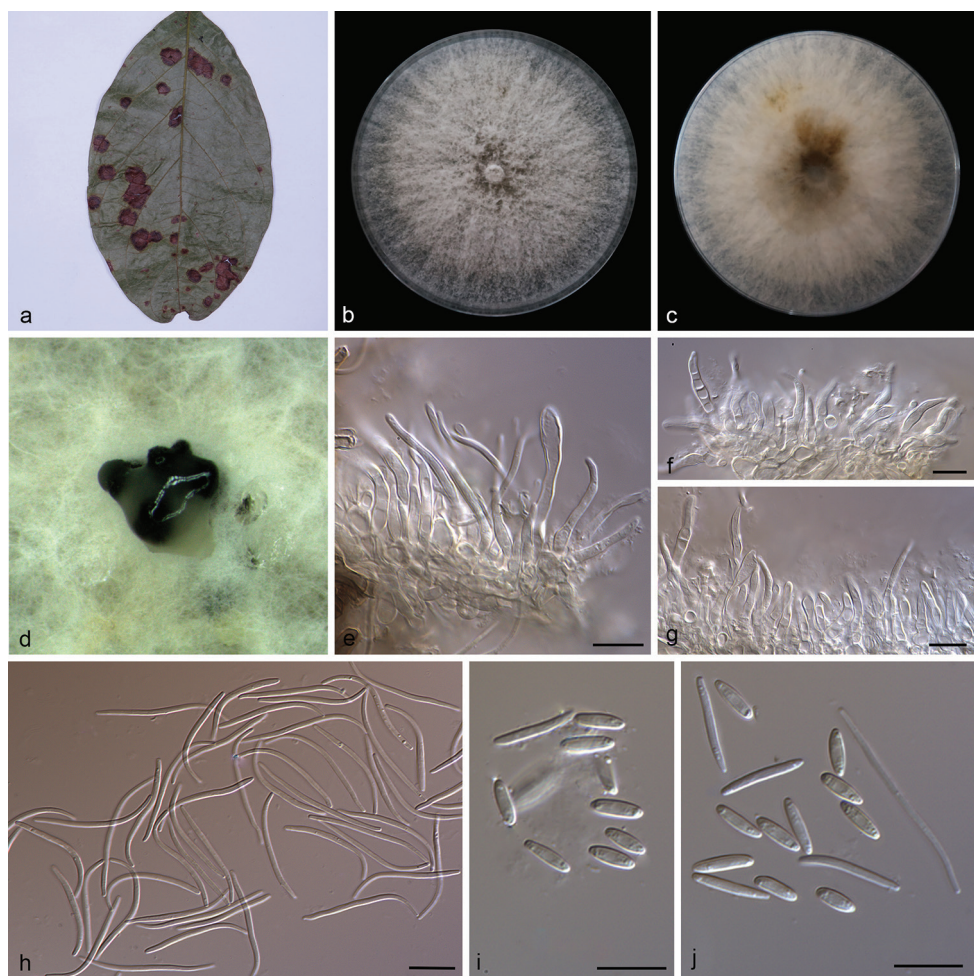


Figure 8. *Diaporthe pandanicola* (SAUCC194.82) **a** infected leaf of *Millettia reticulata* **b, c** surface and reverse of colony after 15 days on PDA **d** conidiomata **e–g** conidiophores and conidiogenous cells **h** beta conidia **i** alpha conidia and gamma conidia **j** alpha conidia, beta conidia and gamma conidia. Scale bars: 10 μ m (**e–j**).

Notes. *Diaporthe pandanicola* was originally described by Tibpromma et al. (2018) on healthy leaves of *Pandanus* sp. (Pandanaceae) as an endophytic fungus. Our strain (SAUCC194.82) is closely related to *Diaporthe pandanicola* based on phylogenetic analyses (Fig. 1). The differences of nucleotides in the concatenated alignment (19/533 in the ITS region and 11/351 in the TUB region) are less than 3%. Morphologically, our strain produces alpha conidia, beta conidia and gamma conidia, while *Diaporthe pandanicola* did not sporulate. We therefore identify our strains as *Diaporthe pandanicola*.

***Diaporthe pometiae* S.T. Huang, J.W. Xia, W.X. Sun, & X.G. Zhang, sp. nov.**

MycoBank No: 837815

Figure 9

Etymology. Named after the host genus on which it was collected, *Pometia pinnata*.

Diagnosis. *Diaporthe pometiae* is similar to *D. biconispora* but differs in having smaller alpha conidia ($5.7\text{--}8.3 \times 2.2\text{--}3.0$ vs. $6.0\text{--}10.5 \times 2\text{--}3.5$ μm) and types of conidia (*D. pometiae* produces beta conidia unlike *D. biconispora*).

Type. China, Yunnan Province: Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, on diseased leaves of *Pometia pinnata* (Sapindaceae). 19 April 2019, S.T. Huang, HSAUP194.72 holotype, ex-type living culture SAUCC194.72.

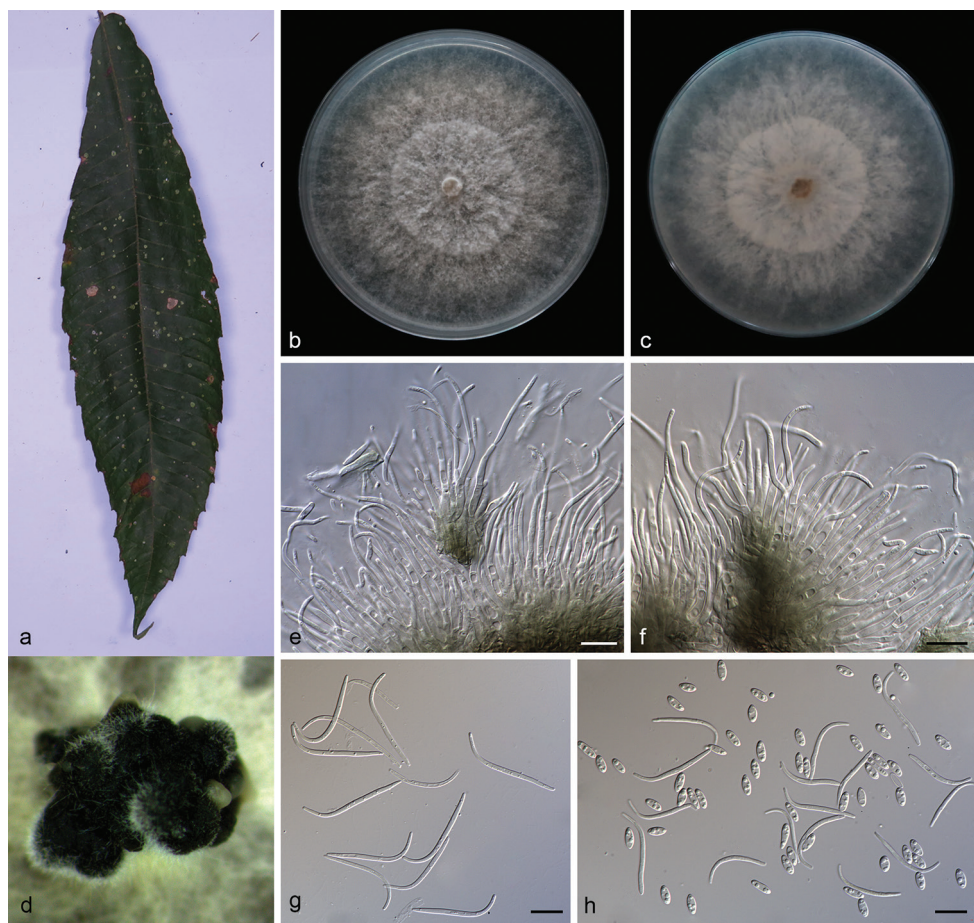


Figure 9. *Diaporthe pometiae* (SAUCC194.72) **a** infected leaf of *Pometia pinnata* **b, c** surface and reverse of colony after 15 days on PDA **d** conidiomata **e, f** conidiophores and conidiogenous cells **g** beta conidia **h** alpha conidia and beta conidia. Scale bars: 10 μm (**e–h**).

Description. Asexual morph: Leaf spots subcircular, fawn to dark brown. Conidiomata pycnidial, subglobose to globose, aggregated in groups, black, coated with white hyphae, thick-walled, exuding creamy droplets from ostioles. Conidiophores hyaline, smooth, slightly septate, branched, densely aggregated, cylindric-clavate, straight to slightly sinuous, $22.5\text{--}32.5 \times 1.0\text{--}2.0 \mu\text{m}$. Conidiogenous cells $15.0\text{--}22.5 \times 1.0\text{--}1.5 \mu\text{m}$, phialidic, cylindrical, multi-guttulate, terminal, tapering towards apex. Alpha conidia abundant in culture, 2–4 guttulate, hyaline, smooth, aseptate, ellipsoidal to oblong ellipsoidal, with both ends obtuse, $5.7\text{--}8.3 \times 2.2\text{--}3.0 \mu\text{m}$ (mean = $6.7 \times 3.1 \mu\text{m}$, $n = 20$). Beta conidia, hyaline, aseptate, filiform, multi-guttulate, slightly curved, tapering towards to apex, $27.8\text{--}34.5 \times 1.0\text{--}1.7 \mu\text{m}$ (mean = $21.7 \times 1.4 \mu\text{m}$, $n = 20$). Gamma conidia not observed. Sexual morph not observed.

Culture characteristics. Cultures incubated on PDA at 25 °C in darkness, growth rate 11.5–13.0 mm diam/day, cottony with abundant aerial mycelium, with a concentric zonation, white on surface, white to grayish on reverse.

Additional specimens examined. China, Yunnan Province: Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, 19 April 2019, S.T. Huang. On diseased leaves of *Persea americana* (Lauraceae), HSAUP194.19 paratype, ex-paratype culture SAUCC194.19; on diseased leaves of *Heliconia metallica* (Musaceae), HSAUP194.73 paratype, ex-paratype culture SAUCC194.73.

Notes. *Diaporthe pometiae* is introduced based on the multi-locus phylogenetic analysis, with three isolates clustering separately in a well-supported clade (ML/Bi = 100/1). *Diaporthe pometiae* is most closely related to *D. biconispora*, but distinguished based on ITS, TUB, TEF and HIS loci by 74 nucleotide differences in the concatenated alignment, in which 2/492 are distinct in the ITS region, 8/353 in the TUB region, 49/370 in the TEF region and 15/471 in the HIS region. Morphologically, *Diaporthe pometiae* differs from *D. biconispora* in its smaller alpha conidia ($5.7\text{--}8.3 \times 2.2\text{--}3.0$ vs. $6.0\text{--}10.5 \times 2\text{--}3.5 \mu\text{m}$). Furthermore, *Diaporthe pometiae* produces beta conidia unlike *D. biconispora* (Huang et al. 2015).

Discussion

The Yunnan Province in southeastern China has a unique geography where three climatic regions meet: the eastern Asia monsoon region, the Tibetan plateau region, and the tropical monsoon region of southern Asia and Indo-China. The environment is conducive to growth of unusual microbial species. Species diversity in Yunnan Province is high compared to other parts of China.

Previously, species identification of *Diaporthe* relied on the assumption of host-specificity, leading to the proliferation of names. The morphological characters of *Diaporthe* could be changeable, as most taxa in culture do not produce all spore states of the asexual (alpha, beta and gamma conidia) or the sexual morph (Gomes et al. 2013). Based on a polyphasic approach and morphology, more than one species of

Diaporthe can colonize a single host, while one species can be associated with several hosts (Gomes et al. 2013; Gao et al. 2017; Guarnaccia and Crous 2017; Guarnaccia et al. 2018; Guo et al. 2020). These studies revealed a high diversity of *Diaporthe* species from different hosts. Our study supports this phenomenon. For example, *Diaporthe arecae* (SAUCC194.18) and *D. pometiae* (SAUCC194.19) were collected from *Persea americana*; In addition, isolates of *D. middletonii* were obtained from three hosts (*Litchi chinensis*, *Lithocarpus craibianus*, *L. glaber*). As for host specificity, in our study, four species of *Diaporthe*, *D. machili* (SAUCC194.69), *D. middletonii* (SAUCC194.27), *D. osmanthi* (SAUCC194.21), and *D. pometiae* (SAUCC194.72) were isolated from *Litchi chinensis* and *Pometia pinnata* belong to the Sapindaceae, and *D. litchiicola* also was reported from *Litchi chinensis* in Queensland (Tan et al. 2013); however, *D. machili* (SAUCC194.111) also was isolated from *Machilus pingii* (Lauraceae), *D. middletonii* (SAUCC194.45) from *Lithocarpus glaber* (Fagaceae), *D. osmanthi* (GUCC 9165) from leaves of *Osmanthus fragrans* (Oleaceae) (Long et al. 2019), and *D. pometiae* (SAUCC194.19 and SAUCC194.73) from *Persea americana* (Lauraceae) and *Heliconia metallica* (Musaceae). These results provide evidence that many species are able to colonise diverse hosts and several different species could co-occur on the same host. It seems obvious that specificity does not occur at the family level.

For the current study, sixteen strains isolated from ten host genera represented three new species and five known species, based on morphological characters and phylogenetic analyses of the five combined loci (ITS, TUB, TEF, CAL and HIS). The descriptions and molecular data for species of *Diaporthe* represent an important resource for plant pathologists, plant quarantine officials and taxonomists.

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References

- Cai L, Hyde KD, Taylor PWJ, Weir B, Waller J, Abang MM, Zhang ZJ, Yang YL, Phoulivong S, Liu ZY, Prihastuti H, Shivas RG, McKenzie EHC, Johnston PR (2009) A polyphasic approach for studying *Colletotrichum*. Fungal Diversity 39: 183–204.
- Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous Ascomycetes. Mycologia 91(3): 553–556. <https://doi.org/10.1080/00275514.1999.12061051>
- Crous PW, Groenewald JZ, Risede JM, Simoneau P, Hywel-Jones NL (2004) *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. Studies in Mycology 50: 415–430.

- Crous PW, Groenewald JZ, Shivas RG, Edwards J, Seifert KA, Alfenas AC, Alfenas RF, Burgess TI, Carnegie AJ, Hardy GEstJ (2011a) Fungal planet description sheets: 69–91. *Persoonia* 26(1): 108–156. <https://doi.org/10.3767/003158511X581723>
- Crous PW, Summerell BA, Swart L, Denman S, Taylor JE, Bezuidenhout CM, Palm ME, Marincowitz S, Groenewald JZ (2011b) Fungal pathogens of Proteaceae. *Persoonia* 27(1): 20–45. <https://doi.org/10.3767/003158511X606239>
- Crous PW, Wingfield MJ, Richardson DM, Roux JJJ, Strasberg D, Edwards J, Roets F, Hubka V, Taylor PWJ, Heykoop M (2016) Fungal planet description sheets: 400–468. *Persoonia* 36(1): 316–458. <https://doi.org/10.3767/003158516X692185>
- Crous PW, Wingfield MJ, Schumacher RK, Akulov A, Bulgakov TS, Carnegie AJ, Jurjević Ž, Decock C, Denman S, Lombard L (2020) New and interesting fungi. 3. *Fungal Systematics and Evolution* 6: 157–231. <https://doi.org/10.3114/fuse.2020.06.09>
- Dayarathne MC, Jones EBG, Maharachchikumbura SSN, Devadatha B, Sarma VV (2020) Morpho-molecular characterization of microfungi associated with marine based habitats. *Mycosphere* 11(1): 1–188. <https://doi.org/10.5943/mycosphere/11/1/1>
- Dissanayake AJ, Phillips AJL, Hyde KD, Yan JY, Li XH (2017) The current status of species in *Diaporthe*. *Mycosphere* 8: 1106–1156. <https://doi.org/10.5943/mycosphere/8/5/5>
- Fan XL, Bezerra JDP, Tian CM, Crous PW (2018) Families and genera of diaporthealean fungi associated with canker and dieback of tree hosts. *Persoonia* 40: 119–134. <https://doi.org/10.3767/persoonia.2018.40.05>
- Gao YH, Sun W, Su YY, Cai L (2014) Three new species of *Phomopsis* in Gutianshan Nature Reserve in China. *Mycological Progress* 13(1): 111–121. <https://doi.org/10.1007/s11557-013-0898-2>
- Gao YH, Su YY, Sun W, Cai L (2015) *Diaporthe* species occurring on *Lithocarpus glabra* in China, with descriptions of five new species. *Fungal Biology* 119(5): 295–309. <https://doi.org/10.1016/j.funbio.2014.06.006>
- Gao YH, Liu F, Cai L (2016) Unravelling *Diaporthe* species associated with *Camellia*. *Systematics and Biodiversity* 14(1): 102–117. <https://doi.org/10.1080/14772000.2015.1101027>
- Gao YH, Liu F, Duan W, Crous PW, Cai L (2017) *Diaporthe* is paraphyletic. *IMA fungus* 8: 153–187. <https://doi.org/10.5598/ima fungus.2017.08.01.11>
- Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61(4): 1323–1330. <https://doi.org/10.1128/AEM.61.4.1323-1330.1995>
- Gomes RR, Glienke C, Videira SIR, Lombard L, Groenewald JZ, Crous PW (2013) *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia: Molecular Phylogeny and Evolution of Fungi* 31(1): 1–41. <https://doi.org/10.3767/003158513X666844>
- Grasso FM, Marini M, Vitale A, Firrao G, Granata G (2012) Canker and dieback on *Platanus x acerifolia* caused by *Diaporthe scabra*. *Forest Pathology* 42(6): 510–513. <https://doi.org/10.1111/j.1439-0329.2012.00785.x>
- Guarnaccia V, Vitale A, Cirvilleri G, Aiello D, Susca A, Epifani F, Perrone G, Polizzi G (2016) Characterisation and pathogenicity of fungal species associated with branch cankers and stem-end rot of avocado in Italy. *European Journal of Plant Pathology* 146(4): 963–976. <https://doi.org/10.1007/s10658-016-0973-z>
- Guarnaccia V, Crous PW (2017) Emerging citrus diseases in Europe caused by *Diaporthe* spp. *IMA Fungus* 8: 317–334. <https://doi.org/10.5598/ima fungus.2017.08.02.07>

- Guarnaccia V, Groenewald JZ, Woodhall J, Armengol J, Cinelli T, Eichmeier A, Ezra D, Fontaine F, Gramaje D, Gutierrez-Aguirregabiria A (2018) *Diaporthe* diversity and pathogenicity revealed from a broad survey of grapevine diseases in Europe. *Persoonia* 40(6): 135–153. <https://doi.org/10.3767/persoonia.2018.40.06>
- Guo LD, Hyde KD, Liew ECY (2000) Identification of endophytic fungi from *Livistona chinensis* based on morphology and rDNA sequences. *New Phytologist* 147(3): 617–630. <https://doi.org/10.1046/j.1469-8137.2000.00716.x>
- Guo YS, Crous PW, Bai Q, Fu M, Yang MM, Wang XH, Du YM, Hong N, Xu WX, Wang GP (2020) High diversity of *Diaporthe* species associated with pear shoot canker in China. *Persoonia* 45: 132–162. <https://doi.org/10.3767/persoonia.2020.45.05>
- Huang F, Hou X, Dewdney MM, Fu Y, Chen GQ, Hyde KD, Li HY (2013) *Diaporthe* species occurring on *citrus* in China. *Fungal Diversity* 61(1): 237–250. <https://doi.org/10.1007/s13225-013-0245-6>
- Huang F, Udayanga D, Wang XH, Hou X, Mei XF, Fu YS, Hyde KD, Li HY (2015) Endophytic *Diaporthe* associated with *Citrus*: A phylogenetic reassessment with seven new species from China. *Fungal Biology* 119(5): 331–347. <https://doi.org/10.1016/j.funbio.2015.02.006>
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: bayesian inference of phylogeny. *Bioinformatics* 17(17): 754–755. <https://doi.org/10.1093/bioinformatics/17.8.754>
- Hyde KD, Dong Y, Phookamsak R, Jeewon R, Bhat DJ, Gareth Jones EB, Liu NG, Abeywickrama PD, Mapook A, Wei D (2020) Fungal diversity notes 1151–1276: Taxonomic and phylogenetic contributions on genera and species of fungal taxa. *Fungal Diversity* 100(1): 1–273. <https://doi.org/10.1007/s13225-020-00439-5>
- Katoh K, Rozewicki J, Yamada KD (2017) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*: 1–7. <https://doi.org/10.1093/bib/bbx108>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33(7): 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Li WJ, McKenzie EHC, Liu JK, Bhat DJ, Dai DQ, Camporesi E, Tian Q, Maharachchikumbura SSN, Luo ZL, Shang QJ (2020) Taxonomy and phylogeny of hyaline-spored coelomycetes. *Fungal Diversity* 100(1): 279–801. <https://doi.org/10.1007/s13225-020-00440-y>
- Lombard L, van Leeuwen GCM, Guarnaccia V, Polizzi G, van Rijswijk PCJ, Rosendahl KCHM, Gabler J, Crous PW (2014) *Diaporthe* species associated with *Vaccinium*, with specific reference to Europe. *Phytopathologia Mediterranea* 53(2): 287–299. https://doi.org/10.14601/PHYTOPATHOL_MEDITERR-14034
- Long H, Zhang Q, Hao YY, Shao XQ, Wei XX, Hyde KD, Wang Y, Zhao DG (2019) *Diaporthe* species in south-western China. *MycoKeys* 57: 113–127. <https://doi.org/10.3897/mycokeys.57.35448>
- Ménard L, Brandeis PE, Simoneau P, Poupard P, Sérandat I, Detoc J, Robbes L, Bastide F, Laurent E, Gombert J, Morel E (2014) First report of umbel browning and stem necrosis caused by *Diaporthe angelicae* on carrot in France. *Plant Disease* 98(3): 421–422. <https://doi.org/10.1094/PDIS-06-13-0673-PDN>

- Miller MA, Pfeiffer W, Schwartz T (2012) The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment. Bridging from the extreme to the campus and beyond. Association for Computing Machinery, USA, 8 pp. <https://doi.org/10.1145/2335755.2335836>
- Murali TS, Suryanarayanan TS, Geeta R (2006) Endophytic *Phomopsis* species: host range and implications for diversity estimates. Canadian Journal of Microbiology 52(7): 673–680. <https://doi.org/10.1139/w06-020>
- Nitschke T (1870) Pyrenomycetes Germanici (2nd ed.). Eduard Trewendt, Breslau, 161–320.
- Nylander JAA (2004) MrModeltest v. 2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Rayner RW (1970) A mycological colour chart. CMI and British Mycological Society, Kew.
- Rehner SA, Uecker FA (1994) Nuclear ribosomal internal transcribed spacer phylogeny and host diversity in the coelomycete *Phomopsis*. Botany 72(11): 1666–1674. <https://doi.org/10.1139/b94-204>
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: bayesian phylogenetic inference under mixed models. Bioinformatics 19(12): 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Rossman AY, Adams GC, Cannon PF, Castlebury LA, Crous PW, Gryzenhout M, Jaklitsch WM, Mejia LC, Stoykov D, Udayanga D (2015) Recommendations of generic names in Diaporthales competing for protection or use. IMA Fungus 6(1): 145–154. <https://doi.org/10.5598/imafungus.2015.06.01.09>
- Santos JM, Phillips AJL (2009) Resolving the complex of *Diaporthe* (*Phomopsis*) species occurring on *Foeniculum vulgare* in Portugal. Fungal Diversity 34: 111–125.
- Santos JM, Vrandečić K, Ćosić J, Duvnjak T, Phillips AJL (2011) Resolving the *Diaporthe* species occurring on soybean in Croatia. Persoonia 27(1): 9–19. <https://doi.org/10.3767/003158511X603719>
- Santos L, Alves A, Alves R (2017) Evaluating multi-locus phylogenies for species boundaries determination in the genus *Diaporthe*. PeerJ 5: e3120. <https://doi.org/10.7287/peerj.preprints.2822v1>
- Senanayake IC, Crous PW, Groenewald JZ, Maharachchikumbura SSN, Jeewon R, Phillips AJL, Bhat DJ, Perera RH, Li QR, Li WJ (2017) Families of Diaporthales based on morphological and phylogenetic evidence. Studies in Mycology 86: 217–296. <https://doi.org/10.1016/j.simyco.2017.07.003>
- Senanayake IC, Jeewon R, Chomnunti P, Wanasinghe DN, Norphanphoun C, Karunarathna A, Pem D, Perera RH, Camporesi E, McKenzie EHC (2018) Taxonomic circumscription of Diaporthales based on multigene phylogeny and morphology. Fungal Diversity 93(1): 241–443. <https://doi.org/10.1007/s13225-018-0410-z>

- Srivastava HC, Banu Z, Govindarajan VS (1962) Fruit rot of arecanut caused by a new fungus. *Mycologia* 54(1): 5–11. <https://doi.org/10.1080/00275514.1962.12024974>
- Stamatakis A (2014) RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9): 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Tan YP, Edwards J, Grice KRE, Shivas RG (2013) Molecular phylogenetic analysis reveals six new species of *Diaporthe* from Australia. *Fungal Diversity* 61(1): 251–260. <https://doi.org/10.1007/s13225-013-0242-9>
- Thompson SM, Tan YP, Young AJ, Neate SM, Aitken EAB, Shivas RG (2011) Stem cankers on sunflower (*Helianthus annuus*) in Australia reveal a complex of pathogenic *Diaporthe* (*Phomopsis*) species. *Persoonia* 27(1): 80–89. <https://doi.org/10.3767/003158511X617110>
- Thompson SM, Tan YP, Shivas RG, Neate SM, Morin L, Bissett A, Aitken EAB (2015) Green and brown bridges between weeds and crops reveal novel *Diaporthe* species in Australia. *Persoonia* 35(1): 39–49. <https://doi.org/10.3767/003158515X687506>
- Tibpromma S, Hyde KD, Bhat JD, Mortimer PE, Xu JC, Promputtha I, Doilom M, Yang JB, Tang AMC, Karunaratna SC (2018) Identification of endophytic fungi from leaves of Pandanaceae based on their morphotypes and DNA sequence data from southern Thailand. *MycKeys* 33(33): 25–67. <https://doi.org/10.3897/mycokeys.33.23670>
- Torres C, Camps R, Aguirre R, Besoain XA (2016) First report of *Diaporthe rudis* in Chile causing stem-end rot on hass avocado fruit imported from California, USA. *Plant Disease* 100(9): 1951–1951. <https://doi.org/10.1094/PDIS-12-15-1495-PDN>
- Udayanga D, Liu X, McKenzie EH, Chukeatirote E, Bahkali AH, Hyde KD (2011) The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. *Fungal Diversity* 50(1): 189–225. <https://doi.org/10.1007/s13225-011-0126-9>
- Udayanga D, Liu XZ, Crous PW, McKenzie EHC, Chukeatirote E, Hyde KD (2012) A multi-locus phylogenetic evaluation of *Diaporthe* (*Phomopsis*). *Fungal Diversity* 56(1):157–171. <https://doi.org/10.1007/s13225-012-0190-9>
- Udayanga D, Castlebury LA, Rossman AY, Chukeatirote E, Hyde KD (2015) The *Diaporthe sojae* species complex: Phylogenetic re-assessment of pathogens associated with soybean, cucurbits and other field crops. *Fungal Biology* 119(5): 383–407. <https://doi.org/10.1016/j.funbio.2014.10.009>
- van Rensburg JCJ, Lamprecht SC, Groenewald JZ, Castlebury LA, Crous PW (2006) Characterization of *Phomopsis* spp. associated with die-back of rooibos (*Aspalathus linearis*) in South Africa. *Studies in Mycology* 55: 65–74. <https://doi.org/10.3114/sim.55.1.65>
- Vilka L, Volkova J (2015) Morphological diversity of *Phomopsis vaccinii* isolates from cranberry (*Vaccinium macrocarpon* Ait.) in Latvia. *Proceedings of the Latvia University of Agriculture* 33: 8–18. <https://doi.org/10.1515/plua-2015-0002>
- White T, Bruns T, Lee S, Taylor FJRM, White TJ, Lee SH, Taylor L, Shawe-Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A guide to methods and applications* 18: 315–322. Academic Press, San Diego. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>

- Yang Q, Du Z, Tian CM (2018a) Phylogeny and morphology reveal two new species of *Diaporthe* from Traditional Chinese Medicine in Northeast China. *Phytotaxa* 336(2): 159–170. <https://doi.org/10.11646/phytotaxa.336.2.3>
- Yang Q, Fan XL, Guarnaccia V, Tian CM (2018b) High diversity of *Diaporthe* species associated with dieback diseases in China, with twelve new species described. *MycoKeys* 39(39): 97–149. <https://doi.org/10.3897/mycokeys.39.26914>
- Yang Q, Jiang N, Tian CM (2020) Three new *Diaporthe* species from Shaanxi Province, China. *MycoKeys* 67: 1–18. <https://doi.org/10.3897/mycokeys.67.49483>
- Zapata M, Palma MA, Aninat MJ, Piontelli E (2020) Polyphasic studies of new species of *Diaporthe* from native forest in Chile, with descriptions of *Diaporthe araucanorum* sp. nov., *Diaporthe foikelawen* sp. nov. and *Diaporthe patagonica* sp. nov. *International Journal of Systematic and Evolutionary Microbiology* 70(5): 3379–3390. <https://doi.org/10.1099/ijsem.0.004183>

Novel taxa and species diversity of *Cordyceps* sensu lato (Hypocreales, Ascomycota) developing on wireworms (Elateroidea and Tenebrionoidea, Coleoptera)

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Abstract

Species of *Cordyceps* sensu lato (Hypocreales, Sordariomycetes) have always attracted much scientific attention for their abundant species diversity, important medicinal values and biological control applications. The insect superfamilies Elateroidea and Tenebrionoidea are two large groups of Coleoptera and their larvae are generally called wireworms. Most wireworms inhabit humid soil or fallen wood and are often infected with *Cordyceps* s.l. However, the species diversity of *Cordyceps* s.l. on Elateroidea and Tenebrionoidea is poorly known. In the present work, we summarise taxonomic information of 63 *Cordyceps* s.l. species that have been reported as pathogens of wireworms. We review their hosts and geographic distributions and provide taxonomic notes for species. Of those, 60 fungal species are accepted as natural pathogens of wireworms and three species (*Cordyceps militaris*, *Ophiocordyceps ferruginosa* and *O. variabilis*) are excluded. Two new species, *O. borealis* from Russia (Primorsky Krai) and *O. spicatus* from China (Guizhou), are described and compared with their closest allies. *Polyccephalomycetes formosus* is also described because it is reported as a pathogen of wireworms for the first time. Phylogeny was reconstructed from a combined dataset, comprising SSU, LSU and TEF1- α gene sequences. The results, presented in this study, support the establishment of the new species and confirm the identification of *P. formosus*.

Keywords

Two new species, Elateridae, molecular phylogeny, *Ophiocordyceps*, taxonomy, Tenebrionidae

Introduction

The superfamilies Elateroidea and Tenebrionoidea are two large groups of Coleoptera. Species within these superfamilies are phytophagous, xylophagous, saprophagous or omnivorous and most of them are important agricultural pests (Gullan and Cranston 2010; Ren et al. 2016). Elateroidea larvae are the well-known wireworms, closely resembling Tenebrionoidea larvae which are known as mealworms or pseudo-wireworms (Ren et al. 2016). As a result, in practice, larvae of both Elateroidea and Tenebrionoidea are generally referred to as wireworms. Most wireworms inhabit humid soil, humus layer or decayed wood and are, thus, easily encountered and infected with entomopathogenic fungi (Kabaluk et al. 2017; Rogge et al. 2017).

Cordyceps sensu lato (Hypocreales, Sordariomycetes) is a well-known group of entomopathogenic fungi. Previously, most species of this group were assigned to the previous *Cordyceps* Fr. genus, so they had commonly been called ‘*Cordyceps*’. It was not until 2007 that Sung et al. revised the classification system of this group, based on substantial molecular and morphological data. In the new classification system, all these fungi are assigned to three families (Cordycipitaceae, Ophiocordycipitaceae and, in part, Clavicipitaceae) and only a few species were retained in the revised *Cordyceps* Fr. emend. G.H. Sung et al. genus (Sung et al. 2007). As a result, the concept of ‘*Cordyceps*’ has been extended from the previous genus *Cordyceps* Fr. to *Cordyceps* s.l. So far, more than 1000 *Cordyceps* s.l. species have been reported (Wei et al. 2020) and these entomopathogenic hypocrealean fungi are widely distributed in all terrestrial regions (except Antarctica), especially tropics and subtropics (Kobayasi 1941; Sung et al. 2007).

Ophiocordyceps Petch and *Polycephalomycetes* Kobayasi are two morphologically, phylogenetically and ecologically closely-related genera placed in Ophiocordycipitaceae. They produce rigid, pliant or wiry stipes that are usually darkly coloured; their asexual morphs are mainly *Hirsutella*-like, but phialides of *Polycephalomycetes* lack the swollen base and are concentrated at the tips of synnemata; and they are typically found on hosts buried in soil or in rotting wood, especially wireworms (Sung et al. 2007; Kepler et al. 2013). *Ophiocordyceps* is the largest genus of *Cordyceps* s.l., with *O. blattae* (Petch) Petch as the type species, linking with *Didymobotryopsis*-, *Hirsutella*-, *Hymenostilbe*-, *Sorospora*-, *Synnematium*- and *Troglobiomyces*-like asexual states (Quandt et al. 2014) and currently comprising approximately 200 species (Wei et al. 2020). *Polycephalomycetes*, with *P. formosus* Kobayasi as its type and linking with *Acremonium*-, *Hirsutella*- and *Polycephalomycetes*-like asexual states, includes 19 known species thus far, some of which are found on stromata of *Ophiocordyceps* spp. (Kepler et al. 2013; Wang 2016; Index Fungorum 2021).

In nature, *Cordyceps* s.l. species develop mainly on insects, spiders, other *Cordyceps* s.l. species and hypogeous fungi of the genus *Elaphomyces*. These ascomycetes can reproduce via ascospores, conidia and mycelia that generally inhabit soil, plants, invertebrates, nematodes, mushrooms and other organisms (Zha et al. 2020). The ecology and habits of different host groups are generally different and this often determines the species specificity of *Cordyceps* s.l. on them. As a result, in practice, *Cordyceps* s.l. species

have commonly been classified according to their host groups. With respect to the taxonomy of *Cordyceps* s.l. on insects, early systematic work mainly came from Petch (e.g. 1934), Kobayasi (e.g. 1941) and Shimizu (1997) who all classified *Cordyceps* s.l. species according to their host orders. Later, Shrestha et al. (2016, 2017) reviewed *Cordyceps* s.l. species on their Coleoptera, Lepidoptera, Hymenoptera and Hemiptera hosts. Recently, Zha et al. (2020) systematically studied the Orthoptera hosts and investigated the relationships with their pathogens.

A diverse range of *Cordyceps* s.l. species have been reported as pathogens of wireworms. Due to the difficulties in identifying wireworms, hosts of these fungal species have generally been recorded as Elateridae larvae, Tenebrionidae larvae or Coleoptera larvae (e.g. Petch 1933, 1937; Kobayasi 1941; Kobayasi and Shimizu 1982b, 1983). Shimizu (1997) provided beautiful drawings for many *Cordyceps* s.l. species, which included more than 30 species on wireworms and wireworm-like insects. A recent report for wireworm-infecting *Cordyceps* s.l. involved only 20 species (Shrestha et al. 2016), which is fewer than the number recorded by Shimizu (1997). It should be noticed that these fungi affect the populations of wireworms and have the potential to control these agricultural pests (Barsics et al. 2013; Rogge et al. 2017). Therefore, we need a deeper knowledge of species diversity, taxonomy, distribution and lifestyle of these wireworm-infecting *Cordyceps* s.l.

In this study, the species diversity of wireworm-infecting *Cordyceps* s.l. (Elateroidea and Tenebrionoidea) is reviewed. We discuss their hosts and geographic distribution and provide taxonomic notes for species. In addition, we describe two new members of this group, *Ophiocordyceps borealis* sp. nov. and *O. spicatus* sp. nov. *Polycephalomycetes formosus* Kobayasi is also described because it represents the first report of this species on wireworms (Elateroidea). We reconstructed a multilocus (SSU, LSU and TEF1- α) phylogeny to support morphological results.

Material and methods

Sample collections and morphological studies

Wireworm-infecting species of *Cordyceps* s.l. were collected from south-western China and the Russian Far East. Specimens were placed in plastic boxes and carried to the laboratory for further study. The macro-characteristics and ecology were photographed using a Nikon Coolpix P520 camera in the field. Specimens were examined and photographed using an Optec SZ660 stereo dissecting microscope and a Nikon Eclipse 80i compound microscope connected with a Canon EOS 600D camera. Microscopic measurements were made using Tarosoft (R) Image Framework software. Images were processed using Adobe Photoshop CS v. 8.0.1 (Adobe Systems Incorporated, San Jose, California, USA). Voucher specimens are deposited in the Fungarium of the Centre of Excellence in Fungal Research, Mae Fah Luang University (MFLU), Chiang Rai, Thailand and the Herbarium of Guizhou University (GACP), Guiyang, China.

DNA extraction, sequencing, sequence assembly and alignment

Total DNA was extracted from dried specimens using E.Z.N.A.TM Fungal DNA MiniKit (Omega Biotech, CA, USA). The ribosomal internal transcribed spacers (ITS), small and large subunits (SSU and LSU) and translation elongation factor 1 α (TEF1- α) genes were amplified and sequenced using the PCR programmes and primer pairs listed in Table 1. PCR amplification reactions were performed in an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR products were purified using Bioteke’s Purification Kit (Bioteke Corporation, Beijing, China) and were sequenced using an ABI 3730 DNA analyser and an ABI BigDye 3.1 terminator cycle sequencing kit (Sangon Co., Shanghai, China). Sequences were aligned and assembled visually and manually using Clustalx1.81, Chromas230, ContigExpress and MEGA6 software.

Construction of molecular phylogenetic trees

BLAST searches were performed to reveal the closest matches in the GenBank database that would allow the selection of appropriate taxa for phylogenetic analyses. Each gene region was independently aligned and improved manually, then the SSU, LSU and TEF1- α gene sequences were combined to form a concatenated dataset. The ITS region was not included in our multilocus analyses because of: 1) insufficient ITS sequence data (Table 2) which may lead to inaccurate phylogenetic results; 2) distinct different rate of evolution from SSU, LSU and TEF genes and with many irregular insertions and deletions of bases. Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI) analyses were performed using the concatenated sequence dataset. Sequence information of the three described species and their allies is listed in Table 2.

Maximum Likelihood (ML) analysis was done via the CIPRES Science Gateway platform (Miller et al. 2010) using RAxML-HPC2 on XSEDE (8.2.10) with the GTRGAMMA nucleotide substitution model and 1000 bootstrap iterations (Jeewon et al. 2003; Hongsanan et al. 2017). An MP tree was constructed with PAUP* 4.0b10 (Swofford 2002) using the heuristic search option with TBR branch swapping and bootstrapping with 1,000 replicates (Cai et al. 2006; Tang et al. 2007). BI analysis was conducted using MrBayes v. 3.1.2 with Markov Chain Monte Carlo sampling to

Table 1. Primers and PCR programmes used in this study (White et al. 1990, Spatafora et al. 2006, Ban et al. 2015).

Locus	Primers	PCR programs (optimised)
ITS	ITS4: 5'-TCCTCCGCTTATTGATATGC-3'	(94 °C for 30 s, 51 °C for 50 s, 72 °C for 45 s) × 33 cycles
	ITS5: 5'-GGAAGTAAAGTCGTAACAAGG-3'	
SSU	NS1: 5'-GTAGTCATATGCTTGTCTC-3'	(94 °C for 30 s, 51 °C for 30 s, 72 °C for 2 min) × 33 cycles
	NS4: 5'-CTTCGGTCAATTCCTTTAAG-3'	
LSU	LROR: 5'-ACCCGCTGAACCTTAAGC-3'	(94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min) × 30 cycles
	LR5: 5'-TCCTGAGGGAACTTCG-3'	
TEF1- α	EF1-983F: 5'-GCYCCYGGHCAYCGTGAYTTYAT-3'	(94 °C for 1 min, 55 °C for 30 s, 72 °C for 2 min) × 35 cycles
	EF1-2218R: 5'-ATGACACCRACRCRACRGTYTG-3'	

Table 2. Sequence information of samples used in this study. Our sequencing results are displayed in bold.

Fungal species	Specimen/ strain No.	Host/substratum	ITS	SSU	LUS	TEF1- α	References
<i>Cordyceps militaris</i> (outgroup)	OSC 93623	Lepidoptera (larva)	JN049825	AY184977	AY184966	DQ522332	Kepler et al. (2012)
<i>Ophiocordyceps annulata</i>	CEM303	Coleoptera	–	KJ878915	KJ878881	KJ878962	Quandt et al. (2014)
<i>O. aphodii</i>	ARSEF 5498	Coleoptera	–	DQ522541	DQ518755	DQ522323	Spatafora et al. (2007)
<i>O. borealis</i> sp. nov.	MFLU 18-0163	Coleoptera: Elateroidea (larva)	MK863251	MK863044	MK863051	MK860189	This study
	GACP R16002	Coleoptera: Elateroidea (larva)	MK863252	MK863045	MK863052	MK860190	
	GACP R16003	Coleoptera: Elateroidea (larva)	MK863253	MK863046	MK863053	MK860191	
<i>O. clavata</i>	NBRC 106962	Coleoptera (larva)	JN943328	JN941726	JN941415	AB968587	Schoch et al. (2012)
<i>O. coccidiarum</i>	MFLU 17-0752	Lepidoptera (larva)	–	MF398186	MF398187	MF928403	Hyde et al. (2018)
<i>O. entomorrhiza</i>	KEW 53484	Lepidoptera	JN049850	EF468954	EF468809	EF468749	Quandt et al. (2014)
<i>O. formosana</i>	MFLU 15-3889	Tenebrionioidea (larva)	–	–	–	KU854950	Li et al. (2016)
<i>O. formosana</i>	MFLU 15-3888	Tenebrionioidea (larva)	–	–	–	KU854949	Li et al. (2016)
<i>O. konnoana</i>	EFCC 7315	Coleoptera (larva)	–	KU854951	–	EF468753	Sung et al. (2007)
<i>O. lanpingensis</i>	YHOS0707	Lepidoptera: Hepialidae (larva)	–	EF468959	–	EF468753	Chen et al. (2013)
<i>O. longissima</i>	NBRC 108989	Hemiptera (cicada nymph)	AB968407	AB968394	KC417461	AB968585	Sanjuan et al. (2015)
<i>O. macrocicularis</i>	NBRC 105888	Lepidoptera (larva)	AB968401	AB968389	AB968417	AB968575	Ban et al. (2015)
<i>O. microlonthae</i>	OSC 110993	Coleoptera: Scarabaeidae (larva)	–	DQ522548	DQ518762	DQ522331	Spatafora et al. (2007)
<i>O. nigra</i>	TNS 16252	Hemiptera	–	KJ878941	KJ878906	KJ878986	Quandt et al. (2014)
<i>O. nigrella</i>	EFCC 9247	Lepidoptera (larva)	JN049853	EF468963	EF468818	EF468758	Sung et al. (2007)
<i>O. purpureostromata</i>	TNS F18430	Coleoptera	–	KJ878931	KJ878897	KJ878977	Quandt et al. (2014)
<i>O. ravenelii</i>	OSC 110995	Coleoptera (larva)	–	DQ522550	DQ518764	DQ522334	Spatafora et al. (2007)
<i>O. robertsii</i>	KEW 27083	Lepidoptera: Hepialidae (larva)	AJ309335	–	EF468826	EF468766	Sung et al. (2007)
<i>O. sinensis</i>	EFCC 7287	Lepidoptera (pupa)	JN049854	EF468971	EF468827	EF468767	Sung et al. (2007)
<i>O. solifera</i>	NBRC 106967	Hemiptera (cicada nymph)	AB968409	AB968395	AB968422	AB968590	Ban et al. (2015)
<i>O. spicata</i> sp. nov.	MFLU 18-0164	Coleoptera: Tenebrionioidea (larva)	MK863254	MK863047	MK863054	MK860192	This study
<i>O. variabilis</i>	OSC 111003	Diptera (larva)	–	EF468985	EF468839	EF468779	Sung et al. (2007)
<i>O. xuefangensis</i>	GZUH2012HN19	Lepidoptera: <i>Endolita nodus</i> (larva)	KC631803	KC631788	–	KC631794	Wen et al. (2013)
<i>Pantisiaria amazônica</i>	Ophama2026	Orthoptera: Acrididae (nymph)	–	KJ917562	KJ917571	KM411989	Sanjuan et al. (2015)
<i>P. coenomyiae</i>	NBRC 108993	Diptera: <i>Coenomyia</i> (larva)	AB968396	AB968384	AB968412	AB968570	Ban et al. (2015)
<i>P. gracilis</i>	EFCC 8572	Lepidoptera (larva)	JN049851	EF468956	EF468871	EF468751	Kepler et al. (2012)
<i>P. heteropoda</i>	OSC106404	Hemiptera (cicada nymph)	–	AY489690	AY489722	AY489617	Castlebury et al. (2004)
<i>Polycephalomyces formosus</i>	MFLU 18-0162	<i>Ophiocordyceps</i> sp. (stroma) on an Elateroidea larva	MK863250	MK863043	MK863050	MK860188	This study
<i>P. formosus</i>	ARSEF 1424	Coleoptera	KF049661	KF049615	KF049634	DQ118754	Chaverri et al. (2005)
<i>P. lianzhouensis</i>	GIMYY9603	Lepidoptera	EU149922	KF226249	KF226250	KF226252	Wang et al. (2014)
<i>P. ramosopulvinatus</i>	EFCC 5566	Hemiptera	KF049658	–	KF049627	KF049682	Kepler et al. (2013)
<i>P. sinensis</i>	CN 80-2	<i>O. sinensis</i> (stroma)	HQ832884	HQ832887	HQ832886	HQ832890	Wang et al. (2012)
<i>P. tomentosus</i>	BL 4	Trichiales	KF049666	KF049623	KF049641	KF049697	Kepler et al. (2013)
<i>P. yunnanensis</i>	YHHPY1006	<i>O. nutans</i> (stroma)	KF977849	–	–	KF977851	Wang et al. (2015)

calculate posterior probabilities (PP) (four simultaneous Markov chains running for 1,000,000 generations; sampling every 100 generations, first 25% of sampled trees discarded) (Rannala and Yang 1996).

Results

Molecular phylogeny of the three described species

The combined concatenated dataset included 36 samples including 32 species of Ophiocordycipitaceae (*Ophiocordyceps*, *Paraisaria* and *Polycephalomyces*) as ingroups and *Cordyceps militaris* (L.) Fr. (strain OSC 93623, Kepler et al. 2012) as the outgroup. The aligned dataset was deposited in the TreeBASE database (<http://purl.org/phylo/treebase/phyloids/study/TB2:S26977?x-access-code=cb3474ce0fd0327526b6fd2465d6c53d&format=html>). The aligned dataset was composed of 2,843/2,837 (including/excluding outgroup) characters (including gaps), of which 740/681 were variable and 527/520 were parsimony-informative. ML, MP and BI analyses resulted in phylogenies with similar topologies and the best-scoring ML tree ($-\ln L = 15804.4393$) is shown in Fig. 1.

According to the phylogenetic tree (Fig. 1), three *Ophiocordyceps borealis* sp. nov. samples (specimens MFLU 18-0163, GACP R16002 and GACP R1600) group together (100% ML/100% MP/1.00 PP) and are related to, but phylogenetically distinct from, *O. purpureostromata* (specimen TNS F18430). *Ophiocordyceps spicatus* sp. nov. (specimen MFLU 18-0164) constitutes a strongly supported independent lineage and is related to *O. formosana*. The two *Polycephalomyces formosus* samples (specimens MFLU 18-0162 and ARSEF 1424) group together and are related to *P. sinensis* (specimen CN 80-2) and *P. tomentosus* (specimen BL 4).

New species and new record of *Cordyceps* s.l. developing on wireworms

Ophiocordyceps borealis L.S. Zha & P. Chomnunti, sp. nov.

Index Fungorum number: IF558114

Facesoffungi number: FoF04101

Fig. 2

Etymology. Referring to the region (south of boreal zone of the Russian Far East) from where the species was collected.

Sexual morph. Parasitising Elateroidea larvae (Coleoptera) living in fallen wood. The larvae are cylindrical, 11 mm long and 1.1–1.3 mm thick, yellowish-brown; their body cavity stuffed with milky yellow mycelia and their intersegmental membranes covered with many milky yellow and flocculent funiculi. *Stromata* arising from any part of larval body, single or paired, unbranched. Stipe grey, slender and cylindrical, fibrous and flexible, curved more or less, 10–13 mm long and 0.25–0.6 mm thick, sur-

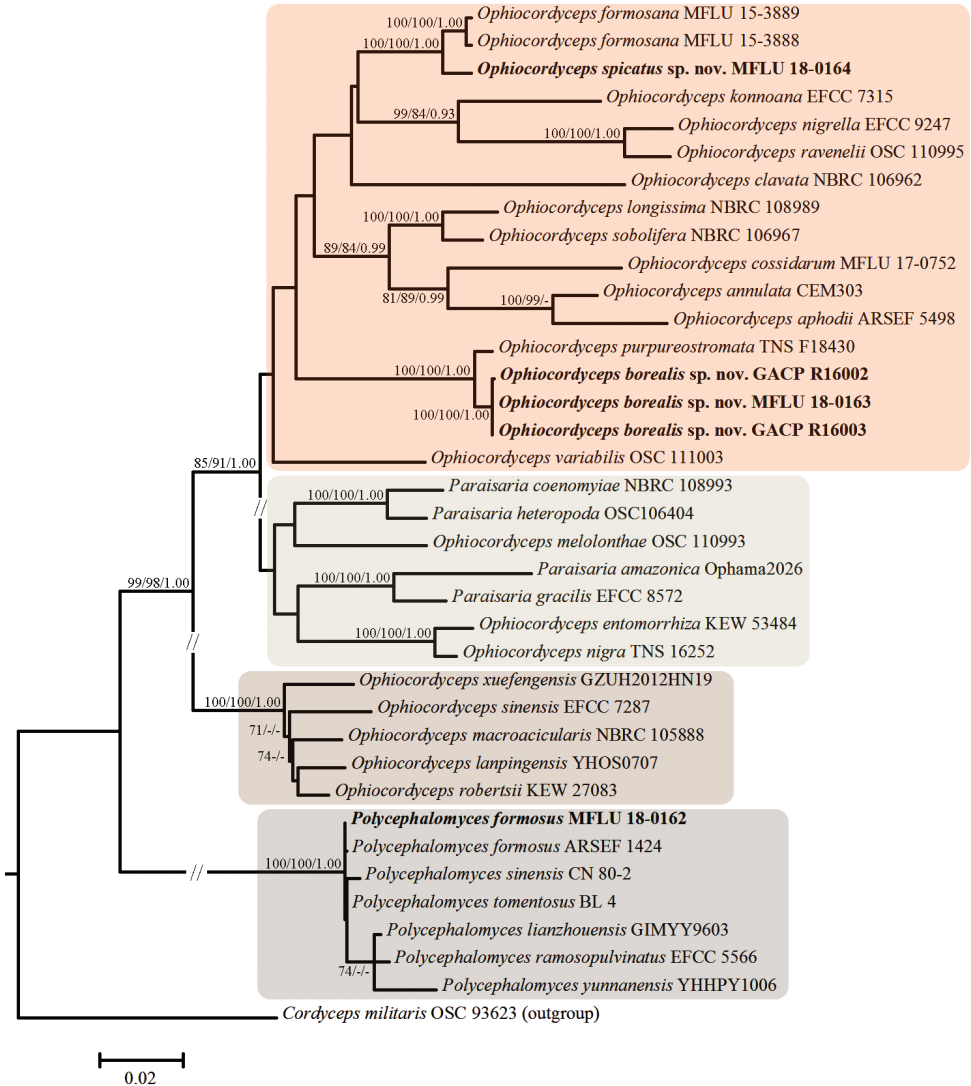


Figure 1. Maximum Likelihood (ML) tree of *Ophiocordyceps borealis* sp. nov., *O. spicatus* sp. nov. and their allies inferred from a combined SSU, LSU and TEF1- α gene dataset. Bootstrap support values of ML and Maximum Parsimony (MP) > 60% and posterior probabilities (PP) of Bayesian Inference > 0.9, are indicated above the nodes and separated by '/' (ML/MP/PP).

face relatively smooth but with many longitudinal wrinkles, apex pointed. *Fertile part* irregularly attached on one side of the surface of distal part of stipe, which resembles a mass of insect eggs that are clustered together or separated into several lumps; substrate layer milky white, surface milky yellow accompanied by lavender and dotted with numerous black ostioles. *Perithecia* immersed, densely arranged, obliquely or at right angles to the surface of stipe, pyriform, neck un conspicuous, 220–290 $\times$ 120–150 μ m

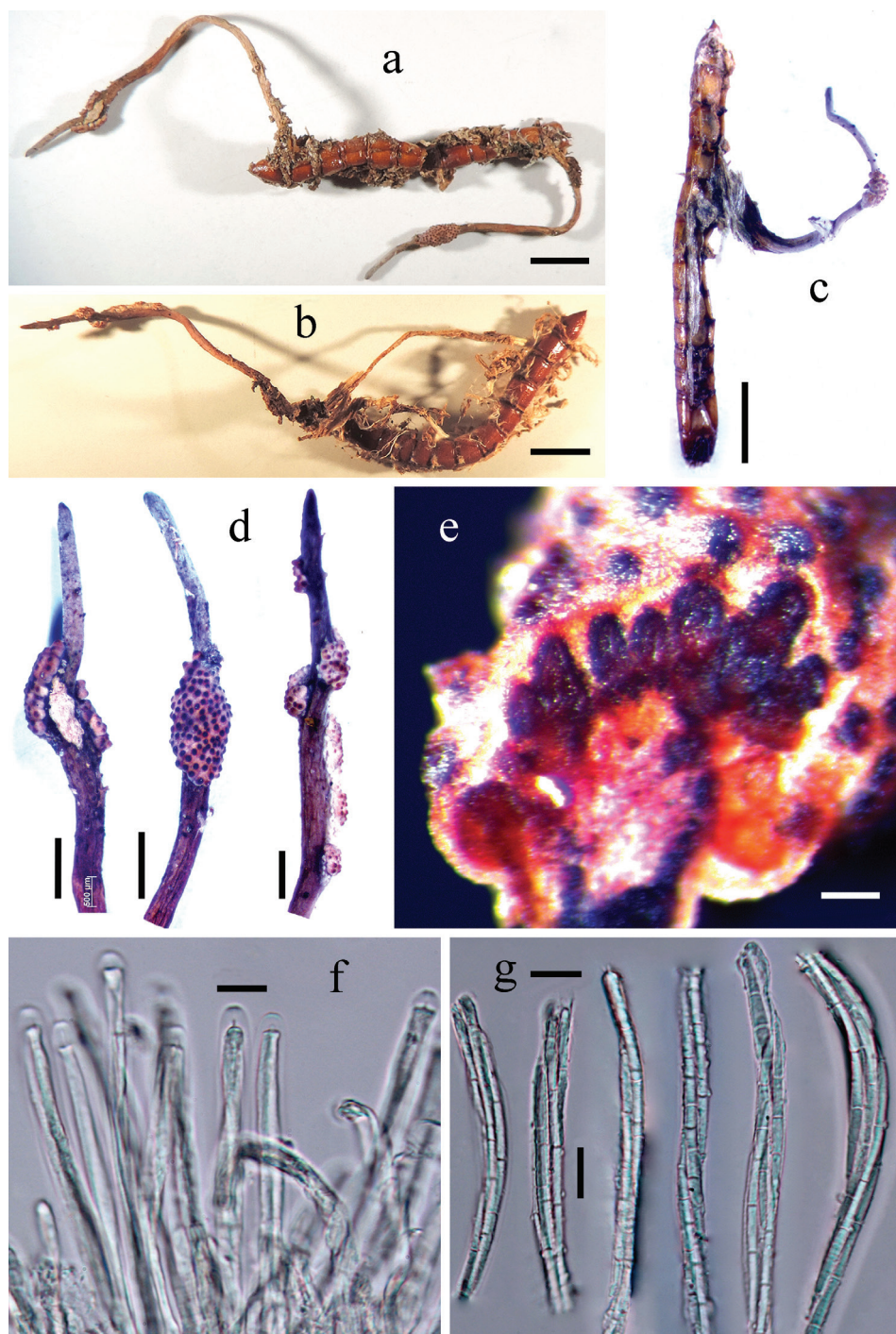


Figure 2. *Ophiocordyceps borealis* **a–c** stromata arising from the different parts of larval bodies **d** apical ends of stromata **e** transverse section of fertile part, on which densely arranged perithecia are shown **f** asci **g** ascospores. Scale bars: 2 mm (**a–c**); 1 mm (**d**); 100 μm (**e**), 10 μm (**f, g**).

and their tops obtuse; walls dark brown and 25–32 μm thick; ostioles slightly thickened and slightly protruding over the surface of fertile part. *Asci* cylindrical, 6–8 μm in diameter; caps hemispherical, 5–6 ($\bar{x}$ = 5.5, n = 30) μm wide and 3.5–5 ($\bar{x}$ = 4.2, n = 30) μm high. *Ascospores* filiform and elongate, multi-septate (far more than 3), not easy to break into part-spores; part-spores cylindrical, truncated at both ends, 10–15 ($\bar{x}$ = 12.2, n = 30) $\times$ 2 μm . **Asexual morph.** Unknown.

Material examined. RUSSIA, the Russian Far East, Primorskiy Krai, National Park Land of the Leopard, Natural Reserve Kedrovaya Pad, 43°05'53.8"N, 131°33'17.8"E, 10 August 2016, Oksana Tomilova & Vadim Yu Kryukov (MFLU 18-0163, **holotype**; GACP R16002 and GACP R16003, **paratypes**).

Known distribution. Russia (Primorskiy Krai).

Hosts. Growing on Elateroidea larvae (Coleoptera) living in fallen wood in a deciduous forest.

Notes. The new species is morphologically similar to *O. purpureostromata* ($\equiv$ *C. purpureostromata*), but their stipes and ascospores are distinct. In *O. purpureostromata*, stipe is thicker (0.6–1 mm in diameter) and has hairs (0.25–0.6 mm in diameter and without hair in *O. borealis*), ascospores are only 65–75 $\times$ 10 μm long and 3-septate (elongate and far more than 3-septate in *O. borealis*) and part-spores are 13–23 μm long (10–15 μm long in *O. borealis*) (Kobayasi and Shimizu 1980b).

Nucleotide sequences of *O. borealis* are most similar to those of *O. purpureostromata* (specimen TNS F18430, Quandt et al. 2014), but there is 2.3% bp difference across the 804 bp in TEF1- α , 0.5% bp difference across the 845 bp in LSU and 0.1% bp difference across 1,061 bp in SSU. ITS of *O. borealis* is > 14.1% different to all ITS available in GenBank (ITS are not available for *O. purpureostromata*). On the phylogenetic tree, the new species is also nearest (100% ML/100% MP/1.00 PP) to *O. purpureostromata*, but they form into two distinct branches which support them being two separate species (Fig. 1).

Ophiocordyceps spicatus L.S. Zha & P. Chomnunti, sp. nov.

Index Fungorum number: IF558115

Facesoffungi number: FoF04102

Fig. 3

Etymology. Referring to the spicate fertile head.

Sexual morph. Parasitising a Tenebrionoidea larva (Coleoptera) living in humid and decayed wood. The larva is cylindrical, 7.5 mm long and 1.0–1.1 mm thick, yellowish-brown. White mycelia stuff the body cavity, also partially cover the intersegmental membranes of the body surface. *Stroma* arising from the first quarter of the larval body, single, fleshy, 5 mm in length. Stipe yellow, cylindrical, 3.5 mm long and 0.35–0.4 mm thick, surface rough and pubescent. *Fertile head* spicate, unbranched, orange, 1.5 mm long and 0.5–0.7 mm thick, obviously differentiated from stipe; its surface rugged and consisting of many humps (outer portions of perithecia), tops of

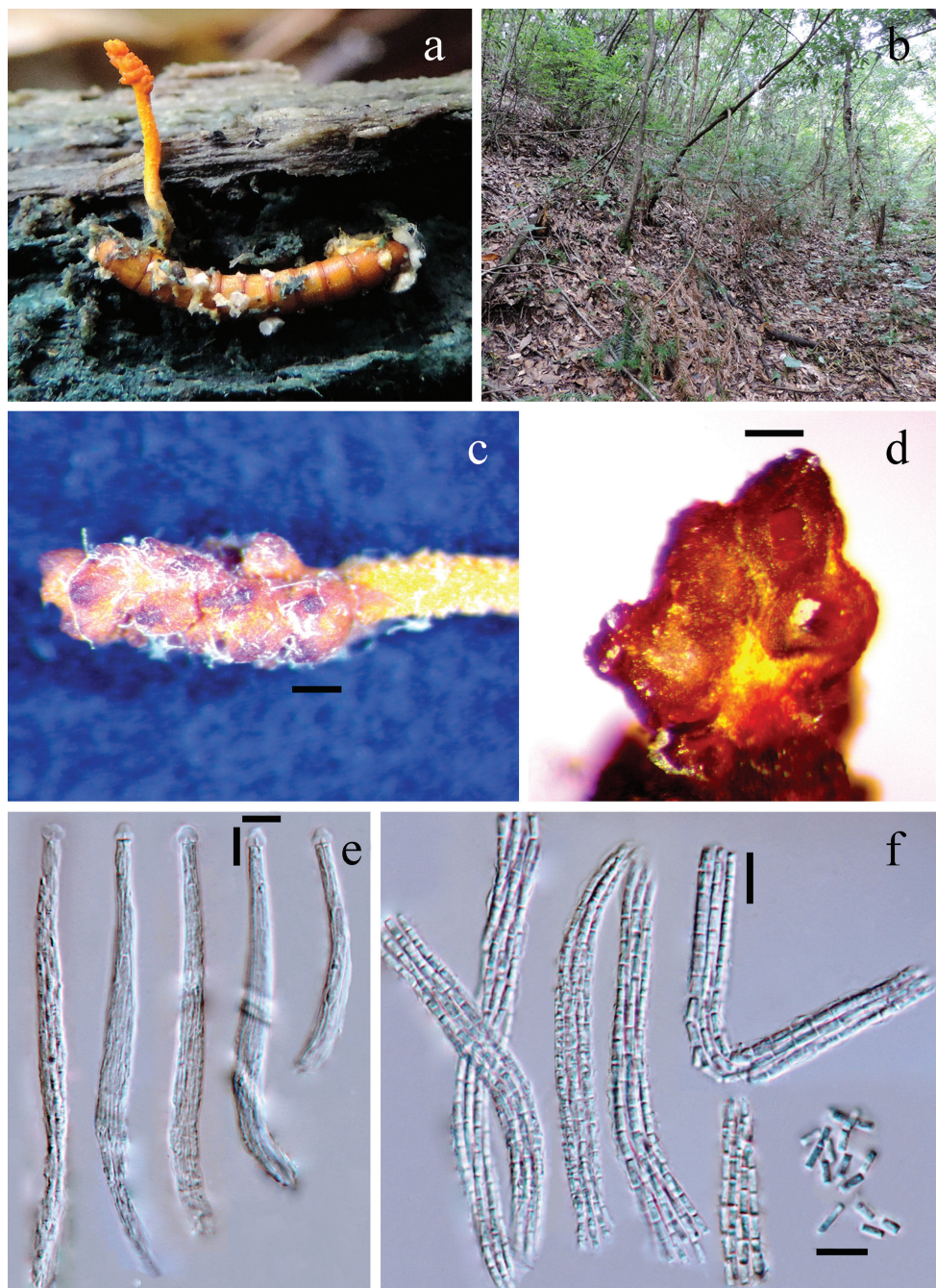


Figure 3. *Ophiocordyceps spicatus* (MFLU 18-0164) **a** infected larva in decayed wood **b** habitat environment **c** fertile head of stroma **d** transverse section of fertile head, on which sparse arranged perithecia are shown **e** Asci **f** Ascospores and part-spores. Scale bars: 200 μm (**c**); 100 μm (**d**) 10 μm (**e**, **f**).

the humps obtuse and with opening ostioles, darker in colour. *Perithecia* partially immersed and obliquely or at right angles to the surface of stipe, broadly pyriform, 200–250 × 170–200 µm; walls 25–35 µm thick. *Asci* cylindrical, 5–9 µm thick, middle part wider than two terminal parts; caps hemispheric, 4.6–5.3 ($\bar{x}$ = 4.9, n = 30) µm wide and 4.0–4.6 ($\bar{x}$ = 4.3, n = 30) µm high. *Ascospores* filiform; part-spores cylindrical, truncated at both ends, 3.5–6.5 ($\bar{x}$ = 4.7, n = 30) µm long and 1.7–2.0 µm thick. **Asexual morph.** Unknown.

Material examined. CHINA, Guizhou Province, Leishan County, Leigongshan Mountain, 26°22'18"N, 108°11'28"E, 1430 m alt., 2 August 2016, Ling-Sheng Zha (MFLU 18-0164, **holotype**).

Known distribution. China (Guizhou).

Host. Growing on a Tenebrionoidea larva (Coleoptera) living in humid and decayed wood in a broad-leaved forest.

Notes. *Ophiocordyceps spicatus* is morphologically somewhat similar to *O. formosana* (Kobayasi and Shimizu 1981; Li et al. 2016), but it has a much smaller stroma (stipes 6–10 (or 19–37) mm long and 1.5–1.7 (or 2–4) mm wide in *O. formosana*), a spicate and rugged fertile head (surface entire and flattened, never spicate or rugged in *O. formosana*) and partially immersed perithecia (immersed in *O. formosana*).

Nucleotide sequences of *O. spicatus* are most similar to those of *O. formosana*, but there is 5.2% bp difference in ITS, 2.0% bp difference in TEF1- α and 0.1% bp difference in SSU (LSU rDNA sequence unavailable for *O. formosana*). LSU of *O. spicatus* is > 5.6% bp different to all LSU available in GeneBank. Additionally, on the phylogenetic tree, *O. spicatus* is closely related (100% ML/100% MP/1.00 PP) to *O. formosana*, but they form into two distinct branches which also support them being two separate species (Fig. 1).

***Polycephalomyces formosus* Kobayasi**

Mycobank No: 289806

Facesoffungi number: FoF04100

Fig. 4

Remarks. *Polycephalomyces formosus* was reported on Coleoptera larvae, stromata of *Ophiocordyceps barnesii* (Thwaites) G.H. Sung et al., *O. falcata* (Berk.) G.H. Sung et al. and *O. cantharelloides* (Samson & H.C. Evans) G.H. Sung et al. and distributed in Ecuador, Japan and Sri Lanka (Kobayasi 1941; Samson and Evans 1985; Wang 2016). We collected a *P. formosus*-like specimen on the stroma of *Ophiocordyceps* sp. on an Elateroidea larva from Guizhou, China. Morphological and phylogenetic data showed that it is *P. formosus*. This is the first report of *P. formosus* on wireworms.

Asexual morph. Growing on the stroma of *Ophiocordyceps* sp. on an Elateroidea larva. Stroma single, arising from the body end of the host larva, unbranched. The larva reddish-brown, cylindrical, 21 × 1.3–1.6 mm, intersegmental membranes conspicuous. Stipe of the stroma shiny black, stiff, band-like, but twisted and deeply wrinkled

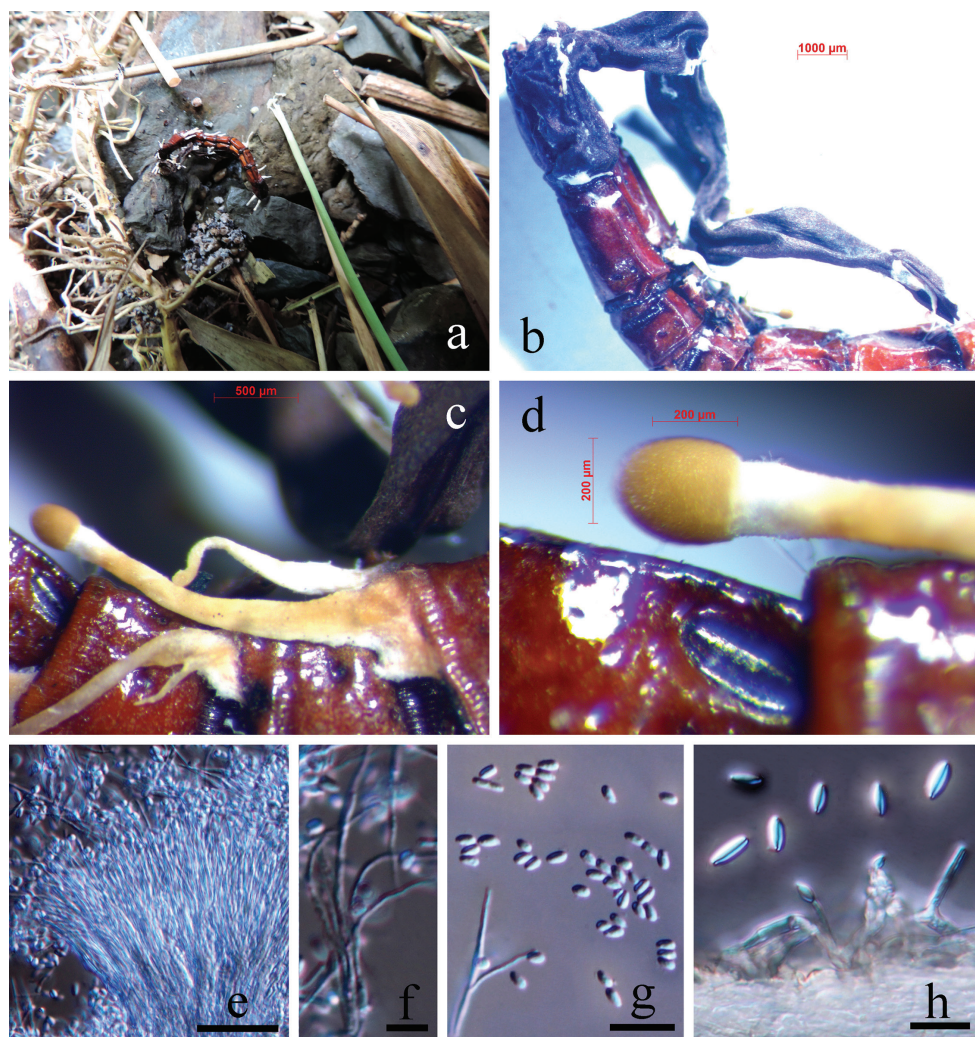


Figure 4. *Polycephalomyces formosus* (MFLU 18-0162) **a** collected on the ground in a bamboo forest **b** produced on the stroma of *Ophiocordyceps* sp. (the fertile head was missing) on an Elateroidea larva **c, d** synnemata **e–g** A-type phialides and A-type conidia **h** B-type phialides and B-type conidia. Scale bars: 20 μm (**e**); 5 μm (**f**); 10 μm (**g, h**).

(dry specimen), more than 20 mm long and 1.0–1.3 mm thick, surface smooth (the fertile head was missing). *Synnemata* solitary or caespitose, arising from the intersegmental membranes of the larva and the surface of the stroma, mostly unbranched, generally straight, capitate, 1–3.5 mm long and 50–600 μm thick. Stipe basally broad and compressed, then gradually cylindrical upwards, white, greyish-white to yellowish-brown, surface smooth. *Fertile head* (including spore mass) abruptly expanded, ellipsoidal, 100–300 $\times$ 80–250 μm , located at the top of every synnemata and distinctly separated from the stipe. Spore mass covers the surface of every fertile head, 15–25 μm

thick, yellowish-brown and composed of hymenia. *Phialides* of two types, A-phialides produced on fertile heads, B-phialides arising laterally along the entire stipe. A-phialides 3–5 in terminal whorl on basal conidiophores, cylindrical to narrowly conical, straight or curved, non-uniform, 10–20 ($\bar{x}$ = 15.1, n = 30) μm long and 1.5–2 μm ($\bar{x}$ = 1.7, n = 30) wide, basally and terminally narrow, neck narrow to 0.5 μm , collarettes and periclinal thickening not visible; *A-conidia* obovate to obpyriform, smooth-walled, hyaline, 2.1–3.2 ($\bar{x}$ = 2.6, n = 30) μm long and 1.5–2.2 ($\bar{x}$ = 1.8, n = 30) μm wide. B-phialides single or in terminal whorls of 2–3 on basal conidiophores, straight, symmetrical or asymmetrical, hyaline, generally cylindrical, 10–25 ($\bar{x}$ = 17, n = 30) μm long, 2–3.5 ($\bar{x}$ = 2.8, n = 30) μm thick at the base, 0.5–0.8 ($\bar{x}$ = 0.65, n = 30) μm thick at the end, collarettes and periclinal thickening not visible; *B-conidia* fusiform, hyaline, smooth-walled, 3.2–6.0 ($\bar{x}$ = 4.6, n = 30) μm long and 1–1.8 ($\bar{x}$ = 1.4, n = 30) μm wide. **Sexual morph.** Not observed.

Material examined. CHINA, Guizhou, Tongzi County, Baiqing Natural Reserve, 28°52'31"N, 107°9'10"E, about 1300 m alt., 13 July 2016, Ling-Sheng Zha (MFLU 18-0162).

Notes. *Polycephalomyces formosus* was originally described from Japan as: growing on Coleoptera larvae; synnemata solitary or caespitose, 1–3.5 mm long and 100–250 μm thick; spore mass covering the surface of the fertile head, 15–25 μm thick; A-phialides 3–4 in terminal whorl on basal conidiophores, cylindrical to narrowly conical, 10–20 $\times$ 1.5–2 μm , neck 0.5 μm ; A-conidia obovate to obpyriform, 2.0–2.8 $\times$ 1.6–2.0 μm ; B-conidia fusiform, 3.2–4.8 $\times$ 0.8–1.6 μm (Kobayasi 1941; Wang 2016). These characteristics are all consistent with our specimen. Sequences of SSU, ITS, LSU and TEF1- α are all identical to those of *P. formosus* (specimen ARSEF 1424); and in our phylogenetic tree, these two samples grouped together and have a same branch length (Fig. 1).

Host and ecology. On the stroma of *Ophiocordyceps* sp. on an Elateroidea larva on the ground in a humid bamboo (*Chimonobambusa quadrangularis* (Franceschi) Makino) forest in Guizhou karst regions.

The larva might live in soil or decayed wood at first, but was then infected by *Ophiocordyceps* sp. and produced a sexual stroma. Following heavy rainfall, the host, together with the stroma of *Ophiocordyceps* sp., was washed away and exposed on the ground and at last, was parasitised by *Polycephalomyces formosus*. The fertile head of the stroma might have been lost during the floods.

Annotated list of recorded *Cordyceps* s.l. species developing on wireworms

Order Hypocreales Lindau

Family Cordycipitaceae Kreisel ex G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora

Akanthomyces lecanii (Zimm.) Spatafora, Kepler & B. Shrestha

≡ *Cephalosporium lecanii* Zimm.

≡ *Verticillium lecanii* (Zimm.) Viégas

≡ *Lecanicillium lecanii* (Zimm.) Zare & W. Gams
 = *Cephalosporium lecanii* f. *coccorum* (Petch) Balazy
 = *Sporotrichum lichenicola* Berk. & Broome
 = *Hirsutella confragosa* Mains
 = *Torrubiella confragosa* Mains
 = *Cordyceps confragosa* (Mains) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora
 = *Cephalosporium coccorum* Petch
 = *Verticillium coccorum* (Petch) Westerd.
 = *Cephalosporium coccorum* var. *uredinis* U.P. Singh & Pavgi
 = *Cephalosporium subclavatum* Petch

For further doubtful synonyms, see Zare and Gams (2001).

Hosts. Spiders, insects from various orders, including Coleoptera (e.g. Tenebrionidae: *Alphitobius diaperinus*); inhabiting phytopathogenic fungi and plant-parasitic nematodes (Humber and Hansen 2005; Shinya et al. 2008).

Distribution. Widely distributed in tropical and temperate regions, for example: Dominican Republic, Jamaica, Indonesia, Peru, Sri Lanka, the West Indies, Turkey and USA (Zare and Gams 2001).

Notes. The species was originally and frequently reported on scale insects (Hemiptera: Coccidae (syn. Lecaniidae)) (Zare and Gams 2001). Humber and Hansen (2005) listed its hosts involving spiders, many insect orders and found on the mushroom *Puccinia striiformis* (Pucciniaceae). The species was also found on phytopathogenic fungi and plant-parasitic nematodes (Shinya et al. 2008). Zare and Gams (2001) systematically studied the species and listed its synonyms. Kepler et al. (2017) rejected *Torrubiella* and *Lecanicillium* and transferred the species to *Akanthomyces*.

Beauveria bassiana sensu lato

Hosts. Many insect orders, including Coleoptera (e.g. Elateroidea and Tenebrionoidea spp., Humber and Hansen 2005; Reddy et al. 2014; Sufyan et al. 2017); inhabiting soil, plant surfaces and plant internal tissues (Bamisile et al. 2018).

Distribution. Widely distributed.

Note. *Beauveria bassiana* sensu lato includes a large complex of cryptic species with wide host ranges, including many Coleoptera families (Rehner et al. 2011; Imoulan et al. 2017).

Cordyceps aurantiaca Lohwag

Hosts. Elateridae larvae (Keissler and Lohwag 1937).

Known distribution. China (Keissler and Lohwag 1937).

Note. Taxonomically uncertain species which was described from the previous *Cordyceps* Fr. (differs from the current *Cordyceps* Fr. emend. G.H. Sung et al., same as below).

***Cordyceps chiangdaoensis* Tasanathai, Thanakitpipattana, Khonsanit & Luangsa-ard**

Hosts. Elateroidea or Tenebrionoidea larvae.

Known distribution. Thailand (Tasanathai et al. 2016).

Note. Hosts of the species were recorded as Coleoptera larvae (Tasanathai et al. 2016). According to the picture provided, the hosts are wireworms.

***Cordyceps chishuiensis* Z.Q. Liang & A.Y. Liu**

Host. Elateroidea or Tenebrionoidea larva.

Known distribution. China (Guizhou) (Liang 2007).

Notes. Taxonomically uncertain species from the previous *Cordyceps*. The species was originally reported on a wireworm (Liang 2007).

***Cordyceps farinosa* (Holmsk.) Kepler, B. Shrestha & Spatafora**

≡ *Ramaria farinosa* Holmsk.

≡ *Clavaria farinosa* (Holmsk.) Dicks.

≡ *Corynoides farinosa* (Holmsk.) Gray

≡ *Isaria farinosa* (Holmsk.) Fr.

≡ *Spicaria farinosa* (Holmsk.) Vuill.

≡ *Penicillium farinosum* (Holmsk.) Biourge

≡ *Paecilomyces farinosus* (Holmsk.) A.H.S. Br. & G. Sm.

For further doubtful synonyms, see Index Fungorum (2021).

Hosts. Mites, spiders, insects from various orders, including Coleoptera (e.g. Tenebrionidae spp.); inhabiting soil, humus, plants, fungi and other organisms (Humber and Hansen 2005; Zimmermann 2008).

Distribution. Widely distributed (Zimmermann 2008).

Note. According to Domsch et al. (1980) and Zimmermann (2008), the species is ubiquitous in temperate and tropical zones.

***Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora**

≡ *Isaria fumosorosea* Wize

≡ *Spicaria fumosorosea* (Wize) Vassiljevsky

≡ *Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm.

= *Paecilomyces fumosoroseus* var. *beijingensis* Q.X. Fang & Q.T. Chen

Hosts. Mites, insects from various orders (e.g. Lagriidae and Tenebrionidae spp. in Tenebrionoidea) (Humber and Hansen 2005; Zimmermann 2008).

Distribution. Widely distributed (Zimmermann 2008).

Note. The species was previously confused with *C. farinosa* or regarded as a complex species (Zimmermann 2008).

Cordyceps huntii Giard [as ‘hunti’, ‘lunti’]

Host. Elateridae larva (Massee 1899).

Known distribution. Gaul (Massee 1899).

Notes. Taxonomically uncertain species from the previous *Cordyceps*. Sung et al. (2007) treated it as a synonym of *Nigelia martiale* ($\equiv$ *C. martialis*).

Cordyceps militaris (L.) Fr.

$\equiv$ *Clavaria militaris* L.

$\equiv$ *Sphaeria militaris* (L.) J.F. Gmel.

$\equiv$ *Hypoxylon militare* (L.) Mérat

$\equiv$ *Xylaria militaris* (L.) Gray

$\equiv$ *Corynesphaera militaris* (L.) Dumort.

$\equiv$ *Torrubia militaris* (L.) Tul. & C. Tul.

= *Clavaria granulosa* Bull.

= *Sphaeria militaris* var. *sphaerocephala* J.C. Schmidt

= *Cordyceps militaris* f. *sphaerocephala* (J.C. Schmidt) Sacc.

= *Cordyceps militaris* f. *alba* Kobayasi & Shimizu ex Y.J. Yao [as ‘albina’]

Hosts. Commonly on Lepidoptera larvae and pupae, infrequently on Hymenoptera (Kobayasi 1941; Kryukov et al. 2011).

Distribution. Widely distributed.

Note. Under laboratory conditions and injection of hyphal bodies into the haemocoel of insects, *C. militaris* can infect many insect orders (Shrestha et al. 2012), including pupae of *Tenebrio molitor* (Tenebrionidae) (De Bary 1867; Sato and Shimazu 2002). Therefore, the conclusion that wireworms (e.g. *Tenebrio molitor*) are the natural hosts of *C. militaris* is probably untenable and we temporarily reject it.

Cordyceps nanatakiensis Kobayasi & Shimizu

Host. Tenebrionidae larva (Shimizu 1997).

Known distribution. Japan (Kobayasi and Shimizu 1983).

Notes. Taxonomically uncertain species from the previous *Cordyceps*. Its host was originally recorded as a Coleoptera larva (Kobayasi and Shimizu 1983) and then Shimizu (1997) identified it as a Tenebrionidae larva.

***Cordyceps nirtolii* Negi, Koranga, Ranj. Singh & Z. Ahmed**

Host. Larva of Elateridae (*Melanotus communis* (Gyllenhal)).

Known distribution. India (Himalaya) (Negi et al. 2012).

Note. Host of the species was recorded as a larva of *Melanotus communis* (Negi et al. 2012). *Melanotus communis* (Gyllenhal) represents an Elateridae insect, while *Melanotus communis* E. Horak is a mushroom (Agaricales: Strophariaceae).

***Cordyceps roseostromata* Kobayasi & Shimizu**

Host. Tenebrionidae larva (Shimizu 1997).

Known distribution. Japan (Kobayasi and Shimizu 1983).

Note. Host of the species was originally recorded as a Coleoptera larva (Kobayasi and Shimizu 1983) and then Shimizu (1997) identified it as a Tenebrionidae larva.

***Cordyceps rubiginosistipitata* Kobayasi & Shimizu [as ‘rubiginosostipitata’]**

Host. Tenebrionoidea or Elateroidea larva.

Known distribution. Japan (Kobayasi and Shimizu 1983).

Note. Taxonomically uncertain species from the previous *Cordyceps*. Its host was recorded as a Coleoptera larva (Kobayasi and Shimizu 1983; Shimizu 1997). According to the illustration by Shimizu (1997), the host is a wireworm.

***Cordyceps rubra* Möller**

Host. Elateridae larva (Möller 1901).

Known distribution. Brazil (Möller 1901).

Note. Taxonomically uncertain species from the previous *Cordyceps*.

***Cordyceps shanxiensis* B. Liu, Rong & H.S. Jin**

Hosts. Elateridae larvae (*Melanotus caudex*? and *Pleonomus canaliculatus*?) (Liu et al. 1985).

Known distribution. China (Shanxi) (Liu et al. 1985).

Notes. Taxonomically uncertain species from the previous *Cordyceps*. According to the original description, the species is morphologically similar to *Paraisaria gracilis* (Grev.) Luangsa-ard et al. on Lepidoptera larvae. Notably, the two host names provided by Liu et al. (1985) cannot be retrieved in GBIF (2021).

***Cordyceps submilitaris* Henn.**

Hosts. Elateroidea or Tenebrionoidea larvae.

Known distribution. South America (Petch 1933).

Notes. Taxonomically uncertain species from the previous *Cordyceps*. Hosts of the species were recorded as beetle larvae in rotten wood (Petch 1933). Petch (1933) considered the species as a synonym of *Nigelia martiale* ($\equiv$ *C. martialis*). According to the information given by Petch (1933), hosts of the species are wireworms.

***Cordyceps velutipes* Masee**

Hosts. Larvae of Elateridae and Scarabaeidae (*Melolontha* sp.) (Masee 1895; Moureau 1949).

Known distribution. Africa (Masee 1895).

Note. Taxonomically uncertain species from the previous *Cordyceps*.

Family Clavicipitaceae (Lindau) Earle ex Rogerson, emend. G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora

***Metarhizium anisopliae* species complex**

Hosts. More than seven insect orders, including Coleoptera (e.g. Elateridae and Tenebrionidae spp., Kabaluk et al. 2005, 2017; Humber and Hansen 2005; Reddy et al. 2014); inhabiting soil, plant surfaces and plant internal tissues (Hu et al. 2014; Bami-sile et al. 2018; Brunner-Mendoza et al. 2019).

Distribution. Widely distributed.

Note. *Metarhizium anisopliae* species complex includes several cryptic species, for example, *M. anisopliae* (Metschn.) Sorokin, *M. brunneum* Petch and *M. robertsii* J.F. Bisch., S.A. Rehner & Humber (Bischoff et al. 2009; Kepler et al. 2014; Mongkol-samrit et al. 2020). Amongst them, *M. brunneum* was most often noted as a wireworm pathogen (e.g. Kabaluk et al. 2017).

***Metarhizium atrovirens* (Kobayasi & Shimizu) Kepler, S.A. Rehner & Humber**

$\equiv$ *Cordyceps atrovirens* Kobayasi & Shimizu

$\equiv$ *Metacordyceps atrovirens* (Kobayasi & Shimizu) Kepler, G.H. Sung & Spatafora

Hosts. Tenebrionidae larvae (Shimizu 1997).

Known distribution. Japan (Kobayasi and Shimizu 1978; Shimizu 1997).

Note. Hosts of the species were originally recorded as Coleoptera larvae (Kobayasi and Shimizu 1978) and then Shimizu (1997) identified them as Tenebrionidae larvae.

Metarhizium brachyspermum* Koh, Yamam., Ohmae & Orihara*Hosts.** Elateridae larvae and pupae (Yamamoto et al. 2020).**Known distribution.** Japan (Yamamoto et al. 2020).***Metarhizium camposterni* (W.M. Zhang & T.H. Li) Kepler, S.A. Rehner & Humber**≡ *Cordyceps camposterni* W.M. Zhang & T.H. Li [as 'camposterna']≡ *Metacordyceps camposterni* (W.M. Zhang & T.H. Li) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**Hosts.** Larva and adult of *Camposternus auratus* (Elateridae) (Zhang et al. 2004).**Known distribution.** China (Guangdong) (Zhang et al. 2004).***Metarhizium clavatum* Luangsa-ard, Mongkolsamrit, Lamlertthon, Thanakitpattana & Samson****Hosts.** Elateridae (*Oxyntopterus*) larvae (Mongkolsamrit et al. 2020).**Known distribution.** Thailand (Mongkolsamrit et al. 2020).***Metarhizium flavum* Luangsa-ard, Mongkolsamrit, Thanakitpattana & Samson****Hosts.** Tenebrionoidea or Elateroidea larvae.**Known distribution.** Thailand (Mongkolsamrit et al. 2020).**Note.** Hosts of the species were originally recorded as Coleoptera larvae (Mongkolsamrit et al. 2020). According to the illustration and the information provided, the hosts are wireworms.***Metarhizium kalasinense* Tasan., Khons., Thanakitp., Mongkols. & Luangsa-ard****Hosts.** Elateroidea larvae.**Known distribution.** Thailand (Luangsa-ard et al. 2017).**Note.** Hosts of the species were originally recorded as elaterid larvae (Coleoptera) (Luangsa-ard et al. 2017).

***Metarhizium pseudoatrovirens* (Kobayasi & Shimizu) Kepler, S.A. Rehner & Humber**

≡ *Cordyceps pseudoatrovirens* Kobayasi & Shimizu

≡ *Metacordyceps pseudoatrovirens* (Kobayasi & Shimizu) Kepler, G.H. Sung & Spatafora

Hosts. Larvae of Tenebrionoidea and/or Elateroidea (Shimizu 1997; Liang 2007).

Known distribution. China (Guizhou), Japan (Kobayasi and Shimizu 1982b; Liang 2007).

Notes. The host of the species was originally recorded as a Coleoptera larva (Kobayasi and Shimizu 1982b), then Shimizu (1997) identified it as a Tenebrionidae larva. Liang (2007) recorded the species with pictures (four specimens) and wireworm hosts.

***Metarhizium purpureonigrum* Luangsa-ard, Tسانathai, Thanakitpipattana & Samson**

Hosts. Elateridae larvae (*Campsosternus* sp.).

Known distribution. Thailand (Mongkolsamrit et al. 2020).

Notes. According to the description and pictures provided (Mongkolsamrit et al. 2020), the species is probably a synonym of *O. jiangxiensis*, a traditional Chinese medicinal mushroom (Zha et al. 2018, also see *O. jiangxiensis* below). Hosts of the species, which were recorded as Coleoptera larvae, are Elateridae larvae (*Campsosternus* sp.).

***Metarhizium purpureum* Luangsa-ard, Mongkolsamrit, Lamlertthon Thanakitpipattana & Samson**

Hosts. Elateridae (*Oxyntopterus*) larvae (Mongkolsamrit et al. 2020).

Known distribution. Thailand (Mongkolsamrit et al. 2020).

***Nigelia martiale* (Speg.) Luangsa-ard & Thanakitp.**

≡ *Cordyceps martialis* Speg.

≡ *Metacordyceps martialis* (Speg.) Kepler, G.H. Sung & Spatafora

≡ *Metarhizium martiale* (Speg.) Kepler, S.A. Rehner & Humber

Hosts. Larvae of Coleoptera (e.g. Elateridae, Shrestha et al. 2016; Cerambycidae, Spegazzini 1889) and Lepidoptera (Liang 2007; Kepler et al. 2012).

Known distribution. Brazil, China (Guangdong, Zhejiang, Taiwan), the West Indies (Kobayasi 1941; Liang 2007).

Family Ophiocordycipitaceae G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora

***Ophiocordyceps acicularis* (Ravenel) Petch**

≡ *Cordyceps acicularis* Ravenel

Hosts. Elateridae larvae (Shimizu 1997).

Known distribution. China (Jiangsu, Guangdong, Guizhou, Hainan, Taiwan), Japan, Russia (Far East), U.S.A. (Carolina) (Massee 1895; Kobayasi and Shimizu 1980a, Koval 1984; Liang 2007).

Note. Hosts of the species were generally identified as wireworms or Coleoptera larvae (Kobayasi and Shimizu 1980a, Liang 2007). Shimizu (1997) identified the hosts of the species from Japan and Taiwan as Elateridae larvae.

***Ophiocordyceps agriotis* (Kawam.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora [as ‘agriotidis’]**

≡ *Cordyceps agriota* Kawam. [as ‘agriotidis’ in Index Fungorum (2021)]

Hosts. Elateridae (e.g. *Agriotes*) larvae (Kobayasi and Shimizu 1980a, Shimizu 1997).

Known distribution. China (Guizhou, Jilin), Japan (Kobayasi and Shimizu 1980a, Yang 2004; Liang 2007).

Notes. The specific epithet of this species was adopted from the generic name of its host insect ‘*Agriotes*’ (Kobayasi and Shimizu 1980a). The epithet ‘agriotidis’, used in Index Fungorum (2021) and related literature (e.g. Sung et al. 2007), is incorrect. Yang (2004) and Liang (2007) also recorded its hosts as Elateridae larvae.

***Ophiocordyceps annulata* (Kobayasi & Shimizu) Spatafora, Kepler & C.A. Quandt [as ‘annulata’ in Index Fungorum (2021)]**

≡ *Cordyceps annulata* Kobayasi & Shimizu [as ‘annulata’ in Index Fungorum (2021)]

Host. Tenebrionoidea or Elateroidea larva.

Known distribution. Japan (Kobayasi and Shimizu 1982a).

Note. Host of the species was originally recorded as a Coleoptera larva (Kobayasi and Shimizu 1982a). According to the illustration by Shimizu (1997), the host is a wireworm.

***Ophiocordyceps appendiculata* (Kobayasi & Shimizu) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Cordyceps appendiculata* Kobayasi & Shimizu

Host. Tenebrionidae larva (Shimizu 1997).

Known distribution. Japan (Kobayasi and Shimizu 1983).

Note. Host of the species was originally recorded as a Coleoptera larva (Kobayasi and Shimizu 1983). Shimizu (1997) identified it as a Tenebrionidae larva.

***Ophiocordyceps asyuensis* (Kobayasi & Shimizu) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora [as ‘asyuënsis’]**

≡ *Cordyceps asyuensis* Kobayasi & Shimizu

Hosts. Elateroidea or Tenebrionoidea larva.

Known distribution. Japan (Kobayasi and Shimizu 1980b).

Note. Host of the species was originally recorded as a Coleoptera larva (Kobayasi and Shimizu 1980b). According to the illustration by Shimizu (1997), the host is a wireworm.

***Ophiocordyceps brunneipunctata* (Hywel-Jones) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Cordyceps brunneipunctata* Hywel-Jones [as ‘brunneapunctata’]

Hosts. Elateridae larvae (Hywel-Jones 1995).

Known distribution. Thailand (Hywel-Jones 1995).

***Ophiocordyceps clavata* (Kobayasi & Shimizu) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Cordyceps clavata* Kobayasi & Shimizu

Hosts. Tenebrionidae larvae (Shimizu 1997).

Known distribution. Japan (Shimizu 1997).

Note. The host of the species was originally recorded as a Coleoptera larva (Kobayasi and Shimizu 1980b). Shimizu (1997) identified the hosts of the species as Tenebrionidae larvae.

***Ophiocordyceps elateridicola* (Kobayasi & Shimizu) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Cordyceps elateridicola* Kobayasi & Shimizu

Host. Elateridae larvae (Kobayasi and Shimizu 1983; Shimizu 1997).

Known distribution. China (Taiwan), Japan (Shimizu 1997).

***Ophiocordyceps entomorrhiza* (Dicks.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Sphaeria entomorrhiza* Dicks.

≡ *Xylaria entomorrhiza* (Dicks.) Gray

≡ *Cordyceps entomorrhiza* (Dicks.) Fr.

= *Isaria eleutheratorum* Nees

= *Torrubia cinerea* Tul. & C. Tul.

= *Cordyceps cinerea* (Tul. & C. Tul.) Sacc.

= *Cordyceps meneristitis* F. Muell. & Berk. [as 'menesteridis']

= *Cordyceps entomorrhiza* var. *meneristitis* (F. Muell. & Berk.) Cooke [as 'mesenteridis']

= *Cordyceps carabi* Quél.

= *Tilachlidiopsis nigra* Yakush. & Kumaz.

= *Hirsutella eleutheratorum* (Nees) Petch

Hosts. Larvae and adults of many Coleoptera families, for example, Tenebrionidae larva (Shrestha et al. 2016) and Lampyridae larvae.

Distribution. Widely distributed.

Note. According to the illustrations by Shimizu (1997), we identify the hosts of the species from Japan as Lampyridae larvae (Elateroidea).

***Ophiocordyceps falcatoidea* (Kobayasi & Shimizu) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Cordyceps falcatoidea* Kobayasi & Shimizu

Host. Tenebrionoidea or Elateroidea larva.

Known distribution. Japan (Kobayasi and Shimizu 1980a).

Note. Host of the species was originally recorded as a Coleoptera larva (Kobayasi and Shimizu 1980a). According to the illustration by Shimizu (1997), the host is a wireworm.

***Ophiocordyceps ferruginosa* (Kobayasi & Shimizu) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Cordyceps ferruginosa* Kobayasi & Shimizu

Hosts. Xylophagidae larvae (Diptera).

Known distribution. Japan (Kobayasi and Shimizu 1980b).

Notes. Hosts of the species were originally identified as Coleoptera larvae living in decayed wood (Kobayasi and Shimizu 1980b, Shimizu 1997). According to the illustrations by Shimizu (1997), the hosts are actually Diptera (Xylophagidae) larvae. Considering the very similar morphology and the same hosts between *O. ferruginosa* and *O. variabilis*, the former might be a synonym of the latter (see notes of *O. variabilis* below). As a result, *O. ferruginosa* is not a pathogen of wireworms.

***Ophiocordyceps formosana* (Kobayasi & Shimizu) Yen W. Wang, S.H. Tsai, Tzean & T.L. Shen**

≡ *Cordyceps formosana* Kobayasi & Shimizu

Hosts. Tenebrionoidea larvae (Li et al. 2002, 2016).

Known distribution. China (Anhui, Fujian, Hunan, Taiwan) (Kobayasi and Shimizu 1981; Li et al. 2002, 2016).

Notes. The host of the species was originally recorded as a Coleoptera larva (Kobayasi and Shimizu 1981). According to the illustration by Shimizu (1997), it appears to be a Tenebrionoidea larva. Li et al. (2002) identified the host of their collection as a Tenebrionidae larva. We cautiously identify these hosts as Tenebrionoidea larvae (used in Li et al. 2016).

***Ophiocordyceps jiangxiensis* (Z.Q. Liang, A.Y. Liu & Yong C. Jiang) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Cordyceps jiangxiensis* Z.Q. Liang, A.Y. Liu & Yong C. Jiang

Hosts. Elateridae larvae (*Campsosternus* sp.) (Liang et al. 2001; Zha et al. 2018).

Known distribution. China (Jiangxi, Fujian, Yunnan) (Zha et al. 2018).

Notes. The species was originally described by Liang et al. (2001) with specimens from Jiangxi, China. Sung et al. (2007) revised it to *O. jiangxiensis* only based on the original morphological description. The species is closely similar to *Metarhizium purpleonigrum*, a recently-described species from Thailand (Mongkolsamrit et al. 2020). Future studies are warranted to clarify its taxonomic placement.

***Ophiocordyceps larvicola* (Quél.) Van Vooren**

≡ *Cordyceps larvicola* Quél.

Hosts. Larvae of Cerambycidae, Scarabaeidae and Tenebrionidae (e.g. *Cylindronotus* sp., *Helops* spp.) (Kobayasi 1941; Shrestha et al. 2016).

Known distribution. France (Kobayasi 1941), the European part of Russia (Koval 1984).

***Ophiocordyceps melolonthae* (Tul. & C. Tul.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Torrubia melolonthae* Tul. & C. Tul.

≡ *Cordyceps melolonthae* (Tul. & C. Tul.) Sacc.

= *Cordyceps rickii* Lloyd

= *Cordyceps melolonthae* var. *rickii* (Lloyd) Mains

= *Ophiocordyceps melolonthae* var. *rickii* (Lloyd) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora

Hosts. Scarabaeidae larvae (Shrestha et al. 2016), Elateridae larvae (Shimizu 1997).

Distribution. North, Central and South America, the West Indies (Kobayasi 1941; Mains 1958), Japan (Shimizu 1997), Belarus, the Russian Far East (Koval 1984).

***Ophiocordyceps nigripoda* (Kobayasi & Shimizu) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora [as ‘nigripes’]**

≡ *Cordyceps nigripoda* Kobayasi & Shimizu

Host. Elateroidea or Tenebrionoidea larva.

Known distribution. Japan (Kobayasi and Shimizu 1982b).

Note. Host of the species was originally recorded as a Coleoptera larva (Kobayasi and Shimizu 1982b). According to the illustration by Shimizu (1997), the host is a wireworm.

***Ophiocordyceps purpureostromata* (Kobayasi) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Cordyceps purpureostromata* Kobayasi

= *Cordyceps purpureostromata* f. *recurvata* Kobayasi

= *Ophiocordyceps purpureostromata* f. *recurvata* (Kobayasi) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora

Hosts. Elateridae larvae (Shimizu 1997).

Known distribution. Japan (Kobayasi and Shimizu 1980b).

***Ophiocordyceps rubiginosiperithecata* (Kobayasi & Shimizu) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Cordyceps rubiginosiperithecata* Kobayasi & Shimizu [as ‘rubiginosoperithecata’]

Hosts. Elateroidea or Tenebrionoidea larvae.

Known distribution. Japan (Shimizu 1997).

Note. The host of the species was originally recorded as a Coleoptera larva (Kobayasi and Shimizu 1983). According to the illustration by Shimizu (1997), hosts of the species are wireworms.

***Ophiocordyceps rubripunctata* (Moreau) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Cordyceps rubripunctata* Moreau

= *Hirsutella rubripunctata* Samson, H.C. Evans & Hoekstra

Hosts. Elateridae larvae (Samson et al. 1982).

Known distribution. Congo, Ghana (Samson et al. 1982).

***Ophiocordyceps salebrosa* (Mains) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Cordyceps salebrosa* Mains

Host. Elateridae adult (Mains 1947).

Known distribution. Panama Canal Zone (Barro Colorado Island) (Mains 1947).

Note. Notably, the host of the species is an adult.

***Ophiocordyceps sporangifera* Y.P. Xiao, T.C. Wen & K.D. Hyde**

Host. Elateroidea or Tenebrionoidea larva.

Known distribution. Thailand (Xiao et al. 2019).

Note. The host of the species was originally identified as an Elateridae larva (Xiao et al. 2019).

***Ophiocordyceps stylophora* (Berk. & Broome) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Cordyceps stylophora* Berk. & Broome
= *Hirsutella stylophora* Mains

Hosts. Larvae of Coleoptera (Cerambycidae, Elateridae, Scarabaeidae) (Shrestha et al. 2016).

Known distribution. Canada (Nova Scotia), China (Guangxi, Jilin, Zhejiang), Japan, Russia (Far East), U.S.A. (Carolina) (Kobayasi 1941; Mains 1941; Koval 1984; Liang 2007).

Note. Liang (2007) recorded the hosts of the species as Lepidoptera larvae, but his provided picture (a specimen collected from Jilin, China) appears to be a wireworm host.

***Ophiocordyceps subflavida* (Mains) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Cordyceps albida* Pat. & Gaillard
≡ *Cordyceps subflavida* Mains

Hosts. Elateridae larvae (Shimizu 1997).

Known distribution. Japan (Shimizu 1997), Venezuela (Mains 1959).

Note. The species was originally reported from Venezuela and its host was recorded as an insect larva (Mains 1959). Shimizu (1997) identified the host of a specimen from Japan as an Elateridae larva.

***Ophiocordyceps variabilis* (Petch) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora**

≡ *Cordyceps variabilis* Petch
= *Cordyceps viperina* Mains

Hosts. Xylophagidae larvae (Diptera) (Hodge et al. 1998; Yaroslavtseva et al. 2019).

Known distribution. China (Shaanxi), Europe, Russia (Far East, Western Siberia), North America (Petch 1937; Liang 2007; Hodge et al. 1998; Yaroslavtseva et al. 2019).

Notes. In early literature, *O. variabilis* was recorded on Coleoptera (e.g. Elateridae) and Diptera larvae in rotten wood (Petch 1937; Mains 1958; Liang 2007). Hodge et al. (1998) checked many samples and confirmed the hosts to be Xylophagidae larvae (Diptera). More than 40 samples of *O. variabilis* were collected in Russia (Far East, Western Siberia) and all of them developed on Xylophagidae larvae (Yaroslavtseva et al. 2019; Kryukov et al., unpublished). Ecological habits and morphology of Xylophagidae larvae and wireworms are closely similar, but their last abdominal segments are distinctly different. As with *O. ferruginosa* listed above, we conclude that *O. variabilis* is not a pathogen of wireworms.

***Paraisaria gracilioides* (Kobayasi) C.R. Li, M.Z. Fan & Z.Z. Li**

≡ *Isaria gracilioides* Kobayasi
 = *Cordyceps gracilioides* Kobayasi
 = *Ophiocordyceps gracilioides* (Kobayasi) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora
 = *Paraisaria gracilioides* (Kobayasi) Luangsa-ard, Mongkolsamrit & Samson, **syn. nov.**

Hosts. Elateridae larvae (Shimizu 1997; Yahagi 2008).

Known distribution. China (Anhui, Fujian), Japan, Russia (Far East) (Kobayasi 1941; Koval 1984; Liang 2007).

Notes. The species is similar to *Paraisaria gracilis* (Grev.) Luangsa-ard et al., but the former grows on Coleoptera larvae (Elateridae), while the latter on Lepidoptera larvae (Kobayasi 1941; Yahagi 2008). Hosts of the sexual *C. gracilioides* and its asexual *Isaria gracilioides* were both originally mistakenly identified as Cossidae larvae (Lepidoptera instead of Coleoptera) (Kobayasi 1941). Fan et al. (2001) collected a sexual specimen of the species on a Coleoptera larva (wireworm); Li et al. (2004) successfully isolated its asexual morph and revised the asexual *Isaria gracilioides* to the asexual *Paraisaria gracilioides* (Kobayasi) C.R. Li et al., linked with the sexual *C. gracilioides*. Later, the sexual *C. gracilioides* has been revised in an orderly manner to *O. gracilioides* (Sung et al. 2007) and *Paraisaria gracilioides* (Kobayasi) Luangsa-ard et al. (Mongkolsamrit et al. 2019). Considering the rules of priority and one fungus, one name (Kepler et al. 2013), we combine *Paraisaria gracilioides* (Kobayasi) Luangsa-ard et al. with *Paraisaria gracilioides* (Kobayasi) C.R. Li et al.

***Paraisaria phuwiangensis* Mongkolsamrit, Noisripoom, Himaman, Jangsantear & Luangsa-ard**

Hosts. Elateridae larvae (Mongkolsamrit et al. 2019).

Known distribution. Thailand (Mongkolsamrit et al. 2019).

***Paraisaria yodhathaii* Mongkolsamrit, Noisripoom, Lamlerthon & Luangsa-ard**

Hosts. Elateridae larva (Mongkolsamrit et al. 2019).

Known distribution. Thailand (Mongkolsamrit et al. 2019).

***Perennicordyceps cuboidea* (Kobayasi & Shimizu) Matočec & I. Kušan**

≡ *Cordyceps cuboidea* Kobayasi & Shimizu
 ≡ *Ophiocordyceps cuboidea* (Kobayasi & Shimizu) S. Ban, Sakane & Nakagiri
 ≡ *Polycephalomyces cuboideus* (Kobayasi & Shimizu) Kepler & Spatafora
 = *Cordyceps alboperitheciata* Kobayasi & Shimizu

Hosts. Tenebrionoidea and/or Elateroidea larvae (Shimizu 1997; Ban et al. 2009); stroma of *O. stylophora* (Ban et al. 2009).

Known distribution. Japan (Kobayasi and Shimizu 1980b).

Note. The host of the species was originally recorded as a Coleoptera larva (Kobayasi and Shimizu 1980b). According to the illustrations by Shimizu (1997) and Ban et al. (2009), hosts of the species are wireworms.

***Perennicordyceps ryogamiensis* (Kobayasi & Shimizu) Matočec & I. Kušan**

≡ *Cordyceps ryogamiensis* Kobayasi & Shimizu

≡ *Ophiocordyceps ryogamiensis* (Kobayasi & Shimizu) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora

≡ *Polycephalomycetes ryogamiensis* (Kobayasi & Shimizu) Kepler & Spatafora

Host. Tenebrionoidea larva.

Known distribution. Japan (Kobayasi and Shimizu 1983).

Note. Host of the species was originally recorded as a Coleoptera larva (Kobayasi and Shimizu 1983). According to the illustration by Shimizu (1997), the host is a Tenebrionoidea larva.

***Polycephalomycetes phaothaiensis* Mongkols., Noisrip., Lamlertthon & Luangsa-ard**

Hosts. Tenebrionoidea or Elateroidea larvae.

Known distribution. Thailand (Crous et al. 2017).

Note. Hosts of the species were recorded as Coleoptera larvae (Crous et al. 2017). According to the picture provided, the hosts are wireworms.

***Tolypocladium cylindrosporum* W. Gams**

≡ *Beauveria cylindrospora* (W. Gams) Arx

Hosts. Coleoptera (e.g. Elateridae sp.), Diptera, Hymenoptera and Lepidoptera (Humber and Hansen 2005); inhabit soil (Scorsetti et al. 2012).

Distribution. Widely distributed.

***Tolypocladium inflatum* W. Gams**

= *Pachybasium niveum* O. Rostr.

= *Tolypocladium niveum* (O. Rostr.) Bissett

- = *Cordyceps subsessilis* Petch
- = *Elaphocordyceps subsessilis* (Petch) G.H. Sung, J.M. Sung & Spatafora
- = *Cordyceps facis* Kobayasi & Shimizu [as ‘Codyceps’]

Hosts. Tenebrionidae larvae (Shimizu 1997).

Distribution. Widely distributed (Petch 1937; Kobayasi 1982; Sung et al. 2007).

Note. Hosts of the species were previously recorded as Coleoptera larvae (Petch 1937; Kobayasi 1982). Shimizu (1997) identified them as Tenebrionidae larvae.

Discussion

The superfamilies Elateroidea and Tenebrionoidea are two very large groups of beetles and comprise more than 50 families of Coleoptera (Catalogue of Life 2021). These include Lampyridae (fireflies), Elateridae (click beetles), Phengodidae (glowworm beetles), Cantharidae (soldier beetles) and their relatives in Elateroidea; and Meloidae (blister beetles), Anthicidae (ant-like flower beetles), Mordellidae (tumbling flower beetles), Tenebrionidae (darkling beetle), Ciidae (the minute tree-fungus beetles), Zopheridae (ironclad beetles) and their relatives in Tenebrionoidea. Most of Elateroidea and Tenebrionoidea larvae (wireworms) are closely similar and morphology alone could hardly distinguish them. In practice, hosts of many wireworm-infecting *Cordyceps* s.l. species are commonly identified as Elateridae (mainly) or Tenebrionidae larvae. Considering the difficulties in identifying wireworms, we suggest to use the superfamily names (Elateroidea or Tenebrionoidea) to record the hosts of the fungi, unless we can definitely know the species identity (e.g. by barcoding techniques).

In present paper, we summarised the data of wireworm-infecting species of *Cordyceps* s.l. To date, a total of 63 species have been reported, including 17 species (*Akanthomyces*, *Beauveria* and *Cordyceps*) in Cordycipitaceae, 11 species (*Metarhizium* and *Nigelia*) in Clavicipitaceae and 35 species (*Ophiocordyceps*, *Paraisaria*, *Perennicordyceps*, *Polycephalomyces* and *Tolypocladium*) in Ophiocordycipitaceae. Amongst these, *C. militaris*, *O. ferruginosa* and *O. variabilis* are rejected; the remaining 60 species are accepted as natural pathogens of wireworms. It is likely that a significant portion of fungi, associated with wireworms, is represented by specialised forms. Thirteen of the reported species (20%) have broad host ranges, that is, they can infect different arthropod taxa and may also parasitise fungi and nematodes. The other 47 species (80%) have, thus far, been registered on wireworms only. Generalist fungi are mostly widespread, whereas specialised fungi are generally reported from warm and humid environments of Southeast Asia (Japan, south-western China and Thailand), the Amazon of South America and the Russian Far East. It should be noted that many animal-associated fungi are awaiting description, especially in groups, such as Hypocreales (Antonelli et al. 2020; Cheek et al. 2020) and many taxonomically-uncertain *Cordyceps* s.l. species infecting Elateroidea and Tenebrionoidea remain to be studied. Apart from the description of novel taxa, further studies should focus on revisions of these uncertain

species and further information of wireworm hosts. Limited by lack of information and taxonomic knowledge of larvae, species diversity of wireworm-infecting *Cordyceps* s.l. may not have been completely accounted for and many wireworm hosts cannot be or are incorrectly assigned to their families.

This is the first study summarising species diversity of wireworm-infecting *Cordyceps* s.l. A checklist of 60 species is provided and two novel species are described. Our work provides basic information for future research on species diversity of *Cordyceps* s.l. associated with wireworms, management and biocontrol of wireworm populations, as well as on edible and medicinal insects and fungi.

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References

- Antonelli A, Fry C, Smith RJ, et al. (2020) State of the World's Plants and Fungi 2020. Royal Botanic Gardens, Kew. <https://doi.org/10.34885/172>
- Bamisile BS, Dash CK, Akutse KS, Keppanan R, Afolabi OG, Hussain M, Qasim M, Wang L (2018) Prospects of endophytic fungal entomopathogens as biocontrol and plant growth promoting agents: an insight on how artificial inoculation methods affect endophytic colonization of host plants. *Microbiological Research* 217: 34–50. <https://doi.org/10.1016/j.micres.2018.08.016>
- Ban S, Sakane T, Toyama K, Nakagiri A (2009) Teleomorph-anamorph relationships and reclassification of *Cordyceps cuboidea* and its allied species. *Mycoscience* 50: 261–272. <https://doi.org/10.1007/S10267-008-0480-Y>
- Ban S, Sakane T, Nakagiri A (2015) Three new species of *Ophiocordyceps* and overview of anamorph types in the genus and the family Ophiocordyceptaceae. *Mycological Progress* 14(1): 1017–1028. <https://doi.org/10.1007/s11557-014-1017-8>
- Barsics F, Haubruge E, Verheggen FJ (2013) Wireworms' management: an overview of the existing methods, with particular regards to *Agriotes* spp. (Coleoptera: Elateridae). *Insects* 4: 117–152. <https://doi.org/10.3390/insects4010117>
- Bischoff JF, Rehner SA, Humber RA (2009) A multilocus phylogeny of the *Metarhizium anisopliae* lineage. *Mycologia* 101(4): 512–530. <https://doi.org/10.3852/07-202>
- Brunner-Mendoza C, Reyes-Montes MDR, Moonjely S, Bidochka MJ, Toriello C (2019) A review on the genus *Metarhizium* as an entomopathogenic microbial biocontrol agent with emphasis on its use and utility in Mexico. *Biocontrol Science and Technology* 29(1): 83–102. <https://doi.org/10.1080/09583157.2018.1531111>

- Cai L, Jeewon R, Hyde KD (2006) Molecular systematics of *Zopfiella* and allied genera: evidence from multigene sequence analyses. *Mycological Research* 110: 359–368. <https://doi.org/10.1016/j.mycres.2006.01.007>
- Catalogue of Life (2021) [accessed 1 March 2021]
- Chaverri P, Bischoff JF, Evans HC, Hodge KT (2005) *Regiocrella*, a new entomopathogenic genus with a pycnidial anamorph and its phylogenetic placement in the Clavicipitaceae. *Mycologia* 97(6): 1225–1237. <https://doi.org/10.1080/15572536.2006.11832732>
- Cheek M, Lughadha EN, Kirk P, Lindon H, Carretero J, Looney B, Douglas B, Haelewaters D, Gaya E, Llewellyn T, Ainsworth AM, Gafforov Y, Hyde K, Crous P, Hughes M, Walker BE, Forzza RC, Wong KM, Niskanen T (2020) New scientific discoveries: Plants and fungi. *Plants, People, Planet* 2(5): 371–388. <https://doi.org/10.1002/ppp3.10148>
- Chen ZH, Dai YD, Yu H, Yang K, Yang ZL, Yuan F, Zeng WB (2013) Systematic analyses of *Ophiocordyceps lanpingensis* sp. nov., a new species of *Ophiocordyceps* in China. *Microbiological Research* 168(8): 525–532. <https://doi.org/10.1016/j.micres.2013.02.010>
- Crous PW, Wingfield MJ, Burgess TI, Carnegie AJ, StJ Hardy GE, Smith D, Summerell BA, Cano-Lira JF, Guarro J, Houbraken J, Lombard L, Martín MP, Sandoval-Denis M, Alexandrova AV, Barnes CW, Baseia IG, Bezerra JDP, Guarnaccia V, May TW, Hernández-Restrepo M, Stchigel AM, Miller AN, Ordoñez ME, Abreu VP, Accioly T, Agnello C, Agustíncolmán A, Albuquerque CC, Alfredo DS, Alvarado P, Araújo-Magalhães GR, Arauzo S, Atkinson T, Barili A, Barreto RW, Bezerra JL, Cabral TS, Rodríguez FC, Cruz RHSF, Daniëls PP, da Silva BDB, de Almeida DAC, de Carvalho Júnior AA, Decock CA, Delgat L, Denman S, Dimitrov RA, Edwards J, Fedosova AG, Ferreira RJ, Firmino AL, Flores JA, García D, Gené J, Giraldo A, Góis JS, Gomes AAM, Gonçalves CM, Gouliamova DE, Groenewald M, Guéorguiev BV, Guevara-Suarez M, Gusmão LFP, Hosaka K, Hubka V, Huhndorf SM, Jadan M, Jurjevi Kraak B, Kuera V, Kumar TKA, Kušan I, Lacerda SR, Lamlertthon S, Lisboa WS, Loizides M, Luangsa-Ard JJ, Lysková P, McCormack WP, Macedo DM, Machado AR, Malysheva EF, Marinho P, Matoc N, Meijer M, Meši A, Mongkolsamrit S, Moreira KA, Morozova OV, Nair KU, Nakamura N, Noisripoom W, Olariaga I, Oliveira RJV, Paiva LM, Pawar P, Pereira OL, Peterson SW, Prieto M, Rodríguez-Andrade E, Rojodeblas C, Roy M, Santos ES, Sharma R, Silva GA, Souza-Motta CM, Takeuchi-Kaneko Y, Tanaka C, Thakur A, Smith MTH, Tkalec Z, Valenzuela-Lopez N, Vanderkleij P, Verbeken A, Viana MG, Wang XW, Groenewald JZ (2017) Fungal Planet description sheets: 625–715. *Persoonia -Molecular Phylogeny and Evolution of Fungi* 39: 270–467. <https://doi.org/10.3767/persoonia.2017.39.11>
- De Bary A (1867) Zur Kenntniss insectentoedender Pilze. *Botanische Zeitung* 25: 17–21. <https://doi.org/10.1007/BF01650457>
- Domsch KH, Gams W, Anderson TH (1980) *Compendium of Soil Fungi*. London: Academic Press, 136–140.
- Fan MZ, Li CR, Chen YY, Li ZZ (2001) *Cordyceps gracilioides*, a new record for China. *Mycosystema* 20: 273–274.
- GBIF (2021) (GBIF = Global Biodiversity Information Facility) [accessed 1 March 2021]
- Gullan PJ, Cranston PS (2010) *The Insects: An Outline of Entomology* (4th edition). Wiley-Blackwell, Oxford.

- Hu X, Xiao G, Zheng P, Shang Y, Su Y, Zhang X, Liu X, Zhan S, St Leger RJ, Wang C (2014) Trajectory and genomic determinants of fungal-pathogen speciation and host adaptation. *Proceedings of the National Academy of Sciences, USA* 111: 16796–16801. <https://doi.org/10.1073/pnas.1412662111>
- Humber RA, Hansen KS (2005) USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF), ARSEF-Index: Host by Fungus. <http://arsef.fpsnl.cornell.edu/>
- Hodge KT, Humber RA, Wozniak AC (1998) *Cordyceps variabilis* and the genus *Syngliocladium*. *Mycologia* 90(5): 743–753. <https://doi.org/10.1080/00275514.1998.12026966>
- Hongsanan S, Maharachchikumbura SSN, Hyde KD, Samarakoon MC, Jeewon R, Zhao Q, Al-Sadi AM, Bahkali AH (2017) An updated phylogeny of Sordariomycetes based on phylogenetic and molecular clock evidence. *Fungal Diversity* 84: 25–41. <https://doi.org/10.1007/s13225-017-0384-2>
- Hyde KD, Chaiwan N, Norphanphoun C, Boonmee S, Camporesi E, Chethana KWT, Dayaratne MC, de Silva NI, Dissanayake AJ, Ekanayaka AH, Hongsanan S, Huang SK, Jayasiri SC, Jayawardena RS, Jiang HB, Karunarathna A, Lin CG, Liu JK, Liu NG, Lu YZ, Luo ZL, Maharachchimbura SSN, Manawasinghe IS, Pem D, Perera RH, Phukhamsakda C, Samarakoon MC, Senwana C, Shang QJ, Tennakoon DS, Thambugala KM, Tibpromma S, Wanasinghe DN, Xiao YP, Yang J, Zeng XY, Zhang JF, Zhang SN, Bulgakov TS, Bhat DJ, Cheewangkoon R, Goh TK, Jones EBG, Kang JC, Jeewon R, Liu ZY, Lumyong S, Kuo CH, McKenzie EHC, Wen TC, Yan JY, Zhao Q (2018) *Mycosphere* notes 169–224. *Mycosphere* 9(2): 271–430. <https://doi.org/10.5943/mycosphere/9/2/8>
- Hywel-Jones NL (1995) *Cordyceps brunneapunctata* sp. nov. infecting beetle larvae in Thailand. *Mycological Research* 99(10): 1195–1198. [https://doi.org/10.1016/S0953-7562\(09\)80277-3](https://doi.org/10.1016/S0953-7562(09)80277-3)
- Imoulan A, Hussain M, Kirk PM, Meziane AE, Yao YJ (2017) Entomopathogenic fungus *Beauveria*: host specificity, ecology and significance of morpho-molecular characterization in accurate taxonomic classification. *Journal of Asia-Pacific Entomology* 20(4): 1204–1212. <https://doi.org/10.1016/j.aspen.2017.08.015>
- Index Fungorum (2021) [accessed 1 March 2021]
- Jeewon R, Liew ECY, Simpson JA, Hodgkiss IJ, Hyde KD (2003) Phylogenetic significance of morphological characters in the taxonomy of *Pestalotiopsis* species. *Molecular Phylogenetics and Evolution* 27: 372–383. [https://doi.org/10.1016/S1055-7903\(03\)00010-1](https://doi.org/10.1016/S1055-7903(03)00010-1)
- Kabaluk T, Goettel M, Erlandson M, Ericsson J, Duke G, Vernon B (2005) *Metarhizium anisopliae* as a biological control for wireworms and a report of some other naturally-occurring parasites. *IOBC/WPRS Bulletin* 28: 109–115.
- Kabaluk T, Li-Leger E, Nam S (2017) *Metarhizium brunneum* – an enzootic wireworm disease and evidence for its suppression by bacterial symbionts. *Journal of Invertebrate Pathology* 150: 82–87. <https://doi.org/10.1016/j.jip.2017.09.012>
- Keissler K, Lohwag H (1937) Part II, Fungi. In: Handel-Mazzetti H (Ed.) *Symbolae Sinicae*. Springer, Wien, Germany.
- Kepler RM, Sung GH, Ban S, Nakagiri A, Chen MJ, Huang B, Li Z, Spatafora JW (2012) New teleomorph combinations in the entomopathogenic genus *Metacordyceps*. *Mycologia* 104(1): 182–197. <https://doi.org/10.3852/11-070>

- Kepler RM, Ban S, Nakagiri A, Bischoff JF, Hywel-Jones NL, Owensby CA, Spatafora JW (2013) The phylogenetic placement of hypocrealean insect pathogens in the genus *Polycephalomycetes*: an application of one fungus one name. *Fungal Biology* 117: 611–622. <https://doi.org/10.1016/j.funbio.2013.06.002>
- Kepler RM, Humber RA, Bischoff JF, Rehner SA (2014) Clarification of generic and species boundaries for *Metarhizium* and related fungi through multigene phylogenetics. *Mycologia* 106(4): 811–829. <https://doi.org/10.3852/13-319>
- Kepler RM, Luangsa-ard JJ, Hywel-Jones NL, Quandt CA, Sung GH, Rehner SA, Aime MC, Henkel TW, Sanjuan T, Zare R, Chen MJ, Li ZZ, Rossman AY, Spatafora JW, Shrestha B (2017) A phylogenetically-based nomenclature for Cordycipitaceae (Hypocreales). *IMA Fungus* 8: 335–353. <https://doi.org/10.5598/imafungus.2017.08.02.08>
- Kobayasi Y (1941) The genus *Cordyceps* and its allies. *Science Reports of the Tokyo Bunrika Daigaku* (Section B, No. 84) 5: 53–260.
- Kobayasi Y (1982) Key to the taxa of the genera *Cordyceps* and *Torrubiella*. *Transactions of the Mycological Society of Japan* 23: 329–364.
- Kobayasi Y, Shimizu D (1978) *Cordyceps* species from Japan. *Bulletin of the National Science Museum, Tokyo, Series B, Botany* 4(2): 43–63.
- Kobayasi Y, Shimizu D (1980a) *Cordyceps* species from Japan 2. *Bulletin of the National Science Museum, Tokyo, Series B, Botany* 6(3): 77–96.
- Kobayasi Y, Shimizu D (1980b) *Cordyceps* species from Japan 3. *Bulletin of the National Science Museum, Tokyo, Series B, Botany* 6(4): 125–145.
- Kobayasi Y, Shimizu D (1981) The genus *Cordyceps* and its allies from Taiwan (Formosa). *Bulletin of the National Science Museum, Tokyo, Series B, Botany* 7: 113–122
- Kobayasi Y, Shimizu D (1982a) *Cordyceps* species from Japan 4. *Bulletin of the National Science Museum, Tokyo, Series B, Botany* 8(3): 79–91.
- Kobayasi Y, Shimizu D (1982b) *Cordyceps* species from Japan 5. *Bulletin of the National Science Museum, Tokyo, Series B, Botany* 8(4): 111–123.
- Kobayasi Y, Shimizu D (1983) *Cordyceps* species from Japan 6. *Bulletin of the National Science Museum, Tokyo B, Botany* 9(1): 1–21.
- Koval EZ (1984) *Clavicipitalean Fungi of the USSR*. Kiev, Naukova Dumka.
- Kryukov VY, Yaroslavtseva ON, Lednev GR, Borisov BA (2011) Local epizootics caused by teleomorphic cordycipitoid fungi (Ascomycota: Hypocreales) in populations of forest lepidopterans and sawflies of the summer-autumn complex in Siberia. *Microbiology* 80(2): 286–295. <https://doi.org/10.1134/S0026261711020093>
- Li CR, Fan MZ, Huang B, Wang SB, Li ZZ (2002) The genus *Cordyceps* and its allies from Anhui I. *Mycosystema* 21(2): 167–171.
- Li CR, Ming L, Fan MZ, Li ZZ (2004) *Paraisaria gracilioides* comb. nov., the anamorph of *Cordyceps gracilioides*. *Mycosystema* 23(1): 165–166.
- Li GJ, Hyde KD, Zhao RL, Hongsan S, Abdel-Aziz F, Abdel-Wahab M, Alvarado P, Alves-Silva G, Ammirati J, Ariyawansa H, Baghela A, Bahkali A, Beug MW, Bhat DJ, Bojantchev D, Boonpratuang T, Bulgakov T, Erio C, Boro MC, Ceska O, Chakraborty D, Chen JJ, Kandawatte TC, Chomnunti P, Consiglio G, Cui BK, Dai DQ, Dai YC, Daranagama DA, Das K, Dayarathne M, Crop ED, Oliveira R, Fragoso de Souza CA, Ivanildo de Souza J,

- Dentinger BTM, Dissanayake AJ, Doilom M, Drechsler-Santos ER, Ghobad-Nejhad M, Gilmore SP, Góes-Neto A, Gorczak M, Haitjema CH, Hapuarachchi K, Hashimoto A, He MQ, Henske JK, Hirayama K, Iribarren MJ, Jayasiri S, Jayawardena RS, Jeon SJ, Jerônimo GH, Lucia de Jesus A, Jones EBG, Kang JC, Karunarathna SC, Kirk PM, Konta S, Kuhnert E, Langer EJ, Lee HS, Lee HB, Li WJ, Li XH, Liimatainen K, Lima D, Lin CG, Liu JK, Liu X, Liu ZY, Luangsa-Ard JJ, Lücking R, Lumbsch T, Lumyong S, Leano E, Marano AV, Matsumura M, McKenzie EHC, Mongkolsamrit S, Mortimer PE, Nguyen TTT, Niskanen T, Norphanphoun C, O'Malley MA, Parnmen S, Pawłowska J, Perera RH, Phookamsak R, Phukhamsakda C, Zottarelli C, Raspé O, Reck MA, Rocha SCO, Santiago A, Senanayake I, Setti L, Shang QJ, Singh S, Sir EB, Solomon KV, Song J, Srikitikulchai P, Stadler M, Suetrong S, Takahashi H, Takahashi T, Tanaka K, Tang LP, Thambugala K, Thanakitpipattana D, Theodorou M, Thongbai B, Thummarukcharoen T, Tian Q, Tibpromma S, Verbeken A, Vizzini A, Vlasák J, Voigt K, Wanasinghe DN, Wang Y, Weerakoon G, Wen HA, Wen TC, Wijayawardene N, Wongkanoun S, Wrzosek M, Xiao YP, Xu JC, Yan JY, Yang J, Yang SD, Hu Y, Zhang JF, Zhao J, Zhou LW, Persoh D, Phillips AJL, Maharachchikumbura S, Amoozegar MA (2016) Fungal diversity notes 253–366: taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* 78(1): 1–237. <https://doi.org/10.1007/s13225-016-0366-9>
- Liang ZQ (2007) *Flora Fungorum Sinicorum*, Vol. 32, *Cordyceps*. Science Press, Beijing.
- Liang ZQ, Liu AY, Jiang YC (2001) Two new species of *Cordyceps* from Jinggang Mountains. *Mycosystema* 20(3): 306–309.
- Liu B, Rong F, Jin H (1985) A new species of the genus *Cordyceps*. *Journal of Wuhan Botanical Research* 3(1): 23–24.
- Luangsa-ard J, Mongkolsamrit S, Thanakitpipattana D, Khonsanit A, Tasanathai K, Noisripoom W, Humber RA (2017) Clavicipitaceous entomopathogens: new species in *Metarhizium* and a new genus *Nigelia*. *Mycological Progress* 16: 369–391. <https://doi.org/10.1007/s11557-017-1277-1>
- Mains EB (1941) *Cordyceps stylophora* and *Cordyceps ravenelii*. *Mycologia* 33: 611–617. <https://doi.org/10.1080/00275514.1941.12020857>
- Mains EB (1947) New and interesting species of *Cordyceps*. *Mycologia* 39(5): 535–545. <https://doi.org/10.1080/00275514.1947.12017632>
- Mains EB (1958) North American entomogenous species of *Cordyceps*. *Mycologia* 50: 169–222. <https://doi.org/10.1080/00275514.1958.12024722>
- Mains EB (1959) *Cordyceps* species. *Bulletin of the Torrey Botanical Club* 86(1): 46–58. <https://doi.org/10.2307/2482660>
- Massee G (1895) A revision of the genus *Cordyceps*. *Annals of Botany* 9(33): 1–44. <https://doi.org/10.1093/oxfordjournals.aob.a090724>
- Massee G (1899) Revision du genre *Cordyceps*. *Revue Mycologique* 21: 1–16.
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: 2010 Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, 1–8. <https://doi.org/10.1109/GCE.2010.5676129>
- Möller A (1901) *Phycomyceten und Ascomyceten, Untersuchungen aus Brasilien*, Botanische Mittheilungen aus den Tropen 9 (edited by Schimper AFW). Gustav Fischer, Jena, Germany, 378 pp. <https://doi.org/10.5962/bhl.title.31997>

- Mongkolsamrit S, Khonsanit A, Thanakitpipattana D, Tasanathai K, Noisripoom W, Lamlerthon S, Himaman W, Houbraken J, Samson RA, Luangsa-ard J (2020) Revisiting *Metarhizium* and the description of new species from Thailand. *Studies in Mycology* 95: 171–251. <https://doi.org/10.1016/j.simyco.2020.04.001>
- Mongkolsamrit S, Noisripoom W, Arnarnart N, Lamlerthon S, Himaman W, Jangsantear P, Samson RA, Luangsa-ard JJ (2019) Resurrection of *Paraisaria* in the Ophiocordycipitaceae with three new species from Thailand. *Mycological Progress* 18(9): 1213–1230. <https://doi.org/10.1007/s11557-019-01518-x>
- Moureaux J (1949) *Cordyceps* du Congo Belge. *Mémoires de l'Institut Royal Colonial Belge* 7: 1–58.
- Negi PS, Singh R, Koranga PS, Ahmed Z (2012) Two new for science species of genus *Cordyceps* Fr. (Ascomycetes) from Indian Himalaya. *International Journal of Medicinal Mushrooms* 14(5): 501–506. <https://doi.org/10.1615/IntJMedMushr.v14.i5.80>
- Petch T (1933) Notes on entomogenous fungi. *Transactions of the British Mycological Society* 18(1): 48–75. [https://doi.org/10.1016/S0007-1536\(33\)80026-X](https://doi.org/10.1016/S0007-1536(33)80026-X)
- Petch T (1934) Notes on entomogenous fungi. *Transactions of the British Mycological Society* 19: 160–194. [https://doi.org/10.1016/S0007-1536\(35\)80008-9](https://doi.org/10.1016/S0007-1536(35)80008-9)
- Petch T (1937) Notes on entomogenous fungi. *Transactions of the British Mycological Society* 21(1–2): 34–67. [https://doi.org/10.1016/S0007-1536\(37\)80005-4](https://doi.org/10.1016/S0007-1536(37)80005-4)
- Quandt CA, Kepler RM, Gams W, Araújo JP, Ban S, Evans HC, Hughes D, Humber R, Hywel-Jones N, Li Z, Luangsa-Ard JJ, Rehner SA, Sanjuan T, Sato H, Shrestha B, Sung GH, Yao YJ, Zare R, Spatafora JW (2014) Phylogenetic-based nomenclatural proposals for Ophiocordycipitaceae (Hypocreales) with new combinations in *Tolypocladium*. *IMA Fungus* 5(1): 121–134. <https://doi.org/10.5598/imafungus.2014.05.01.12>
- Rannala B, Yang Z (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43: 304–311. <https://doi.org/10.1007/BF02338839>
- Reddy GVP, Tangtrakulwanich K, Wu SH, Miller JH, Ophus VL, Prewett J, Jaronski ST (2014) Evaluation of the effectiveness of entomopathogens for the management of wireworms (Coleoptera: Elateridae) on spring wheat. *Journal of Invertebrate Pathology* 120: 43–49. <https://doi.org/10.1016/j.jip.2014.05.005>
- Rehner SA, Minnis AM, Sung GH, Luangsa-ard JJ, Devotto L, Humber RA (2011) Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. *Mycologia* 103(5): 1055–1073. <https://doi.org/10.3852/10-302>
- Ren GD, Ba YB, Liu HY, Niu YP, Zhu XC, Li Z, Shi AM (2016) *Fauna Sinica. Insecta. Vol. 63. Coleoptera: Tenebrionidae (I)*. Science Press, Beijing, 532 pp.
- Rogge SA, Mayerhofer J, Enkerli J, Bacher S, Grabenweger G (2017) Preventive application of an entomopathogenic fungus in cover crops for wireworm control. *BioControl* 62(5): 613–623. <https://doi.org/10.1007/s10526-017-9816-x>
- Samson RA, Evans HC, Hoekstra S (1982) Notes on entomogenous fungi from Ghana, VI. *Mycology* 85(4): 589–605.
- Samson RA, Evans HC (1985) New and rare entomogenous fungi from Amazonia. *Proceedings of the Indian Academy of Sciences (Plant Science)* 94: 309–317.

- Sanjuan TI, Franco-Molano AE, Kepler RM, Spatafora JW, Tabima J, Vasco-Palacios AM, Restrepo S (2015) Five new species of entomopathogenic fungi from the Amazon and evolution of neotropical *Ophiocordyceps*. *Fungal Biology* 119(10): 901–916. <https://doi.org/10.1016/j.funbio.2015.06.010>
- Sato H, Shimazu M (2002) Stromata production for *Cordyceps militaris* (Clavicipitales: Clavicipitaceae) by injection of hyphal bodies to alternative host insects. *Applied Entomology and Zoology* 37(1): 85–92. <https://doi.org/10.1303/aez.2002.85>
- Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Bolchacova E, Voigt K, Crous PW, Miller AN (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences* 109(16): 6241–6246. <https://doi.org/10.1073/pnas.1117018109>
- Scorsetti AC, Eliades LA, Stenglein SA, Cabello MN, Pelizza SA, Saparrat MC (2012) Pathogenic and enzyme activities of the entomopathogenic fungus *Tolyposcladium cylindrosporium* (Ascomycota: Hypocreales) from Tierra del Fuego, Argentina. *Revista de Biología Tropical* 60(2): 833–841. <https://doi.org/10.15517/rbt.v60i2.4006>
- Shimizu D (1997) *Illustrated Vegetable Wasps and Plant Worms in Color*. Ie-No-Hikari Association, Tokyo.
- Shinya R, Watanabe A, Aiuchi D, Tani M, Kuramochi K, Kushida A, Koike M (2008) Potential of *Verticillium lecanii* (*Lecanicillium* spp.) hybrid strains as biological control agents for soybean cyst nematode: is protoplast fusion an effective tool for development of plant-parasitic nematode control agents. *Japanese Journal of Nematology* 38(1): 9–18. <https://doi.org/10.3725/jjn.38.9>
- Shrestha B, Zhang WM, Zhang YJ, Liu XZ (2012) The medicinal fungus *Cordyceps militaris*: research and development. *Mycological Progress* 11(3): 599–614. <https://doi.org/10.1007/s11557-012-0825-y>
- Shrestha B, Tanaka E, Hyun MW, Han JG, Kim CS, Jo JW, Han SK, Oh J, Sung GH (2016) Coleopteran and Lepidopteran hosts of the entomopathogenic genus *Cordyceps* sensu lato. *Journal of Mycology* 2016: e7648219. <https://doi.org/10.1155/2016/7648219>
- Shrestha B, Tanaka E, Hyun MW, Han JG, Kim CS, Jo JW, Han SK, Oh J, Sung JM, Sung GH (2017) Mycosphere Essay 19, *Cordyceps* species parasitizing hymenopteran and hemipteran insects. *Mycosphere* 8: 1424–1442. <https://doi.org/10.5943/mycosphere/8/9/8>
- Spatafora JW, Sung GH, Johnson D, Hesse C, O'Rourke B, Serdani M, Spotts R, Lutzoni F, Hofstetter V, Miadlikowska J, Reeb V, Gueidan C, Fraker E, Lumbsch T, Lücking R, Schmitt I, Hosaka K, Aptroot A, Roux C, Miller AN, Geiser DM, Hafellner J, Hestmark G, Arnold AE, Büdel B, Rauhut A, Hewitt D, Untereiner WA, Cole MS, Scheidegger C, Schultz M, Sipman H, Schoch CL (2006) A five gene phylogeny of Pezizomycotina. *Mycologia* 98: 1018–1028. <https://doi.org/10.1080/15572536.2006.11832630>
- Spatafora JW, Sung GH, Sung JM, Hywel-Jones NL, White JJJ (2007) Phylogenetic evidence for an animal pathogen origin of ergot and the grass endophytes. *Molecular Ecology* 16(8): 1701–1711. <https://doi.org/10.1111/j.1365-294X.2007.03225.x>
- Spegazzini C (1889) Fungi Puiggariani, Pugillus 1. *Boletín De La Academia Nacional De Ciencias De Córdoba* 11(4): 381–622. <https://doi.org/10.5962/bhl.title.3624>

- Sufyan M, Abbasi A, Gogi MD, Arshad M, Nawaz A, Neuhoﬀ D (2017) Efficacy of *Beauveria bassiana* for the management of economically important wireworm species (Coleoptera: Elateridae) in organic farming. *Gesunde Pflanzen* 69(4): 197–202. <https://doi.org/10.1007/s10343-017-0406-8>
- Sung GH, Hywel-Jones NL, Sung JM, Luangsa-ard JJ, Shrestha B, Spatafora JW (2007) Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. *Studies in Mycology* 57: 5–69. <https://doi.org/10.3114/sim.2007.57.01>
- Tang AMC, Jeewon R, Hyde KD (2007) Phylogenetic utility of protein (RPB2, β -tubulin) and ribosomal (LSU, SSU) gene sequences in the systematics of Sordariomycetes (Ascomycota, Fungi). *Antonie van Leeuwenhoek* 91: 327–349. <https://doi.org/10.1007/s10482-006-9120-8>
- Tasanathai K, Thanakitpipattana D, Noisripoom W, Khonsanit A, Kumsao J, Luangsa-ard JJ (2016) Two new *Cordyceps* species from a community forest in Thailand. *Mycological Progress* 15: e28. <https://doi.org/10.1007/s11557-016-1170-3>
- Wang WJ, Wang XL, Li Y, Xiao SR, Kepler RM, Yao YJ (2012) Molecular and morphological studies of *Paecilomyces sinensis* reveal a new clade in clavicipitaceous fungi and its new systematic position. *Systematics and Biodiversity* 10: 221–232. <https://doi.org/10.1080/14772000.2012.690784>
- Wang L, Li HH, Chen YQ, Zhang WM, Qu LH (2014) *Polycephalomyces lianzhouensis* sp. nov., a new species, co-occurs with *Ophiocordyceps crinalis*. *Mycological Progress* 13: 1089–1096. <https://doi.org/10.1007/s11557-014-0996-9>
- Wang YB, Yu H, Dai YD, Chen ZH, Zeng WB, Yuan F, Liang ZQ (2015) *Polycephalomyces yunnanensis* (Hypocreales), a new species of *Polycephalomyces* parasitizing *Ophiocordyceps nutans* and stink bugs (hemipteran adults). *Phytotaxa* 208(1): 34–44. <https://doi.org/10.11646/phytotaxa.208.1.3>
- Wang YB (2016) Studies on Phylogeny of Polycephalomycetaceae Fam. Nov., with Microbial Diversities of Polycephalomyces Multiramosus and its Host. Doctorate Dissertation, Yunnan University, Kunming, China.
- Wei DP, Wanasinghe DN, Hyde KD, Mortimer PE, Xu JC, To-Anun C, Yu FM, Zha LS (2020) *Ophiocordyceps tianshanensis* sp. nov. on ants from Tianshan mountains, PR China. *Phytotaxa* 464(4): 277–292. <https://doi.org/10.11646/phytotaxa.464.4.2>
- Wen TC, Zhu RC, Kang JC, Huang MH, Tan DB, Ariyawansa H, Hyde KD, Liu H (2013) *Ophiocordyceps xuefengensis* sp. nov. from larvae of *Phassus nodus* (Hepialidae) in Hunan Province, southern China. *Phytotaxa* 123(1): 41–50. <https://doi.org/10.11646/phytotaxa.123.1.2>
- White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) *PCR Protocols: A Guide to Methods and Applications*. Academic Press, New York, 315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Xiao YP, Hongsan S, Hyde KD, Brooks S, Xie N, Long FY, Wen TC (2019) Two new entomopathogenic species of *Ophiocordyceps* in Thailand. *MycoKeys* 47: 53–74. <https://doi.org/10.3897/mycokeys.47.29898>

- Yahagi N (2008) Illustrated catalogue of Japanese *Cordyceps* (entomogenous fungi): the Yahagi collection of Japanese *Cordyceps* stored in the Tohoku University Museum. Bulletin of the Tohoku University Museum (8): 29–89.
- Yamamoto K, Ohmae M, Orihara T (2020) *Metarhizium brachyspermum* sp. nov. (Clavicipitaceae), a new species parasitic on Elateridae from Japan. Mycoscience 61: 37–42. <https://doi.org/10.1016/j.myc.2019.09.001>
- Yang SB (2004) The Taxonomic Diversity and Conservation of *Cordyceps* Fr. from Changbai Mountains. Masters Dissertation, Jilin Agricultural University, Changchun, China.
- Zare R, Gams W (2001) A revision of *Verticillium* section *Prostrata*, IV, the genera *Lecanicillium* and *Simplicillium* gen. nov. Nova Hedwigia 73: 1–50. <https://doi.org/10.1127/nova.hedwigia/73/2001/1>
- Yaroslavtseva ON, Ageev DV, Bulyonkova TM, Kryukov VY (2019) First records of the entomopathogenic fungus *Ophiocordyceps variabilis* (Petch) G.H. Sung, J.M. Sung, Hywel-Jones et Spatafora from Siberia. Euroasian Entomological Journal 18(6): 379–381. <https://doi.org/10.15298/euroasentj.18.6.01>
- Zha LS, Huang SK, Xiao YP, Boonmee S, Eungwanichayapant PD, McKenzie EHC, Kryukov V, Wu XL, Hyde KD, Wen TC (2018) An evaluation of common cordyceps species found in Chinese markets. International Journal of Medicinal Mushrooms 20(12): 1149–1162. <https://doi.org/10.1615/IntJMedMushrooms.2018027330>
- Zha LS, Ye L, Huang SK, Boonmee S, Eungwanichayapant PD, Hyde KD, Wen TC (2020) Species diversity and host associations of cordyceps (Hypocreales) parasitic on Orthoptera insects. Mycosystema 39(4): 407–722. <https://doi.org/10.13346/j.mycosystema.190195>
- Zhang WM, Li TH, Chen VQ, Qu LH (2004) *Cordyceps campsosterna*, a new pathogen of *Campsosternus auratus*. Fungal Diversity 17: 239–242.
- Zimmermann G (2008) The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology and use in biological control. Biocontrol Science and Technology 18(9): 865–901. <https://doi.org/10.1080/09583150802471812>

The lichens of the Majella National Park (Central Italy): an annotated checklist

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Abstract

The botanical exploration of the Majella National Park has a long tradition dating back to the eighteenth century. However, the lichen biota of this area is still poorly investigated. To provide a baseline for future investigations, in this annotated checklist, we summarised all available information on the occurrence of lichens in the Majella National Park, retrieved from previous literature, herbarium material and original data produced by recent research.

The checklist includes 342 infrageneric taxa. However, seven taxa are considered as dubious, thus setting the number of accepted taxa at 335, i.e. 45.8% of those currently known to occur in the Abruzzo Region. This checklist provides a baseline of the lichens known to occur in the Majella National Park, highlighting the potential of this area as a hotspot of lichen biodiversity, especially from a biogeographical point of view as indicated by the occurrence of several arctic-alpine species that form disjunct populations in the summit area of the massif.

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Keywords

Abruzzo, arctic-alpine species, biodiversity hotspot, climate change, lichen biota, Mediterranean mountains, steppic species

Introduction

The botanical exploration of the Majella National Park has a long tradition dating back to the eighteenth century, which has provided the basis for the compilation of a recent checklist of vascular plants including 2286 infrageneric taxa (Conti et al. 2019). This massif clearly is a hotspot of plant diversity due to the interaction of physical, climatic and biogeographic factors. In particular, the flora of high-elevation habitats consists of many endemic taxa of high phytogeographic relevance.

On the other hand, the lichen biota of this area is still poorly investigated. Historical data are scanty, the main contribution being that by Nimis and Tretiach (1999), who carried out intensive lichen collections along the eastern part of the Italian peninsula. These authors collected several specimens, currently stored in the TSB herbarium, in at least five localities distributed along a steep elevational gradient, from 500 to 2500 m, in the Majella National Park. Ten years later, Cucchi et al. (2009) studied the microtopography of carbonatic rocks, reporting several endolithic taxa. Overall, these collections revealed several interesting species that were either new to the Abruzzo region or indicative of the biogeographic importance of the Majella massif also for lichens. For example, several arctic-alpine lichens occur there in small and disjunct areas at the southernmost limit of their European distribution, the nearest populations being in the Alps (Nimis 2016).

In 2017, a scientific collaboration started between the administration of the Majella National Park (with its botanical office) and the University of Bologna, under the project “Lichen biodiversity in the Majella National Park”, with the aim of contributing to fill this knowledge gap. Besides pure floristic explorations (e.g. Nascimbene et al. 2019), the research project also included ecological investigations, mainly focused on high elevation areas, for example, a lichen survey on the four GLORIA summits (Di Cecco et al. 2019) and along an elevational transect across the whole main ridge of the massif (Di Nuzzo et al. 2021).

To provide a baseline for future investigations, in this annotated checklist, we have summarised all available information on the occurrence of lichens in the Majella National Park, retrieved from previous literature, herbarium material and original data produced by our research. In this checklist, very few lichenicolous fungi are included. To be treated exhaustively, this component would require specific investigations.

Materials and methods**Study area**

The Majella National Park (MNP) is located in the central Apennines, Italy, and was established in 1995 by National Law 1991, n. 394, to preserve, protect and

enhance the high value of the inherent natural, historical and cultural resources of the area. The Park consists mainly of carbonate mountains, separated by valleys and karst high plateaus, with a broad altitudinal range (130–2,793 m a.s.l.). The Majella massif has more than 60 peaks, with half of them rising above 2,000 m and includes the second highest peak in the Apennines, Mount Amaro (2,793 m). From a bioclimatic point of view, the study area is included in the alpine biogeographical region (Cervellini et al. 2020) and the climate corresponds to the subalpine-alpine humid type as far as the lower summit is concerned, whereas the other summits belong to the alpine humid type (Blasi et al. 2005). The Park's territories are part of the Natura 2000 network. The boundaries coincide with a Special Protection Area (SPA) for the conservation of wild birds (established by the Birds Directive 79/409/EEC). Furthermore, within the Park, there are four Special Areas of Conservation (SAC), established by the Habitats Directive 92/43/EEC (Di Cecco et al. 2020).

The data

Occurrence data were retrieved from multiple sources, for a total of 1625 records:

- 1) critical evaluation of literature records (463 records from 10 publications);
- 2) 217 records stored in on-line available herbaria, mainly from TSB (Herbarium of the University of Trieste);
- 3) reliable field observations related to our research project (e.g. only in the case of easily-identifiable species) recorded between 2017 and 2019 (100 records);
- 4) 845 herbarium records (personal herbarium of JN and GG) related to our research project collected between 2017 and 2019.

All these records were georeferenced and stored in a database.

The following abbreviations were used for the sources of occurrence data: C09 (Cucchi et al. 2009), C73 (Cesati 1873), C86 (Coassini Lokar et al. 1986), GG (personal herbarium and field observations by Gabriele Gheza), J74 (Jatta 1874), J11 (Jatta 1909–1911), JN (personal herbarium and field observations by Juri Nascimbene and collaborators), N19 (Nascimbene et al. 2019), NAP (Herbarium of the University of Naples), NT99 (Nimis and Tretiach 1999), R20 (Ravera et al. 2020), RV96 (Recchia and Villa 1996), T15 (Tretiach 2015), TSB (Herbarium of the University of Trieste).

The specimens collected during our project were identified in the laboratory using a dissecting and a compound microscope. Routine chemical spot tests were performed for most specimens. The identification of sterile crustose lichens (e.g. *Lepraria*-species) was based on standardised thin-layer chromatography (TLC), following the protocols of Orange et al. (2001).

Lichen nomenclature, as well as synonymisation of old records, follow ITALIC 6.0 – The information system on Italian Lichens (Nimis and Martellos 2020), which is mainly based on the checklist of the Italian lichens by Nimis (2016). This source was

used also for retrieving information on biological traits, ecological requirements and geographic distribution for each taxon.

Taxa are listed alphabetically. For each taxon, the accepted name, all available records, the altitudinal distribution, habitat preference and/or substrate are reported, whenever information is available. A short note (on ecology, distribution and/or taxonomy) is associated with each noteworthy taxon (e.g. taxa which are new to the region and/or of particular biogeographic or conservation importance). Dubious records are reported at the end of the checklist. For each record, a critical note accounting for the “dubious status” is reported.

Results

General overview

The checklist includes 342 infrageneric taxa. However, seven taxa are considered as dubious, thus setting the number of accepted taxa at 335, i.e. 45.8% of the those currently known to occur in the Abruzzo Region. In the following, the main traits of the lichen biota are detailed:

1) growth forms: five taxa (1.5%) are leprose, 199 (59.4%) are crustose (161 crustose, 13 placodiomorph, 25 endolithic), 16 (4.9%) are squamulose, 80 (23.9%) are foliose (52 broad-lobed, 25 narrow-lobed, three umbilicate) and 34 (10.2%) are fruticose (31 fruticose, three filamentous). Only four taxa (1.2%) are non-lichenised, lichenicolous fungi: *Arthonia galactinaria*, *Carbonea vitellinaria*, *Merismatium decolorans* and *Opegrapha rupestris*.

2) photobionts (only lichenised taxa): 296 taxa (88.6%) are chlorolichens (282 with a chlorococcoid photobiont and 16 with a trentepohlioid photobiont), 37 taxa (11.2%) are cyanolichens (35 with a filamentous cyanobacterium and two with a coccaceous cyanobacterium), and one species (0.2%), *Peltigera leucophlebia*, is a cephalolichen with both a chlorococcoid and a cyanobacterial (in external cephalodia) photobiont.

3) main reproductive strategies: 257 taxa (76.8%) mainly disperse by sexual reproduction, forming ascospores in apothecia or perithecia, while 77 taxa (23.2%) disperse by asexual reproduction (22 by means of isidia or isidia-like structures, 47 by means of soredia or soredia-like structures and seven mainly by means of thallus fragmentation).

4) substrates: 122 taxa (36.6%) are mainly epiphytic, four (1.2%) mainly lignicolous, 126 (38.1%) mainly saxicolous, 78 (22.8%) mainly terricolous and five (1.3%) are lichenicolous on saxicolous lichens: *Placocarpus schaereri* on *Protoparmeliopsis versicolor*, *Placopyrenium canellum* on *Circinaria calcarea*, *Verrucula biatorinaria* on *Calogaya biatorina*, *Verrucula coccinearia* on *Caloplaca coccinea* and *Verrucula granulosaria* on *Flavoplaca granulosa*. Some species can occur on more than one substrate.

Noteworthy taxa

The checklist includes several noteworthy taxa, especially from a biogeographical perspective. Twenty taxa are new to the Abruzzo Region: *Arthrorhaphis citrinella*, *Biatorella hemisphaerica*, *Blastenia ammiospila*, *Blastenia subathallina*, *Calogaya bryochryson*, *Circinaria hispida*, *Cladonia cariosa*, *Gyalolechia bracteata*, *Heppia adglutinata*, *Myriolecis perpruinosa*, *Peltigera elisabethae*, *Peltigera lepidophora*, *Ramonia luteola*, *Rinodina roscida*, *Rostania ceranisca*, *Scytinium imbricatum*, *Solorina bispora* subsp. *macrospora*, *Toninia subnitida*, *Toniniopsis coelestina* and *Trapeliopsis gelatinosa*. Additionally, *Scoliciosporum umbrinum* var. *corticicolum* is formally new to Italy (see note below), while *Halecania lecanorina* is formally new to Abruzzo on the basis of an old literature record that is accepted here (see note).

One taxon is known to occur in Italy only for the record reported here (*Thelidium dionantense*), while 16 taxa are the only records for both peninsular and central Italy (*Agonimia gelatinosa*, *Caloplaca cacuminum*, *Circinaria hispida*, *Cladonia polycarpoides*, *Lecidea speirodes*, *Parabagliettoa disjuncta*, *Polyblastia dermatodes*, *Polyblastia verrucosa*, *Rhizocarpon atroflavescens*, *Rinodina roscida*, *Rostania ceranisca*, *Scytinium imbricatum*, *Solorina bispora* subsp. *macrospora*, *Thelidium dionantense*, *Toniniopsis coelestina* and *Verrucula coccinearia*).

Twenty-six taxa (*Allocetraria madreporiformis*, *Arthrorhaphis citrinella*, *Aspicilia verrucosa* var. *verrucosa*, *Athallia saxifragarum*, *Bilimbia microcarpa*, *Blastenia ammiospila*, *Caloplaca cacuminum*, *Caloplaca stillicidiorum*, *Candelariella commutata*, *Cetraria erectorum*, *Farnoldia hypocrita*, *Farnoldia micropsis*, *Flavocetraria nivalis*, *Lecanora epibryon* var. *epibryon*, *Lecidella wulfenii*, *Myriolecis zosteriae* var. *palanderi*, *Ochrolechia upsaliensis*, *Ophioparma ventosa*, *Parvoplaca tirolensis*, *Phaeorrhiza nimbosea*, *Physconia muscigena* var. *muscigena*, *Rhizocarpon umbilicatum*, *Rinodina roscida*, *Rostania ceranisca*, *Rusavskia sorediata* and *Solorina bispora* subsp. *bispora*) have an arctic-alpine distribution, several of them being at their southernmost distribution limit in Italy, or even in Europe, as in the case of *Allocetraria madreporiformis* and *Caloplaca cacuminum*.

The record of the steppic lichen *Circinaria hispida* provides a connection between the main area of distribution of this taxon (Eastern Europe and Central Asia) and its scattered Western European populations (Northern Italy, Spain).

Finally, thirteen epiphytic taxa are of conservation interest, being included in the Red List of epiphytic lichens of Italy (Nascimbene et al. 2013): *Calogaya lobulata* (VU), *Cetrelia olivetorum* (NT), *Enchylium ligerinum* (NT), *Eopyrenula leucoplaca* (NT), *Gyalecta ulmi* (NT), *Heterodermia speciosa* (NT), *Leptogium hildenbrandii* (NT), *Lobaria pulmonaria* (LC), *Melaspilea enteroleuca* (NT), *Nephroma resupinatum* (NT), *Parmeliella triptophylla* (NT), *Ramonia luteola* (VU) and *Sclerophora pallida* (VU).

Annotated checklist

Acarospora cervina A. Massal.

Roccacaramanico (NT99); Anticima Femmina Morta (JN: 2017). – From the montane (1000 m: NT99) to the alpine (2420 m: JN) belt. On rock (JN).

***Acarospora glaucocarpa* (Ach.) Körb.**

Valle dell'Orfento (J74). – This record is the only one available from Abruzzo (Nimis 1993, 2016); despite the fact that it was not confirmed by recent exploration, it is considered as reliable, since this is a widespread, common species (Nimis 2016).

***Acarospora macrospora* (Hepp) Bagl.**

M. Focalone, near Bivacco Fusco (NT99). – In the alpine belt (2500 m: NT99). – Previously reported from Abruzzo only by Grillo and Romano (1987) from the Abruzzo National Park.

Acrocordia conoidea* (Fr.) Körb. var. *conoidea

Pretoro, Colle dell'Angelo (NT99); below the Maielletta (TSB: 2005); road between Lettomanoppello and Passo Lanciano (TSB: 2005). – In the montane belt (1080–1350 m: TSB). In a beech forest (TSB). On calcareous rock (TSB).

Acrocordia gemmata* (Ach.) A. Massal. var. *gemma

Valico della Forchetta (TSB: 1996); Val di Foro (JN: 2018). – From the lower (970 m: JN) to the upper montane belt (1360 m: TSB). On bark of *Fagus* (TSB; JN).

***Agonimia gelatinosa* (Ach.) M. Brand & Diederich**

Trail between Blockhaus and M. Focalone (T15; TSB: 2005); Femmina Morta (JN: 2017). – In the subalpine belt (2300–2420 m: T15; TSB; JN). In calcareous grasslands (T15; TSB; JN). On organic soil (T15; TSB; JN). – These are the only known records for Abruzzo and peninsular Italy and, thus, also the southernmost ones in Italy (Nimis 2016).

***Agonimia tristicula* (Nyl.) Zahlbr.**

Roccacaramanico (NT99); at 20 sites along the main ridge of the Majella massif between 2139 and 2664 m (JN: 2018, 2019). – From the montane (1000 m: NT99) to the alpine (2664 m: JN) belt. In high-altitude open habitats (JN). On soil (JN).

***Allocetraria madreporiformis* (Ach.) Kärnefelt & A. Thell**

M. Amaro (C73; J11); Tavola Rotonda (JN: 2017); Vetta Femmina Morta (JN: 2017); Colle d'Acquaviva (JN: 2017); Anticima M. Acquaviva (JN: 2016); M. Macellaro (JN: 2017, 2018); between Iaccione and Piano Amaro (JN: 2017); Piano Amaro (JN: 2017); Grotta Canosa (JN: 2017); Sella di Grotta Canosa (JN: 2017); between Grotta Canosa and M. Amaro (JN: 2017); M. Amaro (JN: 2017); between M. Acquaviva and M. Focalone (JN: 2017); Cima dell'Altare (JN: 2017); at five sites along the main ridge of Majella between 2322 and 2664 m (JN: 2018, 2019). – From the subalpine (2207 m: JN) to the alpine (2750 m: JN) belt. In high-altitude open habitats (JN). On soil (JN). – These records are the southernmost ones in Europe (cf. Nimis 2016) and confirm the old record by Cesati (1873) from M. Amaro (Jatta 1909–1911, Nimis 1993). According to Nimis (1993), the other old record from Majella by Jatta (1874)

from pine bark probably refers to another species. Widespread in the Alps, this species is known from the Apennines only for Abruzzo (Campo Imperatore, in the Gran Sasso massif, Nimis and Tretiach 1999).

***Alyxoria varia* (Pers.) Ertz & Tehler**

Guesthouse of Lama dei Peligni (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – From the colline (650 m: JN) to the montane (1350 m: JN) belt. On bark of *Acer campestre* (N19; JN), *Fagus* (N19; JN) and *Quercus pubescens* (JN).

***Amandinea punctata* (Hoffm.) Coppins & Scheid.**

Majella (C73); at two sites along the main ridge of Majella between 1825 and 2091 m (JN: 2019). – In the subalpine belt (1825–2091 m: JN). In high-altitude open habitats (JN). On plant debris (JN).

***Anaptychia ciliaris* (L.) A. Massal.**

Majella (C73; J74); Valico della Forchetta (TSB: 1996); Monti Pizzi near S. Domenico (JN: 2017); Lama dei Peligni (JN: 2017); along the highway Strada Statale 164 (JN: 2018); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018); Valle di Mario (JN: 2018); Piano Cerreto near Campo di Giove (JN: 2018); Cansano (JN: 2018). – From the colline (650 m: JN) to the montane (1434 m: JN) belt. On bark of *Acer campestre* (N19), *Acer pseudoplatanus* (N19; JN), *Fagus* (N19; JN) and *Quercus cerris* (JN).

***Arthonia apatetica* (A. Massal.) Th. Fr.**

Hermitage of M. Morrone (NT99; TSB: 1997). – In the colline belt (500 m: NT99; TSB). On bark of *Fraxinus ornus* (TSB). – This is the only known record for Abruzzo (Nimis 2016).

***Arthonia atra* (Pers.) A. Schneid.**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350 m: JN). On bark of *Fagus* (N19; JN).

***Arthonia calcarea* (Sm.) Ertz & Diederich**

Pretoro, Colle dell'Angelo (NT99). – In the montane belt (1200 m: NT99). – This is the only known record for Abruzzo (Nimis 2016).

***Arthonia calcicola* Nyl.**

Trail between Lettomanoppello and Passo Lanciano (T15; TSB: 2005). – In the montane belt (1080 m: T15). On calcareous rock (T15). – This is the only known record for Abruzzo (Nimis 2016).

***Arthonia fusca* (A. Massal.) Hepp**

Anticima M. Acquaviva (JN: 2017). – In the alpine belt (2700 m: JN). On calcareous rock (JN).

***Arthonia galactinaria* Leight.**

Roccacaramanico (NT99). – In the montane belt (1000 m: NT99). Parasite on *Myriolecis dispersa* (NT99). – This record was reported under *Arthonia clemens* (Tul.) Th. Fr. by Nimis and Tretiach (1999), but later Nimis (2016) moved it under *A. galactinaria*, since *A. clemens* is recognised to parasitise only species of *Rhizoplaca*.

***Arthonia mediella* Nyl.**

Pescocostanzo, Bosco di S. Antonio (J19; JN: 2018). – In the montane belt (1350 m: JN). On bark of *Acer campestre* (N19; JN) and *Fagus* (N19).

***Arthonia radiata* (Pers.) Ach.**

Pretoro, Colle dell'Angelo (NT99); Valico della Forchetta (TSB: 2016); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018); along the highway Strada Statale 164 (JN: 2018); Centiata, Villaggio Mirastelle (JN: 2018). – In the montane belt (1200–1420 m: JN). On bark of *Fagus* (N19; JN).

***Arthrorhaphis citrinella* (Ach.) Poelt**

At two sites along the main ridge of Majella between 2582 and 2592 m (JN: 2019). – In the alpine belt (2582–2592 m: JN). In high-altitude open habitats (JN). On soil (JN). – New to Abruzzo. These records are located between the main Italian range of the species on the Alps and the disjunct populations occurring on the highest mountains of Calabria and Sicily (Nimis 2016).

Aspicilia verrucosa* (Ach.) Körb. subsp. *verrucosa

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); near Bivacco Fusco (JN: 2016); Anticima M. Acquaviva (JN: 2016); Sella di Grotta Canosa (JN: 2017); Anticima Femmina Morta (JN: 2017); Femmina Morta (JN: 2017); at 13 sites along the main ridge of Majella between 1997 and 2664 m (JN: 2018, 2019). – From the submontane (1997 m: JN) to the alpine (2664 m: JN) belt. In high-altitude open habitats (JN). On bryophytes and plant debris (JN).

***Aspicilia verrucosa* (Ach.) Körb. subsp. *mutabilis* (Ach.) Cl. Roux**

Caramanico (TSB). – In the lower montane belt (820 m: TSB). On bark of deciduous *Quercus* sp. (TSB).

***Athallia holocarpa* (Hoffm.) Arup, Frödén & Søchting**

Majella (C73; J74). – On calcareous rock (J74). – The historical records were not confirmed recently, but the record is considered as reliable, since this is a widespread species (Nimis 2016).

***Athallia inconnexa* (Nylander) S.Y. Kondr. & L. Lökös**

Roccacaramanico (NT99; TSB: 1996); near Martellose (JN: 2017). – From the montane (1000 m: NT99; TSB) to the subalpine (2065 m: JN) belt. On calcareous rock (TSB; JN).

***Athallia pyracea* (Ach.) Arup, Frödén & Søchting**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350 m: JN). On bark of *Fagus* (N19; JN).

***Athallia saxifragarum* (Poelt) Arup, Frödén & Søchting**

M. Focalone near Bivacco Fusco (NT99); Grotte di Celano near M. Blockhaus (NT99); Anticima M. Acquaviva (JN: 2016); Anticima Femmina Morta (JN: 2017); Femmina Morta (JN: 2017); at five sites along the main ridge of Majella between 2322 and 2634 m (JN: 2018, 2019). – From the subalpine (2150 m: NT99) to the alpine (2634 m: JN) belt. On plant debris (JN).

***Bacidia igniarii* (Nyl.) Oxner**

Vallone Grascito (R20). – In the colline belt (568 m: R20). On bark of *Quercus pubescens* (R20). – This record is the only one available from Abruzzo (Nimis and Martellos 2020).

***Bacidia rubella* (Hoffm.) A. Massal.**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350 m: JN). On bark of *Acer campestre* (N19), *Fagus* (N19; JN).

***Bacidina arnoldiana* (Körb.) V. Wirth & Vězda**

Pretoro, Colle dell'Angelo (NT99; TSB: 1996). – In the montane belt (1200 m: NT99; TSB: 1996). On calcareous rock (NT99; TSB: 1996).

***Bagliettoa calciseda* (DC.) Gueidan & Cl. Roux**

Passo S. Leonardo (C09); Passo Lanciano (C09); Caramanico (C09); Lettomanoppello (C09); M. Blockhaus (C09). – From the colline (570 m: C09) to the subalpine (2170 m: C09) belt. In open shrublands (C09) and pastures (C09). On calcareous rock (C09).

***Bagliettoa marmorea* (Scop.) Gueidan & Cl. Roux**

Majella (C73; J74); Roccacaramanico (NT99); Passo S. Leonardo (C09); Caramanico (C09). – From the colline (570 m: C09) to the montane (1200 m: C09) belt. In pastures (C09) and open shrublands (C09). On calcareous rock (C09).

***Bagliettoa parmigera* (J. Steiner) Vězda & Poelt**

Roccacaramanico (NT99). – In the montane belt (1000 m: NT99).

***Bagliettoa parmigerella* (Zahlbr.) Vězda & Poelt**

Pretoro, Colle dell'Angelo (NT99). – In the montane belt (1200 m: NT99).

***Biatorea beckhausii* (Körb.) Tuck.**

Val di Foro (JN: 2018). – In the montane belt (1200 m: JN). On bark of *Fagus* (JN).

***Biatorella hemisphaerica* Anzi**

Anticima M. Acquaviva (JN: 2016). – In the alpine belt (2600 m: JN). In high-altitude open habitats (JN). On soil (JN). – New to Abruzzo. This is the southernmost record in Italy (Nimis 2016).

***Bilimbia lobulata* (Sommerf.) Hafellner & Coppins**

M. Focalone near Bivacco Fusco (NT99); at three sites along the main ridge of Majella between 2073 and 2664 m (JN: 2018, 2019). – From the subalpine (2073 m: JN) to the alpine (2664 m: JN) belt. In high-altitude open habitats (JN). On soil (JN).

***Bilimbia microcarpa* (Th. Fr.) Th. Fr.**

At one site along the main ridge of Majella (JN: 2019). – In the alpine belt (2634 m: JN). In high-altitude open habitats (JN). On soil (JN).

***Bilimbia sabuletorum* (Schreb.) Arnold**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996). – In the subalpine belt (2150 m: NT99; TSB). On calcareous soil (TSB).

***Blastenia ammiospila* (Ach.) Arup, Søchting & Frödén**

Tavola Rotonda (JN: 2017). – In the alpine belt (2400 m: JN). In high-altitude open habitats (JN). On plant debris with *Ochrolechia androgyna* and *Lecidella wulfenii* (JN). This is a mainly arctic-alpine to boreal-montane, bipolar lichen and the record reported here is the southernmost in Italy. New to Abruzzo.

***Blastenia ferruginea* (Huds.) A. Massal.**

Hermitage of M. Morrone (NT99; TSB: 1997). – In the colline belt (500 m: NT99; TSB). On bark of *Fraxinus ornus* (TSB).

***Blastenia subathallina* (H. Magn.) Arup & Vondrák**

At two sites along the main ridge of Majella between 2025 and 2085 m (JN: 2019). – In the subalpine belt (2025–2028 m: JN). In high-altitude open habitats (JN). On plant debris (JN). – New to Abruzzo. These are the first records from the Apennines and from peninsular Italy (cf. Nimis 2016).

***Blennothallia crispa* (Huds.) Otálora, P.M. Jørg. & Wedin**

Above Bivacco Fusco (JN: 2016); at one site along the main ridge of Majella (JN: 2018). – In the alpine belt (2490–2579 m: JN). In high-altitude open habitats (JN). On soil (JN).

***Bryoplaca sinapisperma* (DC.) Søchting, Frödén & Arup**

Majella (C73); Campo di Giove (J74); at four sites along the main ridge of Majella between 2560 and 2640 m (JN: 2019). – In the alpine belt (2560–2640 m: JN). In high-altitude open habitats (JN). On soil (JN). – These records from the Majella massif are the southernmost in Italy (cf. Nimis 2016).

***Bryoria fuscescens* (Gyeln.) Brodo & D. Hawksw.**

Majella (C73); Bosco di Pacentro (J74). – On bark (J74). – The historical record was not confirmed recently, but it is considered as reliable, since the ecological requirements of this species (Nimis 2016) occur within the study area.

***Buellia griseovirens* (Sm.) Almb.**

Val di Foro (JN: 2018). – In the montane belt (1200 m: JN). On bark of *Fagus* (JN).

***Buellia spuria* (Schaer.) Anzi**

Majella (C73); Campo di Giove (J74). – This is a silicicolous lichen (Nimis 2016) that likely meets its substrate requirements in the Majella massif on flint limestone.

***Calicium salicinum* Pers.**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350 m: JN). On bark of *Fagus* (N19; JN).

***Calogaya biatorina* (A. Massal.) Arup, Frödén & Söchting**

Roccacaramanico (NT99; TSB: 1996); Anticima Femmina Morta (JN: 2017). – From the montane (1000 m: NT99; TSB) to the alpine (2420 m: JN) belt. On calcareous rock (TSB).

***Calogaya bryochryson* (Poelt) Vondrák**

Above Bivacco Fusco (JN: 2016). – In the alpine belt (2490: JN). On soil (JN). – New to Abruzzo. This species is currently known from the Alps and this is the southernmost record in Italy (Nimis 2016).

***Calogaya lobulata* (Flörke) Arup, Frödén & Söchting**

Majella (C73). – The historical record was not confirmed recently, but it is considered as reliable since the ecological requirements of this species (Nimis 2016) occur within the study area. This old record is the only one from the Majella massif. The other records from Abruzzo were collected elsewhere (Nimis 2016). The species is included in the Italian Red List of epiphytic lichens as “vulnerable” (Nascimbene et al. 2013).

***Calogaya pusilla* (A. Massal.) Arup, Frödén & Söchting**

Majella (C73; J74). – The historical records were not confirmed recently, but they are considered as reliable, since this is a widespread species (Nimis 2016).

***Calogaya rouxii* (Gaya, Nav.-Ros. & Llimona) – provisionally placed here, ICN Art. 36.1b**

M. Focalone near Bivacco Fusco (NT99; TSB: 1996); Grotte di Celano near M. Blockhaus (NT99; TSB: 1996). – From the subalpine (2150 m: NT99; TSB) to the alpine (2500 m: NT99; TSB) belt. On calcareous rock (TSB). – These records were reported under *Caloplaca arnoldii* subsp. *arnoldii* by Nimis and Tretiach (1999), but later Nimis (in Nimis and Martellos 2020) revised the material, which proved to belong to *C. rouxii*.

***Calogaya schistidii* (Anzi) Arup, Frödén & Söchting**

Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On saxicolous mosses (TSB).

***Caloplaca cacuminum* Poelt**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996). – In the subalpine belt (1250 m: NT99; TSB). On calcareous rock (TSB). – This is the only known record for Abruzzo and peninsular Italy (Nimis 2016) and the southernmost in Europe (Nimis and Tretiach 1999; Nimis 2016).

***Caloplaca cerina* (Hedw.) Th. Fr. s.lat.**

Roccacaramanico (NT99; TSB: 1996); Valico della Forchetta (TSB: 1996); hermitage of M. Morrone (NT99; TSB: 1997); Lama dei Peligni (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018); Campo di Giove, Piano Cerreto (JN: 2018); along the highway Strada Statale 164 (JN: 2018). – From the colline (500 m: NT99; TSB) to the montane (1420 m: JN) belt. On bark of *Fagus* (N19; TSB; JN), *Fraxinus ornus* (TSB), *Quercus cerris* (JN) and *Ulmus minor* (JN). – *Caloplaca cerina* s. str. is an epiphytic species; the record from M. Blockhaus by Nimis and Tretiach (1999) could refer to *C. stillicidiorum* (see) and is not reported here.

***Caloplaca coccinea* (Müll. Arg.) Poelt**

M. Focalone near Bivacco Fusco (NT99; TSB: 1996); Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); above Bivacco Fusco (JN: 2016); M. d’Ugni (JN: 2017); Anticima Femmina Morta (JN: 2017); M. Macellaro (JN: 2018). – From the subalpine (1770 m: JN) to the alpine (2635 m: JN) belt. On calcareous rock (TSB; JN).

***Caloplaca erythrocarpa* (Pers.) Zwackh**

Majella (C73; J74); Roccacaramanico (NT99); Lama dei Peligni (JN: 2019). – From the colline (635 m: JN) to the montane (1000 m: NT99) belt. On concrete (JN).

***Caloplaca haematites* (Chaub.) Zwackh**

Hermitage of M. Morrone (NT99; TSB: 1997). – In the colline belt (500 m: NT99; TSB). On bark of *Fraxinus ornus* (TSB).

***Caloplaca nubigena* (Kremp.) Dalla Torre & Sarnth. var. *keissleri* (Servít) Clauzade & Cl. Roux**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996). – In the subalpine belt (2150 m: NT99; TSB). On calcareous rock (TSB).

***Caloplaca stillicidiorum* (Vahl) Lynge**

M. Focalone near Bivacco Fusco (NT99; TSB: 1996); Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); Anticima M. Acquaviva (JN: 2016); above Bivacco Fusco (JN: 2016); Sella di Grotta Canosa (JN: 2017); Anticima Femmina Morta (JN:

2017); at 30 sites along the main ridge of Majella between 1958 and 2681 m (JN: 2018, 2019). – From the subalpine (1958 m: JN) to the alpine (2681 m: JN) belt. In high-altitude open habitats (JN). On plant debris (JN) and calcareous soil (TSB).

***Caloplaca teicholyta* (Ach.) J. Steiner**

Lama dei Peligni (JN: 2019). – In the colline belt (635 m: JN). On concrete (JN).

***Candelaria concolor* (Dicks.) Stein**

Majella (C73; J74). – The historical records were not confirmed recently, but they are considered as reliable, since this is a very widespread species (Nimis 2016), which is probably common in the study area at low elevations.

***Candelariella aurella* (Hoffm.) Zahlbr.**

Majella (C73); Roccacaramanico (NT99; TSB: 1996); M. Focalone near Bivacco Fusco (NT99; TSB: 1996); Anticima Femmina Morta (JN: 2017). – From the montane (1000 m: NT99; TSB) to the alpine (2500 m: NT99; TSB) belt. On calcareous rock (TSB).

***Candelariella commutata* Otte & M. Westb.**

At two sites along the main ridge of Majella between 2634 and 2664 m (JN: 2019). – In the alpine belt (2634–2664 m: JN). In high-altitude open habitats (JN). On soil (JN). – This species was previously reported from Abruzzo, as *C. unilocularis*, only from the Gran Sasso massif by Nimis and Tretiach (1999).

***Candelariella faginea* Nimis, Poelt & Puntillo**

Along the Strada Statale 164 (JN: 2018); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350–1420 m: JN) belt. On bark of *Fagus* (N19; JN).

***Candelariella medians* (Nyl.) A.L. Sm.**

Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On calcareous rock (TSB).

***Candelariella reflexa* (Nyl.) Lettau**

Tocco da Casauria, Osservanza (RV96). – In the colline belt (370 m: RV96). On bark of *Quercus pubescens* (RV96).

***Candelariella vitellina* (Hoffm.) Müll. Arg.**

M. Focalone near Bivacco Fusco (NT99; TSB: 1996). – In the alpine belt (2500 m: NT99; TSB). On decalcified calcareous rock (TSB).

***Candelariella xanthostigma* (Ach.) Lettau**

Valico della Forchetta (TSB: 1996); Valle di Mario (JN: 2018); at three sites along the main ridge of Majella between 1825 and 2350 m (JN: 2019). – From the montane

(1360 m: TSB) to the alpine (2350 m: JN) belt. In high-altitude open habitats (JN). On bark of *Acer pseudoplatanus* (JN), *Fagus* (TSB) and on plant debris (JN).

***Carbonea vitellinaria* (Nyl.) Hertel**

M. Focalone near Bivacco Fusco (NT99; TSB: 1996). – In the alpine belt (2500 m: NT99; TSB). On decalcified calcareous rock (TSB). – A lichenicolous fungus growing on *Candelariella vitellina* (Nimis 2016).

***Catapyrenium cinereum* (Pers.) Körb.**

Anticima M. Acquaviva (JN: 2016); Sella di Grotta Canosa (JN: 2017); Grotta Canosa (JN: 2017); Cima dell'Altare (JN: 2017); at 20 sites along the main ridge of Majella between 2001 and 2660 m (JN: 2018, 2019). – From the upper montane (1535 m: JN) to the alpine (2660 m: JN) belt. In high-altitude open habitats (JN). On soil (JN).

***Catapyrenium daedaleum* (Kremp.) Stein**

At two sites along the main ridge of Majella between 2018 and 2119 m (JN: 2019). – In the subalpine belt (2018–2119 m: JN). In high-altitude open habitats (JN). On soil (JN). – This species was previously reported from Abruzzo only from the Gran Sasso massif by Nimis and Tretiach (1999).

***Catillaria lenticularis* (Ach.) Th. Fr.**

Pretoro, Colle dell'Angelo (NT99; TSB: 1996). – In the montane belt (1200 m: NT99; TSB). On calcareous rock (TSB).

***Catillaria nigroclavata* (Nyl.) J. Steiner**

Hermitage of M. Morrone (NT99; TSB: 1997). – In the colline belt (500 m: NT99; TSB). On bark of *Fraxinus ornus* (TSB).

***Cerothallia luteoalba* (Turner) Arup, Frödén & Söchting**

Vallone Grascito (R20). – In the colline belt (564 m: R20). On bark of *Quercus pubescens* (R20). – This record is the only one available from Abruzzo (Nimis and Martellos 2020).

***Cetraria aculeata* (Schreb.) Fr.**

At one site along the main ridge of Majella (JN: 2019). – In the alpine belt (2322 m: JN). In high-altitude open habitats (JN). On soil (JN).

***Cetraria ericetorum* Opiz**

Femmina Morta (J74); M. Rapina (JN: 2017); Iaccione (JN: 2017); near Campo di Giove (JN: 2018); at two sites along the main ridge of Majella between 1995 and 2020 m (JN: 2018, 2019). – From the montane (1250 m: JN) to the alpine (2367 m: JN) belt. In dry grasslands (JN) and high-altitude open habitats (JN). On soil (JN).

Cetraria islandica* (L.) Ach. subsp. *islandica

Majella (C73); M. Amaro (J74; JN: 2017); Femmina Morta (J74; JN: 2017); M. Focalone near Bivacco Fusco (NT99); above Bivacco Fusco (JN: 2016); Anticima M. Acquaviva (JN: 2016); Anfiteatro Murelle (JN: 2017); Guado di Coccia (JN: 2017); Tavola Rotonda (JN: 2017); Valle di Taranta (JN: 2017); Fondo di Femmina Morta (JN: 2017); trail “Sentiero P1” (JN: 2017); M. Macellaro (JN: 2017); between Iaccione and Piano Amaro (JN: 2017); between M. Amaro and Grotta Canosa (JN: 2017); Sella di Grotta Canosa (JN: 2017); Cima dell’Altare (JN: 2017); Valle Cannella (JN: 2017); Rava del Ferro (JN: 2017); M. Focalone (JN: 2017); M. Pescofalcone (JN: 2017); between M. Pescofalcone and M. Rapina (JN: 2017); M. Rapina (JN: 2017); La Carozza (JN: 2017); Cima Murelle (JN: 2017); Bivacco Fusco (JN: 2017); Martellese (JN: 2017); M. Blockhaus (JN: 2017); at 19 sites along the main ridge of Majella between 1847 and 2765 m (JN: 2018, 2019). – From the subalpine (1623 m: JN) to the alpine (2765 m: JN) belt. In high-altitude open habitats (JN). On soil (JN) and plant debris (JN).

***Cetraria muricata* (Ach.) Eckfeldt**

Anticima M. Acquaviva (JN: 2016); near Campo di Giove (JN: 2018). – From the montane (1250 m: JN) to the alpine (2600 m: JN) belt. In a dry grassland (JN). On soil (JN).

***Cetrelia olivetorum* (Nyl.) W. L. Culb. & C. F. Culb.**

M. Morrone, Impianezza (RV96). – In the colline belt (630 m: RV96). On bark of *Quercus pubescens* (RV96). – One of the few confirmed records from central Italy (Nimis 2016). The species is included in the Italian Red List of epiphytic lichens as “near-threatened” (Nascimbene et al. 2013).

***Circinaria calcarea* (L.) A. Nordin, Savić & Tibell**

Majella (J74); Roccamaramanico (NT99; TSB: 1996); hermitage of M. Morrone (NT99; TSB: 1997). – From the colline (500 m: NT99; TSB) to the montane (1000 m: NT99; TSB) belt. On calcareous rock (TSB).

***Circinaria hispida* (Mereschk.) A. Nordin, Savić & Tibell**

At one site along the main ridge of Majella (JN: 2019). – In the subalpine belt (1997 m: JN). In open habitats (JN). On soil (JN). – New to Abruzzo and to peninsular Italy (cf. Nimis 2016). The only other known Italian record is from Alpi Cozie (Piedmont), not far from the only known French locality in the Maritime Alps (Hafellner et al. 2004; Roux et al. 2017). This is a species typical of cold steppes and deserts which occurs in Eastern Europe, Near Asia, Central Asia and North America; it is found also in *Juniperus* steppes of Central Spain and the scattered occurrences in Italy, France and Greece represent natural connections between the two European disjunctions (Hafellner et al. 2004).

***Circinaria hoffmanniana* (S. Ekman & Fröberg ex R. Sant.) A. Nordin**

Roccacaramanico (NT99; TSB: 1996); Anticima Femmina Morta (JN: 2017). – From the montane (1000 m: NT99; TSB) to the alpine (2420 m: JN) belt. On calcareous rock (TSB).

***Circinaria viridescens* (A. Massal.) – provisionally placed here, ICN Art. 36.1b**

Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On decalcified calcareous rock (TSB).

***Cladonia cariosa* (Ach.) Spreng.**

At one site along the main ridge of Majella (JN: 2019). – In the subalpine belt (1847 m: JN). In open grasslands (JN). On calcareous soil (JN). – New to Abruzzo.

***Cladonia chlorophaea* (Sommerf.) Spreng.**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350 m: JN). In a beech forest (JN). On bark of *Fagus* (N19; JN).

***Cladonia coniocraea* (Flörke) Spreng.**

Valico della Forchetta (TSB: 1996). – In the montane belt (1360 m: TSB). On dead wood (TSB).

***Cladonia fimbriata* (L.) Fr.**

Pretoro, Colle dell'Angelo (NT99); summit ridge of M. Majella (JN: 2019). – From the montane (1200 m: NT99) to the subalpine (2091 m: JN) belt. On soil (JN).

***Cladonia foliacea* (Huds.) Willd.**

Roccacaramanico (NT99; TSB: 1996); Valle di Fara (JN: 2017); Capo Le Macchie (JN: 2017); Campo di Giove (JN: 2018); Cansano (JN: 2018); Palena (GG: 2018). – From the lower (800 m: JN) to the upper montane (1250 m: JN) belt. In dry grasslands (NT99; JN; GG). On calcareous soil (NT99; JN; GG). – The calciphilous ecotype, which occurs in the study area, has been considered for long as a separate species, *Cladonia convoluta* (Lam.) Anders, but recent studies proved that it belongs to the same species as the acidophilous ecotype (Pino Bodas et al. 2018).

Cladonia furcata* (Huds.) Schrad. subsp. *furcata

Near Campo Giove (JN); at two sites along the main ridge of Majella between 1995 and 2322 m (JN: 2019). – From the montane (1250 m: JN) to the subalpine (2322 m: JN) belt. In dry grasslands (JN) and high-altitude open habitats (JN). On soil (JN).

***Cladonia furcata* (Huds.) Schrad. subsp. *subrangiformis* (L. Scriba ex Sandst.) Pišút**

Roccacaramanico (NT99; TSB: 1996); Capo Le Macchie (JN: 2017). – In the montane belt (875–1000 m: NT99; JN). In calcareous dry grasslands (JN). On calcareous soil (NT99; JN).

***Cladonia humilis* (With.) J.R. Laundon**

Maiellone (C86; NAP: 1872). – This is the only known record for Abruzzo (Nimis 2016).

***Cladonia ochrochlora* Flörke**

Valle dell’Orfento (J74). – On soil (J74).

***Cladonia pocillum* (Ach.) Grognot**

Blockhaus, Grotte di Celano (NT99; TSB: 1996); near Bivacco Fusco (JN: 2016); near Femmina Morta (JN: 2017); Anticima Femmina Morta (JN: 2017); Tavola Rotonda (JN: 2017); trail between Rifugio Pomilio and M. Blockhaus (GG: 2018); at seven sites along the main ridge of Majella between 2025 and 2640 m (JN: 2018, 2019). – From the subalpine (1499 m: JN) to the alpine (2640 m: JN) belt. In open habitats, for example, grasslands (JN; GG). On calcareous soil (TSB; JN; GG).

***Cladonia polycarpoides* Nyl.**

Blockhaus, Grotte di Celano (NT99; TSB: 1996). – In the subalpine belt (2150 m: NT99; TSB). On calcareous soil (NT99; TSB). – This is the only known record for Abruzzo and peninsular Italy (Nimis 2016).

***Cladonia pyxidata* (L.) Hoffm.**

Majella (J74); Campo di Giove (JN: 2017, 2018); at 13 sites along the main ridge of Majella between 1812 and 2645 m (JN: 2018, 2019). – From the montane (1250 m: JN) to the alpine (2645 m: JN) belt. In open habitats, for example, grasslands (JN). On soil (JN).

***Cladonia rangiformis* Hoffm.**

Capo Le Macchie (JN: 2017); trail between Lama dei Peligni and Rifugio Fonte Tarì (JN: 2017); Cansano (JN: 2018); Campo di Giove (JN: 2018). – From the lower (875 m: JN) to the upper montane (1250 m: JN) belt. In dry grasslands (JN). On soil (JN).

***Cladonia symphycarpa* (Flörke) Fr.**

Fara San Martino, Vallone di Santo Spirito (RV96); Anticima Femmina Morta (JN: 2017); Grotta Canosa (JN: 2017); Sella di Grotta Canosa (JN: 2017); Valle di Taranta (JN: 2017); M. Blockhaus (GG: 2018); at 38 sites along the main ridge of Majella between 1847 and 2640 m (JN: 2018, 2019). – From the subalpine (1650 m: JN) to the alpine (2640 m: JN) belt. In calcareous grasslands (JN; GG) and high-altitude open habitats (JN). On calcareous soil (JN; GG).

***Clauzadea metzleri* (Körb.) D. Hawksw.**

Pretoro, Colle dell’Angelo (NT99; TSB: 1996); Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000–1200 m: NT99; TSB). On calcareous rock (TSB).

***Clauzadea monticola* (Schaer.) Hafellner & Bellem.**

Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On calcareous rock (TSB). – The only other record of this common species from Abruzzo is from the Gran Sasso massif (Nimis and Tretiach 1999).

***Collema flaccidum* (Ach.) Ach.**

Valico della Forchetta (TSB: 1996); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2016); Val di Foro (JN: 2018). – In the montane belt (1200–1350: JN). On bark of *Acer campestre* (N19) and *Fagus* (N19; TSB; JN).

***Collema furfuraceum* Du Rietz**

Lama dei Peligni (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – From the colline (650 m: JN) to the montane (1350 m: JN) belt. On bark of *Acer campestre* (N19; JN), *Acer pseudoplatanus* (N19) and *Fagus* (N19; JN).

***Collema nigrescens* (Huds.) DC.**

Caramanico, S. Tommaso (TSB). – In the colline belt (468 m: TSB). On bark of *Quercus* sp. (TSB).

***Collema subflaccidum* Degel.**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350 m: JN). On bark of *Acer campestre* (N19) and *Fagus* (N19; JN).

***Collema subnigrescens* Degel.**

Lama dei Peligni (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2016, 2018). – From the colline (650 m: JN) to the montane (1350 m: JN) belt. On bark of *Acer campestre* (N19) and *Fagus* (N19; JN).

***Dacampia hookeri* (Borrer) A. Massal.**

Trail between Blockhaus and M. Focalone (T15; TSB: 2005); Majella, Bivacco Fusco (JN: 2016). – In the subalpine belt (2290–2300 m: T15; TSB; JN). On organic soil (T15; TSB; JN). – This is the only known record for Abruzzo and central Italy (Nimis 2016).

***Dermatocarpon miniatum* (L.) W. Mann**

Majella (C73); ford of S. Antonio (J74); M. Focalone near Bivacco Fusco (NT99); Grotte di Celano near M. Blockhaus (NT99); between Grotta Canosa and M. Amaro (JN: 2017); M. Amaro (JN: 2017). – From the subalpine (2150 m: NT99) to the alpine (2700 m: JN) belt. On rock (JN).

***Diploschistes gypsaceus* (Ach.) Zahlbr.**

Majella (C73; J74). – The historical records were not confirmed recently, but they are considered as reliable, since the ecological conditions required by this species (Nimis 2016) occur within the study area.

***Diplotomma alboatrum* (Hoffm.) Flot.**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350 m: JN). On bark of *Acer campestre* (N19), *Fagus* (JN).

***Diplotomma hedinii* (H. Magn.) P. Clerc & Cl. Roux**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); Anticima Femmina Morta (JN: 2017). – From the subalpine (2150 m: NT99; TSB) to the alpine (2420 m: JN) belt. On calcareous rock (TSB; JN).

***Diplotomma venustum* (Körb.) Körb.**

Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On calcareous rock (NT99; TSB).

***Enchylium ligerinum* (Hy) Otálora, P.M. Jørg. & Wedin**

Lama dei Peligni (JN: 2017). – In the colline belt (600 m: JN). On bark of *Quercus pubescens* (JN). – The species is included in the Italian Red List of epiphytic lichens as “near-threatened” (Nascimbene et al. 2013).

***Enchylium limosum* (Ach.) Otálora, P.M. Jørg. & Wedin**

Majella (C73); Valle dell’Orfento (J74). – The historical records were not confirmed recently, but they are considered as reliable, since the ecological conditions required by this species (Nimis 2016) occur within the study area.

Enchylium polycarpon* (Hoffm.) Otálora, P.M. Jørg. & Wedin subsp. *polycarpon

Anticima M. Acquaviva (JN: 2016); Cima dell’Altare (JN: 2017). – From the subalpine (1535 m: JN) to the alpine (2600 m: JN) belt. On calcareous rock (JN).

***Enchylium tenax* (Sw.) Gray**

Majella (C73); Valle dell’Orfento (J74); M. Focalone near Bivacco Fusco (NT99; TSB: 1996); hermitage of M. Morrone (NT99; TSB: 1997); Anticima Femmina Morta (JN: 2017); M. Macellaro (JN: 2018); at 17 sites along the main ridge of Majella between 1812 and 2664 m (JN: 2018, 2019). – From the colline (500 m: NT99; TSB) to the alpine (2664 m: JN) belt. In high-altitude open habitats (JN). On soil (TSB; JN).

***Eopyrenula leucoplaca* (Wallr.) R.C. Harris**

Lama dei Peligni (JN: 2017). – In the colline belt (650 m: JN). On bark of *Quercus pubescens* (JN). – The species is included in the Italian Red List of epiphytic lichens as “near-threatened” (Nascimbene et al. 2013).

***Evernia divaricata* (L.) Ach.**

Ridge beneath Cima Macirenelle (JN: 2020). – In the subalpine belt (1825 m: JN). In a rocky high-altitude habitat (JN). On soil (JN). – This is the only known record for the Majella massif. The species has a scattered distribution on the highest mountains

of the Apennines (Nimis 2016) and was reported previously from Abruzzo only by Recchia and Villa (1996).

***Evernia prunastri* (L.) Ach.**

Monti Pizzi near S. Domenico (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018); Valle di Mario (JN: 2018); Piano Cerreto near Campo di Giove (JN: 2018); along the Strada Statale 164 (JN: 2018). – In the montane belt (1350–1434 m: JN). On bark of *Acer campestre* (N19), *Acer pseudoplatanus* (JN), *Fagus* (N19; JN) and *Quercus cerris* (JN).

Farnoldia hypocrita* (A. Massal.) Fröberg var. *hypocrita

Anticima M. Acquaviva (JN: 2016). – In the alpine belt (2600 m: JN). On calcareous rock (JN). – Previously reported from Abruzzo only by Jatta (1889).

Farnoldia jurana* (Schaer.) Hertel subsp. *jurana

M. Focalone near Bivacco Fusco (NT99; TSB: 1996); Anticima Femmina Morta (JN: 2017); M. Macellaro (JN: 2018). – In the alpine belt (2420–2635 m: JN). On calcareous rock (TSB; JN).

***Farnoldia micropsis* (A. Massal.) Hertel**

M. Focalone near Bivacco Fusco (NT99; TSB: 1996); Anticima M. Acquaviva (JN: 2017); M. Macellaro (JN: 2018). – In the alpine belt (2500–2700 m: NT99; TSB; JN). On calcareous rock (TSB; JN). – Previously reported from Abruzzo only by Hertel (1967) from the Majella and the Gran Sasso massifs, where it was recorded also by Nimis and Tretiach (1999).

***Flavocetraria nivalis* (L.) Kärnefelt & A. Thell**

Femmina morta (J74; JN: 2017); ridge beneath Cima Macirenelle (JN: 2020). – From the subalpine (1825 m: JN) to the alpine (2408 m: JN) belt. In open habitats (JN). On soil (J74; JN). – These are the only known records for the Majella massif. Common in the Alps, this species occurs only in a few sites of the central Apennines (Nimis 2016); it was previously reported from Abruzzo only from the Gran Sasso massif by Nimis and Tretiach (1999).

***Flavoparmelia caperata* (L.) Hale**

Majella (C73; J74); Pretoro, Colle dell'Angelo (NT99; TSB: 1996). – In the montane belt (1200 m: NT99; TSB). On bark of broadleaved trees (TSB).

***Flavoparmelia soredians* (Nyl.) Hale**

M. Morrone, Impianezza (RV96). – In the colline belt (630 m: RV96). On bark of *Quercus pubescens* (RV96).

***Flavoplaca granulosa* (Müll. Arg.) Arup, Frödén & Søchting**

Roccacaramanico (NT99; TSB: 1996); hermitage of M. Morrone (NT99; TSB: 1997). – From the colline (500 m: NT99; TSB) to the montane (1000 m: NT99; TSB) belt. On calcareous rock (NT99; TSB).

***Gyalecta jenensis* (Batsch) Zahlbr.**

Pretoro, Colle dell'Angelo (NT99; TSB: 1996; S: 1996); below the Majelletta (TSB: 2005). – In the montane belt (1200–1350 m: NT99; TSB). On calcareous rock (TSB).

***Gyalecta ulmi* (Sw.) Zahlbr.**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350 m: JN). On bark of *Acer campestre* (N19; JN) and *Acer pseudoplatanus* (N19). – The species is included in the Italian Red List of epiphytic lichens as “near-threatened” (Nascimbene et al. 2013).

***Gyalolechia aurea* (Schaer.) A. Massal.**

Majella (C73); Grotte di Celano near M. Blockhaus (NT99; TSB: 1996). – In the sub-alpine belt (2150 m: NT99; TSB). On soil (TSB). – This species has been previously reported from other localities in the Apennines only from Abruzzo (Gran Sasso massif) by Nimis and Tretiach (1999). The record from M. Blockhaus is the southernmost in Europe (Nimis 2016).

***Gyalolechia bracteata* (Hoffm.) A. Massal.**

Campo di Giove (JN: 2018); at one site along the main ridge of Majella (JN: 2019). – From the montane (1200 m: JN) to the alpine (2634 m: JN) belt. In high-altitude open habitats (JN). On soil (JN). – New to Abruzzo. This is the southernmost record in Italy (Nimis 2016).

Gyalolechia flavorubescens* (Huds.) Søchting, Frödén & Arup var. *flavorubescens

Lama dei Peligni (JN: 2017); Grotta di S. Angelo (JN: 2018). – From the colline (650 m: JN) to the lower montane (850 m: JN) belt. On bark of *Fagus* (JN) and *Quercus pubescens* (JN).

***Gyalolechia flavorubescens* (Huds.) Søchting, Frödén & Arup var. *quercina* (Flagey) Nimis**

Caramanico, S. Tommaso (TSB: 1990). – In the colline belt (468 m: TSB). On bark of *Quercus* sp. (TSB).

***Gyalolechia fulgens* (Sw.) Søchting, Frödén & Arup**

Majella (C73); Valle di Fara (JN: 2017). – In the lower montane belt (800 m: JN). On soil (JN).

***Gyalolechia subbracteata* (Nyl.) Søchting, Frödén & Arup**

Near Bivacco Fusco (JN: 2016); Sella di Grotta Canosa (JN: 2017). – In the alpine belt (2290–2552 m: JN). On soil (JN).

***Halecania lecanorina* (Anzi) M. Mayrhofer & Poelt**

Majella (C73). – This is the only record from Abruzzo, Apennines and peninsular Italy and the southernmost in Italy (Nimis 2016).

***Heppia adglutinata* (Kremp.) A. Massal.**

Femmina Morta (JN: 2019). – In the alpine belt (2330 m: JN). On calciferous soil (JN). New to Abruzzo. This is a cool-temperate to boreal-montane, circumpolar, ephemeral lichen growing in dry, open grasslands.

***Hertelidea botryosa* (Fr.) Printzen & Kantvilas**

Bolognano, Madonna del M. (RV96). – In the colline belt (330 m: RV96). On bark of *Quercus pubescens* (RV96). – This is the only record from Abruzzo and the southernmost for Italy (Nimis 2016).

***Heterodermia speciosa* (Wulfen) Trevis.**

Majella (C73). – The historical record was not confirmed recently, but it is considered as reliable, since the ecological conditions required by this species (Nimis 2016) occur within the study area. It is included in the Italian Red List of epiphytic lichens as “near-threatened” (Nascimbene et al. 2013).

***Heteroplacidium fuscum* (Nyl.) Gueidan & Cl. Roux**

Roccacaramanico (NT99). – In the montane belt (1000 m: NT99). On calcareous rock, lichenicolous on *Circinaria calcarea* (NT99).

***Hyperphyscia adglutinata* (Flörke) H. Mayrhofer & Poelt**

Lama dei Peligni (JN: 2017). – In the colline belt (650 m: JN). On bark of *Ulmus minor* (JN).

***Lathagrium auriforme* (With.) Otálora, P.M. Jørg. & Wedin**

Majella (C73); Caramanico (J74); Roccacaramanico (NT99; TSB: 1996); at three sites in Val di Foro (JN: 2018); below Villaggio Mirastelle (JN: 2018). – From the lower (970 m: JN) to the upper montane (1250 m: JN) belt. On calcareous rock (TSB), terricolous mosses (J74; JN).

***Lathagrium cristatum* (L.) Otálora, P.M. Jørg. & Wedin**

Majella (C73); Caramanico (J74); Roccacaramanico (NT99; TSB: 1996); hermitage of M. Morrone (NT99; TSB: 1996); at one site along the main ridge of Majella (JN: 2019). – From the montane (1000 m: NT99; TSB) to the subalpine (2081 m: JN) belt. In high-altitude open habitats (JN). On calcareous rock (TSB), soil (JN).

***Lathagrium fuscovirens* (With.) Otálora, P.M. Jørg. & Wedin**

Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On calcareous rock (TSB).

***Lathagrium undulatum* (Flot.) Poetsch**

Majella (C73); at five sites along the main ridge of Majella between 2380 and 2664 m (JN: 2019). – In the alpine belt (2380–2664 m: JN). In high-altitude open habitats (JN). On calcareous rock (JN).

***Lecania cyrtella* (Ach.) Th. Fr.**

Roccacaramanico (NT99; TSB: 1996); at three sites along the main ridge of Majella between 2210 and 2461 m (JN: 2018, 2019). – From the montane (1000 m: NT99; TSB) to the alpine (2461 m: JN) belt. In high-altitude open habitats (JN). On bark of broadleaved trees (TSB) and on small shrubs (JN).

Lecanora allophana* (Ach.) Nyl. f. *allophana

Majella (C73); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350 m: JN). On bark of *Acer campestre* (N19; JN).

***Lecanora argentata* (Ach.) Malme**

Pretoro, Colle dell'Angelo (NT99; TSB: 1996); Valico della Forchetta (TSB: 1996); Monti Pizzi near S. Domenico (JN: 2017). – In the montane belt (1200–1434 m: NT99; TSB; JN). On bark of *Fagus* (TSB; JN).

***Lecanora carpinea* (L.) Vain.**

Roccacaramanico (NT99; TSB: 1996); Pretoro, Colle dell'Angelo (NT99; TSB: 1996); Piano Cerreto near Campo di Giove (JN: 2018); below Villaggio Mirastelle (JN: 2018); along the Strada Statale 164 (JN: 2018). – In the montane belt (1000–1420 m: NT99; TSB; JN). On bark of *Fagus* (TSB; JN), *Quercus cerris* (JN).

Lecanora chlarotera* Nyl. subsp. *chlarotera

Pretoro, Colle dell'Angelo (NT99; TSB: 1996); Lama dei Peligni (JN: 2017); Monti Pizzi near S. Domenico (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018); Valle di Mario (JN: 2018); Piano Cerreto near Campo di Giove (JN: 2018); at one site along the main ridge of Majella (JN: 2019). – From the colline (650 m: JN) to the alpine (2350 m: JN) belt. On bark of *Acer pseudoplatanus* (JN), *Fagus* (N19; TSB; JN) and *Quercus cerris* (JN).

Lecanora epibryon* (Ach.) Ach. var. *epibryon

M. Focalone near Bivacco Fusco (NT99; TSB: 1996); Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); near Bivacco Fusco (JN: 2016); Tavola Rotonda (JN: 2017); Femmina Morta (JN: 2017); at one site along the main ridge of Majella (JN: 2019). – From the subalpine (2150 m: NT99; TSB) to the alpine (2634 m: JN) belt. In high-altitude open habitats (JN). On plant debris (TSB; JN).

***Lecanora horiza* (Ach.) Linds.**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350 m: JN). On bark of *Fagus* (N19; JN).

***Lecanora intumescens* (Rebent.) Rabenh.**

Valico della Forchetta (TSB: 1996); Monti Pizzi near S. Domenico (JN: 2017); Valle di Mario (JN: 2018); Val di Foro (JN: 2018). – From the lower (970 m: JN) to the upper montane (1434 m: JN) belt. On bark of *Fagus* (TSB; JN).

***Lecanora leptyroides* (Nyl.) Degel.**

Pretoro, Colle dell'Angelo (NT99; TSB: 1996); Lama dei Peligni (JN: 2017); Monti Pizzi near S. Domenico (JN: 2017); Valle di Mario (JN: 2018). – From the lower (650 m: JN) to the upper montane (1434 m: JN) belt. On bark of *Fagus* (TSB; JN).

***Lecanora pulicaris* (Pers.) Ach.**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996). – In the subalpine belt (2150 m: NT99; TSB). On bark of conifers (TSB).

***Lecanora rouxii* S. Ekman & Tønsberg**

Hermitage of M. Morrone (NT99; TSB: 1997). – In the colline belt (500 m: NT99; TSB). On calcareous soil (TSB). – This is the only known record for Abruzzo and the southernmost in Italy (Nimis 2016).

***Lecanora subcarpineae* Szatala**

Roccacaramanico (NT99; TSB: 1996); Valico della Forchetta (TSB: 1996); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1000–1360 m: NT99; TSB). On bark of *Fagus* (N19; TSB; JN).

***Lecanora varia* (Hoffm.) Ach.**

Majella (C73; J74). This old record was not confirmed by recent surveys, but it can be considered reliable since this cool-temperate to circumboreal-montane lichen is common on hard lignum in upland areas, including Mediterranean mountains (Nimis 2016).

***Lecidea berengeriana* (A. Massal.) Nyl.**

M. Focalone near Bivacco Fusco (NT99; TSB: 1996). – In the alpine belt (2500 m: NT99; TSB). On bryophytes and plant debris (TSB).

***Lecidea confluens* (Weber) Ach.**

Majella (C73); Monte Amaro (J74). – This is a silicolous lichen (Nimis 2016) that likely meets its substrate requirements in the Majella massif on flint limestone.

***Lecidea speirodes* Nyl.**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996). – In the subalpine belt (2150 m: NT99; TSB). On calcareous rock (TSB). – This is the only known record for Abruzzo and peninsular Italy and the southernmost in Europe (Nimis 2016).

***Lecidella carpathica* Körb.**

Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On calcareous rock (TSB).

Lecidella elaeochroma* (Ach.) M. Choisy var. *elaeochroma* f. *elaeochroma

Majella (C73); Roccacaramanico (NT99; TSB: 1996); Pretoro, Colle dell'Angelo (NT99; TSB: 1996); Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); Valico della Forchetta (TSB: 1996); hermitage of M. Morrone (NT99; TSB 1997); Lama dei Peligni (JN: 2017); Monti Pizzi near S. Domenico (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018); Valle di Mario (JN: 2018); Piano Cerreto near Campo di Giove (JN: 2018); below Villaggio Mirastelle (JN: 2018); along Strada Statale 164 (JN: 2018); at 9 sites along the main ridge of Majella between 2001 and 2533 m (JN: 2018, 2019). – From the colline (500 m: NT99; TSB) to the alpine (2533 m: JN) belt. In high-altitude open habitats (JN). On bark of *Acer pseudoplatanus* (JN), *Fagus* (N19; TSB; JN), *Fraxinus ornus* (TSB), *Quercus cerris* (JN) and on plant debris (JN).

***Lecidella euphorea* (Flörke) Hertel**

At twenty sites along the main ridge of Majella between 1812 and 2350 m (JN: 2019). – From the subalpine (1812 m: JN) to the alpine (2350 m: JN) belt. In high-altitude open habitats (JN). On *Juniperus* twigs (JN). – It was previously reported from Abruzzo only by Grillo and Romano (1987) from the Abruzzo National Park.

***Lecidella patavina* (A. Massal.) Knoph & Leuckert**

Pretoro, Colle dell'Angelo (NT99; TSB: 1996); M. Focalone near Bivacco Fusco (NT99; TSB: 1996); Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); Anticima Femmina Morta (JN: 2017); Anticima M. Acquaviva (JN: 2017); M. Macellaro (JN: 2018). – From the montane (1200 m: NT99; TSB) to the alpine (2700 m: JN) belt. On calcareous rock (TSB; JN).

***Lecidella wulfenii* (Hepp) Körb.**

Tavola Rotonda (JN: 2017); at one site along the main ridge of Majella (JN: 2019). – In the alpine belt (2322–2398 m: JN). In high-altitude open habitats (JN). On plant debris (JN). – This species was previously reported from Abruzzo only from the Gran Sasso massif by Nimis and Tretiach (1999).

***Leptra albescens* (Huds.) Hafellner**

Lama dei Peligni (JN: 2017); Monti Pizzi near S. Domenico (JN: 2017); Grotta di S. Angelo (JN: 2018); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – From the

colline (650 m: JN) to the montane (1440 m: JN) belt. On bark of *Fagus* (N19; JN), *Quercus cerris* (N19) and *Quercus pubescens* (JN).

***Lepraria eburnea* J.R. Laundon**

At one site along the main ridge of Majella (JN: 2019). – In the alpine belt (2573 m: JN). In high-altitude open habitats (JN). On soil (JN).

***Lepraria nivalis* J.R. Laundon**

Lettomanoppello, Fontana del Papa (TSB: 2005). – In the colline belt (500 m: TSB). On calcareous rock (TSB).

***Lepraria vouauxii* (Hue) R.C. Harris**

Anticima M. Acquaviva (JN: 2016). – In the alpine belt (2600 m: JN). On soil (JN).

***Leproplaca xantholyta* (Nyl.) Hue**

Pretoro, Colle dell'Angelo (NT99; TSB: 1996); Valle di Fara (JN: 2017). – In the montane belt (800–1200 m: NT99; TSB; JN). On calcareous rock (TSB; JN).

***Leptogium hildenbrandii* (Garov.) Nyl.**

Majella (C73); Piano dei Mulini (J74); Lama dei Peligni (JN: 2017). – In the colline belt (650 m: JN). On bark of *Quercus pubescens* (JN). – The species is included in the Italian Red List of epiphytic lichens as “near-threatened” (Nascimbene et al. 2013).

***Leptogium saturninum* (Dicks.) Nyl.**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2016, 2018); Valle di Mario (JN: 2018); at one site along the main ridge of Majella (JN: 2018). – In montane belt (1350 m: JN). In beech-dominated forests (JN). On bark of *Acer pseudoplatanus* (JN) and *Fagus* (N19; JN).

***Lobaria pulmonaria* (L.) Hoffm.**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2016, 2018). – In the montane belt (1350 m: JN). On bark of *Fagus* (N19; JN) and *Quercus cerris* (N19). – The species is included in the Italian Red List of epiphytic lichens as “least concern” (Nascimbene et al. 2013).

***Lobothallia controversa* Cl. Roux & A. Nordin**

Majella (C73). This old record was not confirmed by recent survey, but it seems reliable since this is a mainly southern species in Europe, found on hard rocks with optimum in the montane belt (Nimis 2016).

***Lobothallia radiosa* (Hoffm.) Hafellner**

Majella (C73); Valle dell'Orfento (J74); Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On calcareous rock (TSB).

***Melanelixia glabra* (Schaer.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch**

Roccacaramanico (NT99; TSB: 1996); Lama dei Peligni (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – From the colline (650 m: JN) to the montane (1350 m: JN) belt. On bark of *Acer campestre* (N19), *Fagus* (N19; JN) and *Ulmus minor* (JN).

***Melanelixia glabratula* (Lamy) Sandler & Arup**

Valico della Forchetta (TSB: 1996); Lama dei Peligni (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018); Valle di Mario (JN: 2018); Piano Cerreto near Campo di Giove (JN: 2018); below Villaggio Mirastelle (JN: 2018); along the highway Strada Statale 164 (JN: 2018). – From the colline (650 m: JN) to the montane (1420 m: JN) belt. On bark of *Acer campestre* (N19), *Acer pseudoplatanus* (JN), *Fagus* (N19; TSB; JN), *Quercus cerris* (JN) and *Ulmus minor* (JN).

***Melanelixia subargentifera* (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch**

Lama dei Peligni (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – From the colline (650 m: JN) to the montane (1350 m: JN) belt. On bark of *Acer campestre* (N19; JN), *Fagus* (N19; JN) and *Ulmus minor* (JN).

***Melanelixia subaurifera* (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch**

Bosco di S. Antonio (RV96); San Domenico, Monti Pizzi (JN: 2017); Valle di Mario (JN: 2018); along the highway Strada Statale 164 (JN: 2018). – In the montane belt (1340–1434 m: RV96; JN). On bark of *Acer pseudoplatanus* (JN) and *Fagus* (RV96; JN).

***Melanohalea elegantula* (Zahlbr.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch**

Lama dei Peligni (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – From the colline (650 m: JN) to the montane (1350 m: JN) belt. On bark of *Acer campestre* (N19), *Fagus* (N19; JN) and *Quercus pubescens* (JN).

***Melanohalea exasperata* (De Not.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch**

Roccacaramanico (NT99; TSB: 1996); Pretoro, Colle dell'Angelo (NT99; TSB: 1996); Piano Cerreto near Campo di Giove (JN: 2018). – In the montane belt (1000–1200 m: NT99; TSB). On bark of *Quercus cerris* (JN).

***Melaspilea enteroleuca* (Ach.) Ertz & Diederich**

Guesthouse of Lama dei Peligni (JN: 2017). – In the colline belt (650 m: JN). On bark of *Quercus pubescens* (JN). – The species is included in the Italian Red List of epiphytic lichens as “near-threatened” (Nascimbene et al. 2013).

***Merismatium decolorans* (Rehm) Triebel**

Anticima Femmina Morta (JN: 2017). – In the alpine belt (2420 m: JN). In open high-altitude habitat (JN). – A lichenicolous fungus growing on *Cladonia symphycarpa* (JN).

Micarea lignaria* (Ach.) Hedl. var. *lignaria

Bosco di Pacentro (J74). – On bark of *Fagus* (J74). – The historical record was not confirmed recently, but it is considered as reliable, since the ecological conditions required by this species (Nimis 2016) occur within the study area.

***Mycobilimbia pilularis* (Körb.) Hafellner & Türk**

Majella, S. Antonino (J74). – On mosses (J74). – The historical record was not confirmed recently, but is considered as reliable, since the ecological conditions required by this species (Nimis 2016) occur within the study area.

Myriolecis agardhiana* (Ach.) Sliwa, Zhao Xin & Lumbsch subsp. *agardhiana

M. Focalone near Bivacco Fusco (NT99; TSB: 1996); Anticima Femmina Morta (JN: 2017); Anticima M. Acquaviva (JN: 2017); M. Macellaro (JN: 2018). – In the alpine belt (2420–2700 m: JN). On calcareous rock (TSB; JN).

***Myriolecis agardhiana* (Ach.) Sliwa, Zhao Xin & Lumbsch subsp. *sapaudica* (Cl. Roux) Nimis & Cl. Roux**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); Anticima Femmina Morta (JN: 2017). – From the subalpine (2150 m: NT99; TSB) to the alpine (2420 m: JN) belt. On calcareous rock (TSB; JN). – The only other record of this taxon from Abruzzo is from the Gran Sasso massif (Nimis and Tretiach 1999). Those from Majella are the southernmost records for Italy (Nimis 2016).

***Myriolecis dispersa* (Pers.) Sliwa, Zhao Xin & Lumbsch**

Majella (C73); Roccamaramanico (NT99). – In the montane belt (1000 m: NT99).

***Myriolecis hagenii* (Ach.) Sliwa, Zhao Xin & Lumbsch**

Popoli, Impianezza (RV96); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018); Campo di Giove, Piano Cerreto (JN: 2018). – From the colline (630 m: RV96) to the montane (1350 m: JN) belt. On bark of *Acer campestre* (N19), *Fagus* (N19; JN), *Quercus cerris* (JN) and *Quercus pubescens* (RV96).

***Myriolecis perpruinosa* (Fröberg) Sliwa, Zhao Xin & Lumbsch**

Anticima Femmina Morta (JN: 2017). – In the alpine belt (2420 m: JN). On calcareous rock (JN). – New to Abruzzo. This is the southernmost record of the species in Italy (Nimis 2016).

***Myriolecis reuteri* (Schaer.) Sliwa, Zhao Xin & Lumbsch**

M. Focalone near Bivacco Fusco (NT99; TSB: 1996); Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); M. d'Ugni (JN: 2017). – From the subalpine (1770 m: JN) to the alpine (2500 m: NT99; TSB) belt. On calcareous rock (TSB; JN).

***Myriolecis semipallida* (H. Magn.) Sliwa, Zhao Xin & Lumbsch**

Majella (C73); M. Focalone near Bivacco Fusco (NT99; TSB: 1996); Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); Anticima M. Acquaviva (JN: 2017). – From the subalpine (2150 m: NT99; TSB) to the alpine (2700 m: JN) belt. On calcareous rock (TSB; JN).

***Myriolecis zosteræ* (Ach.) Šliwa, Zhao Xin & Lumbsch var. *palanderi* (Vain.) Šliwa**

M. Focalone near Bivacco Fusco (NT99); Bivacco Fusco (JN: 2016); Anticima M. Acquaviva (JN: 2016); Anticima Femmina Morta (JN: 2017); Sella di Grotta Canosa (JN: 2017); at 11 sites along the main ridge of Majella between 1997 and 2681 m (JN: 2018, 2019). – From the subalpine (1997 m: JN) to the alpine (2681 m: JN) belt. In high-altitude open habitats (JN). On plant debris (JN).

***Nephroma resupinatum* (L.) Ach.**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2016, 2018); Monti Pizzi, Valle del Sole (JN: 2017). – In the montane belt (1350–1455 m: JN). On bark of *Fagus* (N19; JN). – The species is included in the Italian Red List of epiphytic lichens as “near-threatened” (Nascimbene et al. 2013).

***Ochrolechia arborea* (Kreyer) Almb.**

Majella (J74). – On bark of *Fagus* (J74). – The historical record was not confirmed recently, but it is considered as reliable, since this is a widespread species (Nimis 2016).

***Ochrolechia pallescens* (L.) A. Massal.**

Majella (C73); Monti Pizzi near S. Domenico (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350–1434 m: JN). On bark of *Fagus* (N19; J74) and *Quercus cerris* (N19).

***Ochrolechia upsaliensis* (L.) A. Massal.**

Tavola Rotonda (JN: 2017). – In the alpine belt (2398 m: JN). On soil (JN). – The only other record of this taxon from Abruzzo is from the Gran Sasso massif (Nimis and Tretiach 1999). Those from Majella are the southernmost records of this taxon for Italy (Nimis 2016).

***Opegrapha rupestris* Pers.**

Majella (C73; J74). – The historical records were not confirmed recently, but they are considered as reliable, since this is a widespread species. Lichenicolous in various verrucarialean crustose lichens (Nimis 2016).

***Ophioparma ventosa* (L.) Norman**

Majella (C73); Campo di Giove (J74). – This is a silicicolous, arctic-alpine circumpolar lichen (Nimis 2016) that likely meets its substrate requirements in the Majella massif on flint limestone. The southernmost records in Italy are those of Calabria (Nimis 2016).

***Parabagliettoa disjuncta* (Arnold) Krzewicka**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996). – In the subalpine belt (2150 m: NT99; TSB). On calcareous rock (TSB). – This is the only known record of the species from Abruzzo, peninsular Italy and the Apennines and the southernmost in Italy (Nimis 2016).

***Parabagliettoa dufourii* (DC.) Gueidan & Cl. Roux**

M. Focalone near Bivacco Fusco (NT99; TSB: 1996). – In the alpine belt (2500 m: NT99; TSB). On calcareous rock (TSB). – This is the only known record of the species from Abruzzo (Nimis 2016).

***Parmelia saxatilis* (L.) Ach.**

Majella (C73); S. Antonio (J74). – The historical records were not confirmed recently, but they are considered as reliable, since this is a widespread species (Nimis 2016). However, different cryptic species may occur in the group of *P. saxatilis* (e.g. Molina et al. 2004) and further research is required to clarify which of them occurs in the study area.

***Parmelia submontana* Hale**

Monti Pizzi near S. Domenico (JN: 2017); below Villaggio Mirastelle (JN: 2018); along the highway Strada Statale 164 (JN: 2018). – In the montane belt (1230–1434 m: JN). On bark of *Fagus* (JN).

***Parmelia sulcata* Taylor**

Pretoro, Colle dell'Angelo (NT99; TSB: 1996); Valico della Forchetta (TSB: 1996); Pescocostanzo (TSB); Monti Pizzi near S. Domenico (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018); Valle di Mario (JN: 2018); Piano Cerreto near Campo di Giove (JN: 2018); below Villaggio Mirastelle (JN: 2018); along the highway Strada Statale 164 (JN: 2018). – In the montane belt (1200–1434 m: NT99; TSB; JN). On bark of *Acer campestre* (N19), *Acer pseudoplatanus* (N19; JN), *Fagus* (N19; TSB; JN) and *Quercus cerris* (N19; JN).

***Parmeliella triptophylla* (Ach.) Müll. Arg.**

Majella (C73). – The historical record was not confirmed recently, but it is considered as reliable, since the ecological conditions required by this species (Nimis 2016) occur within the study area. It is included in the Italian Red List of epiphytic lichens as “near-threatened” (Nascimbene et al. 2013).

***Parmelina carporrhizans* (Taylor) Poelt & Vězda**

Cerro, Popoli (RV96). – In the colline belt (350 m: RV96). On bark of *Quercus pubescens* (RV96). – This is the only known record from Abruzzi (Nimis 2016).

***Parmelina pastillifera* (Harm.) Hale**

Monti Pizzi near S. Domenico (JN: 2017); Grotta di S. Angelo near Lama dei Peligni (JN: 2018). – From the lower (850 m: JN) to the upper montane (1440 m: JN) belt. On bark of *Fagus* (JN).

***Parmelina quercina* (Willd.) Hale**

Valico della Forchetta (TSB: 1996); Lama dei Peligni (JN: 2017); Piano Cerreto near Campo di Giove (JN: 2018). – From the colline (650 m: JN) to the montane (1350 m: TSB) belt. On bark of *Fagus* (TSB), *Quercus cerris* (JN) and *Ulmus minor* (JN).

***Parmelina tiliacea* (Hoffm.) Hale**

Majella (J74); Lama dei Peligni (JN: 2017); Monti Pizzi near S. Domenico (JN: 2017); Grotta di S. Angelo near Lama dei Peligni (JN: 2018); Valle di Mario (JN: 2018); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – From the colline (650 m: JN) to the montane (1434 m: JN) belt. On bark of *Acer campestre* (N19), *Acer pseudoplatanus* (N19; JN), *Fagus* (N19; JN), *Quercus cerris* (N19) and *Quercus pubescens* (JN).

***Parmotrema perlatum* (Huds.) M. Choisy**

Majella (C73; J74). – The historical records were not confirmed recently, but they are considered as reliable, since this is a widespread species (Nimis 2016).

***Parvoplaca tirolensis* (Zahlbr.) Arup, Søchting & Frödén**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); near Bivacco Fusco (JN: 2016); Anticima M. Acquaviva (JN: 2016); Anticima Femmina Morta (JN: 2017); at 11 sites along the main ridge of Majella between 2322 and 2664 m (JN: 2018, 2019). – From the subalpine (2150 m: NT99; TSB) to the alpine (2664 m: JN) belt. In high-altitude open habitats (JN). On plant debris (TSB; JN). – The only other record of this taxon from Abruzzo is from the Gran Sasso massif (Nimis and Tretiach 1999). Those from Majella are the southernmost records for Italy (Nimis 2016).

***Peccania coralloides* (A. Massal.) Arnold**

Guado di S. Antonio (J74). – On rock (J74). – The historical record was not confirmed recently, but it is considered as reliable, since this is a widespread species (Nimis 2016).

***Peltigera canina* (L.) Willd.**

Majella (C73; J74). – The historical record was not confirmed recently, but it is considered as reliable, since this is a widespread species (Nimis 2016).

***Peltigera collina* (Ach.) Schrad.**

Pescocostanzo (TSB); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350 m: JN). On bark of *Fagus* (N19; TSB; JN).

***Peltigera elisabethae* Gyeln.**

M. Rapina (JN: 2017); Val di Foro (JN: 2018). – From the montane (970 m: JN) to the subalpine (1920 m: JN) belt. On soil (JN) and terricolous mosses (JN). – New to Abruzzo.

***Peltigera horizontalis* (Hudson) Baumg.**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2016); near Campo di Giove (JN: 2018); Val di Foro (JN: 2018). – In the montane belt (1200–1450 m: JN). In beech woods (JN). On soil (JN), terricolous mosses (JN) and epiphytic mosses on *Fagus* (N19; JN).

***Peltigera lepidophora* (Vain.) Bitter**

Above Bivacco Fusco (JN: 2016). – In the alpine belt (2490 m: JN). On soil (JN). – New to Abruzzo.

***Peltigera leucophlebia* (Nyl.) Gyeln.**

Fara San Martino, Vallone di Santo Spirito (RV96); Blockhaus, Grotte di Celano (NT99). – From the montane (1100 m: RV96) to the subalpine (2150 m: NT99) belt. On soil above calcareous rock (RV96). – These are the only known records for Abruzzo (Nimis 2016).

***Peltigera neckeri* Müll.Arg.**

Valle dell'Orfento (RV96); near Passo Lanciano (JN: 2017); Val di Foro (JN: 2018); at one site along the main ridge of Majella (JN: 2019). – From the colline (530 m: RV96) to the subalpine (2119 m: JN) belt. In high-altitude open habitats (JN). On soil (JN), mosses (RV96).

***Peltigera polydactylon* (Neck.) Hoffm.**

Above Bivacco Fusco (JN: 2016). – In the alpine belt (2490 m: JN). On soil (JN).

***Peltigera praetextata* (Sommerf.) Zopf**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2016, 2018); Monti Pizzi near S. Domenico (JN: 2017); near Campo di Giove (JN: 2018); Val di Foro (JN: 2018); below Villaggio Mirastelle (JN: 2018). – In the montane belt (970–1450 m: JN). In beech woods (JN). On soil (JN), terricolous mosses (JN), epiphytic mosses on *Fagus* (JN) and bark of *Fagus* (N19; JN).

***Peltigera rufescens* (Weiss) Humb.**

M. Focalone near Bivacco Fusco (NT99; TSB: 1996); near Bivacco Fusco (JN: 2016); Anticima M. Acquaviva (JN: 2016); Campo di Giove (JN: 2017); Colle d'Acquaviva

(JN: 2017); Sella di Grotta Canosa (JN: 2017); Campo di Giove (JN: 2018); near Campo di Giove (JN: 2018); trail between Rifugio Pomilio and M. Blockhaus (GG); M. Blockhaus (GG); at nine sites along the main ridge of Majella between 2149 and 2637 m (JN: 2018, 2019). – From the subalpine (1715 m: JN) to the alpine (2637 m: JN) belt. In dry grasslands (JN; GG) and high-altitude open habitats (JN). On soil (TSB; JN; GG).

***Pertusaria coronata* (Ach.) Th. Fr.**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350 m: JN). On bark of *Fagus* (N19; JN).

***Petractis clausa* (Hoffm.) Kremp.**

Roccacaramanico (NT99; TSB: 1996); below the Maielletta (TSB: 2005). – In the montane belt (1000 m: NT99; TSB). On calcareous rock (TSB). – These records from Majella are the only recent ones from Abruzzo, the others date back to the 19th Century (see literature cited by Nimis 1993).

***Phaeophyscia ciliata* (Hoffm.) Moberg**

Roccacaramanico (NT99; TSB: 1996); Lama dei Peligni (JN: 2017). – From the colline (650 m: JN) to the montane (1000 m: NT99; TSB) belt. On bark of *Ulmus minor* (JN).

***Phaeophyscia orbicularis* (Neck.) Moberg**

Majella (C73); S. Antonio (J74); Caramanico S. Tommaso (TSB: 1990); Roccacaramanico (NT99; TSB: 1996); Lama dei Peligni (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018); Piano Cerreto near Campo di Giove (JN: 2018). – From the colline (468 m: TSB) to the montane (1350 m: JN) belt. On bark of *Acer campestre* (JN), *Acer pseudoplatanus* (N19), *Fagus* (N19), *Quercus cerris* (JN) and *Quercus* sp. (TSB).

***Phaeophyscia sciastra* (Ach.) Moberg**

Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On calcareous rock (TSB).

***Phaeorrhiza nimbosa* (Fr.) H. Mayrhofer & Poelt**

M. Focalone near Bivacco Fusco (NT99; TSB: 1996; JN: 2016). – In the alpine belt (2490–2500 m: NT99; TSB; JN). On calcareous soil (NT99; TSB; JN). – The only other records from Abruzzo are from the Gran Sasso massif (Nimis and Tretiach 1999). Those from Majella are the southernmost records for Italy (Nimis 2016).

***Phlyctis argena* (Spreng.) Flot.**

Lama dei Peligni (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018); below Villaggio Mirastelle (JN: 2018). – From the colline (650 m: JN) to the montane (1350 m: JN) belt. On bark of *Acer campestre* (N19), *Acer pseudoplatanus* (N19), *Fagus* (N19; JN), *Quercus cerris* (N19) and *Quercus pubescens* (JN).

***Physcia adscendens* H. Olivier**

Lama dei Peligni (JN: 2017); Monti Pizzi near S. Domenico (JN: 2017); Piano Cerreto near Campo di Giove (JN: 2018); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018); Valle di Mario (JN: 2018); along the highway Strada Statale 164 (JN: 2018). – From the colline (650 m: JN) to the montane (1434 m: JN) belt. On bark of *Acer campestre* (N19), *Acer pseudoplatanus* (N19; JN), *Fagus* (N19; JN), *Quercus cerris* (JN) and *Ulmus minor* (JN).

***Physcia aipolia* (Humb.) Fűrnr.**

Roccacaramanico (NT99; TSB: 1996); Lama dei Peligni (JN: 2017); Monti Pizzi near S. Domenico (JN: 2017); Piano Cerreto near Campo di Giove (JN: 2018); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018); Valle di Mario (JN: 2018); along the highway Strada Statale 164 (JN: 2018). – From the colline (650 m: JN) to the montane (1434 m: JN) belt. On bark of *Acer campestre* (N19), *Acer pseudoplatanus* (JN), *Fagus* (N19; JN), *Quercus cerris* (JN) and *Ulmus minor* (JN).

Physcia biziana* (A. Massal.) Zahlbr. var. *biziana

Roccacaramanico (NT99; TSB: 1996); Lama dei Peligni (JN: 2017). – From the colline (650 m: JN) to the montane (1000 m: NT99; TSB) belt. On bark of *Ulmus minor* (JN).

***Physcia dubia* (Hoffm.) Lettau**

Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On calcareous rock (TSB).

***Physcia leptalea* (Ach.) DC.**

Roccacaramanico (NT; TSB: 1996); Pretoro, Colle dell'Angelo (NT99; TSB: 1996); Lama dei Peligni (JN: 2017); Piano Cerreto near Campo di Giove (JN: 2018). – From the colline (650 m: JN) to the montane (1200 m: NT99; TSB) belt. On bark of *Fagus* (TSB), *Quercus cerris* (JN) and *Ulmus minor* (JN).

***Physcia stellaris* (L.) Nyl.**

Majella (C73); Pretoro, Colle dell'Angelo (TSB: 1996); Piano Cerreto near Campo di Giove (JN: 2018); along the highway Strada Statale 164 (JN: 2018). – In the montane belt (1200–1420 m: NT99; TSB; JN). On bark of *Fagus* (TSB; JN) and *Quercus cerris* (JN).

***Physcia tenella* (Scop.) DC.**

Caramanico S. Tommaso (TSB: 1990). – In the colline belt (468 m: TSB). On bark of *Quercus* sp. (TSB).

***Physconia detersa* (Nyl.) Poelt**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350 m: JN). On bark of *Fagus* (N19; JN).

***Physconia distorta* (With.) J.R. Laundon**

Majella (C73); Roccacaramanico (NT99; TSB: 1996); Lama dei Peligni (JN: 2017); Piano Cerreto near Campo di Giove (JN: 2018); Valle di Mario (JN: 2018); along the highway Strada Statale 164 (JN: 2018); Pescocostanzo, Bosco di S. Antonio (N19). – From the colline (650 m: NT99; TSB) to the montane (1420 m: JN) belt. On bark of *Acer campestre* (N19), *Acer pseudoplatanus* (N19; JN), *Fagus* (N19; JN), *Quercus cerris* (JN) and *Ulmus minor* (JN).

***Physconia enteroxantha* (Nyl.) Poelt**

Monti Pizzi near S. Domenico (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350–1434 m: JN). On bark of *Acer campestre* (N19) and *Fagus* (N19; JN).

Physconia muscigena* (Ach.) Poelt var. *muscigena

M. Focalone near Bivacco Fusco (TSB: 1996; JN: 2016); Anticima M. Acquaviva (JN: 2016). – In the alpine belt (2490–2600 m: JN). On soil (TSB; JN).

***Physconia perisidiosa* (Erichsen) Moberg**

Caramanico, S. Tommaso (TSB: 1990); Valico della Forchetta (TSB: 1996); Lama dei Peligni (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – From the colline (468 m: TSB) to the montane (1360 m: JN) belt. On bark of *Acer campestre* (N19; JN), *Fagus* (N19; TSB; JN), deciduous *Quercus* sp. (TSB) and *Ulmus minor* (JN).

***Physconia servitii* (Nádv.) Poelt**

S. Tommaso (TSB: 1990). – In the colline belt (580 m: TSB). On bark of deciduous *Quercus* sp. (TSB).

***Physconia venusta* (Ach.) Poelt**

S. Tommaso (TSB: 1990); Valico della Forchetta (TSB: 1996); Lama dei Peligni (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – From the colline (580 m: TSB) to the montane (1360 m: JN) belt. On bark of *Fagus* (N19; TSB; JN), *Quercus cerris* (N19) and deciduous *Quercus* sp. (TSB).

***Placidium lachneum* (Ach.) B. de Lesd.**

At six sites along the main ridge of Majella between 2018 and 2620 m (JN: 2019). – From the subalpine (2018 m: JN) to the alpine (2620 m: JN) belt. In high-altitude open habitats (JN). On soil (JN). – The only other records of this taxon from Abruzzo are from the Gran Sasso massif (Nimis and Tretiach 1999). Those from Majella are the southernmost records for Italy (Nimis 2016).

***Placidium squamulosum* (Ach.) Breuss**

Anticima Femmina Morta (JN: 2017); near Campo di Giove (JN: 2018); at 12 sites along the main ridge of Majella between 1812 and 2640 m (JN: 2018, 2019). – From

the montane (1250 m: JN) to the alpine (2640 m: JN) belt. In dry grasslands (JN) and high-altitude open habitats (JN). On soil (JN).

***Placocarpus schaeferi* (Fr.) Breuss**

Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On calcareous rock (TSB). – A lichenicolous lichen occurring on *Protoparmeliopsis versicolor*.

***Placopyrenium canellum* (Nyl.) Gueidan & Cl. Roux**

Roccacaramanico (NT99). – In the montane belt (1000 m: NT99). – A lichenicolous lichen occurring on *Circinaria calcarea*.

***Placopyrenium fuscillum* (Turner) Gueidan & Cl. Roux**

Roccacaramanico (NT99; TSB: 1996); hermitage of M. Morrone (NT99; TSB: 1997). – From the colline (500 m: NT99; TSB) to the montane (1000 m: NT99; TSB) belt. On calcareous rock (TSB).

***Placynthiella icmalea* (Ach.) Coppins & P. James**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2016); at one site along the main ridge of Majella (JN: 2018). – From the montane (1350: JN) to the alpine (2595 m: JN) belt. In high-altitude open habitats (JN). On soil (JN), dead wood (JN) and bark of *Fagus* (N19).

***Placynthium nigrum* (Hudson) Gray**

M. d'Ugni (JN: 2017). – In the subalpine belt (1770 m). On calcareous rock (JN).

***Pleurosticta acetabulum* (Neck.) Elix & Lumbsch**

Majella (C73); Bosco di Pacentro (J74); Pretoro, Colle dell'Angelo (NT99; TSB: 1996); Valico della Forchetta (TSB: 1996); Monti Pizzi near S. Domenico (JN: 2017); Pescocostanzo, Bosco di S. Domenico (N19; JN: 2018); Piano Cerreto near Campo di Giove (JN: 2018); Valle di Mario (JN: 2018); along the highway Strada Statale 164 (JN: 2018). – In the montane belt (1200–1434 m: NT99; TSB; JN). On bark of *Acer campestre* (N19), *Acer pseudoplatanus* (N19; JN), *Fagus* (N19; TSB; JN) and *Quercus cerris* (N19; JN).

***Polyblastia albida* Arnold**

M. Blockhaus (C09). – In the subalpine belt (2170 m: C09). In a pasture (C09). On calcareous rock (C09).

***Polyblastia dermatodes* A. Massal.**

Majelletta (C09); M. Blockhaus (C09); Lettomanoppello (C09). – From the colline (750 m: C09) to the subalpine (2170 m: C09) belt. In open shrublands (C09) and pastures (C09). On calcareous rock (C09). – These records from Majella are the only ones for Abruzzo and peninsular Italy and the southernmost in Italy (Nimis 2016).

***Polyblastia nidulans* (Stenh.) Arnold**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); Majelletta (C09). – In the subalpine belt (1850–2150 m: NT99; C09; TSB). On calcareous rock (C09; TSB). – Those from the Majella massif are the only records from Abruzzo (Nimis 2016).

***Polyblastia sendtneri* Kremp.**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); above Bivacco Fusco (JN: 2016). – From the subalpine (2150 m: NT99; TSB) to the alpine (2490 m: JN) belt. On calcareous soil (TSB; JN). – Those from the Majella massif are the southernmost records in Italy (Nimis 2016).

***Polyblastia sepulta* A. Massal.**

Pretoro, Colle dell'Angelo (NT99; TSB: 1996); Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); Passo S. Leonardo (C09); Lettomanoppello (C09); Passo Lanciano (C09). – From the colline (750 m: C09) to the subalpine (2150 m: NT99; TSB) belt. In open shrublands (C09) and pastures (C09). On calcareous rock (C09; TSB). – Records from Majella are the only ones from Abruzzo (Nimis 2016).

***Polyblastia verrucosa* (Ach.) Lönnr.**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996). – In the subalpine belt (2150 m: NT99; TSB). On calcareous rock (TSB). – Records from Majella are the only one for Abruzzo, Apennines and peninsular Italy and the southernmost in Italy (Nimis 2016).

***Polycauliona polycarpa* (Hoffm.) Frödén, Arup & Søchting**

Pretoro, Colle dell'Angelo (NT99; TSB: 1996). – In the montane belt (1200 m: NT99; TSB). On bark of broad-leaved trees (TSB).

***Polysporina urceolata* (Anzi) Brodo**

M. Focalone near Bivacco Fusco (NT99; TSB: 1996); Anticima M. Acquaviva (JN: 2017); M. Macellaro (JN: 2018). – In the alpine belt (2500–2700 m: NT99; TSB; JN). On calcareous rock (TSB; JN).

***Porina oleriana* (A. Massal.) Lettau**

Below the Majelletta (T15; TSB: 2005). – In the montane belt (1350 m: T15; TSB). In a beech forest (T15; TSB). On limestone (T15; TSB). – These are the only known records for Abruzzo (Nimis 2016).

***Porpidia cinereoatra* (Ach.) Hertel & Knoph**

Majella (C73). – This is a silicicolous lichen (Nimis 2016) that likely meets its substrate requirements in the Majella massif on flint limestone.

***Protoblastenia cyclospora* (Körb.) Poelt**

Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On calcareous rock (TSB).

Protoblastenia incrustans* (DC.) J. Steiner var. *incrustans

Roccacaramanico (NT99; TSB: 1996); Grotte di Celano near M. Blockahus (NT99; TSB: 1996); Anticima Femmina Morta (JN: 2017); M. Macellaro (JN: 2018). – From the montane (1000 m: NT99; TSB) to the alpine (2635 m: JN) belt. On calcareous rock (TSB; JN).

***Protoblastenia rupestris* (Scop.) J. Steiner**

Roccacaramanico (NT99; TSB: 1996); Pretoro, Colle dell'Angelo (NT99; TSB: 1996; S: 1996). – In the montane belt (1000–1200 m: NT99; TSB). On calcareous rock (TSB).

***Protoparmeliopsis admontensis* (Zahlbr.) Hafellner**

M. d'Ugni (JN: 2017). – In the subalpine belt (1770 m: JN). On calcareous rock (JN). – The only other records of this taxon from Abruzzo come from the Gran Sasso massif (Poelt 1958; Poelt and Leuckert 1976).

***Protoparmeliopsis versicolor* (Pers.) M. Choisy**

Roccacaramanico (NT99; TSB: 1996); Anticima Femmina Morta (JN: 2017). – From the montane (1000 m: NT99; TSB) to the alpine (2420 m: JN) belt. On calcareous rock (TSB; JN).

Pseudevernia furfuracea* (L.) Zopf var. *furfuracea

Majella (C73); Bosco di Pacentro (J74); Popoli (TSB: 1986); Macchialunga (JN: 2017). – In the montane belt (1100–1249 m: TSB; JN). On bark of *Fagus* (JN).

***Pseudosagedia aenea* (Körb.) Hafellner & Kalb**

Pretoro, Colle dell'Angelo (NT99; TSB: 1996); Val di Foro (JN: 2018). – In the montane belt (1200 m: NT99; TSB; JN). On bark of *Fagus* (NT99; TSB; JN).

***Psora decipiens* (Hedw.) Hoffm.**

Majella (C73); Majellone (J74); near Bivacco Fusco (JN: 2016); between Grotta Canosa and M. Amaro (JN: 2017); at two sites along the main ridge of Majella between 2560 and 2620 m (JN: 2019). – In the alpine belt (2290–2622 m: JN). In high-altitude open habitats (JN). On soil (JN).

***Punctelia borreri* (Sm.) Krog**

M. Morrone, Osservanza (RV96). – In the colline belt (335 m: RV96). On bark of *Quercus pubescens* (RV96).

***Pyrenodesmia albopruinosa* (Arnold) S.Y. Kondr.**

Roccacaramanico (NT99; TSB: 1996); M. Focalone near Bivacco Fusco (NT99; TSB: 1996). – From the montane (1000 m: NT99; TSB) to the alpine (2500 m: NT99; TSB) belt. On calcareous rock (TSB).

***Pyrenodesmia alociza* (A. Massal.) Arnold**

Roccacaramanico (NT99; TSB: 1996); Anticima M. Acquaviva (JN: 2016); Anticima Femmina Morta (JN: 2017); M. Macellaro (JN: 2018). – From the montane (1000 m: NT99; TSB) to the alpine (2635 m: JN) belt. On calcareous rock (TSB; JN).

***Pyrenodesmia chalybaea* (Fr.) A. Massal.**

Roccacaramanico (NT99; TSB: 1996); Eremo di M. Morrone (NT99; TSB: 1997); between Lettomanoppello and Passo Lanciano (TSB: 2005). – From the colline (500 m: NT99; TSB) to the montane (1080 m: TSB) belt. On calcareous rock (TSB).

***Pyrenodesmia erodens* (Tretiach, Pinna & Grube) Søchting, Arup & Frödén**

Anticima M. Acquaviva (JN: 2016); Anticima Femmina Morta (JN: 2017). – In the alpine belt (2420–2600 m: JN). On calcareous rock (JN).

***Pyrenodesmia variabilis* (Pers.) A. Massal.**

Hermitage of M. Morrone (NT99; TSB: 1997); near Martellose (JN: 2017). – From the colline (500 m: NT99; TSB) to the alpine (2065 m: JN) belt. On calcareous rock (TSB; JN).

***Pyrenula nitida* (Weigel) Ach.**

Valle di Mario (JN: 2018); Val di Foro (JN: 2018); below Villaggio Mirastelle (JN: 2018). – In the montane belt (1200–1230 m: JN). On bark of *Fagus* (JN).

***Ramalina farinacea* (L.) Ach.**

Majella (J74); Monti Pizzi near S. Domenico (JN: 2017); Valle di Mario (JN: 2018); Piano Cerreto near Campo di Giove (JN: 2018); along the highway Strada Statale 164 (JN: 2018). – In the montane belt (1420–1440 m: JN). On bark of *Acer pseudoplatanus* (JN), *Fagus* (JN) and *Quercus cerris* (JN).

***Ramalina fastigiata* (Pers.) Ach.**

Popoli (TSB: 1986); Monti Pizzi near S. Domenico (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350–1440 m). On bark of *Fagus* (N19; JN).

***Ramalina fraxinea* (L.) Ach.**

Majella (C73; J74); Popoli (TSB: 1986); Pretoro, Colle dell'Angelo (NT99; TSB: 1996); Valico della Forchetta (TSB: 1996); Monti Pizzi near S. Domenico (JN: 2017);

Palena, Fontana delle Rose (JN: 2018); Cansano (JN: 2018); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018); Valle di Mario (JN: 2018); along the highway Strada Statale 164 (JN: 2018). – In the montane belt (1100–1440 m: JN). On bark of *Acer pseudoplatanus* (JN), *Fagus* (N19; JN) and *Quercus cerris* (N19).

***Ramonia luteola* Vězda**

Sella di Grotta Canosa (JN: 2018). – In the montane belt (1200 m: JN). On bark of *Fagus* (JN). – First record for Abruzzo and southernmost record in Italy (cf. Nimis 2016). The species is included in the Italian Red List of epiphytic lichens as “vulnerable” (Nascimbene et al. 2013).

***Rhizocarpon atroflavescens* Lynge**

Grotte di Celano (NT99; TSB: 1996). – In the subalpine belt (2150 m: NT99; TSB). On calcareous rock (TSB). – This is the only record for Abruzzo and peninsular Italy and the southernmost one in Europe (Nimis 2016). This is a slightly silicicolous lichen (Nimis 2016) that meets its substrate requirements in the Majella massif on flint limestone.

***Rhizocarpon badioatrum* (Spreng.) Th. Fr.**

Majella (C73); Valle dell’Orfento (J74). – This is a silicicolous lichen (Nimis 2016) that likely meets its substrate requirements in the Majella massif on flint limestone.

***Rhizocarpon umbilicatum* (Ramond) Flagey**

Majella (C73); Valle dell’Orfento (J74); trail between Blockhaus and M. Focalone (TSB: 2005). – In the alpine belt (2300 m: TSB). On calcareous rock (TSB).

***Rinodina bischoffii* (Hepp) A. Massal.**

Roccacaramanico (NT99; TSB: 1996); hermitage of M. Morrone (NT99; TSB: 1997). – From the colline (500 m: NT99; TSB) to the montane (1000 m: NT99; TSB) belt. On calcareous rock (TSB).

***Rinodina guzzinii* Jatta**

Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On calcareous rock (TSB).

***Rinodina immersa* (Körb.) J. Steiner**

Roccacaramanico (NT99; TSB: 1996); Passo San Leonardo (C09); Majelletta (C09); Anticima M. Acquaviva (JN: 2017); M. Macellaro (JN: 2018). – From the montane (1000 m: NT99; TSB) to the alpine (2635 m: JN) belt. On calcareous rock (TSB; JN).

***Rinodina lecanorina* (A. Massal.) A. Massal.**

Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On calcareous rock (TSB).

***Rinodina roscida* (Sommerf.) Arnold**

At ten sites along the main ridge of Majella between 2250 and 2664 m (JN: 2018, 2019). – From the subalpine (2250 m: JN) to the alpine (2664 m: JN) belt. In high-altitude open habitats (JN). On bryophytes and plant debris (JN). – New to Abruzzo. These are the only records for Apennines and peninsular Italy and the southernmost in Italy (Nimis 2016).

***Rinodina sophodes* (Ach.) A. Massal.**

Roccacaramanico (NT99; TSB: 1996); Pretoro, Colle dell'Angelo (NT99; TSB: 1996); hermitage of M. Morrone (NT99; TSB: 1997); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – From the colline (500 m: NT99; TSB) to the montane (1350 m: JN) belt. On bark of *Fagus* (N19; TSB; JN) and *Fraxinus ornus* (TSB).

***Romjularia lurida* (Ach.) Timdal**

Majella (C73); Majellone (J74); Roccacaramanico (NT99; TSB: 1996); at one site along the main ridge of Majella (JN: 2019). – From the montane (1000 m: NT99; TSB) to the subalpine (1958 m: JN) belt. In high-altitude open habitats (JN). On calcareous soil (TSB; JN).

***Rostania ceranisca* (Nyl.) Otálora, P.M. Jørg. & Wedin**

At one site along the main ridge of Majella (JN: 2019). – In the alpine belt (2662 m: JN). In high-altitude open habitats (JN). On soil (JN). – New to Abruzzo. This is the only record for Apennines and peninsular Italy and the southernmost in Italy (Nimis 2016).

Rusavskia elegans* (Link) S.Y. Kondr. & Kärnefelt subsp. *elegans

Femmina Morta (J74; JN: 2017); Roccacaramanico (NT99; TSB: 1996); M. Focalone near Bivacco Fusco (NT99; TSB: 1996); Anticima M. Acquaviva (JN: 2017); Sella di Grotta Canosa (JN: 2017); at one site along the main ridge of Majella (JN: 2018). – From the montane (1000 m: NT99; TSB) to the alpine (2669 m: JN) belt. In high-altitude open habitats (JN). On calcareous rock (TSB; JN).

***Rusavskia sorediata* (Vain.) S.Y. Kondr. & Kärnefelt**

M. Focalone near Bivacco Fusco (NT99; TSB: 1996); Grotta Canosa (JN: 2017). – In the alpine belt (2500–2559 m: TSB; JN). On calcareous rock (TSB; JN).

***Sarcogyne hypophaea* (Nyl.) Arnold**

Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On calcareous rock (TSB).

Sarcogyne regularis* Körb. var. *regularis

Hermitage of M. Morrone (NT99; TSB: 1997). – In the colline belt (500 m: NT99; TSB). On calcareous rock (TSB).

***Sclerophora pallida* (Pers.) Y.J. Yao & Spooner**

Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018). – In the montane belt (1350 m: JN). On bark of *Acer campestre* (N19; JN) and *Fagus* (N19; JN). – The species is included in the Italian Red List of epiphytic lichens as “vulnerable” (Nascimbene et al. 2013).

Scoliciosporum umbrinum* (Ach.) Arnold var. *corticolum

Pretoro, Colle dell’Angelo (NT99; TSB: 1996); Valico della Forchetta (TSB: 1996). – In the montane belt (1200–1360 m: NT99; TSB). On bark of *Fagus* (TSB).

This name, whose taxonomic value is uncertain, is applied to corticolous populations of a *Scoliciosporum* with the hymenial characters of *S. umbrinum* (Nimis et al. 2018). In Nimis (2016) this taxon is not reported and therefore it is formally new to Italy.

***Scytinium gelatinosum* (With.) Otálora, P.M. Jørg. & Wedin**

Hermitage of M. Morrone (NT99; TSB: 1997); Val di Foro (JN: 2018). – From the colline (500 m: NT99; TSB) to the montane (1250 m: JN) belt. On calcareous soil (TSB) and terricolous mosses (JN).

***Scytinium imbricatum* (P.M. Jørg.) Otálora, P.M. Jørg. & Wedin**

Near Campo di Giove (JN: 2018); at 23 sites along the main ridge of Majella between 1990 and 2664 m (JN: 2018, 2019). – From the montane (1250 m: JN) to the alpine (2664 m: JN) belt. In dry grasslands (JN) and high-altitude open habitats (JN). On soil (JN). – New to Abruzzo. These are the only records for Apennines and peninsular Italy and the southernmost in Italy (Nimis 2016).

***Scytinium lichenoides* (L.) Otálora, P.M. Jørg. & Wedin**

Majella (C73; J74); Roccacaramanico (NT99; TSB: 1996); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2016, 2018); Lama dei Peligni (JN: 2017); Val di Foro (JN: 2018); Centiata, Villaggio Mirastelle (JN: 2018). – From the colline (650 m: JN) to the montane (1350 m: JN) belt. On bark of *Fagus* (N19; JN) and calcareous soil (TSB).

***Scytinium schraderi* (Ach.) Otálora, P.M. Jørg. & Wedin**

At four sites along the main ridge of Majella (JN: 2019). – From the subalpine (2081 m: JN) to the alpine (2300 m: JN) belt. In high-altitude open habitats (JN). On soil (JN).

***Seiophora contortuplicata* (Ach.) Frödén**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); Anticima Femmina Morta (JN: 2017). – From the subalpine (2150 m: NT99; TSB) to the alpine (2420 m: JN) belt. On calcareous rock (TSB; JN).

Solorina bispora* Nyl. subsp. *bispora

Anticima M. Acquaviva (JN: 2016). – In the alpine belt (2600 m: JN). On soil (JN).

***Solorina bispora* subsp. *macrospora* (Harm.) Burgaz & I. Martínez**

Near Bivacco Fusco (JN: 2016); at one site along the main ridge of Majella (JN: 2019). – In the subalpine belt (2230–2290 m: JN). In high-altitude open habitats (JN). On soil (JN). – New to Abruzzo. These are the only records for Apennines and peninsular Italy and the southernmost in Italy (Nimis 2016).

Squamarina cartilaginea* (With.) P. James var. *cartilaginea

Majella (C73; J74); hermitage of M. Morrone (NT99; TSB: 1997); Valle di Fara (JN: 2017); trail between Lama dei Peligni and Rifugio Fonte Tari (JN: 2017). – From the colline (500 m: NT99; TSB) to the montane (1135 m: JN) belt. On calcareous soil (TSB) and rock (JN).

***Squamarina gypsacea* (Sm.) Poelt**

Near Martellose (JN: 2017). – In the subalpine belt (2065 m: JN). On calcareous rock (JN).

***Squamarina lentigera* (Weber) Poelt**

Campo di Giove (JN: 2018); near Campo di Giove (JN: 2018). – In the montane belt (1200–1250 m: JN). In dry grasslands (JN). On soil (JN).

***Squamarina stella-petraea* Poelt**

Roccacaramanico (NT99; TSB: 1996); hermitage of M. Morrone (NT99; TSB: 1997). – From the colline (500 m: NT99; TSB) to the montane (1000 m: NT99; TSB) belt. On calcareous rock (TSB) and soil (TSB).

***Staurothele orbicularis* (A. Massal.) Th. Fr.**

Caramanico (C09). – In the colline belt (570 m: C09). In a pasture with scattered shrubs (C09). On calcareous rock (C09).

***Synalissa ramulosa* (Bernh.) Fr.**

Roccacaramanico (NT99; TSB: 1996). – In the montane belt (1000 m: NT99; TSB). On calcareous rock (TSB).

***Tephromela atra* (Huds.) Hafellner var. *torulosa* (Flot.) Hafellner**

Monti Pizzi near S. Domenico (JN: 2017). – In the montane belt (1434 m: JN). On bark of *Fagus* (JN).

***Tetramelas geophilus* (Sommerf.) Norman**

Majella (C73). – The historical record was not confirmed recently, but is considered as reliable, since this is a widespread species (Nimis 2016).

***Thalloidima candidum* (Weber) A. Massal.**

Majella (C73; J74); Grotte di Celano near M. Blockhaus (NT99). – In the subalpine belt (2150 m: NT99).

***Thalloidima diffractum* (A. Massal.) A. Massal.**

Grotte di Celano near M. Blockhaus (TSB: 1996); near Bivacco Fusco (JN: 2016); Sella di Grotta Canosa (JN: 2017); Rava della Vespa (JN: 2017). – From the subalpine (2150 m: TSB) to the alpine (2643 m: JN) belt. On calcareous rock (TSB; JN) and soil (JN).

***Thalloidima sedifolium* (Scop.) Kistenich, Timdal, Bendiksby & S. Ekman**

Majella (C73; J74); near Bivacco Fusco (JN: 2016); Cima dell'Altare (JN: 2017); Campo di Giove (JN: 2018); near Campo di Giove (JN: 2018); at one site along the main ridge of Majella (JN: 2019). – From the montane (1250 m: JN) to the alpine (2490 m: JN) belt. In dry grasslands (JN) and high-altitude open habitats (JN). On calcareous soil (JN).

***Thelidium decipiens* (Nyl.) Kremp.**

Anticima M. Acquaviva (JN: 2017); M. Macellaro (JN: 2018). – In the alpine belt (2635–2700 m: JN). On calcareous rock (JN).

***Thelidium dionantense* (Hue) Zschacke**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996). – In the subalpine belt (2150 m: NT99; TSB). On calcareous rock (TSB). – This is the only known record from Italy (Nimis 2016).

***Thelidium incavatum* Mudd**

M. Focalone (C09). – In the alpine belt (2600 m: C09). On calcareous rock (C09). – This is the only record from Abruzzo (Nimis 2016).

***Thelidium papulare* (Fr.) Arnold**

Pretoro, Colle dell'Angelo (NT99; TSB: 1996); Anticima M. Acquaviva (JN: 2017). – From the montane (1200 m: NT99; TSB) to the alpine (2700 m: JN) belt. On calcareous rock (TSB; JN).

***Toninia subnitida* (Hellb.) Hafellner & Türk**

Anticima Femmina Morta (JN: 2017). – In the alpine belt (2420 m: JN). On rock (JN). – New to Abruzzo. This is the only record for central Italy (Nimis 2016).

***Toniniopsis coelestina* (Anzi) Kistenich, Timdal, Bendiksby & S. Ekman**

Below Bivacco Fusco (JN: 2016). – In the subalpine belt (2290 m: JN). On soil (JN). – New to Abruzzo. This is the only record for peninsular Italy and the Apennines and the southernmost record in Italy (Nimis 2016).

***Trapeliopsis gelatinosa* (Flörke) Coppins & P. James**

At two sites along the main ridge of Majella between 2579 and 2634 m (JN: 2019). – In the alpine belt (2579–2634 m: JN). In high-altitude open habitats (JN). On soil (JN). – New to Abruzzo. This is the southernmost record in Italy (Nimis 2016).

***Umbilicaria cylindrica* (L.) Delise**

Majella (J74). – This is a silicicolous lichen (Nimis 2016) that likely meets its substrate requirements in the Majella massif on flint limestone. This is the only record from Abruzzo (Nimis 2016).

***Usnea barbata* (L.) F.H. Wigg.**

Majella (C73). – Most Italian records of the genus *Usnea* would require accurate revision and this historical record was not confirmed recently. However, we considered it as reliable, since the ecological conditions required by this species (Nimis 2016) occur within the study area.

***Usnea dasopoga* (Ach.) Nyl.**

Bosco di Pacentro (J74). – Most Italian records of the genus *Usnea* would require accurate revision, and this historical record was not confirmed recently. However, we considered it as reliable, since the ecological conditions required by this species (Nimis 2016) occur within the study area.

***Variospora aurantia* (Pers.) Arup, Frödén & Søchting**

Majella (C73). – The historical record was not confirmed recently, but is considered as reliable, since this is a widespread species (Nimis 2016).

***Variospora velana* (A. Massal.) Arup, Søchting & Frödén**

Roccacaramanico (NT99; TSB: 1996); hermitage of M. Morrone (NT99; TSB: 1997). – From the colline (500 m: NT99; TSB) to the montane (1000 m: NT99; TSB) belt. On calcareous rock (TSB).

***Verrucaria hochstetteri* Fr.**

M. Focalone (C09); M. d'Ugni (JN: 2017); M. Macellaro (JN: 2018). – From the sub-alpine (1770 m: JN) to the alpine (2635 m: JN) belt. On calcareous rock (C09; JN).

Verrucaria nigrescens* Pers. f. *nigrescens

Pretoro, Colle dell'Angelo (NT99; TSB: 1996); hermitage of M. Morrone (NT99; TSB: 1997). – From the colline (500 m: NT99; TSB) to the montane (1200 m: NT99; TSB) belt. On calcareous rock (TSB).

***Verrucula biatorinaria* (Zehetl.) Nav.-Ros. & Cl. Roux**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996). – In the subalpine belt (2150 m: NT99; TSB). On calcareous rock (TSB). – A lichenicolous lichen occurring on *Calogaya biatorina*.

***Verrucula coccinearia* (Zehetl.) Nav.-Ros. & Cl. Roux**

Grotte di Celano near M. Blockhaus (NT99; TSB: 1996); Anticima Femmina Morta (JN: 2017). – From the subalpine (2150 m: NT99; TSB) to the alpine (2420 m: JN) belt. On calcareous rock (TSB; JN). – A lichenicolous lichen occurring on *Caloplaca*

coccinea. Those from Majella are the only records for Abruzzo, Apennines and peninsular Italy and the southernmost in Italy (Nimis 2016).

***Verrucula granulosaria* (Clauzade & Zehetl.) Nav.-Ros. & Cl. Roux**

Roccacaramanico (NT99). – In the montane belt (1000 m: NT99). – This is a lichenicolous lichen occurring on *Flavoplaca granulosa*. In Nimis and Tretiach (1999), it was reported under *Verrucula latericola*.

***Vulpicida pinastri* (Scop.) J.-E. Mattsson & M.J. Lai**

Majella (C73); M. Amaro (J74). – On bark of *Pinus* sp. (J74). – The historical records were not confirmed recently, but are considered as reliable, since the ecological conditions required by this species (Nimis 2016) occur within the study area.

***Xanthocarpia lactea* (A. Massal.) A. Massal.**

Hermitage of M. Morrone (NT99; TSB: 1997); Anticima M. Acquaviva (JN: 2017); M. Macellaro (JN: 2018). – From the colline (500 m: NT99; TSB) to the alpine (2700 m: JN) belt. On calcareous rock (TSB; JN).

***Xanthocarpia marmorata* auct.**

Hermitage of M. Morrone (NT99; TSB: 1997). – In the colline belt (500 m: NT99; TSB). On calcareous rock (TSB).

***Xanthoria parietina* (L.) Th. Fr.**

Majella (C73; J74); Roccacaramanico (NT99; TSB: 1996); Pretoro, Colle dell'Angelo (NT99; TSB: 1996); Lama dei Peligni (JN: 2017); Pescocostanzo, Bosco di S. Antonio (N19; JN: 2018); Piano Cerreto near Campo di Giove (JN: 2018); Valle di Mario (JN: 2018); along the highway Strada Statale 164 (JN: 2018). – From the colline (650 m: JN) to the montane (1420 m: JN) belt. On bark of *Acer campestre* (N19), *Acer pseudo-platanus* (N19; JN), *Fagus* (N19; TSB; JN), *Quercus cerris* (JN) and *Ulmus minor* (JN).

Dubious records

***Caloplaca subochracea* auct.**

Femmina Morta (J74). – This is a mainly coastal species (Nimis 2016) whose occurrence in the study area is dubious.

***Cladonia scabriuscula* (Delise) Nyl.**

Majella (C73). – This is a rare species in Italy and is not reported by other sources in central-southern Apennines. The record would require confirmation.

***Dermatocarpon complicatum* (Lightf.) W. Mann**

Majella (C73); Femmina Morta (J74). – This is a critical taxon mainly growing on periodically inundated siliceous rocks (Nimis 2016) and it would need to be confirmed in the study area. This would be the only record for Abruzzo.

***Leptogium brebissonii* Mont.**

Valle dell'Orfento (RV96). – In the colline belt (570 m: RV96). On sandstone (RV96). This species is usually epiphytic (Nimis 2016) and therefore this record would require confirmation.

***Lichina confinis* (O.F. Müll.) C. Agardh**

Majella (C73); Valle dell'Orfento (J74). – This is a coastal species occurring on rocks at the interface between the littoral and the mesic supralittoral belts (Nimis 2016) whose occurrence in the study area is very dubious. The record was reported by Jatta under the name *Lichina elisabethae* A. Massal.

***Lobaria linita* (Ach.) Rabenh.**

Majella (C73); Bosco di Pacentro (J74). – This record reported by Jatta was collected “ad terram inter muscos in sylva Pacentri”. According to Nimis (1993), old records from central-southern Italy should be referred to *L. pulmonaria*, since *L. linita* is restricted to the Alps in Italy.

***Rinodina oxydata* (A. Massal.) A. Massal.**

Valle dell'Orfento (J74). – This is a silicicolous taxon whose occurrence in the Majella massif would be related to flint limestone. Since this would be the only record for Abruzzo, it requires confirmation.

Discussion

This checklist provides a baseline of the lichens known to occur in the Majella National Park, highlighting the potential of this area as a hotspot of lichen biodiversity, especially from a biogeographical point of view. On one hand, the high number of regionally-new taxa discovered during our recent investigations suggests that further research is needed to reach a more exhaustive picture of the lichen biota of Abruzzo, as well as of the Majella massif. In particular, a more intensive collection in rocky and forest habitats, as well as in high elevation ranges, is likely to produce a relevant increase in the number of species.

On the other hand, the occurrence of many arctic-alpine taxa (see Nimis 1997; Nimis and Tretiach 1995) that reach here their southernmost Italian or European distribution limit and the occurrence of steppic chorotypes, as in the case of *Circinaria hispida*, confirm the phytogeographical peculiarity of this area also for lichens (see Conti et al. 2019 for vascular plants; Nimis 2016 for lichens). In the core of the Mediterranean Region, small, disjunct populations of arctic-alpine taxa that are disjunct from those of the Alps are currently restricted to the highest 200 m of the Majella massif. In a global change perspective, this cold-adapted, disjunct component of the lichen biota is strongly exposed to the impact of warming conditions, as in the emblematic case of *Allocetraria madreporeiformis* whose main local populations almost exclusively occur within *Salix retusa* islands in the Macellaro summit. The past establishment and cur-

rent persistence of these cold-adapted taxa are likely related to the great extension of the high altitude area characterised by vast plateaus that may provide microrefugia (e.g. small-scale cold refugia) suitable for these small-sized organisms (Conti et al. 2019).

In addition, the epiphytic lichen biota is noteworthy, including several species of conservation concern that are Red-listed in Italy (Nascimbene et al. 2013; a national Red List is currently available only for epiphytic species). This indicates that the forests of the Majella National Park effectively contribute to the conservation of endangered epiphytic species of the Italian lichen biota. The best conserved part of the “Bosco di S. Antonio” forest is an emblematic example of this situation, hosting species sensitive to human disturbance as *Lobaria pulmonaria* or rare calicioid lichens (Nascimbene et al. 2019).

References

- Blasi C, Di Pietro R, Pelino G (2005) The vegetation of alpine belt karst-tectonic basins in the Central Apennines. *Plant Biosystems* 139: 357–385. <https://doi.org/10.1080/11263500500350150>
- Cervellini M, Zannini P, Di Musciano M, Fattorini S, Jiménez-Alfaro B, Rocchini D, Field R, Vetaas OR, Severin DHI, Beierkuhnlein C, Hoffmann S, Fischer JC, Casella L, Angelini P, Genovesi P, Nascimbene J, Chiarucci A (2020) A grid-based map for the Biogeographical Regions of Europe. *Biodiversity Data Journal* 8: e53720. <https://doi.org/10.3897/BDJ.8.e53720>
- Cesati V (1873) Congresso degli alpinisti in Chieti – Escursioni alla Majella. *Relazione botanica. Bollettino del Club Alpino Italiano* 21: 157–187.
- Coassini Lokar L, Nimis PL, Ciconi G (1986) Chemistry and chorology of the *Cladonia chlorophaea-pxydata* complex (Lichens, Cladoniaceae) in Italy. *Webbia* 39: 259–273. <https://doi.org/10.1080/00837792.1986.10670373>
- Conti F, Ciaschetti G, Di Martino L, Bartolucci F (2019) An annotated checklist of the vascular flora of the Majella National Park (Central Italy). *Phytotaxa* 412: 1–90. <https://doi.org/10.11646/phytotaxa.412.1.1>
- Cucchi F, Furlani S, Burelli G, Zini L, Tretiach M (2009) Variazioni microtopografiche di superfici carbonatiche colonizzate da licheni endolitici (Carso Giuliano, Maiella). *Atti e Memorie della Commissione Grotte “E. Boegan”* 42: 67–84.
- Di Cecco V, Di Martino L, Stanisci A, Nascimbene J (2019) Lichen monitoring on the GLORIA summits of the Majella National Park (Italy). *NOTIZIARIO della Società Lichenologica Italiana* 32: e47.
- Di Cecco V, Di Santo M, Di Musciano M, Manzi A, Di Cecco M, Ciaschetti G, Marcantonio G, Di Martino L (2020) The Majella National Park: a case study for the conservation of plant biodiversity in the Italian Apennines. *Italian Botanist* 10: 1–24. <https://doi.org/10.3897/italianbotanist.10.52952>
- Di Nuzzo L, Vallese C, Benesperi R, Giordani P, Chiarucci A, Di Cecco V, Di Martino L, Di Musciano M, Gheza G, Lelli C, Spitale D, Nascimbene J (2021) Contrasting multitaxon responses to climate change in Mediterranean mountains. *Scientific Reports* 11: e4438. <https://doi.org/10.1038/s41598-021-83866-x>

- Hafellner J, Nimis PL, Tretiach M (2004) New records of *Aspicilia hispida* from Italy and Greece. *Herzogia* 17: 95–102.
- Hertel H (1967) Revision einiger calciphiler Formenkreise der Sammelgattung *Lecidea*. Beihefte Nova Hedwigia 24: 1–155.
- Jatta A (1874) Lichenum inferioris Italiae manipulus primus, quem collegit et ordinavit A. Jatta. *Nuovo Giornale Botanico Italiano* 6: 1–58.
- Jatta A (1889) *Monographia Lichenum Italiae Meridionalis*. Tip. Vecchi Trani.
- Jatta A (1909–1911) *Flora Italica Cryptogama. Pars III. Lichenes*. Tip. Cappelli Rocca di S. Casciano.
- Molina MC, Crespo A, Blanco O, Lumbsch HT, Hawksworth DL (2004) Phylogenetic relationships and species concepts in *Parmelia* s. str. (*Parmeliaceae*) inferred from nuclear ITS rDNA and β -tubulin sequences. *The Lichenologist* 36: 37–54. <https://doi.org/10.1017/S0024282904013933>
- Nascimbene J, Nimis PL, Ravera S (2013) Evaluating the conservation status of epiphytic lichens of Italy: a Red List. *Plant Biosystems* 147: 898–904. <https://doi.org/10.1080/11263504.2012.748101>
- Nascimbene J, Di Cecco V, Di Martino L, Frascaroli F, Giordani P, Lelli C, Vallese C, Zannini P, Chiarucci A (2019) Epiphytic lichens of the sacred natural site “Bosco di Sant’Antonio” (Majella National Park – Abruzzo). *Italian Botanist* 7: 149–156. <https://doi.org/10.3897/italianbotanist.7.34639>
- Nimis PL (1993) The lichens of Italy. An annotated catalogue. Museo Regionale di Scienze Naturali. Torino Monografie XII, 897 pp.
- Nimis PL (1997) The arctic-alpine element in the lichen flora of the Alps. *Revue Valdotaïne Histoire Naturelle, Suppl.* 51: 361–369.
- Nimis PL (2016) The lichens of Italy. A second annotated catalogue. EUT, Trieste, 740 pp.
- Nimis PL, Martellos S (2020, continuously updated) ITALIC – The Information System on Italian Lichens. Version 6.0. University of Trieste, Dept. of Biology. [Available at:] <http://dryades.units.it/italic>
- Nimis PL, Tretiach M (1995) The lichens of Italy. A phytoclimatic outline. *Cryptogamic Botany* 5: 199–208.
- Nimis PL, Tretiach M (1999) Itinera Adriatica – lichens from the eastern part of the Italian peninsula. *Studia Geobotanica* 18: 51–106.
- Nimis PL, Hafellner J, Roux C, Clerc P, Mayrhofer H, Martellos S, Bilovitz PO (2018) The lichens of the Alps – an annotated checklist. *MycoKeys* 31: 1–634. <https://doi.org/10.3897/mycokeys.31.23568>
- Poelt J (1958) Die lobaten Arten der Flechtengattung *Lecanora* Ach. *sens. ampl.* in der Holarktis. *Mitteilungen der Botanischen Staatssammlung München* 2: 411–589.
- Poelt J, Leuckert C (1976) *Lecanora* (Plac.) *sphalera* spec. nov. (Lichenes, Lecanoraceae) und die Frage, die sie stellt. *Herzogia* 4: 25–31.
- Ravera S, Puglisi M, Vizzini A, Totti C, Barberis G, Bianchi E, Boemo A, Bonini I, Bouvet D, Coccozza C, Dagnino D, Di Nuzzo L, Fačková Z, Gheza G, Gianfreda S, Giordani P, Hilpold A, Hurtado P, Köckinger H, Isocrono D, Loppi S, Malíček J, Martino C, Minuto L, Nascimbene J, Pandeli G, Paoli L, Puntillo D, Puntillo M, Rossi A, Sguazzin F, Spitale D, Stifter S, Turcato C, Vazzola S (2020) Notulae to the Italian flora of algae, bryophytes,

- fungi and lichens: 10. Italian Botanist 10: 83–99. <https://doi.org/10.3897/italianbotanist.10.59352>
- Recchia F, Villa S (1996) A first contribution to the lichen flora of Abruzzi (C. Italy). *Flora Mediterranea* 6: 5–9.
- Tretiach M (2015) Notulae Cryptogamicae 10 (72–97): Notulae 77–96. *Informatore Botanico Italiano* 47: 93–96.

Multiple-marker phylogeny and morphological evidence reveal two new species in Steccherinaceae (Polyporales, Basidiomycota) from Asia

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Abstract

Two new wood-inhabiting fungi, *Mycorrhaphium subadustum* **sp. nov.** and *Trullella conifericola* **sp. nov.**, are proposed and described from Asia based on ITS, nrLSU and *tef1* molecular phylogeny and morphological characteristics. *Mycorrhaphium subadustum* is characterized by a stipitate basidiocarp, velutinate pileal surface concentrically zoned, hydroid hymenophore, a dimitic hyphal system in spine trama and monomitic in context, absence of gloecystidia, presence of cystidioles and the non-amyloid, cylindrical to ellipsoid basidiospores. *Trullella conifericola* is characterized by a laterally stipitate basidiocarp with flabelliform to semicircular pileus, hirtellous pileal surface with appressed coarse hair and concentrically zoned and sulcate, tiny pores (10–12 per mm), a dimitic hyphal system, absence of any type of cystidia, short clavate basidia and thin-walled, smooth, cylindrical to allantoid basidiospores. Phylogenetic analyses based on a three-marker dataset were performed using maximum likelihood and Bayesian inference methods. The two new species formed isolated lineages with full support in Steccherinaceae. The distinguishing characters of the two new species as well as allied species are discussed, and a key to species of *Mycorrhaphium* is provided.

Keywords

Hydnaceous fungi, molecular phylogeny, polypores, taxonomy, wood-inhabiting fungi

Introduction

Steccherinaceae Parmasto was typified by the genus *Steccherinum* Gray (1968). It belongs to the residual polyporoid clade of the Polyporales Gäum. (Basidiomycota). It is a distinct and well-defined group based on phylogenetic evidence (Miettinen et al. 2012; Binder et al. 2013). Steccherinaceae includes around 23 genera according to Zmitrovich (2018). The taxa in this family show highly variable morphological and anatomical features. For instance, the basidiocarps range from resupinate (e.g. *Junghuhnia* Corda.) to pileate (e.g. *Austeria* Miettinen and *Flabellophora* G. Cunn.), and the hymenophore can be poroid (e.g. *Citripora* Miettinen) or hydroid (e.g. *Mycorrhaphium* Maas Geest. and *Steccherinum* Gray). The hyphal system ranges from monomitic (e.g. *Caudicicola* Miettinen, M. Kulju & Kotir. and *Elaphroporia* Z.Q. Wu & C.L. Zhao), dimitic (e.g. *Antrodiella* Ryvarden & I. Johans.) to trimitic (e.g. *Metuloidea* G. Cunn.). Any type of cystidia can be absent (e.g. *Frantisekia* Spirin & Zmitr.) or take the form of gloeocystidia (e.g. *Antella* Miettinen and *Butyrea* Miettinen) or encrusted cystidia (e.g. *Flaviporus* Murrill). The basidiospores are typically cylindrical, allantoid (e.g. *Nigroporus* Murrill and *Trullella* Zmitr.) or ellipsoid (e.g. *Steccherinum* Gray). Nevertheless, the members of the family also share several characters including the white-rot nutritional mode, small pores or densely arranged spines, smooth and relatively small basidiospores, and mainly cyanophilic but inamyloid hyphae (Gray 1821; Corda 1842; Murrill 1905; Maas Geesteranus 1962; Cunningham 1965; Ryvarden and Johansen 1980; Spirin et al. 2007; Yuan and Dai 2009; Yuan and Wu 2012; Yuan et al. 2012; Yuan 2014; Miettinen and Ryvarden 2016; Kotiranta et al. 2017; Wu et al. 2018; Zmitrovich 2018).

Morphological and phylogenetic analyses have provided more accurate identification and contributed to the definition of the taxonomic status of the genera in Steccherinaceae. In recent years, phylogenetic analysis based on multi-marker data has been widely used in the taxonomy of these fungi (He and Dai 2012; Miettinen et al. 2012; Binder et al. 2013; Dai et al. 2014; Miettinen and Ryvarden 2016; Justo et al. 2017; Kotiranta et al. 2017; Westphalen et al. 2018; Yuan et al. 2018; Yuan et al. 2020).

The species of the Steccherinaceae are widely distributed all over the world. During the investigation of specimens in Steccherinaceae from Asia, several specimens which represent two undescribed species were found. The morphological and molecular features showed that they belong to the genus *Mycorrhaphium* and *Trullella*. In this study, we describe them as two new species based on morphological characteristics and three-marker phylogenetic analyses.

Material and methods

Morphological studies

The studied specimens were deposited at the herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP). Microscopic procedures followed Yuan

and Qin (2018). Microscopic observations were made on tissue sections mounted in cotton blue and Melzer's reagent to test for any amyloid and/or dextrinoid reactions (cotton blue: 0.1 mg Methyl blue (SIGMA, PCode: 1001545602) dissolved in 60 g pure lactic acid; Melzer's reagent: 1.5 g KI (potassium iodide), 0.5 g I (crystalline iodine), 22 g chloral hydrate, distilled water 20 mL). The following abbreviations are used in the text: KOH = 2.5% potassium hydroxide; CB = cotton blue; CB+/- = cyanophilous/acyanophilous; IKI = Melzer's reagent; IKI- = neither amyloid nor dextrinoid; L_m = mean spore length (arithmetic average of all spores); W_m = mean spore width (arithmetic average of all spores); Q = variation in the ratios of L_m/W_m between specimens studied, and n = total number of spores measured from a given number of specimens. Sections were studied at magnifications up to $\times 1000$ using a Nikon Eclipse E600 microscope (Tokyo, Japan) with phase-contrast illumination, and dimensions were estimated subjectively with an accuracy of 0.1 μm . Microscopic drawings were made with the aid of a drawing tube. The spores' measurements excluded the apiculus, and 5% of the measurements at each end of the range are given in parentheses. The spores' measurements were made with a Nikon SMZ 645 stereomicroscope. Special colour terms are from Kornerup and Wanscher (1981).

Molecular procedures and phylogenetic analyses

DNA was extracted from dried herbarium specimens with a Thermo Scientific Phire Plant Direct PCR kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA) according to the manufacturer's instructions and was used for the polymerase chain reaction (PCR). Nuclear ribosomal RNA markers were used to determine the phylogenetic position of the new species. The internal transcribed spacer (ITS) was amplified with the primers ITS4 (5' TCCTCCGCTTATTGATATGC 3') and ITS5 (5' GGAAG-TAAAAGTCGTAACAAGG 3'); LR0R (5' ACCCGCTGAACTTAAGC 3') and LR7 (5' TACTACCACCAAGATCT 3') for partial nrLSU; 983F (5' GCYCCYGGHCAY-CGTGAYTTYAT 3') and 2218R (5' ATGACACCRACRGCACRGTGTG 3') for *tef1* (White et al. 1990; Gardes and Bruns 1993; Rehner and Buckley 2005; Matheny et al. 2007).

PCR reactions were performed in 30 μL reaction mixtures containing 15 μL of 2 $\times$ Phire Plant PCR buffer, 0.6 μL Phire Hot Start II DNA Polymerase, 1.5 μL of each PCR primer (10 μM), 10.5 μL double deionized H_2O (ddH_2O), and 0.9 μL template DNA. The PCR thermal cycling program condition was set as follows: initial denaturation at 95 $^{\circ}\text{C}$ for 5 min, followed by 34 cycles at 95 $^{\circ}\text{C}$ for 30 s, the annealing temperatures were as follows: 58.9 $^{\circ}\text{C}$ for ITS4/ITS5, 47.2 $^{\circ}\text{C}$ for LR0R/LR7, 57.6 $^{\circ}\text{C}$ for 983F/2218R, then 72 $^{\circ}\text{C}$ for 20 s, and a final extension at 72 $^{\circ}\text{C}$ for 7 min. PCR amplification was confirmed on 1% agarose electrophoresis gel stained with ethidium bromide (Stöger et al. 2006) and sequenced at the Beijing Genomics Institute (BGI) with the same primers as used in PCR. The newly generated DNA sequences were assembled and manually modified with the software DNAMAN8 (Lynnon Biosoft, Quebec, Canada). The sequences quality control followed the guidelines by Nilsson

et al. (2012). All newly obtained sequences were submitted to GenBank (Sayers et al. 2020). Sequences from allied genera were based on the studies of Miettinen et al. (2012), Yuan (2014) and Westphalen et al. (2019) or found in GenBank (<http://www.ncbi.nlm.gov>) using the BLAST option and downloaded (Table 1). DNA alignments were performed using the MAFFT v.7.471 online service (<https://mafft.cbrc.jp/alignment/server/index.html>; Katoh et al. 2019). Intron regions of *tefl* as well as low-homology regions of ITS1 and ITS2 were removed before phylogenetic analyses, and the sequence datasets were combined using BioEdit v 7.2.6 (Hall 2005).

Bayesian analysis and Maximum likelihood were applied to the ITS + nrLSU + *tefl* dataset. All characters were weighted, and gaps were treated as missing data. Bayesian analysis with MrBayes 3.2.7 (Ronquist et al. 2012) implemented the Markov Chain Monte Carlo (MCMC) technique. The combined dataset was divided into seven partitions: ITS1, 5.8S, ITS2, nrLSU and *tefl* 1st, 2nd as well as 3rd codon positions. The best-fit models selected were K80+G for ITS1, GTR+I+G for 5.8S, JC+G for ITS2, GTR+I+G for nrLSU, JC for *tefl* 1st, TrNef+G for *tefl* 2nd and GTR+I+G *tefl* 3rd which were determined by the jModelTest 2.1.10 (Darriba et al. 2012) based on the Corrected Akaike Information Criterion (AICc). Four simultaneous Markov chains were run with 10 million generations and starting from random trees and keeping one tree every 100th generation until the average standard deviation of split frequencies was below 0.01. The value of burn-in was set to discard 25% of trees when calculating the posterior probabilities. Bayesian posterior probabilities were obtained from the 50% majority rule consensus of the trees kept. A Maximum Likelihood (ML) analysis uses the seven-partitions' database which is the same as Bayesian analysis and performed in RAXML v8.2.4 (Stamatakis 2014). The best tree was obtained by performing 1000 rapid bootstrap inferences followed by a thorough search for the most likely tree (Stamatakis et al. 2008). Phylogenetic trees were checked and modified in FigTree 1.4 (Rambaut 2012). The combined dataset and trees were deposited in TreeBASE (No. S27633).

Results

Phylogenetic analyses

Multiple-marker analyses provide an advantage of accurately and promptly discovering a new species or genus (Taylor et al. 2000). Therefore, we used three markers in our dataset which included 75 ITS, 68 nrLSU and 20 *tefl*. The combined dataset includes two species belonging to the genera *Mycorrhaphium* and *Trullella* respectively, and other 69 samples from 23 allied genera. *Climacocystis borealis* (Fr.) Kotl. & Pouzar was used as the outgroup. The data matrix comprised 163 sequences and had an aligned length of 2121 bases. Bayesian analysis resulted in an average standard deviation of split frequencies = 0.004878. The maximum likelihood and Bayesian analyses produced similar topologies and therefore, only the ML tree is shown in Figure 1.

Table 1. Specimens and sequences used in this study. Type specimens are indicated as superscript T and the newly generated sequences in this study are in bold.

Species	GenBank No.			Specimen/culture voucher	Locality	References
	ITS	nrLSU	<i>tefl</i>			
<i>Antella americana</i> (Ryvarden & Gilb.) Ryvarden	JN710509	JN710509	JN710711	KHL 11949	Sweden	Miettinen et al. 2012
<i>A. americana</i>	EU232186	EU232270	–	HHB 4100-Sp	USA	GenBank Database
<i>A. chinensis</i> (H.S. Yuan) Miettinen	JX110844	KC485542	–	Dai 9019 ^T	China	Yuan 2013
<i>A. chinensis</i>	JX110843	KC485541	–	Dai 8874 ^T	China	Yuan 2013
<i>A. niemelaii</i> (Vampola & Vlasák) Miettinen	AF126876	–	–	Renvall 3218	Finland	Johannesson et al. 2000
<i>A. niemelaii</i>	AF126877	–	–	Haikonen 14727	Finland	Johannesson et al. 2000
<i>A. lactea</i> H.S. Yuan	KC485530	KC485548	–	Yuan 5720 ^T	China	Yuan 2014
<i>A. lactea</i>	KC485532	KC485550	–	Yuan 5757 ^T	China	Yuan 2014
<i>A. semisupina</i> (Berk. & M.A. Curtis) Ryvarden	JN710521	JN710521	–	X242	Canada	Miettinen et al. 2012
<i>Antrodiella</i> sp.	JN710523	JN710523	–	Núñez 1040	Japan	Miettinen et al. 2012
<i>A. stipitata</i> H.S. Yuan & Y.C. Dai	KC485525	KC485544	–	Yuan 5640	China	Yuan 2014
<i>Arraporiella neotropica</i> Ryvarden	HQ659221	HQ659221	–	Miettinen X1021	Belize	Miettinen et al. 2012
<i>Austeria citrea</i> (Berk.) Miettinen	JN710511	JN710511	–	X1171	New Zealand	Miettinen et al. 2012
<i>Butyrea luteoalba</i> (P. Karst.) Miettinen	JN710558	JN710558	JN710719	isolate 5403	Estonia	Miettinen et al. 2012
<i>B. japonica</i> (Núñez & Ryvarden) Miettinen & Ryvarden	JN710556	JN710556	JN710718	isolate 10202 ^T	Japan	Miettinen et al. 2012
<i>B. japonica</i>	KC485536	KC485553	–	Li 1648	China	Yuan 2014
<i>Cabalodontia queletii</i> (Bourdote & Galzin) Piątek	AF141626	AF141626	–	FCUG 722	Sweden	GenBank Database
<i>Citripora bannaensis</i> Miettinen	JN710526	JN710526	–	OM9999 ^T	China	Miettinen et al. 2012
<i>Climacocystis borealis</i> (Fr.) Kotl. & Pouzar	JN710527	JN710527	–	KHL 13318	Estonia	Miettinen et al. 2012
<i>Elaphroporia ailaoshanensis</i> Z.Q. Wu & C.L. Zhao	MG231568	MG748854	–	CLZhao 595 ^T	China	Wu et al. 2018
<i>E. ailaoshanensis</i>	MG231572	MG748855	–	CLZhao 596	China	Wu et al. 2018
<i>Enheirodon fimbriatum</i> (Pers.) Banker	JN710530	JN710530	–	KHL 11905	Sweden	Miettinen et al. 2012
<i>Flabellophora</i> sp1	JN710533	JN710533	–	Miettinen 14305	Indonesia	Miettinen et al. 2012
<i>Flabellophora</i> sp2	JN710534	JN710534	–	Miettinen 11443	Indonesia	Miettinen et al. 2012
<i>Flabellophora</i> sp3	JN710535	JN710535	–	Syamsi NOM677	Indonesia	Miettinen et al. 2012
<i>Flabellophora</i> sp4	JN710536	JN710536	–	Ryvarden 34508	USA	Miettinen et al. 2012
<i>Flabellophora</i> sp.	MT269765	MT259330	MT793111	Yuan 12794	China	This study
<i>F.</i> sp.	MT269766	MT259331	MT793112	Yuan 12796	China	This study
<i>Flaviporus brownii</i> (Humb.) Donk	KY175008	KY175008	KY175022	MCW 362/12	Ecuador	Westphalen et al. 2018
<i>F. brownie</i>	JN710538	JN710538	–	X462	Australia	Miettinen et al. 2012
<i>F. liebmanni</i> (Fr.) Ginns	JN710539	JN710539	–	X249	China	Miettinen et al. 2012
<i>F. liebmanni</i>	KC502914	–	–	Yuan 1766	China	Yuan 2014
<i>Frantisekia mentschulensis</i> (Pilát ex Pilát) Spirin	FJ496670	FJ496728	–	BRNM 710170	Czech Republic	Tomšovský et al. 2010
<i>F. mentschulensis</i>	JN710544	JN710544	–	isolate 1377	Australia	Miettinen et al. 2012
<i>F. ussuri</i> Y.C. Dai & Niemelä	KC485526	–	–	Dai 8249	China	Yuan 2014
<i>F. ussuri</i>	KC485527	KC485545	–	Wei 3081	China	Yuan 2014
<i>Jungibubnia crustacea</i> (Jungb.) Ryvarden	JN710553	JN710553	–	X626	Indonesia	Miettinen et al. 2012
<i>J. micropora</i> Spirin, Zmitr. & Malysheva	JN710559	JN710559	JN710720	Spirin 2652	Russia	Miettinen et al. 2012
<i>Lamelloporus americanus</i>	JN710567	JN710567	–	Læssøe 10119	Ecuador	Miettinen et al. 2012
<i>Loweomyces fractipes</i> (Berk. & M.A. Curtis) Jülich	KX378866	KX378866	–	MT 13/2012	Brazil	Westphalen et al. 2016

Species	GenBank No.			Specimen/culture voucher	Locality	References
	ITS	nrLSU	<i>tefl</i>			
<i>L. spissus</i> Westph., Tomšovský & Rajchenb.	KX378869	KX378869	–	MCW 488/14	Brazil	Westphalen et al. 2016
<i>L. tomentosus</i> Westph., Tomšovský & Rajchenb.	KX378870	KX378870	–	MCW 366/12 ^f	Brazil	Westphalen et al. 2016
<i>L. wynneae</i> (Berk. & Broome) Jülich	JN710604	JN710604	–	X1215	Denmark	Miettinen et al. 2012
<i>Metuloidea cinnamomea</i> (Iturr. & Ryvarden) Miettinen & Ryvarden	KU926963	–	–	X1228 ^T	Venezuela	Miettinen and Ryvarden 2016
<i>M. fragrans</i> (A. David & Tortic) Miettinen	KC858281	–	–	LE295277	Russia	GenBank Database
<i>M. murashkinskyi</i> (Burt) Miettinen & Spirin	JN710588	JN710588	–	X449	Russia	Miettinen et al. 2012
<i>M. rhinocephala</i> (Berk.) Miettinen	JN710562	JN710562	–	X460	Australia	Miettinen et al. 2012
<i>Mycorrhaphium adustum</i> (Schwein.) Maas Geest.	JN710573	JN710573	JN710727	KHL12255	USA	Miettinen et al. 2012
<i>M. hispidum</i> Westph. & Miettinen	MH475306	MH475306	MH475317	MCW 363/12 ^f	Brazil	Westphalen et al. 2019
<i>M. hispidum</i>	MH475307	MH475307	MH475318	MCW 429/13	Brazil	Westphalen et al. 2019
<i>M. subadustum</i>	KC485537	KC485554	–	Dai 10173 ^T	China	Yuan 2014
<i>M. subadustum</i>	MW491378	MW488040	MW495253	Yuan 12976 ^T	China	This study
<i>Nigroporus vinosus</i> (Berk.) Murrill	JX109857	JX109857	JX109914	BHS2008-100	USA	Binder et al. 2013
<i>N. vinosus</i>	JN710575	JN710575	–	X839	Indonesia	Miettinen et al. 2012
<i>N. cf. vinosus</i>	MT681923	MT675108	MT793113	Yuan 12916	China	This study
<i>N. stipitatus</i> Douanla-Meli & Ryvarden	JN710574	JN710574	–	X546 ^f	Cameroon	Miettinen et al. 2012
<i>Skeletocutis novae-zelandiae</i> (G. Cunn.)P.K. Buchanan & Ryvarden	JN710582	JN710582	–	Ryvarden 38641	New Zealand	Miettinen et al. 2012
<i>Steccherinum aridum</i> Svrček	JN710583	JN710583	–	Bureid 110510	Norway	Miettinen et al. 2012
<i>S. cf. ciliolatum</i>	JN710585	JN710585	–	Ryvarden 47033	Estonia	Miettinen et al. 2012
<i>S. meridionale</i> (Rajchenb.) Westphalen, Tomšovský & Rajchenberg	KY174992	KY174992	KY175019	MR 284	Chile	Westphalen et al. 2018
<i>S. neonitidum</i> Westphalen & Tomšovský	KY174990	KY174990	KY175017	MCW 371/12 ^f	Brazil	Westphalen et al. 2018
<i>S. ochraceum</i> (Pers. ex J.F. Gmel.) Gray	JN710590	JN710590	JN710730	KHL 11902	Brazil	Miettinen et al. 2012
<i>S. robustius</i> (J. Erikss. & S. Lundell) J. Erikss.	JN710591	JN710591	–	G1195	Sweden	Miettinen et al. 2012
<i>S. straminellum</i> (Bres.) Melo	JN710597	JN710597	–	KH Larsson 13849	France	Miettinen et al. 2012
<i>Trullella conifericola</i>	MT269764	–	–	Cui 2851 ^T	China	This study
<i>T. conifericola</i>	MT269760	MT259326	MT793109	Yuan 12655 ^T	Vietnam	This study
<i>T. conifericola</i>	MT269761	MT259327	MT793110	Yuan 12657 ^T	Vietnam	This study
<i>T. dentipora</i> (Ryvarden & Iturr.) Zmitr.	JN710512	JN710512	–	X200 ^f	Venezuela	Miettinen et al. 2012
<i>T. duracina</i> (Pat.) Zmitr.	MH475309	MH475309	–	MCW 410/13	Brazil	Westphalen et al. 2019
<i>T. duracina</i>	MH475310	MH475310	–	RP 96	Brazil	Westphalen et al. 2019
<i>T. meridae</i> (Miettinen & Ryvarden) Zmitr.	KY980668	KY980676	–	AS 2150	Brazil	GenBank Database
<i>T. meridae</i>	JN710513	JN710513	–	X290 ^f	Venezuela	Miettinen et al. 2012
<i>T. polyporoides</i> (Ryvarden & Iturr.) Zmitr.	JN710602	JN710602	–	X510 ^f	Venezuela	Miettinen et al. 2012
<i>Xanthoporus syringae</i> (Parmasto) Audet	JN710607	JN710607	–	Jeppson 2264	Sweden	Miettinen et al. 2012
<i>X. syringae</i>	AY789078	AY684166	DQ059049	AFTOL-ID 774	China	Miettinen et al. 2012

The two new species *Mycorrhaphium subadustum* and *Trullella conifericola* were both defined with three markers and they form full-support (100% ML and 1.00 BPP) isolated lineages respectively in this study. The new species *M. subadustum*

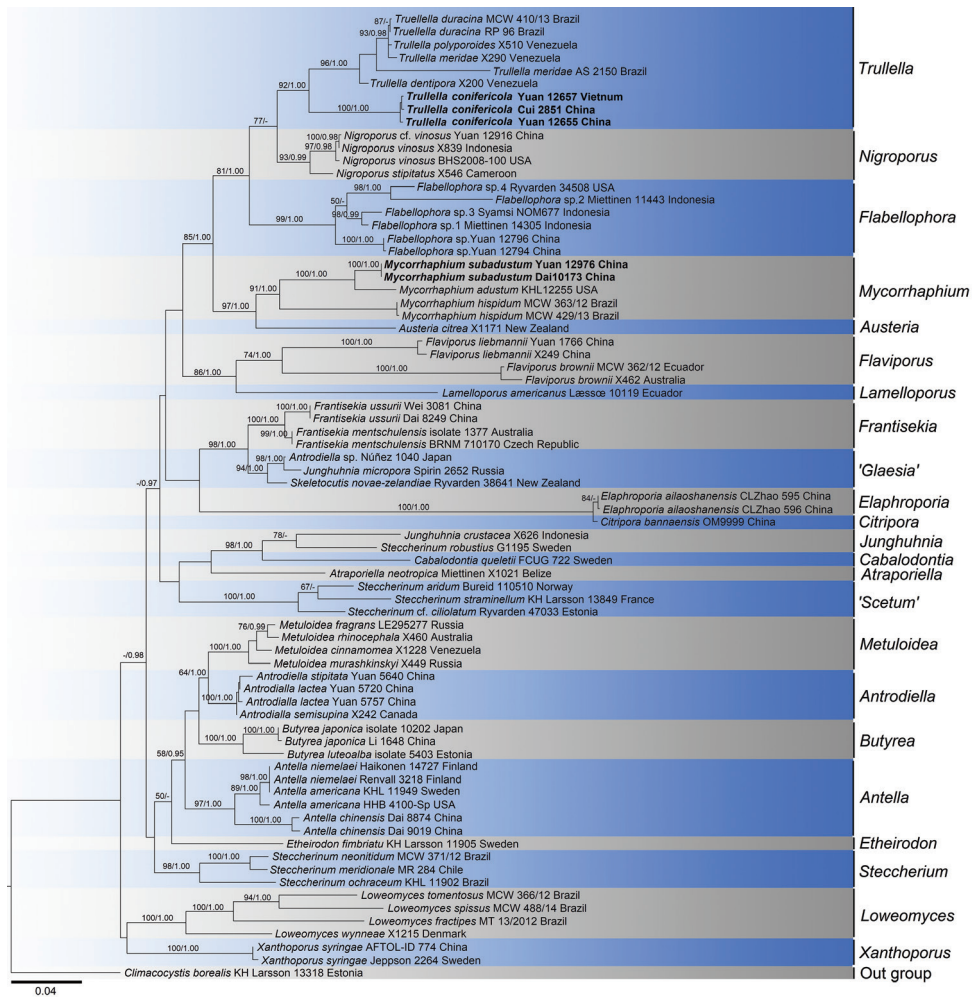


Figure 1. Maximum likelihood tree based on the combined ITS + nrLSU + *tefl* sequence dataset illustrating the phylogeny of *Mycorrhaphium subadustum* and *Trullella confiericola* and related taxa in Steccherinaceae. The new species are in bold. Branches are labelled with maximum likelihood bootstrap higher than 50% and Bayesian posterior probabilities more than 0.95.

clustered together with *Mycorrhaphium* spp. and form a subclade with American *M. adustum*. In case of another new species *T. confiericola*, although the material of *T. confiericola* Cui 2851 was only provided with ITS sequences, it showed a high similarity of ITS to the other two samples (Yuan 12657 and Yuan 12655) with 99.59% and 98.77% respectively. Furthermore, the morphological and anatomical features, distribution and the coniferous-saprophytic habit suggested it represented an individual which belongs to *T. confiericola*. Three samples of *T. confiericola* get together with another six samples from the *Trullella* clade with support 92% in ML and 1.00 BPP. The phylogenetic tree obtained in this study is similar to that of Miettinen et al. (2012). All the species were divided into 23 main clades which include *Antella*,

Antrodiella, *Atraporrella*, *Austeria*, *Butyrea*, *Cabalodontia*, *Citripora*, *Elaphroporia*, *Etheiaron*, *Flabellophora*, *Flaviporus*, *Frantisekia*, 'Glaesia', *Junghuhnia*, *Lamelloporus*, *Loweomyces*, *Metuloidae*, *Mycorrhaphium*, *Nigroporus*, 'Scetum', *Steccherinum*, *Trullella* and *Xanthoporus*. It is notable that the genera *Austeria*, *Flabellophora*, *Mycorrhaphium*, *Nigroporus* and *Trullella* formed a large clade in *Steccherinaceae* with a strong support (85% ML and 1.00 BPP).

Taxonomy

Mycorrhaphium subadustum T. Cao & H.S. Yuan, sp. nov.

MycoBank No: 838509

Figures 2, 3

Diagnosis. Basidiocarps stipitate; pileus semicircular to dimidiate; pileal surface velutinate, concentrically zonate, pileal margin yellowish white; hymenophore hydroid. Hyphal system dimitic in spine trama and monomitic in context; generative hyphae with clamp connections; cystidia and gloecystidia absent, cystidiols present. Basidiospores cylindrical to allantoid, CB–, IKI–.

Holotype. CHINA. Liaoning Province, Huanren County, Laotudingzi Nature Reserve, on fallen branch of angiosperm, 4.VIII.2018, *Yuan 12976* (**holotype** IFP 019374).

Etymology. *Subadustum* (Lat.), referring to the affinity with *M. adustum*.

Description. *Basidiocarps* annual, stipitate, solitary or imbricate, corky to soft fibrous, without odor and taste when fresh, light in weight when dry. *Pilei* semicircular to dimidiate, 2.5–4.5 cm wide and 0.3 cm thick. *Pileal surface* velutinate, smooth, concentrically zonate, yellowish white to greyish orange (4A2–5B4); margin acute, yellowish white (4A2). Hymenophore hydroid; spines crowded, evenly distributed, greyish orange (5B4), fibrous, subulate to terete, straight to somewhat flexuous, solitary or confluent, up to 1 mm long, 5–7 per mm; sterile margin smooth, yellowish grey (4B2), up to 2 mm wide. Context yellowish white (3A2), leathery, azonate, homogeneous, up to 0.5 mm thick. Stipe up to 3 cm long, 1 cm wide, straight and base inflated, surface tomentum eventually glabrous, brownish orange (5C4).

Hyphal structure. Hyphal system monomitic in context, dimitic in spine trama; generative hyphae often with clamp connections and simple septate occasionally present; skeletal hyphae thick-walled to subsolid, CB+, IKI–; tissues pale yellow in KOH.

Context. Generative hyphae with clamp connections, colorless, thin- to slightly thick-walled, frequently branched, 3–5 µm diam; skeletal hyphae absent.

Spines. Generative hyphae often with clamp connections, simple-septate occasionally present, colorless, thin- to slightly thick-walled, moderately branched, 2.5–4 µm diam; skeletal hyphae thick-walled to subsolid, unbranched, subparallel along the spine, 3–5 µm diam. Gloecystidia absent; cystidioles present among the basidia, fusiform, 8–12 × 1.5–3 µm. Basidia clavate, with a basal clamp and four sterigmata, 8–13.5 × 2–3.5 µm; basidioles in shape similar to basidia, but slightly smaller.



Figure 2. Basidiocarps of *Mycorrhaphium subadustum* (IFP 019374, holotype). Scale bar: 10 mm.

Basidiospores cylindrical to ellipsoid, colorless, thin-walled, smooth, CB–, IKI–, $(3.5\text{--}3.8\text{--}4.0(4.2) \times (1.5\text{--}1.8\text{--}1.9(-2.0) \mu\text{m}$, $L_m = 3.89 \mu\text{m}$, $W_m = 1.83 \mu\text{m}$, $Q = 2.13\text{--}2.17$ ($n = 60/2$).

Type of rot. White rot.

Distribution. In temperate zones.

Additional specimen examined. CHINA. Jilin Province, Antu Country, Changbai Mountain Nature Reserve, Huangsongpu, on fallen branch of angiosperm, 2.VIII.2008, *Dai 10173* (IFP 008336).

***Trullella conifericola* T. Cao & H.S. Yuan, sp. nov.**

Mycobank No: 836287

Figures 4, 5

Diagnosis. Basidiocarps annual, sessile or laterally stipitate; pileus flabelliform to semi-circular; pileal surface hirtellous, with appressed coarse hair, concentrically zonate and sulcate; pores round to angular. Hyphal system dimitic; generative hyphae with clamp connections; skeletal hyphae CB+, IKI–. Basidiospores cylindrical to allantoid, thin-walled.

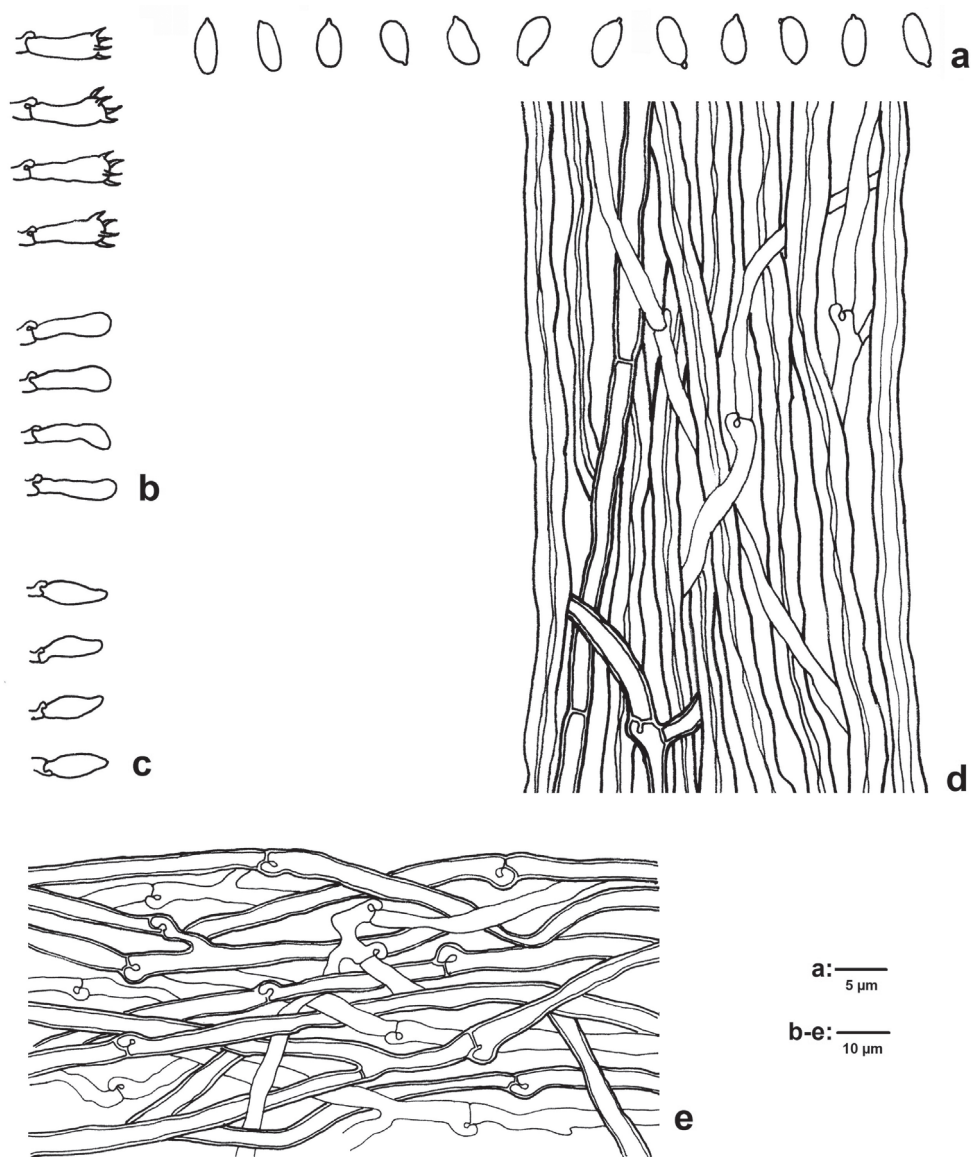


Figure 3. Microscopic structures of *Mycorrhaphium subadustum* (IFP 019374, holotype) **a** Basidiospores **b** Basidia and basidioles **c** cystidioles **d** Hyphae from spine trama **e** Hyphae from context.

Holotype. VIETNAM. Lam dong Province, Lac Duong District, Lac Duong District, Bidoup Nui Ba National Park, on fallen branch of *Pinus kesiya*, 15.X.2017, *Yuan 12655* (holotype IFP 019372).

Etymology. *Conifericola* (Lat.), referring to growth on the coniferous substrate.

Description. *Basidiocarps* annual, sessile or laterally stipitate, solitary to imbricate, without special odor or taste, leathery when fresh, shrinking, hard corky and



Figure 4. Basidiocarps of *Trullella conifericola* (IFP 019372, holotype). Scale bar: 10 mm.

light in weight upon drying. *Pileus* flabelliform to semi-circular, applanate, projecting 4–10 cm and 1 cm thick at the base; pileal surface hirtellous, with appressed coarse hair, concentrically zonate and sulcate, alternating white and greyish orange (6A1–6B3) when fresh, yellowish white (2A2/3A2/4A2) and nearly azonate when dry; margin acute, drying involute and wavy. *Pore surface* light orange (5A4), shiny; pores round to angular, tiny, 10–12 per mm, hardly visible to the naked eye; dissepiments entire; sterile margin ca. 1 mm wide. *Context* color paler than pores and upper surface, yellowish white (2A2–3A2), soft corky, azonate, 0.5–1.5 mm thick. *Tubes* non-stratified, concolorous with pore surface, dense, ca. 1.5 mm thick when dry. *Stipe* round, glabrous and smooth, light yellow to greyish yellow (4A4–4B5), 0.5–2 cm long and 2–4 mm in diam, dense and homogenous.

Hyphal structure. *Hyphal system* dimitic: generative hyphae bearing clamp connections, skeletal hyphae CB+, IKI–; tissues unchanged in KOH.

Context. Dominated by generative hyphae, interwoven; generative hyphae hyaline, thin- to slightly thick-walled, clamp connections abundant, frequently branched, 2.5–5.5 μm diam; skeletal hyphae hyaline, thick-walled with a wide lumen, unbranched, 1.5–5 μm diam.

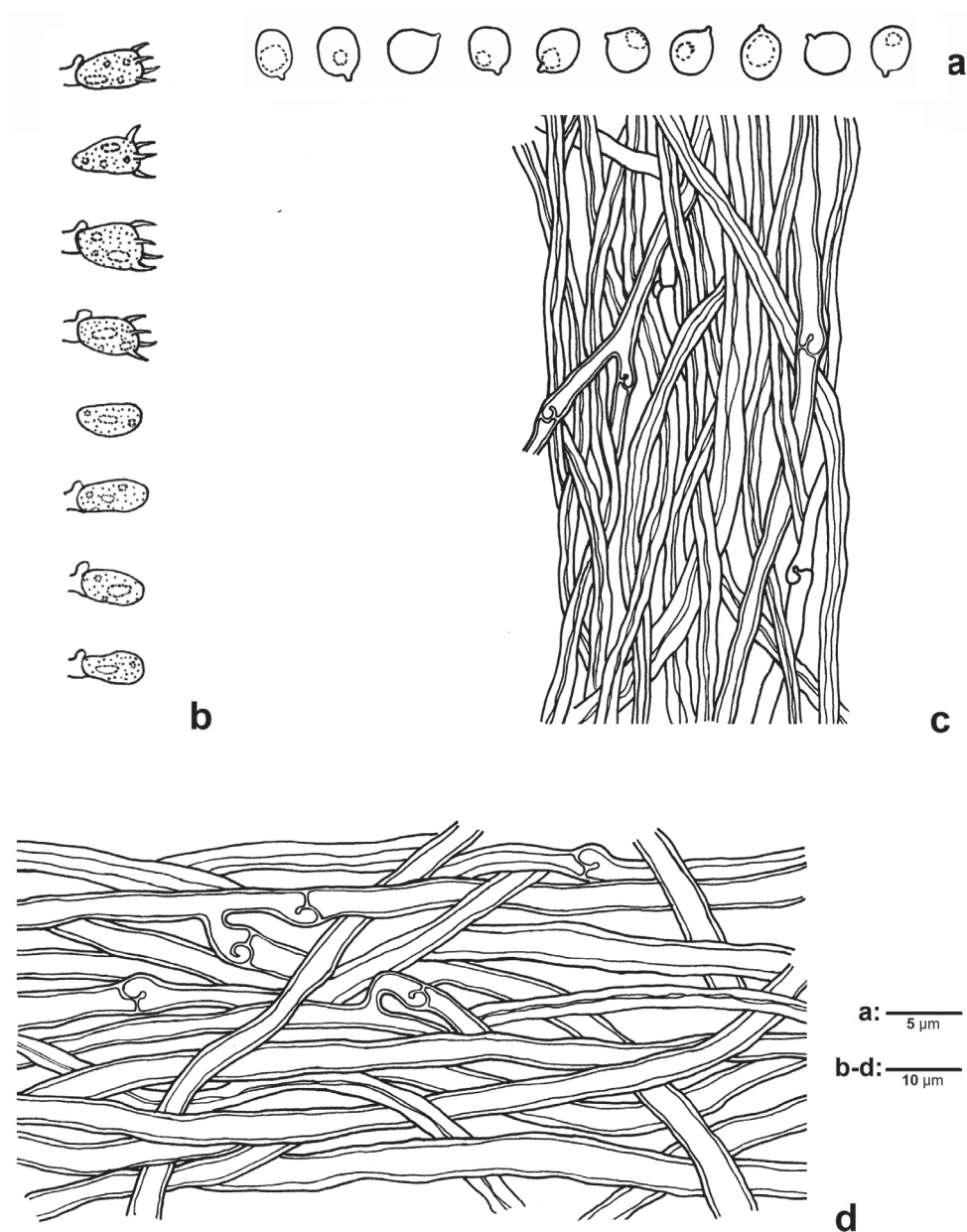


Figure 5. Microscopic structures of *Trullella conifericola* (IFP 019372, holotype) **a** basidiospores **b** basidia and basidioles **c** hyphae from trama **d** hyphae from context.

Tubes. Dominated by skeletal hyphae, interwoven; generative hyphae hyaline, thin- to slightly thick-walled, moderately branched, 2–4 µm diam; skeletal hyphae hyaline, thick-walled to semisolid, straight to flexuous, unbranched, 1.5–3.5 µm diam. *Cystidia* or other sterile hymenial elements absent. *Basidia* short 8–15 × 4–5.5 µm,

clavate, 4-sterigmata of 0.5–1 μm in length, with a clamp connection at base; basidioles similar to basidia in shape, but slightly smaller.

Basidiospores. Cylindrical to allantoid, slightly curved, hyaline, thin-walled, smooth, CB–, IKI–, (4.0–)4.1–5.5(–5.8) $\times$ (1.6–)1.8–2.3(–2.5) μm , $L_m = 4.94 \mu\text{m}$, $W_m = 2.09 \mu\text{m}$, $Q = 2.36\text{--}2.45$ ($n = 60/2$).

Ecology. On fallen gymnosperm branch, causing a white rot.

Distribution. In high altitude area of subtropical to tropical zones.

Additional specimens examined. CHINA. Fujian Prov., Wuyishan Forest Park, on fallen trunk of *Pinus kesiya*, 16.IX.2005, *Cui 2851* (IFP 000645). VIETNAM. Lam dong Province, Lac Duong District, Bidoup Nui Ba National Park, on fallen branch of *Pinus kesiya*, 15.X.2017, *Yuan 12657* (IFP 019373).

Discussion

The phylogenetic profiling showed that the new species *Mycorrhaphium subadustum* as well as *Trullella conifericola* are nested in the Steccherinaceae which belongs to the residual polyporoid clade (Miettinen et al. 2012; Binder et al. 2013; Zmitrovich 2018; Westphalen et al. 2019) where they emerge robustly supported isolated lineages. Furthermore, these lineages are supported by morphological characteristics.

Mycorrhaphium was recommended by Maas Geesteranus (1962) and typified by *M. adustum*. The two samples of the new species *M. subadustum* (Yuan 12976 and Dai 10173) clustered in *Mycorrhaphium* clade, were both collected on fallen branches of angiosperm from northeast of China. The similarity of ITS and nrLSU sequences between the two samples of *M. subadustum* are 99.00% as well as 99.64% respectively, and they form a full-support isolated lineage which is closely related to *M. adustum*, the type species of the genus. The type material of *M. subadustum* Yuan 12976 have a 95.56% similarity of ITS sequences with the American *M. adustum* KHL12255. Morphologically, *M. subadustum* is similar to *M. adustum* in having the velutinate and concentrically zonate pileal surface, presence of clamps and simple septa, a dimitic hyphae system in spine trama and monomitic in context, absence of cystidia as well as gloeocystidia and the non-amyloid basidiospores. However, *M. adustum* often have a dark-colored pileal margin, which is distinctly different from the yellowish white ones of *M. subadustum*. Anatomically, the new species can be differentiated from *M. adustum* by the slender generative hyphae in context (3–5 μm vs. 4–6.3 μm), cyanophilous hyphae and presence of cystidiols (Maas Geesteranus 1962; Ryvarden 1989; Westphalen et al. 2019).

Mycorrhaphium embraced nine species (<http://www.indexfungorum.org>, 2020) and among which there are others two species described from Asia: *Mycorrhaphium sessile* H.S. Yuan & Y.C and *M. stereoides* Maas Geest. *M. sessile* is a species described from China, but the characteristics such as the sessile basidiocarps and presence of gloeocystidia can differentiate it from *M. subadustum* (Yuan and Dai 2009). *Mycorrhaphium stereoides* is related to *M. subadustum* in having stipitate basidiocarps, hyd-

noid hymenophore, a monomitic hyphal system in context and dimitic in spines, but differs from it by the presence of gloeocystidia and the larger basidiospores ($4\text{--}6.3 \times 2.5\text{--}3.8 \mu\text{m}$) (Maas Geesteranus 1971). The North Europe *Mycorrhaphium pusillum* (Brot.) Maas Geest. is closely related to *M. subadustum* in having the stipitate basidiocarps as well as pale colored and zonate pileal surface, but differs it by the presence of gloeocystidia, absence of clamps and the broader basidiospores ($Q = 1.52$ in *M. pusillum* vs. $2.13\text{--}2.17$ in *M. subadustum*) (Tervonen et al. 2015). *Mycorrhaphium ursinum* Decock & Ryvardeen is a new species from African; its habit of growing on the soil can be distinguished from *M. subadustum*. Ryvardeen (1989) as well as Mossebo and Ryvardeen (2003) have provided keys to a part of species in *Mycorrhaphium* and after which several new taxa have been described. We provide a new key to the whole described species (except *M. ursinum*) of the genus in this study.

In the phylogenetic tree, nine samples of *Trullella* species which include the new species *T. conifericola* form the clade with strong support (92% ML and 1.00 BPP). *Trullella* is a genus which was originally proposed as ‘*Trulla*’ by Miettinen and Ryvardeen (2016) and renamed by Zmitrovich (2018). *Trullella conifericola* is quite an extraordinary species in the genus because of its coniferous-saprophytic habit. The type species of *Trullella*, *T. dentipora* (Ryvardeen & Iturr.) Zmitr., was described from South America. *Trullella dentipora*, together with the other species of the genus, inhabits dead angiosperm trees (e.g. *Quercus* and *Cecropia peltata*) (Patouillard 1902; Murrill 1907; Miettinen and Ryvardeen 2016). Morphologically and anatomically, *T. conifericola* resembles others *Trullella* spp. in having sessile or laterally stipitate basidiocarps, mostly small and regular pores, a dimitic hyphal structure, nearly monomitic in the context, and curved cylindrical spores. However, the new species can be distinctly differentiated from others species by having a hirtellous pileal surface with appressed coarse hair, larger spores than those of previous *Trullella* species ($L_m = 4.94 \mu\text{m}$ and $W_m = 2.09 \mu\text{m}$ in *T. conifericola* vs $L_m = 4.00\text{--}4.77 \mu\text{m}$ and $W_m = 1.39\text{--}1.91 \mu\text{m}$ in others *Trullella* spp.), and inhabiting fallen gymnosperm branches. *Trullella* composed of six species as of now, and the key to these species was provided by Miettinen and Ryvardeen (2016).

Besides, the genera *Mycorrhaphium* and *Trullella* together with *Austeria*, *Flabellophora* and *Nigroporus* form a large clade in the phylogenetic tree with strong support (85% ML and 1.00 BPP), and share similar morphological features, including zonate or sulcate pileal surfaces, tiny pores or dense spines and a context that is entirely or almost monomitic. They form a distinct subgroup in the Steccherinaceae.

Key to species of worldwide *Mycorrhaphium*

1	Hymenophore hydroid	2
–	Hymenophore poroid	<i>M. hispidum</i> Westph. & Miettinen
2	Spores less than $3.5 \mu\text{m}$ long.....	3
–	Spores more than $3.5 \mu\text{m}$ long	4
3	Stipe present, spines less than 2 mm long.....	<i>M. adustulum</i> (Banker) Ryvardeen
–	Stipe absent, spines up to 4 mm long.....	<i>M. sessile</i>

4	Spines less than 5 mm long, spores less than 5 μ m long	5
–	Spines up to 10 mm long, spores up to 6.3 μ m long	<i>M. stereoides</i>
5	Pileal less than 2 cm wide, gloecystidia present	
	 <i>M. pusillum</i> (Brot.) Maas Geest.	
–	Pileal more than 2 cm wide, gloecystidia absent	6
6	Habit on the ground	7
–	Habit on the fallen branch of hard wood	8
7	Spines more than 3 mm long	<i>M. africanum</i> Mossebo & Ryvarden
–	Spines less than 3 mm long	<i>M. citrinum</i> Ryvarden
8	Pileal margin black, hyphae acyanophilous	<i>M. adustum</i>
–	Pileal margin yellowish white, hyphae cyanophilous	<i>M. subadustum</i>

Acknowledgements

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References

- Binder M, Justo A, Riley R, Salamov A, Lopez-Giraldez F, Sjökvist E, Copeland A, Foster B, Sun H, Larsson E, Larsson KH, Townsend J, Grigoriev IV, Hibbett DS (2013) Phylogenetic and phylogenomic overview of the Polyporales. *Mycologia* 105: 1350–1373. <https://doi.org/10.3852/13-003>
- Corda ACJ (1842) Anleitung zum Studium der Mykologie. Prague, 384 pp.
- Cunningham GH (1965) Polyporaceae of New Zealand. New Zealand Department of Scientific and Industrial Research Bulletin 164: 1–304.
- Dai YC, Xue HJ, Vlasák J, Rajchenberg M, Wang B, Zhou LW (2014) Phylogeny and global diversity of *Polyporus* group *Melanopus* (Polyporales, Basidiomycota). *Fungal Diversity* 64: 133–144. <https://doi.org/10.1007/s13225-013-0248-3>
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: e772. <https://doi.org/10.1038/nmeth.2109>
- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Gray SF (1821) A natural arrangement of British plants 1: 1–824.
- Hall T (2005) BioEdit: biological sequence alignment editor for Win95/98/NT/2K/XP. Ibis Therapeutic, Carlsbad.
- He SH, Dai YC (2012) Taxonomy and phylogeny of *Hymenochaete* and allied genera of Hymenochaetaceae (Basidiomycota) in China. *Fungal Diversity* 56: 77–93. <https://doi.org/10.1007/s13225-012-0174-9>

- Johannesson H, Renvall P, Stenlid J (2000) Taxonomy of *Antrodiella* inferred from morphological and molecular data. *Mycological Progress* 104: 92–99. <https://doi.org/10.1017/S0953756299008953>
- Justo A, Miettinen O, Floudas D, Ortiz-Santana B, Sjökvist E, Lindner D, Nakasone K, Niemelä T, Larsson KH, Ryvarden L, Hibbett DS (2017) A revised family-level classification of the Polyporales (Basidiomycota). *Fungal Biology* 121: 798–824. <https://doi.org/10.1016/j.funbio.2017.05.010>
- Karoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20: 1160–1166. <https://doi.org/10.1093/bib/bbx108>
- Kornerup A, Wanscher J (1981) *Methuen Handbook of Colour* Fletcher. Norwich, 252 pp.
- Kotiranta H, Kulju M, Miettinen O (2017) *Caudicicola gracilis* (Polyporales, Basidiomycota), a new polypore species and genus from Finland. *Annales Botanici Fennici* 54: 159–167. <https://doi.org/10.5735/085.054.0325>
- Maas Geesteranus RA (1962) Hyphal structures in *Hydnum*. *Persoonia* 2: 377–405.
- Maas Geesteranus RA (1971) Hydneous fungi of the eastern old world. *Verhandelingen Koninklijke Nederlandse Akademie van Wetenschappen Afdeling Natuurkunde* 60: 1–176.
- Matheny PB, Wang Z, Binder M, Curtis JM, Lim YW, Nilsson RH, Hughes KW, Hofstetter V, Ammirati JF, Schoch CL, Langer E, Langer G, McLaughlin DJ, Wilson AW, Froslev T, Ge ZW, Kerrigan RW, Slot JC, Yang ZL, Baroni TJ, Fischer M, Hosaka K, Matsuura K, Seidl MT, Vauras J, Hibbett DS (2007) Contributions of *rpb2* and *tef1* to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). *Molecular Phylogenetics and Evolution* 43: 430–451. <https://doi.org/10.1016/j.ympev.2006.08.024>
- Miettinen O, Larsson E, Sjökvist E, Larsson KH (2012) Comprehensive taxon sampling reveals unaccounted diversity and morphological plasticity in a group of dimitic polypores (Polyporales, Basidiomycota). *Cladistics* 28: 251–270. <https://doi.org/10.1111/j.1096-0031.2011.00380.x>
- Miettinen O, Ryvarden L (2016) Polypore genera *Antella*, *Austeria*, *Butyrea*, *Citripora*, *Metuloidea* and *Trulla* (Steccherinaceae, Polyporales). *Annales Botanici Fennici* 53: 157–172. <https://doi.org/10.5735/085.053.0403>
- Mossebo DC, Ryvarden L (2003) The genus *Mycorrhaphium* in Africa. *Mycotaxon* 88: 229–232.
- Murrill WA (1905) The Polyporaceae of North America: XI. A synopsis of the brown pileate species. *Bulletin of the Torrey Botanical Club* 32: 353–371. <https://doi.org/10.2307/2478499>
- Murrill WA (1907) Polyporaceae, Part 1. *North American Flora* 9: 1–72.
- Nilsson RH, Tedersoo L, Abarenkov K, Ryberg M, Kristiansson E, Hartmann M, Schoch CL, Nylander JAA, Bergsten J, Porter TM, Jumpponen A, Vaishampayan P, Ovaskainen O, Hallenberg N, Bengtsson-Palme J, Eriksson KM, Larsson KH, Larsson E, Kõljalg U (2012) Five simple guidelines for establishing basic authenticity and reliability of newly generated fungal ITS sequences. *MycoKeys* 4: 37–63. <https://doi.org/10.3897/mycokeys.4.3606>
- Parmasto, E (1968) *Conspectus systematis coriciacearum*. Institutum Zoologicum & Botanicum Academiae Scientiarum R.P.S.S. Estonicae, Tartu.
- Patouillard NT (1902) Champignons de la Guadeloupe, recueillis par le R.P. Duss. *Bulletin de la Société Mycologique de France* 18: 171–186.

- Rambaut A (2012) FigTree version 1.4.0. <http://tree.bio.ed.ac.uk/software/figtree/>
- Rehner SA, Buckley EP (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1- α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97: 84–98. <https://doi.org/10.3852/mycologia.97.1.84>
- Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Ryvarden L (1989) *Mycorrhaphium citrinum* sp. nov. (Basidiomycetes, Aphyllophorales). *Memoirs of the New York Botanical Garden* 49: 344–347.
- Ryvarden L, Johansen I (1980) A preliminary polypore flora of East Africa, 636 pp.
- Sayers EW, Cavanaugh M, Clark K, Ostell J, Pruitt KD, Karsch-Mizrachi I (2020) GenBank. *Nucleic Acids Research* 48: 84–86. <https://doi.org/10.1093/nar/gkaa1023>
- Spirin W, Zmitrovich I, Malysheva V (2007) *Steccherinum tenuispinum* (Polyporales, Basidiomycota), a new species from Russia, and notes on three other species. *Annales Botanici Fennici* 44: 298–302. <https://www.jstor.org/stable/23727802>
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology* 75: 758–771. <https://doi.org/10.1080/10635150802429642>
- Stöger A, Schaffer J, Ruppitsch W (2006) A rapid and sensitive method for direct detection of *Erwinia amylovora* in symptomatic and asymptomatic plant tissues by polymerase chain reaction. *Journal of Phytopathology* 154: 469–473. <https://doi.org/10.1111/j.1439-0434.2006.01130.x>
- Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* 31: 21–32. <https://doi.org/10.1006/fgbi.2000.1228>
- Tervonen K, Spirin V, Halme P (2015) Redescription of *Mycorrhaphium pusillum*, a poorly known hydroid fungus. *Mycotaxon* 130: 549–554. <https://doi.org/10.5248/130.549>
- Tomšovský M, Menkis A, Vasaitis R (2010) Phylogenetic relationships in European *Ceriporiopsis* species inferred from nuclear and mitochondrial ribosomal DNA sequences. *Fungal Biology* 114: 350–358. <https://doi.org/10.1016/j.funbio.2010.02.004>
- Westphalen MC, Tomšovský M, Rajchenberg M, Gugliotta MA (2016) Morphological and phylogenetic studies of two new neotropical species of *Loweomyces* (Polyporales, Basidiomycota). *Mycological Progress* 15: 967–975. <https://doi.org/10.1007/s11557-016-1223-7>
- Westphalen MC, Rajchenberg M, Tomšovský M, Gugliotta AM (2018) A re-evaluation of Neotropical *Junghuhnia* s.l. (Polyporales, Basidiomycota) based on morphological and multi-gene analyses. *Persoonia* 41: 130–141. <https://doi.org/10.3767/persoonia.2018.41.07>
- Westphalen MC, Tomšovský M, Gugliotta AM, Rajchenberg M (2019) An overview of *Antrodia* and related genera of Polyporales from the Neotropics. *Mycologia* 111: 813–831. <https://doi.org/10.1080/00275514.2019.1633895>
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ

- (Eds) PCR Protocols, a Guide to Methods an Applications. Academic Press, SanDiego, 315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Wu ZQ, Xu TM, Shen Sh, Liu XF, Luo KY, Zhao CL (2018) *Elaphroporia ailaoshanensis* gen. et sp. nov. in Polyporales (Basidiomycota). MycoKeys 29: 81–95. <https://doi.org/10.3897/mycokeys.29.22086>
- Yuan HS (2013) *Antrodiella chinensis* sp. nov., a Chinese representative of the *Antrodiella americana* complex. Mycological Progress 12: 437–443. <https://doi.org/10.1007/s11557-012-0852-8>
- Yuan HS (2014) Molecular phylogenetic evaluation of *Antrodiella* and morphologically allied genera in China. Mycological Progress 13: 353–364. <https://doi.org/10.1007/s11557-013-0921-7>
- Yuan HS, Dai YC (2009) Hydneous fungi of China 2. *Mycorrhaphium sessile* sp. nov. Nova Hedwigia 88: 205–209. <https://doi.org/10.1127/0029-5035/2009/0088-0205>
- Yuan HS, Dai YC, Wu SH (2012) Two new species of *Junghuhnia* (Basidiomycota, Polyporales) from Taiwan and a key to all species known worldwide of the genus. Sydowia 64: 137–145.
- Yuan HS, Qin WM (2018) Multiple genes phylogeny and morphological characters reveal *Dextrinoporus aquaticus* gen. et sp. nov. (Polyporales, Basidiomycota) from southern China. Mycological Progress 17: 773–780. <https://doi.org/10.1007/s11557-018-1392-7>
- Yuan HS, Wu SH (2012) Two new species of *Steccherinum* (Basidiomycota, Polyporales) from Taiwan. Mycoscience 53: 133–138. <https://doi.org/10.1007/S10267-011-0139-Y>
- Yuan HS, Lu X, Decock C (2018) Molecular and morphological evidence reveal a new genus and species in Auriculariales from tropical China. MycoKeys 35: 27–39. <https://doi.org/10.3897/mycokeys.35.25271>
- Yuan HS, Lu X, Dai YC, Hyde KD, Kan YH, Kušan I, He SH, Liu NG, Sarma VV, Zhao CL, Cui BK, Yousaf N, Sun GY, Liu SY, Wu F, Lin CG, Dayarathne MC, Gibertoni TB, Conceição LB, Garibay-Orijel R, Villegas-Ríos M, Salas-Lizana R, Wei TZ, Qiu JZ, Yu ZF, Phookamsak R, Zeng M, Paloi S, Bao DF, Abeywickrama PD, Wei DP, Yang J, Manawasinghe IS, Harishchandra D, Brahmanage RS, de Silva NI, Tennakoon DS, Karunaratna A, Gafforov Y, Pem D, Zhang SN, de Azevedo Santiago ALCM, Bezerra JDP, Dima B, Acharya K, Alvarez-Manjarrez J, Bahkali AH, Bhatt VK, Brandrud TE, Bulgakov TS, Camporesi E, Cao T, Chen YX, Chen YY, Devadatha B, Elgorban AM, Fan LF, Du X, Gao L, Gonçalves CM, Gusmão LFP, Huanraluek N, Jadan M, Jayawardena RS, Khalid AN, Langer E, Lima DX, de Lima-Júnior NC, de Lira CRS, Liu JK, Liu S, Lumyong S, Luo ZL, Matočec N, Niranjana M, Oliveira-Filho JRC, Papp V, Pérez-Pazos E, Phillips AJL, Qiu PL, Ren YH, Castañeda-Ruiz RF, Semwal KC, Soop K, de Souza CAF, Souza-Motta CM, Sun LH, Xie ML, Yao YJ, Zhao Q, Zhou LW (2020) Fungal diversity notes 1277–1386: taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 104: 1–266. <https://doi.org/10.1007/s13225-020-00461-7>
- Zmitrovich IV (2018) Conspectus systematis Polyporacearum v. 1.0. Folia Cryptogamica Petropolitana 6: 3–145.

Morphological and molecular identification of two new *Alternaria* species (Ascomycota, Pleosporaceae) in section *Radicina* from China

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Abstract

The fungal genus *Alternaria* was distributed widely and found in different habitats such as plant or indoor environment. During an investigation into this genus in China, two new *Alternaria* species, *Alternaria vulgarae* and *A. divaricatae* were respectively isolated from diseased leaves of *Foeniculum vulgare* and *Saposhnikovia divaricata*, which both belonged to Umbelliferae. Phylogenetically, they were determined as new species belonging in the section *Radicina* of *Alternaria* based on the combined four gene fragments of ITS, *TEF1*, *GAPDH* and *RPB2*. Morphologically, the two species were illustrated and compared with other relevant *Alternaria* species in section *Radicina*.

Keywords

Alternaria, new taxon, phylogeny, Pleosporaceae, taxonomy

Introduction

Alternaria Nees (1816) was typified by *Alternaria tenuis* (the synonym of *A. alternata*), a species with muriform and catenulate conidia. Since then, hundreds of new species were proposed in the genus. Meanwhile, because of unstable taxonomic standards

(morphological characteristics, host and growing environment, etc.), the controversies about species boundary started and never stopped (Elliott 1917; Fries 1832; Neergaard 1945; Joly 1964; Wiltshire 1933; Simmons 1967, 1992). In 1992, Simmons introduced reasonable standards to get unified taxonomic concepts on *Alternaria* species based on colony and conidial morphology. At the same time, the concept of species-group was introduced, the small-spored, catenulate taxa of *Alternaria* were divided into six morphological groups by Simmons and Roberts (1993). More recently, around 300 *Alternaria* morphospecies have been accepted based on the shape, size, septation of conidia, as well as sporulation patterns. Small-spored *Alternaria* species were also re-defined and divided into 10 subsections characterized by short (>50 – 60 μm) or medium (50 – 100 – 105 μm) conidia produced in various patterns of branched and unbranched chains or solitary (Simmons 2007). However, the identification remained challenging due to the impact of environmental conditions and other unknown factors.

On the other hand, multigene phylogenetic analyses have provided strong support for the re-definition of the *Alternaria* genus. Many sequences of gene regions such as the internal transcribed spacer region of rDNA (ITS), large subunit ribosomal DNA (*LSU*), mitochondrial small subunit (*mtSSU*), *Alternaria* major allergen (*ALT*), glyceraldehydes-3-phosphate dehydrogenase (*GAPDH*), translation elongation factor 1-alpha (*TEF1*), RNA polymerase second largest subunit (*RPB2*), and ATPase etc. were applied to delimit the genus (Pryor and Gilbertson 2000; Hong et al. 2005; Lawrence et al. 2012, 2013; Woudenberg et al. 2013, 2014; Poursafar et al. 2018). In recent studies, both morphological and molecular analyses were used for the delimitation of the genus *Alternaria*, which has been divided into 28 sections and eight monotypic lineages (Woudenberg et al. 2013; Lawrence et al. 2016; Ghafri et al. 2019; Marin-Felix et al. 2019). The number of *Alternaria* species has been continuously growing after re-descriptions and new discovery (Deng et al. 2018; Ahmadvpour 2019; Liu et al. 2019; Tao et al. 2019; Bessadat et al. 2020; He et al. 2020). Coincidentally, several phylogenetic lineages have strongly supported morphology-based sections but others not (Simmons 2007; Woudenberg et al. 2015).

During the investigation into *Alternaria* species in China, two new taxa were isolated from umbelliferous plants, *Foeniculum vulgare* and *Saposhnikovia divaricata*. The study was designed to determine them based on a polyphasic approach including morphology and phylogenetic analyses.

Materials and methods

Isolation and morphological studies

Leaves of *Foeniculum vulgare* and *Saposhnikovia divaricata* with necrotic spots were respectively collected from Wenjiang district (Chengdu, Sichuan in June, 2015) and Badong county (Yichang, Hubei in July, 2016) in China. For fungal isolation, the samples were stored in sterile plastic bags and transported to the laboratory. The tissues

were cut into small segments and placed on moist filter papers within Petri dishes then incubated at 25 °C to stimulate sporulation. After 24 h, the samples were examined under a stereomicroscope. *Alternaria*-like spores were picked up and inoculated to potato dextrose agar (PDA: Difco, Montreal, Canada) using sterilized glass needles. All isolated pure cultures were inoculated to test-tube slants and stored at 4 °C. Dried cultures from the single spore and ex-type strains were deposited in the Fungi Herbarium of Yangtze University (YZU), Jingzhou, Hubei, China.

To determine colonial characteristics (size, color and texture of colony), the strains were cultured on PDA at 25 °C for 7 days in darkness. To analyze the morphological features of conidia (conidial size, shape, sporulation, etc.), fresh mycelia were transferred on potato carrot agar (PCA) and V8 juice agar (V8A) then incubated at 22 °C under an 8 hour photoperiod for 7 days (Simmons 2007). Conidia were mounted into a lactophenol picric acid solution and digital images were captured under a Nikon ECLIPSE Ni-U microscope system (Nikon, Japan). Conidia (n = 50) were randomly selected for determining the morphology and sporulation patterns were also photographed at the same time.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fresh mycelia growing on PDA after 3–5 days of growth following the CTAB method described in Watanabe et al. (2010). For amplification of the ITS, *TEF1*, *GAPDH* and *RPB2* gene fragments, the primer pairs ITS5/ITS4 (White et al. 1990), EF1-728F/EF1-986R (Carbone and Kohn 1999), gpd1/gpd2 (Berbee et al. 1999) and RPB2-5F/RPB2-7cR (Liu et al. 1999) were used, respectively. A total of 25 µL of a PCR reaction mixture containing 21 µL of 1.1×Taq PCR Star Mix (TSINGKE, Beijing, China), 2 µL template DNA and 1 µL of each primer was prepared and the PCR was performed in an Eppendorf Mastercycler following the protocols described by Woudenberg et al. (2013). Successful products were purified and sequenced by TSINGKE company (Beijing, China). All sequences were assembled with BioEdit (Hall 1999) and deposited at GenBank (<https://www.ncbi.nlm.nih.gov/>) (Table 1).

Phylogenetic analyses

Preliminary BLAST searches in GenBank with ITS and *TEF1* sequences of the present isolates indicated that they had a close phylogenetic relationship with species from section *Radicina* of *Alternaria*. Subsequently, sequence data of 19 *Alternaria* species and *Stemphylium herbarum* CBS 191.86 (outgroup) were retrieved from National Center for Biotechnology Information (NCBI), mostly published in Marin-Felix et al. (2019), Woudenberg et al. (2013), and Tao et al. (2019) (Table 1). The gene sequences were concatenated and edited manually according to ITS+*TEF1*+*GAPDH*+*RPB2* for YZU 161234 and YZU 161235 and ITS+*TEF1*+*RPB2* for YZU 151055 and YZU 151059 with equal weight in MEGA v.7.0.26 (Kumar et al. 2016). Maximum parsimony (MP)

Table 1. *Alternaria* strains and their accession numbers used in the phylogenetic analysis.

Section	Species	Strain	Host/Substrate	Country	GenBank accession numbers			
					ITS	GAPDH	TEFI	RPB2
<i>Alternaria</i>	<i>A. alternata</i>	CBS 916.96 T	<i>Anachis hypogaea</i>	India	AF347031	AY278808	KC584634	KC584375
	<i>A. tenuissima</i>	CBS 918.96 R	<i>Dianthus</i> sp.	UK	AF347032	AY278809	KC584693	KC584435
<i>Althernantherae</i>	<i>A. althernantherae</i>	CBS 124392	<i>Solanum melongena</i>	China	KC584179	KC584096	KC584633	KC584374
	<i>A. perpunctulata</i>	CBS 115267 T	<i>Alternanthera philoxeroides</i>	USA	KC584210	KC584129	KC584676	KC584418
<i>Gypsophilae</i>	<i>A. gypsophilae</i>	CBS 107.41 T	<i>Gypsophila elegans</i>	USA	KC584199	KC584118	KC584660	KC584401
	<i>A. nobilis</i>	CBS 116490 R	<i>Dianthus caryophyllus</i>	New Zealand	KC584208	KC584127	KC584673	KC584415
	<i>A. vaccariae</i>	CBS 116533 R	<i>Vaccaria hispanica</i>	USA	KC584223	KC584146	KC584696	KC584438
	<i>A. vaccariicola</i>	CBS 118714 T	<i>Vaccaria hispanica</i>	USA	KC584224	KC584147	KC584697	KC584439
<i>Radicina</i>	<i>A. carotiunculae</i>	CBS 109381 T	<i>Daucus carota</i>	USA	KC584188	KC584106	KC584645	KC584386
	<i>A. chlamydosporifera</i>	FMR 17360 T	Rabbit dung	Spain	LR133924	LR133927	LR133929	LR133926
	<i>A. divaricatae</i> sp. nov.	YZU 151055 T	<i>Saposhnikovia divaricata</i>	China	MW541932	–	MW579314	MW579316
		YZU 151059	<i>Saposhnikovia divaricata</i>	China	MW541933	–	MW579315	MW579317
	<i>A. glehniae</i>	YZU 161149 T	<i>Glehnia littoralis</i>	China	MK279385	–	MK279392	MK279394
	<i>A. petroselinii</i>	CBS 112.41 T	<i>Petroselinum sativum</i>	Unknown	KC584211	KC584130	KC584677	KC584419
	<i>A. radicina</i>	CBS 245.67 T	<i>Daucus carota</i>	USA	KC584213	KC584133	KC584681	KC584423
	<i>A. selini</i>	CBS 109382 T	<i>Petroselinum crispum</i>	Saudi Arabia	AF229455	AY278800	KC584684	KC584426
	<i>A. smyrnii</i>	CBS 109380 R	<i>Smyrniolum olusatrum</i>	UK	AF229456	KC584138	KC584687	KC584429
	<i>A. vulgariae</i> sp. nov.	YZU 161234 T	<i>Foeniculum vulgare</i>	China	MW541936	MW579308	MW579310	MW579312
YZU 161235		<i>Foeniculum vulgare</i>	China	MW541937	MW579309	MW579311	MW579313	
<i>Porri</i>	<i>A. dauci</i>	CBS 117097 R	<i>Daucus carota</i>	USA	KC584192	KC584111	KC584651	KC584392
	<i>A. porri</i>	CBS 116698 R	<i>Allium cepa</i>	USA	DQ323700	KC584132	KC584679	KC584421
<i>Sonchi</i>	<i>A. cinerariae</i>	CBS 116495 R	<i>Ligularia</i> sp.	USA	KC584190	KC584109	KC584648	KC584389
	<i>A. sonchi</i>	CBS 119675 R	<i>Sonchus asper</i>	Canada	KC584220	KC584142	KC584691	KC584433
Out-group	<i>Stemphylium herbarum</i>	CBS 191.86 T	<i>Medicago sativa</i>	India	KC584239	AF443884	KC584731	KC584471

Notes: *Alternaria* strains of the present study are marked in bold. Type strains are marked 'T'. Representative strains are marked 'R'.

analysis was performed in PAUP 4.0 (Swofford 2002) using the heuristic search option of 1000 random-addition sequences and tree bisection and reconnection (TBR) as the branch-swapping algorithm. Gaps were treated as missing data. The bootstrap values (BS) with 1000 replicates were performed to determine branch support. Parsimony scores of tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency (RC) were calculated for each generated tree. The Bayesian inference (BI) analysis was performed with a Markov Chain Monte Carlo (MCMC) algorithm with Bayesian posterior probabilities in MrBayes v. 3.2.1 (Ronquist et al. 2012). The best-fit evolutionary models (GTR+I+G) were determined in MrModel-test v. 2.3 (Posada and Crandall 1998) using the Akaike Information Criterion (AIC). Two independent analyses with four chains each were run for 10,000,000 generations. Trees were

sampled every 100th generation. The run was stopped until the standard deviation of split frequencies reaches < 0.01 and the initial 25 % of the trees were discarded as the burn-in phase of each analysis. Maximum likelihood (ML) analysis was performed using RAxML v.7.2.8 (Stamatakis 2006), implementing GTRCAT model and executing 1000 rapid ML bootstrap replications. Branch support equal to or above 0.70/70%/70% for PP (posterior probability of BI analysis) and BS (bootstrap for ML and MP analyses) values were shown at the nodes in the phylogram.

Results

Phylogenetic analyses

The combined dataset of twenty-four strains (including 20 references and present four strains) had a length of 2166 characters with gaps after alignment, 536 characters for ITS, 247 for *TEF1*, 537 for *GAPDH* and 846 for *RPB2*. Of these characters, 1555 were constant and 198 were variable and parsimony-uninformative. MP analysis of the remaining 413 parsimony-informative characters resulted in one parsimonious tree of 995 lengths (CI = 0.739, RI = 0.815, RC = 0.602); Tree topologies computed from the MP, BI, and ML analysis were similar and the ML tree was shown in Fig. 1. The results indicated that all strains in the present study fell into the section *Radicina* with PP/BS (BI/ML/MP) values of 1/100%/100%. The strains YZU 161234 and YZU 161235 were clustered with *A. petroselini* and *A. selini* in a clade supported by values of 1.0/91%/90% (BI/ML/MP). This clade was sister to a separate clade containing the other two strains (YZU 151055, YZU 151059) supported by PP value of 0.95 and BS values of 77%/70% (ML/MP) (Fig. 1).

Taxonomy

Alternaria divaricatae L. He & J.X. Deng, sp. nov.

Mycobank No: 838893

Figure 2

Type. China, Sichuan Province, Chengdu City, Wenjiang District, Herb Garden of Chengdu University of Traditional Chinese Medicine, from leaf spot of *Saposhnikovia divaricata*. 17 June, 2015, J.X. Deng, (YZU-H-0029, holotype), ex-type culture YZU 151055.

Etymology. In reference to the host species name, *divaricata*.

Description. Colonies on PDA (Fig. 2A) vinaceous buff, hazel in the center, velvety, cottony, dark mouse grey to pale mouse grey in reverse, 56–64 mm in diam.; On PCA, *conidiophores* arising directly from lateral or apical of aerial hyphae or medium, lightly flexuous, sometimes geniculate at apex, smooth-walled, 9–36 × 3.5–6 µm, 1–3 transverse septa, the aerial hyphae sometimes up to 82–400 × 4–6 µm; *conidia* solitary

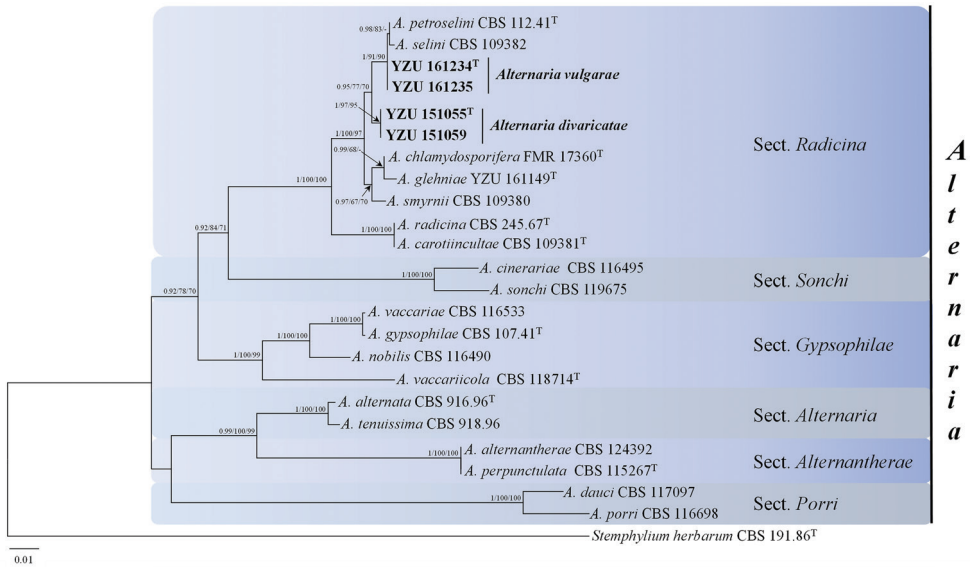


Figure 1. Phylogenetic tree based on the combined gene sequences of ITS, *TEF1*, *GAPDH*, and *RPB2*. The Bayesian posterior probabilities >0.70 (PP), maximum likelihood and maximum parsimony bootstrap support values >70 (BS) are given at the nodes (PP/BS). Examined strains are in bold.

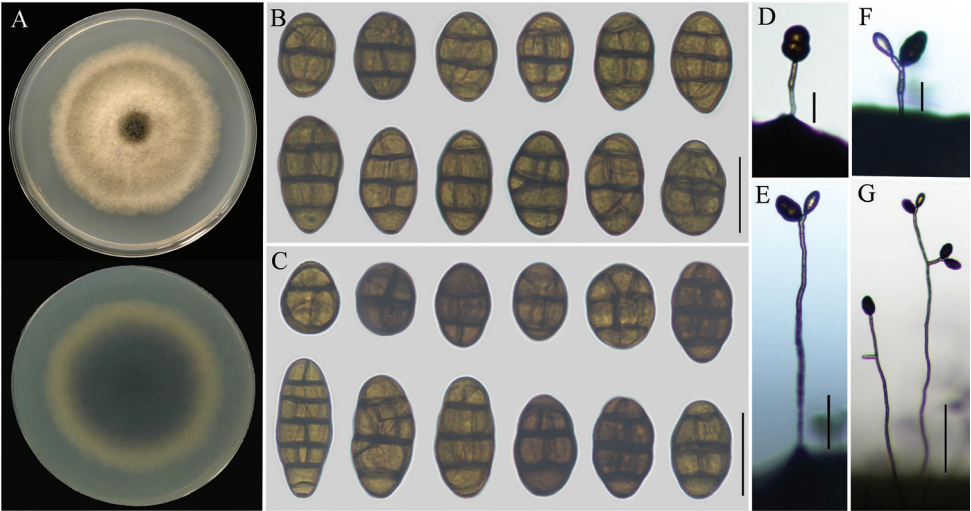


Figure 2. Morphological characteristics of *Alternaria divaricatae* (strain: YZU 151055). Colony on PDA for 7 days at 25 °C (A); Conidia on PCA and V8A (B, C); Sporulation patterns from PCA and V8A (D–G: D, E from PCA F, G from V8A); Scale bars: 25 µm (B, C); 2 µm (D, F); 50 µm (E); 100 µm (G).

from apex or geniculate loci, short-ovoid, subglobose, ellipsoid, 21–38 × 12–26 µm, with 1–4 transverse septa and 1–4 longitudinal septa (Fig. 2B, D, E); On V8A, *conidiophores* 10–26 (–53) × 3–4 µm, 1–7 transverse septa, *conidia* 22–39 × 13–24 µm, 1–4

transverse septa, 1–3 longitudinal or oblique between septa (Fig. 2C, F, G). There was no secondary conidium production observed on PCA and V8A medium.

Additional isolate examined. China, Sichuan Province, Chengdu City, Wenjiang District, Herb Garden of Chengdu University of Traditional Chinese Medicine, from leaf spot of *Saposhnikovia divaricata*. 17 June, 2015, L He, living culture YZU 151059.

Notes. Phylogenetically, *Alternaria divaricatae* forms a distinct clade in section *Radicina*, which appears to be sister to a clade including *A. petroselini*, *A. selini* and *A. vulgarae* (Fig. 1). Morphologically, *A. divaricatae* was different from *A. petroselini*, *A. selini* and *A. vulgarae* by producing smaller conidia (Table 2) and special sporulation from apex or geniculate loci of lateral or apical of aerial hyphae. Moreover, *A. chlamydosporifera*, *A. glehniae* and *A. smyrnii* grouped together and clustered as a sister clade with *A. divaricatae*, *A. petroselini*, *A. selini* and *A. vulgarae* (Fig. 1). Obviously, the conidia of *A. divaricatae* was smaller than *A. smyrnii* (Table 2) and *A. divaricatae* could be also easily differentiated from *A. chlamydosporifera* by the lack of chlamydospores in culture (Marin-Felix et al. 2019). Meanwhile, *A. glehniae* was distinguished from *A. divaricatae* by its single conidium on apex of conidiophore (there was no geniculate sporulation loci) and production of secondary conidium (Tao et al. 2019). In addition, *A. radicina* and *A. carotiincultae* were distinguished from present species by distant phylogenetic relationship in section *Radicina*.

***Alternaria vulgarae* L. He & J.X. Deng, sp. nov.**

Mycobank No: 838892

Figure 3

Type. China, Hubei Province, Yichang city, Badong county on infected leaves of *Foeniculum vulgare*. 19 July, 2016, J.X Deng, (YZU-H-0040, holotype), ex-type culture YZU 161234.

Etymology. In reference to the host species name, *vulgare*.

Description. Colonies on PDA (Fig. 3A) hazel in center and vinaceous buff at the edge, greenish black to mouse gray in reverse, surface velvety or floccose, 79–82 mm in diam.; On PCA, conidiophores straight or curved, 12–80 × 4–6 µm, 1–4 transverse septa; conidia solitary arising from the apex or near the apex of the conidiophores or terminal hyphae, rare from lateral of wire-like hyphae, ovoid, short-ovoid or ellipsoid, 25–50 (–70) × 16–27 µm, with 1–5 transverse septa and 1–4 longitudinal septa (Fig. 3B, C, F); On V8A, conidiophores 24–93 × 4–7 µm, 1–4 transverse septa, wire-like hyphae up to 200–400 × 4–6 µm; conidia short-ovoid, ovoid, ellipsoid or long-ellipsoid, 24–55 (–77) × 13–26 µm, 1–8 transverse septa, 1–4 longitudinal or oblique between septa (Fig. 3D, E, G). There was no secondary conidium production observed on PCA and V8A medium.

Additional isolate examined. China, Hubei Province, Yichang city, Badong county on infected leaves of *Foeniculum vulgare*. 19 July, 2016, L He, living culture YZU 161235.

Table 2. Morphological comparison of the present species and other *Alternaria* species in section *Radicina*

Species	Conidia			Conidia per chain	Medium
	Shape	Size (µm)	Septa		
<i>A. atrocaris</i>	Ovoid, ellipsoid	50–100×25–38	3–12	1–2	Host ^a
<i>A. divaricatae</i> sp. nov.	Short-ovoid, subglobose, ellipsoid	21–38×12–26 22–39×13–24	1–4 1–4	1	PCA^d V8A^d
<i>A. carotiincultae</i>	Long ovoid or ellipsoid	40–80×15–23	5–7 (–11)	1–3	PCA ^a
<i>A. chlamydsporifera</i>	ellipsoidal or ovoid, occasionally, subglobose	12–41×7–20	1–3 (–4)	1, occasionally 2	PCA ^b
<i>A. glehniae</i>	Long ovoid, ellipsoid	20–40 (–48)×10–20	3–7	1, occasionally 2	PCA ^c
<i>A. petroselini</i>	Short-ovoid to subsphaeroid	35–62 (–66)×20–26	6–8	1, rarely to 2	PCA ^a
<i>A. radicina</i>	Short-broad or long-narrow ellipsoid and ovoid	42–63×15–20	4–8	1, seldom up to 2	PCA ^a
<i>A. selini</i>	Short-ovoid	32–42 (–50)×22–27	3–5	1–3	PCA ^a
	Long-ellipsoid	48–65 (–50)×15–20	Up to 7		
<i>A. smyrnii</i>	Ovoid, obovoid	40–58×18–22	7–8 (–10)	1–2	PCA ^a
	Narrower ellipsoid	67–96×13–16			
<i>A. vulgarae</i> sp. nov.	Short-ovoid, ovoid or long-ellipsoid	25–50 (–70)×16–27 24–55 (–77)×13–26	1–5 1–8	1	PCA^d V8A^d

^a referenced from Simmons (2007); ^b referenced from Marin-Felix et al. (2019); ^c referenced from Tao et al. (2019); ^d determined in the present study.

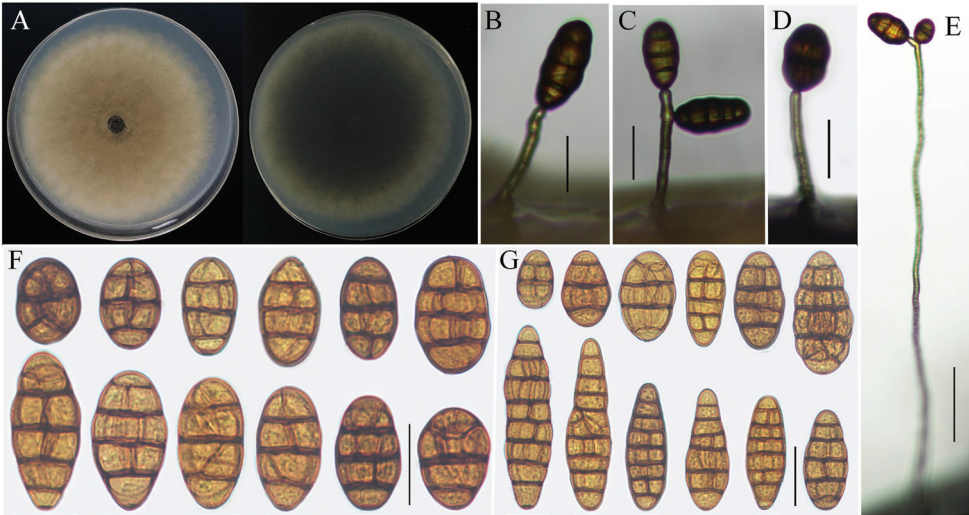


Figure 3. Morphological characteristics of *Alternaria vulgarae* (strain: YZU 161234). Colony on PDA for 7 days at 25 °C (**A**); Sporulation patterns on PCA and V8A (**B–E**; **B, C** from V8A **D, E** from PCA); Conidia from PCA and V8A (**F–G**). Scale bars: 25 µm (**B, C, D, F, G**); 50 µm (**E**).

Notes. Phylogenetic analysis based on combining four gene fragments indicated that *Alternaria vulgarae* fell in an individual branch in section *Radicina* of *Alternaria* and displayed a close relationship with *A. petroselini* and *A. selini* with high supported values (Fig. 1). Morphologically, *A. vulgarae* could be easily distinguished from *A. petroselini* and *A. selini* by their sporulation and length of conidiophores. Conidia of *A. petroselini* were solitary or cluster a small clump with 2–4 spores near the tips or lateral of conidiophores. Occasionally, the secondary conidium could be observed. Meanwhile, the single conidium or conidial chains (1–3) of *A. selini* grew from numerous lateral conidiophores, which produced from wire-like hyphae (Simmons 2007). Differently, conidia

of *A. vulgarae* were erected from apex of conidiophores or terminal hyphae. There were no small conidial clumps and secondary conidium formed (Fig. 3B, C, D, E). Moreover, the conidiophores of *A. vulgarae* ($12\text{--}80 \times 4\text{--}6\ \mu\text{m}$) was longer than *A. petroselini* ($30\text{--}60 \times 5\text{--}6.5\ \mu\text{m}$) and shorter than *A. selini* ($200\text{--}400 \times 4\text{--}6\ \mu\text{m}$) (Simmons 2007). Besides, *A. vulgarae* differed from *A. petroselini* in conidial shape. Conidium populations of *A. petroselini* were dominated by shot-ovoid to subsphaerical spores though, the shapes of *A. vulgarae* were mainly ovoid, ellipsoid or long-ellipsoid (Simmons 2007).

Discussion

Morphologically, *Alternaria radicina* species-group was one of the 10 subsections (A–I) and comprised 8 species described by Simmons (2007): *A. atrocariis*, *A. carotincultae*, *A. japonica*, *A. petroselini*, *A. radicina*, *A. selini*, *A. smyrnii* and *A. soliaridae*. With the development of molecular studies, the species-group was re-defined and the section *Radicina* was introduced and perfected (Pryor and Gilbertson 2000; Lawrence et al. 2013; Woudenberg et al. 2013). Uniformly, species in this section had some similar morphological characters, such as conidiophores, sporulation, conidial shape and etc. The phylogenetic analysis showed that only five species were clustered in section *Radicina*. Except for *A. atrocariis*, which had no published sequence data, the two other species were shown to belong to other sections: *A. japonica* felled in the section *Japonicae* and *A. soliaridae* formed a separate monophyletic lineage (Woudenberg et al. 2013). Recently, two more species *A. chlamydosporifera* and *A. glehniae* were reported in the section *Radicina* (Marin-Felix et al. 2019; Tao et al. 2019).

In the current study, two new *Alternaria* species belonged to the section *Radicina* based on morphological and phylogenetic analysis. *Alternaria divaricatae* was identified as a novel species based on unique morphological and well-supported phylogenetic analysis (Fig. 2 and Table 2). Phylogenetically, *A. vulgarae* clustered with *A. petroselini* and *A. selini*. Although its phylogenetic position was not well-supported, *A. vulgarae* can be distinguished from these two species in section *Radicina* by morphological characteristics (Table 2). Except the length of conidiophores, *A. vulgarae* was characterized by its sporulation. Meanwhile, *A. vulgarae* won't form secondary conidium (Table 2). These characters were important standards to identify *Alternaria* species (Simmons 2007). And, according to Jeewon and Hyde (2016), a fungal species can be defined based on the distinctive morphological characters even though the phylogenies were not well-supported, because the phylogeny cannot really reflect all morphologies.

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References

- Ahmadpour A (2019) *Alternaria caricicola*, a new species of *Alternaria* in the section *Nimbya* from Iran. *Phytotaxa* 405: 65–73. <https://doi.org/10.11646/phytotaxa.405.2.1>
- Berbee ML, Pirseyedi M, Hubbard S (1999) *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. *Mycologia* 91: 964–977. <https://doi.org/10.1080/00275514.1999.12061106>
- Bessadat N, Hamon B, Bataillesimoneau N, Mabrouk K, Simoneau P (2020) *Alternaria telliensis* sp. nov., a new species isolated from *Solanaceae* in Algeria. *Phytotaxa* 440(2). <https://doi.org/10.11646/phytotaxa.440.2.1>
- Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91: 553–556. <https://doi.org/10.1080/00275514.1999.12061051>
- Deng JX, Li MJ, Chandra Paul N, Oo MM, Lee HB, Oh SK, Yu SH (2018) *Alternaria brassicifolii* sp. nov. isolated from *Brassica rapa* subsp. *pekinensis* in Korea. *Mycobiology* 46: 172–176. <https://doi.org/10.1080/12298093.2018.1468054>
- Elliott JA (1917) Taxonomic characters of the genera *Alternaria* and *Macrosporium*. *American Journal of Botany* 4: 439–476. <https://doi.org/10.1002/j.1537-2197.1917.tb05467.x>
- Fries EM (1832) *Systema mycologicum* (Vol. 3). Lundae. 210 p.
- Ghafri AA, Maharachchikunbura SS, Hyde KD, Nadiya AAS, Abdullah MAS (2019) A new section and a new species of *Alternaria* encountered from Oman. *Phytotaxa* 405: 279–289. <https://doi.org/10.11646/phytotaxa.405.6.1>
- Hall TA (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- He L, Liu HF, Ge H, Pei DF, Deng JX (2020) *Alternaria vicatiae* sp. nov. (Ascomycota: Pleosporaceae) isolated from *Vicatia thibetica* in China. *Phytotaxa* 439(3), 255–264.
- Hong SG, Cramer RA, Lawrence CB, Pryor BM (2005) *Alt a 1* allergen homologs from *Alternaria* and related taxa: analysis of phylogenetic content and secondary structure. *Fungal Genetics and Biology* 42: 119–129. <https://doi.org/10.1016/j.fgb.2004.10.009>
- Jeewon R, Hyde KD (2016) Establishing species boundaries and new taxa among fungi: recommendations to resolve taxonomic ambiguities. *Mycosphere* 7(11): 1669–357. <https://doi.org/10.5943/mycosphere/7/11/4>
- Joly P (1964) Le Genre *Alternaria*. [Encyclopedie mycologique]. P. Lechevalier, Paris, 250 pp.
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Lawrence DP, Park MS, Pryor BM (2012) *Nimbya* and *Embellisia* revisited, with nov. comb. for *Alternaria celosiae* and *A. perpunctulata*. *Mycological Progress* 11: 799–815. <https://doi.org/10.1007/s11557-011-0793-7>
- Lawrence DP, Gannibal PB, Peever TL, Pryor BM (2013) The sections of *Alternaria*: formalizing species-group concepts. *Mycologia* 105: 530–546. <https://doi.org/10.3852/12-249>
- Lawrence DP, Rotondo F, Gannibal PB (2016) Biodiversity and taxonomy of the pleomorphic genus *Alternaria*. *Mycological Progress* 15: 1–22. <https://doi.org/10.1007/s11557-015-1144-x>

- Liu HF, Liao J, Chen XY, Liu QK, Deng JX (2019) A novel species and a new record of *Alternaria* isolated from two *Solanaceae* plants in china. *Mycological Progress* 18(8). <https://doi.org/10.1007/s11557-019-01504-3>
- Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* 16: 1799–1808. <https://doi.org/10.1093/oxfordjournals.molbev.a026092>
- Marin-Felix Y, Hernández-Restrepo M, Iturrieta-González I, Garcia D, Gene J, Groenewald JZ, Cai L, Chen Q, Quaedvlieg W, Schumacher RK, Taylor PWJ, Ambers C, Bonthond G, Edwards J, Krueger-Hadfield SA, Luangsa-ard JJ, Morton L, Moslemi A, Sandoval-Denis M, Tan YP, Thangavel R, Vaghefi N, Cheewangkoon R, Crous PW (2019) Genera of phytopathogenic fungi: GOPHY 3. *Studies in Mycology* 94: 1–124. <https://doi.org/10.1016/j.simyco.2019.05.001>
- Neergaard P (1945) Danish Species of *Alternaria* and *Stemphylium*. Oxford Univ. Press, London, 560 p.
- Park MS, Romanoski, CE Pryor BM (2008) A re-examination of the phylogenetic relationship between the causal agents of carrot black rot, *Alternaria radicina* and *A. carotiincultae*. *Mycologia* 100: 511–527. <https://doi.org/10.3852/07-186R1>
- Poursafar A, Ghosta Y, Orina AS, Gannibal PB, Javan-Nikkhah M, Lawrence DPF (2018) Taxonomic study on *Alternaria* sections of *Infectoriae* and *Pseudoalternaria* associated with black (sooty) head mold of wheat and barley in Iran. *Mycological Progress* 17: 343–356. <https://doi.org/10.1007/s11557-017-1358-1>
- Posada D, Crandall KA (1998) Model test: Testing the model of DNA substitution. *Bioinformatics* 14: 817–818. <https://doi.org/10.1093/bioinformatics/14.9.817>
- Pryor BM, Gilbertson RL (2000) Molecular phylogenetic relationships amongst *Alternaria* species and related fungi based upon analysis of nuclear ITS and mt SSU rDNA sequences. *Mycological Research* 104: 1312–1321. <https://doi.org/10.1017/S0953756200003002>
- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Simmons EG (1967) Typification of *Alternaria*, *Stemphylium* and *Ulocladium*. *Mycologia* 59: 67–92. <https://doi.org/10.2307/3756943>
- Simmons EG (1992) *Alternaria* taxonomy: current status, viewpoint, challenge. In: Chelkowski J, Visconti A (Eds) *Alternaria* biology, plant diseases and metabolites. Elsevier Science Publishers, Amsterdam, 35 pp.
- Simmons EG, Roberts RG (1993) *Alternaria* themes and variations (73). *Mycotaxon* 48: 109–140.
- Simmons EG (2007) *Alternaria*: An identification manual, CBS Biodiversity Series 6. Centraalbureau voor Schimmelcultures, Utrecht.
- Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690. <https://doi.org/10.1093/bioinformatics/btl446>
- Swofford DL (2002) PAUP, Phylogenetic analysis using parsimony (and other methods). Version 4.0b10. Sinauer Associates, Sunderland.

- Tao YQ, Jia GG, Aung SLL, Wu QL, Lu HX, Deng JX (2019) Multigene phylogeny and morphology of *Alternaria* reveal a novel species and a new record in china. *Phytotaxa* 397(2): 169–176. <https://doi.org/10.11646/phytotaxa.397.2.4>
- Watanabe M, Lee K, Goto K, Kumagai S, Sugita-Konishi Y, Hara-Kudo Y (2010) Rapid and effective DNA extraction method with bead grinding for a large amount of fungal DNA. *Journal of Food Protection* 73(6): 1077–1084. <https://doi.org/10.4315/0362-028X-73.6.1077>
- White TJ, Bruns T, Lee S (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky, JJ (Eds) *PCR Protocols: A Guide to Methods and Applications*. Academic Press Inc, New York, 315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Wiltshire SP (1933) The foundation species of *alternaria* and *macrosporium*. *Transactions of the British Mycological Society* 18: IN1–160. [https://doi.org/10.1016/S0007-1536\(33\)80003-9](https://doi.org/10.1016/S0007-1536(33)80003-9)
- Woudenberg JHC, Groenewald JZ, Binder M, Crous PW (2013) *Alternaria* redefined. *Studies in Mycology* 75: 171–212. <https://doi.org/10.3114/sim0015>
- Woudenberg JHC, Truter M, Groenewald JZ, Crous PW (2014) Large-spored *Alternaria* pathogens in section *Porri* disentangled. *Studies in Mycology* 79: 1–47. <https://doi.org/10.1016/j.simyco.2014.07.003>
- Woudenberg JHC, Seidl MF, Groenewald JZ, De Vries M, Stielow JB, Thomma BP HJ, Crous PW (2015) *Alternaria* section *Alternaria*: Species, formae speciales or pathotypes? *Studies in Mycology* 82: 1–21. <https://doi.org/10.1016/j.simyco.2015.07.001>