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Systematics and evolution of the genus *Torrubiella* (Hypocreales, Ascomycota)

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ARTICLE INFO

Article history:

Received 28 January 2008

Received in revised form

23 June 2008

Accepted 12 September 2008

Published online 4 October 2008

Corresponding Editor: Rajesh Jeewon

Keywords:

Conoideocrella

Gibellula

Orbiocrella

Phylogeny

Scale insect pathogens

Spider

Torrubiella

ABSTRACT

Torrubiella is a genus of arthropod-pathogenic fungi that primarily attacks spiders and scale insects. Based on the morphology of the perithecia, asci, and ascospores, it is classified in *Clavicipitaceae* s. lat. (*Hypocreales*), and is considered a close relative of *Cordyceps* s. 1., which was recently reclassified into three families (*Clavicipitaceae* s. str., *Cordycipitaceae*, *Ophiocordycipitaceae*) and four genera (*Cordyceps* s. str., *Elaphocordyceps*, *Metacordyceps*, and *Ophiocordyceps*). *Torrubiella* is distinguished morphologically from *Cordyceps* s. lat. mainly by the production of superficial perithecia and the absence of a well-developed stipitate stroma. To test and refine evolutionary hypotheses regarding the placement of *Torrubiella* and its relationship to *Cordyceps* s. lat., a multi-gene phylogeny was constructed by conducting ML and Bayesian analyses. The monophyly of *Torrubiella* was rejected by these analyses with species of the genus present in *Clavicipitaceae*, *Cordycipitaceae*, and *Ophiocordycipitaceae*, and often intermixed among species of *Cordyceps* s. lat. The morphological characters traditionally used to define the genus are, therefore, not phylogenetically informative, with the stipitate stromata being gained and/or lost several times among clavicipitaceous fungi. Two new genera (*Conoideocrella*, *Orbiocrella*) are proposed to accommodate two separate lineages of torrubielloid fungi in the *Clavicipitaceae* s. str. In addition, one species is reclassified in *Cordyceps* s. str. and three are reclassified in *Ophiocordyceps*. The phylogenetic importance of anamorphic genera, host affiliation, and stipitate stromata is discussed.

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Introduction

The genus *Torrubiella* Boud. (1885) is classified as a member of *Clavicipitaceae* s. lat. (*Ascomycota*: *Hypocreales*), a group of perithecial ascomycetes well known for their obligate symbioses with animals, plants, and other fungi, as well as for their role in human history (White *et al.* 2003). Most species of the genus, like other members of *Clavicipitaceae* s. lat., possess

cylindrical asci, thickened ascus apices, and filiform ascospores, which in many species disarticulate into part-spores (Kobayasi & Shimizu 1982; Rogerson 1970; Sung *et al.* 2007). *Torrubiella* is characterized as an arthropod pathogen that produces superficial perithecia on a loose mat of hyphae (subiculum) or a highly reduced non-stipitate stroma. It has been hypothesized to be closely related to *Cordyceps* s. lat. based on similarities in perithecia, ascus, and ascospore

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doi:10.1016/j.mycres.2008.09.008

morphology, as well as its life style as a pathogen of arthropods (Kobayasi 1982; Mains 1949; Petch 1923). The type of *Torrubiella* is *T. aranicida* Boud. 1885, an infrequently collected and poorly known pathogen of spiders. Currently *Torrubiella* contains approximately 80 species (Kobayasi & Shimizu 1982; Index Fungorum: <http://www.speciesfungorum.org/Index.htm>).

Nine anamorphic genera have been linked to *Torrubiella* species (Hodge 2003). These include *Gibellula*, *Granulomanus*, *Akanthomyces*, *Paecilomyces*, *Hirsutella*, *Pseudogibellula*, *Lecanicillium*, *Simplicillium*, and *Verticillium*. The anamorphic genera *Gibellula*, *Granulomanus*, and *Pseudogibellula* are known to associate exclusively with *Torrubiella*, while the remaining genera are also potential or confirmed anamorphs of *Cordyceps* s. lat. (Hodge 2003). The taxonomic utility of clavicipitaceous anamorphs varies according to genus (Sung et al. 2007). Some anamorphic genera (e.g. *Aschersonia*, *Beauveria*, *Hymenostilbe*, *Metarhizium*) are restricted in their phylogenetic distribution and are phylogenetically informative in characterizing species of *Cordyceps* s. lat. (Sung et al. 2007). In contrast, other anamorphic forms, such as *Paecilomyces* s. lat. and *Verticillium* s. lat., are morphologically and phylogenetically diverse and previous molecular studies have shown them to be polyphyletic (Zare & Gams 2001; Luangsa-ard et al. 2005; Sung et al. 2007).

Torrubiella species infect a wide range of arthropods, but are primarily pathogens of spiders (Arachnida) and scale insects (Hemiptera) (Kobayasi & Shimizu 1982). Most species are restricted to a particular host order or family, but hosts are often difficult to identify to genus or species level, so the degree to which these fungi are host specific is poorly known (Hywel-Jones 1993, 1997a; Kobayasi & Shimizu 1982). In addition, host specificity of the anamorphic genera varies from narrow (e.g. *Gibellula* restricted to spiders) to broad (e.g. *Paecilomyces*, *Akanthomyces* on at least six orders of Arthropoda). Spiders, especially species of jumping spiders (Salticidae), represent a major host group of *Torrubiella* (Kobayasi & Shimizu 1982), but surprisingly few other teleomorphic taxa of Clavicipitaceae s. lat. parasitize spiders. For example, only ten to 15 species of *Cordyceps* s. lat. are reported from spider hosts (Kobayasi & Shimizu 1977; Mains 1958). *Torrubiella* is also confirmed to parasitize two families of scale insects, the soft scales (Coccidae) and the armoured scales (Diaspididae) (Evans & Prior 1990; Hywel-Jones 1993, 1997a). Many species accounts though, specify only 'scale insect' or the author had a very broad interpretation of 'coccid', which could also include white flies (Aleyrodidae) (Evans & Hywel-Jones 1997). Little reference is made of which life stage or sex of scale insects is parasitized by *Torrubiella*, but based on host life histories, it is assumed that the adult females are the primary hosts, as they are stationary and gregarious (Evans & Hywel-Jones 1997). Finally, in many cases (e.g. *T. tenuis*) the host is almost completely destroyed and definitive identification beyond 'scale insect' is not possible (Hywel-Jones 1993).

Multi-gene phylogenetic analysis of *Hypocreales* demonstrated that neither the Clavicipitaceae nor *Cordyceps* represented monophyletic taxa (Sung et al. 2007). Based on these results, Clavicipitaceae s. lat. was reclassified into three families (Clavicipitaceae s. str., Cordycipitaceae, and Ophiocordycipitaceae) and *Cordyceps* s. lat. was reclassified into four genera (*Cordyceps* s. str., *Elaphocordyceps*, *Metacordyceps*, and *Ophiocordyceps*) (Sung et al. 2007). Importantly, *Torrubiella* was hypothesized

to be closely related to some species of *Cordyceps* s. str. (e.g. *C. tuberculata*), which produce superficial perithecia on reduced pallid stromata (Mains 1958). Although previous phylogenetic studies have shown that *Torrubiella* is not monophyletic and it integrates at multiple points within *Cordyceps* s. lat. (Artjariyasripong et al. 2001; Sung et al. 2007), these studies did not sample a large number of *Torrubiella* species and did not attempt to address the phylogenetic diversity of the genus. Thus, the phylogenetic structure of the genus *Torrubiella* and its anamorphs remain unclear and species of the genus have not been integrated into the phylogenetic classification of *Cordyceps* and related fungi. In our study, we increased the sampling of *Torrubiella* species and associated anamorphic taxa for a more robust multi-gene phylogeny to test the monophyly of *Torrubiella* and its relationship with *Cordyceps* s. lat. In addition, the phylogenetic distribution and taxonomic utility of anamorphs and host affiliation were explored.

Material and methods

Taxon sampling

To understand the phylogenetic placement of species of *Torrubiella* (Fig 1), 29 isolates of *Torrubiella* and associated torrubielloid taxa (e.g. *Cordyceps confragosa*, *C. cf. coccidioperitheciata*, *C. tuberculata*) and anamorphs (e.g. *Akanthomyces*, *Gibellula*) were sampled. These taxa were appended to a dataset representing the taxonomic diversity of the *Hypocreales* based on the classification of Sung et al. (2007), so that a total of 101 taxa were included in a final dataset. Voucher and host information for each taxon are listed in Table 1. *Glomerella cingulata* and *Verticillium dahliae* (Glomeraceae) were used as outgroups as in the previous phylogenetic analyses (Sung et al. 2007; Zhang et al. 2006).

DNA isolation, PCR amplification, and sequencing

Total genomic DNA was extracted from specimens and cultures grown in malt-extract liquid media following the CTAB protocol (Doyle & Doyle 1990). Five nuclear gene regions were amplified and sequenced in order to confidently place the species of *Torrubiella* in the phylogeny of clavicipitaceous fungi reported in the study of Sung et al. (2007). Regions sequenced were from nu-rSSU and nu-rLSU, elongation factor 1 α (*TEF*), and the largest and second largest subunits of RNA polymerase II (*RPB1* and *RPB2*), totalling approximately 5000 base pairs of data.

The PCR amplification of nu-rSSU, nu-rLSU, and *TEF* was performed in a BIORAD iCycler thermocycler (BIORAD, Hercules, CA) programmed as follows: 94 °C for 2 min; 4 cycles of 94 °C for 30 s, 55 °C for 1 min, and 72 °C for 2 min; 35 cycles of 94 °C for 30 s, 50.5 °C for 1 min, and 72 °C for 2 min; 72 °C for 3 min. All primers used in PCR and sequencing are listed in the study of Sung et al. (2007). The nu-rSSU was amplified with the primer pairs NS1 and NS4. All taxa were sequenced using the primers NS1, NS3, SR7, and NS4. The nu-rLSU was amplified and sequenced with the primers LR0R and LR5. The *tef* was amplified and sequenced with the primers 983F and 2218R. The PCR amplification of *RPB1* and *RPB2* was performed in a BIORAD iCycler thermocycler programmed as

follows: 94 °C for 2 min; 4 cycles of 94 °C for 30 s, 47 °C for 1 min, and 72 °C for 2 min; 35 cycles of 94 °C for 30 s, 47 °C for 1 min, and 72 °C for 2 min; 72 °C for 3 min. *RPB1* was amplified and sequenced using the primers *CRPB1A* and *RPB1CR*. For the amplification of *RPB2*, primer pairs *fRPB2-5F* and *fRPB2-7cR* or *RPB2F1*, and *RPB2R2* were used. Sequencing was performed using the same primers as amplification.

All PCR products were purified using GeneClean III purification kits (Qbiogene, Irvine, CA) according to the manufacturer's instructions and eluted in 10 µl TE. Sequencing reactions were conducted on a BIORAD iCycler thermocycler using ABI Big-Dye Ready-reaction kit (Applied Biosystems, Foster City, CA) following manufacturer's instructions; sequencing reactions were run on an ABI 3700.

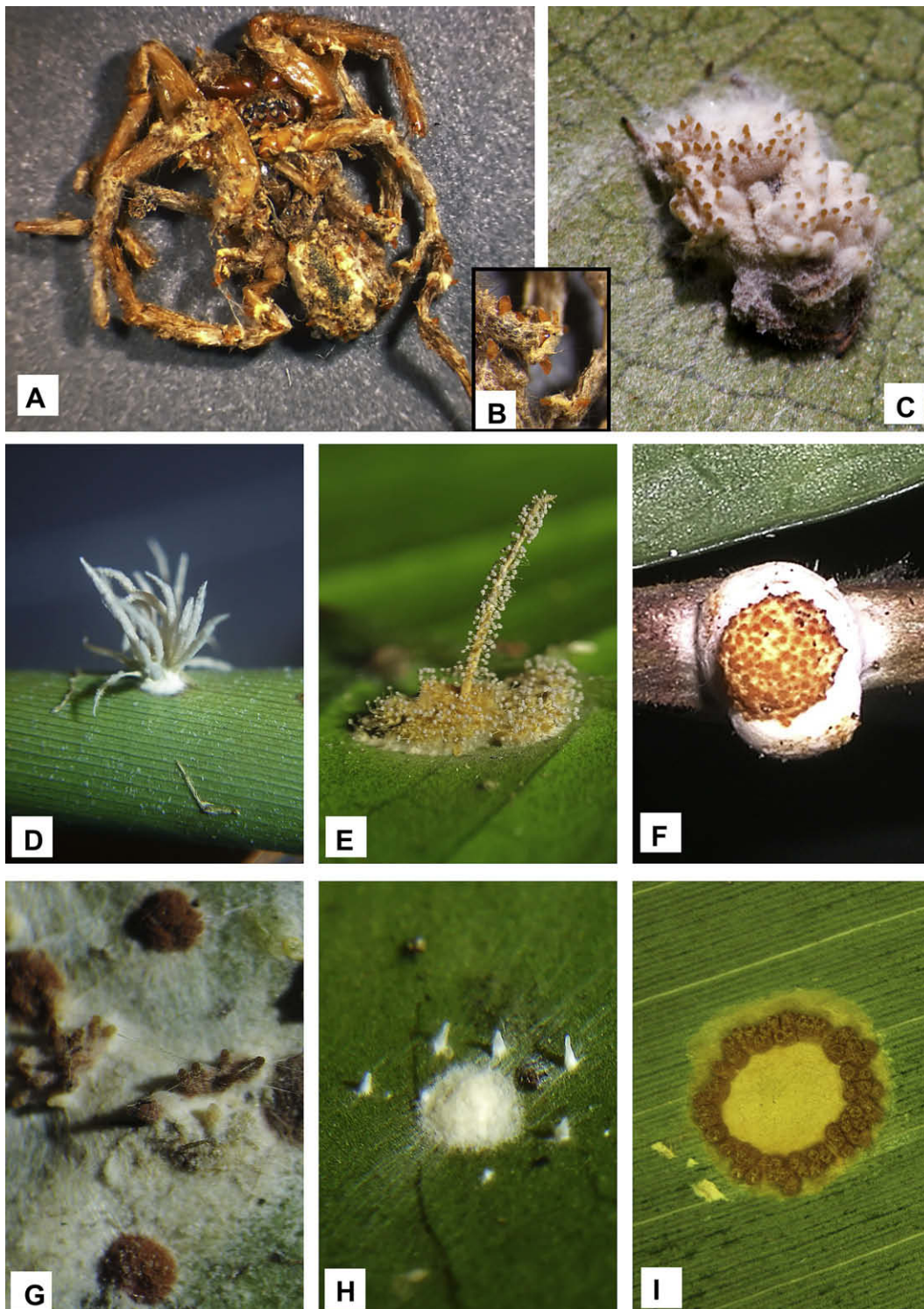


Fig 1 – Representative torrubielloid taxa. (A–B) *Torrubiella aranicida*. (C) *Torrubiella* sp. (D) *Akanthomyces novoguineensis*. (E) *Gibellula* cf. *pulchra*. (F) *T. piperis*. (G) *T. luteorostrata*. (H) *T. tenuis*. (I) *T. petchii*.

Table 1 – The list of cultures and specimens used in this study

Taxon	Specimen voucher ^a	Host/substratum	GenBank accession nos				
			nrSSU	nrLSU	TEF	RBP1	RBP2
<i>Akanthomyces arachnophilus</i>	NHJ 10469	Spider (Arachnida)	EU369090	EU369031	EU369008	EU369047	
<i>A. cinereus</i>	NHJ 3510	Spider (Arachnida)	EU369091		EU369009	EU369048	EU369070
<i>A. novoguineensis</i>	NHJ 13117	Spider (Arachnida)	EU369092		EU369010	EU369049	EU369073
<i>A. novoguineensis</i>	NHJ 11923	Spider (Arachnida)	EU369095	EU369032	EU369013	EU369052	EU369072
<i>A. novoguineensis</i>	NHJ 4314	Spider (Arachnida)	EU369094		EU369012	EU369051	EU369071
<i>A. novoguineensis</i>	NHJ 13161	Spider (Arachnida)	EU369093		EU369011	EU369050	
<i>Aschersonia badia</i>	BCC 8105	Scale insect (Hemiptera)	DQ522573	DQ518752	DQ522317	DQ522363	DQ522411
<i>A. placenta</i>	BCC 7869	Scale insect (Hemiptera)	EF469121	EF469074	EF469056	EF469085	EF469104
<i>Balansia henningsiana</i>	GAM 16112	<i>Panicum</i> sp. (Poaceae)	AY545723	AY545727	AY489610	AY489643	DQ522413
<i>B. pilulaeformis</i>	AEG 94-2	Poaceae	AF543764	AF543788	DQ522319	DQ522365	DQ52241
<i>Bionectria ochroleuca</i>	CBS 114056	On bark	AY489684	AY489716	AY489611	DQ842031	DQ522415
<i>Claviceps fusiformis</i>	ATCC 26019	Poaceae	DQ522539	U17402	DQ522320	DQ522366	
<i>C. paspali</i>	ATCC 13892	Poaceae	U32401	U47826	DQ522321	DQ522367	DQ522416
<i>C. purpurea</i>	GAM 12885	<i>Dactylis glomerata</i> (Poaceae)	AF543765	AF543789	AF543778	AY489648	DQ522417
<i>Cordyceps cardinalis</i>	OSC 93609	Lepidopteran larva	AY184973	AY184962	DQ522325	DQ522370	DQ522422
<i>C. cf. coccidioperitheciata</i>	NHJ 7859	Spider (Arachnida)	EU369107			EU369064	EU369085
<i>C. cf. coccidioperitheciata</i>	NHJ 5112	Spider (Arachnida)	EU369109	EU369043	EU369026	EU369066	
<i>C. gunnii</i>	OSC 76404	Lepidopteran larva	AF339572	AF339522	AY489616	AY489650	DQ522426
<i>C. militaris</i>	OSC 93623	Lepidopteran pupa	AY184977	AY184966	DQ522332	DQ522377	AY545732
<i>C. scarabaeicola</i>	ARSEF 5689	Scarabaeid adult (Coleoptera)	AF339574	AF339524	DQ522335	DQ522380	DQ522431
<i>C. tuberculata</i>	OSC 111002	Lepidoptera	DQ522553	DQ518767	DQ522338	DQ522384	DQ522435
<i>Cosmospora coccinea</i>	CBS 114050	<i>Inonotus nodulosus</i> (Hymenomyces)	AY489702	AY489734	AY489629	AY489667	DQ522438
<i>Elaphocordyceps capitata</i>	OSC 71233	<i>Elaphomyces</i> sp. (Euscomycetes)	AY489689	AY489721	AY489615	AY489649	DQ522421
<i>E. fracta</i>	OSC 110990	<i>Elaphomyces</i> sp. (Euscomycetes)	DQ522545	DQ518759	DQ522328	DQ522373	DQ522425
<i>E. ophioglossoides</i>	OSC 106405	<i>Elaphomyces</i> sp. (Euscomycetes)	AY489691	AY489723	AY489618	AY489652	DQ522429
<i>Engyodontium aranearum</i>	CBS 309.85	Spider (Arachnida)	AF339576	AF339526	DQ522341	DQ522387	DQ522439
<i>Epichloë typhina</i>	ATCC 56429	<i>Festuca rubra</i> (Poaceae)	U32405	U17396	AF543777	AY489653	DQ522440
<i>Gibellula</i> cf. <i>alba</i>	NHJ 11679	Spider (Arachnida)			EU369016	EU369054	
<i>G. longispora</i>	NHJ 12014	Spider (Arachnida)	EU369098		EU369017	EU369055	EU369075
<i>G. pulchra</i>	NHJ 10808	Spider (Arachnida)	EU369099	EU369035	EU369018	EU369056	EU369076
<i>Gibellula</i> sp.	NHJ 5401	Spider (Arachnida)	EU369102			EU369059	EU369079
<i>Gibellula</i> sp.	NHJ 10788	Spider (Arachnida)	EU369101	EU369036	EU369019	EU369058	EU369078
<i>Gibellula</i> sp.	NHJ 13158	Spider (Arachnida)	EU369100	EU369037	EU369020	EU369057	EU369077
<i>Glomerella cingulata</i>	CBS 114054	<i>Fragaria</i> sp. (Rosaceae)	AF543762	AF543786	AF543773	AY489659	DQ522441
<i>Haptocillium balanoides</i>	CBS 250.82	Nematode	AF339588	AF339539	DQ522342	DQ522388	DQ522442
<i>H. sinense</i>	CBS 567.95	Nematode	AF339594	AF339545	DQ522343	DQ522389	DQ522443
<i>Hirsutella</i> sp.	OSC 128575	Hemipteran adult	EF469126	EF469079	EF469064	EF469093	EF469110
<i>Hydropisphaera erubescens</i>	ATCC 36093	<i>Cordyline banksii</i> (Laxmanniaceae)	AY545722	AY545726	DQ522344	DQ522390	AY545731
<i>H. peziza</i>	CBS 102038	On bark	AY489698	AY489730	AY489625	AY489661	DQ522444
<i>Hypocrea lutea</i>	ATCC 208838	On decorticated conifer wood	AF543768	AF543791	AF543781	AY489662	DQ522446
<i>Hypocrella schizostachyi</i>	BCC 14123	Scale insect (Hemiptera)	DQ522557	DQ518771	DQ522346	DQ522392	DQ522447
<i>Hypocrella</i> sp.	GJS 89-104	Scale insect (Hemiptera)	U32409	U47832	DQ522347	DQ522393	DQ522448
<i>Hypomyces polyporinus</i>	ATCC 76479	<i>Trametes versicolor</i> (Hymenomyces)	AF543771	AF543793	AF543784	AY489663	
<i>Isaria farinosa</i>	OSC 111005	Lepidopteran pupa	DQ522558	DQ518772	DQ522348	DQ522394	
<i>I. takamizusanensis</i>	NHJ 3582	Stink bug (Hemiptera)	EU369097	EU369034	EU369015		
<i>I. takamizusanensis</i>	NHJ 3497	Stink bug (Hemiptera)	EU369096	EU369033	EU369014	EU369053	EU369074
<i>I. tenuipes</i>	OSC 111007	Lepidopteran pupa	DQ522559	DQ518773	DQ522349	DQ522395	DQ522449
<i>Lecanicillium antillanum</i>	CBS 350.85	Agaric (Hymenomyces)	AF339585	AF339536	DQ522350	DQ522396	DQ522450
<i>L. psalliotae</i>	CBS 101270	Soil	EF469128	EF469081	EF469066	EF469095	EF469113
<i>L. psalliotae</i>	CBS 532.81	Soil	AF339609	AF339560	EF469067	EF469096	EF469112
<i>Mariannaea pruinosa</i>	ARSEF 5413	<i>Iragoides fasciata</i> (Lepidoptera)	AY184979	AY184968	DQ522351	DQ522397	DQ522451
<i>Metacordyceps chlamydosporia</i>	CBS 101244	Egg of slug (Diplopoda)	DQ522544	DQ518758	DQ522327	DQ522372	DQ522424
<i>M. taii</i>	ARSEF 5714	Lepidoptera	AF543763	AF543787	AF543775	DQ522383	DQ522434
<i>Metarhizium album</i>	ARSEF 2082	<i>Cofana spectra</i> (Hemiptera)	DQ522560	DQ518775	DQ522352	DQ522398	DQ522452

Table 1 – (continued)

Taxon	Specimen voucher ^a	Host/substratum	GenBank accession nos				
			nrSSU	nrLSU	TEF	RPB1	RPB2
<i>M. anisopliae</i>	ARSEF 3145	<i>Oryctes rhinoceros</i> (Coleoptera)	AF339579	AF339530	AF543774	DQ522399	DQ522453
<i>M. flavoviride</i>	ARSEF 2037	<i>Nilaparvata lugens</i> (Hemiptera)	AF339580	AF339531	DQ522353	DQ522400	DQ522454
<i>Myriogenospora atramentosa</i>	AEG 96-32	<i>Andropogon virginicus</i> (Poaceae)	AY489701	AY489733	AY489628	AY489665	DQ522455
<i>Nectria cinnabarina</i>	CBS 114055	<i>Betula</i> sp. (Betulaceae)	U32412	U00748	AF543785	AY489666	DQ522456
<i>Nomuraea atypicola</i>	CBS 744.73	Spider (Arachnida)	EF468987	EF468841	EF468786	EF468892	
<i>Ophiocordyceps</i> cf. <i>acicularis</i>	OSC 128580	Coleoptera	DQ522543	DQ518757	DQ522326	DQ522371	DQ522423
<i>O. agriotidis</i>	ARSEF 5692	Coleoptera	DQ522540	DQ518754	DQ522322	DQ522368	DQ522418
<i>O. aphodii</i>	ARSEF 5498	<i>Aphodius hewitti</i> (Coleoptera)	DQ522541	DQ518755	DQ522323		DQ522419
<i>O. brunneipunctata</i>	OSC 128576	Coleoptera	DQ522542	DQ518756	DQ522324	DQ522369	DQ522420
<i>O. heteropoda</i>	OSC 106404	Nymph of cicada (Hemiptera)	AY489690	AY489722	AY489617	AY489651	
<i>O. melolonthae</i>	OSC 110993	Scarabaeid larva (Coleoptera)	DQ522548	DQ518762	DQ522331	DQ522376	
<i>O. ravenelii</i>	OSC 110995	Coleopteran larva	DQ522550	DQ518764	DQ522334	DQ522379	DQ522430
<i>O. stylophora</i>	OSC 111000	Elaterid larva (Coleoptera)	DQ522552	DQ518766	DQ522337	DQ522382	DQ522433
<i>O. unilateralis</i>	OSC 128574	Ant (Hymenoptera)	DQ522554	DQ518768	DQ522339	DQ522385	DQ522436
<i>O. variabilis</i>	ARSEF 5365	Dipteran larva	DQ522555	DQ518769	DQ522340	DQ522386	DQ522437
<i>Ophionectria trichospora</i>	CBS 109876	On liana	AF543766	AF543790	AF543779	AY489669	DQ522457
<i>Paecilomyces lilacinus</i>	CBS 284.36	Soil	AY624189	AY624227	EF468792		EF468941
<i>P. lilacinus</i>	CBS 431.87	<i>Meloidogyne</i> sp. (Nematoda)	AY624188	EF468844	EF468791	EF468897	EF468940
<i>Pochonia gonioides</i>	CBS 891.72	Nematode	AF339599	AF339550	DQ522354	DQ522401	DQ522458
<i>Pseudonectria rousseleana</i>	CBS 114049	<i>Buxus sempervirens</i> (Buxaceae)	AF543767	U17416	AF543780	AY489670	DQ522459
<i>Regiocrella camerunensis</i>	ARSEF 7682	Scale insect (Hemiptera)		DQ118735	DQ118743	DQ127234	
<i>Rotiferophthora angustispora</i>	CBS 101437	Rotifer (Rotifera)	AF339584	AF339535	AF543776	DQ522402	DQ522460
<i>Roumegueriella rufula</i>	CBS 346.85	<i>Globodera rostochiensis</i> (Nematoda)	DQ522561	DQ518776	DQ522355	DQ522403	DQ522461
<i>Shimizuomyces paradoxus</i>	EFCC 6279	<i>Smilax sieboldi</i> (Smilacaceae)	EF469131	EF469084	EF469071	EF469100	EF469117
<i>Simplicillium lamellicola</i>	CBS 116.25	<i>Agaricus bisporus</i> (Hymenomycetes)	AF339601	AF339552	DQ522356	DQ522404	DQ522462
<i>S. lanosoniveum</i>	CBS 704.86	<i>Hemileia vastatrix</i> (Urediales)	AF339602	AF339553	DQ522358		DQ522464
<i>S. lanosoniveum</i>	CBS 101267	<i>Hemileia vastatrix</i> (Urediales)	AF339603	AF339554	DQ522357	DQ522405	DQ522463
<i>Sphaerostilbella berkeleyana</i>	CBS 102308	Polypore (Hymenomycetes)	AF543770	U00756	AF543783	AY489671	DQ522465
<i>Torrubiella confragosa</i>	CBS 101247	<i>Coccus viridis</i> (Hemiptera)	AF339604	AF339555	DQ522359	DQ522407	DQ522466
<i>T. luteoestrata</i>	NHJ 12516	Scale insect (Hemiptera)	EF468994	EF468849	EF468800		EF468946
<i>T. luteoestrata</i>	NHJ 11343	Scale insect (Hemiptera)	EF468995	EF468850	EF468801	EF468906	
<i>T. petchii</i>	NHJ 6240	Scale insect (Hemiptera)	EU369103	EU369038	EU369022	EU369060	EU369082
<i>T. petchii</i>	NHJ 6209	Scale insect (Hemiptera)	EU369104	EU369039	EU369023	EU369061	EU369081
<i>T. petchii</i>	NHJ 5318	Scale insect (Hemiptera)	EU369105	EU369040	EU369021	EU369062	EU369080
<i>T. piperis</i>	CBS 116719	Scale insect (Hemiptera)		AY466442	DQ118749	DQ127240	EU369083
<i>T. pruinosa</i>	NHJ 12994	Scale insect (Hemiptera)	EU369106	EU369041	EU369024	EU369063	EU369084
<i>T. ratticaudata</i>	ARSEF 1915	Spider (Arachnida)	DQ522562	DQ518777	DQ522360	DQ522408	DQ522467
<i>Torrubiella</i> sp.	NHJ 6709	Spider (Arachnida)	EU369110	EU369042	EU369025	EU369067	EU369086
<i>Torrubiella</i> sp.	DJ 29	Spider (Arachnida)	EU369108		EU369027	EU369065	
<i>T. tenuis</i>	NHJ 6791	Scale insect (Hemiptera)	EU369113	EU369046	EU369028	EU369069	EU369089
<i>T. tenuis</i>	NHJ 6293	Scale insect (Hemiptera)	EU369112	EU369044	EU369029	EU369068	EU369087
<i>T. tenuis</i>	NHJ 345.01	Scale insect (Hemiptera)	EU369111	EU369045	EU369030		EU369088
<i>T. wallacei</i>	CBS 101237	Lepidoptera	AY184978	AY184967	EF469073	EF469102	EF469119
<i>Verticillium dahliae</i>	ATCC 16535	<i>Crataegus crus-galli</i> (Rosaceae)	AY489705	AY489737	AY489632	AY489673	DQ522468
<i>V. epiphytum</i>	CBS 384.81	<i>Hemileia vastatrix</i> (Uredinales)	AF339596	AF339547	DQ522361	DQ522409	DQ522469
<i>V. incurvum</i>	CBS 460.88	<i>Ganoderma lipsiense</i> (Hymenomycetes)	AF339600	AF339551	DQ522362	DQ522410	DQ522470
<i>Viridispora diparietispora</i>	CBS 102797	<i>Crataegus crus-galli</i> (Rosaceae)	AY489703	AY489735	AY489630	AY489668	DQ522471

a AEG, A. E. Glenn personal collection; ARSEF, USDA-ARS Collection of Entomopathogenic Fungal cultures, Ithaca, NY; ATCC, American Type Culture Collection, Manassas, VA; BCC, BIOTEC Culture Collection, Khlong Luang, Thailand; CBS, Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; DJ, D. Johnson personal collection; EFCC, Entomopathogenic Fungal Culture Collection, Chuncheon, Korea; GAM, Julian H. Miller Mycological Herbarium Athens, GA; GJS, G. J. Samuels personal collection; NHJ, Nigel Hywel-Jones personal collection; OSC, Oregon State University Herbarium, Corvallis, OR.

Phylogenetic analyses

Sequences were edited using CodonCode, version 1.4.4 (Dedham, MA). Edited sequences were manually aligned in BioEdit Sequence Alignment Editor, version 7.0.5.2 (Hall 1999). Ambiguously aligned regions were excluded from phylogenetic analyses and gaps were treated as missing data. ML analyses were performed using RAxML-VI-HPC v2.0 using a GTR-GAMMA model of evolution (Stamatakis 2006). The multigene dataset consisted of 11 partitions, which were defined as nu-rSSU, nu-rLSU, and nine codon positions of the three protein-coding genes (*TEF*, *RPB1*, and *RPB2*). A total of 100 multiple runs were conducted and the tree with the best likelihood was selected as an optimal tree given with the combined alignment. Non-parametric BS analyses (Felsenstein 1985) were performed to provide nodal supports with 200 BS replicates. Bayesian analyses were conducted using a parallelized version of MRBAYES v3.1.2 (Ronquist & Huelsenbeck 2003) over four 2X AMD Opteron 285 Dual Cores processors. Bayesian analyses were conducted with a general time reversible (GTR) model of DNA substitution with gamma-distributed rate variation across invariant sites; independent models were applied for each of the 11 partitions (nu-rSSU, nu-rLSU, and nine codon positions of the three protein-coding genes). Bayesian analyses were performed for a total of 5×10^6 generations; analyses were initiated from a random tree and sampled trees every 100th generation. In order to verify the stationarity phase of likelihood values, two additional runs with 5×10^6 generations were conducted.

Results

Sequence alignment and phylogenetic analyses

The combined dataset with five genes and 101 taxa included 4907 characters (nu-rSSU: 1103, nu-rLSU: 945, *TEF*: 1020, *RPB1*: 791, *RPB2*: 1048). After ambiguously aligned sites were excluded, the dataset comprised 4640 characters (nu-rSSU: 1073, nu-rLSU: 829, *TEF*: 999, *RPB1*: 694, *RPB2*: 1045), including 1771 parsimony informative characters (nu-rSSU: 187, nu-rLSU: 217, *TEF*: 1st 83, 2nd 45, 3rd 267, *RPB1*: 1st 112, 2nd 77, 3rd 226, *RPB2*: 1st 144, 2nd 77, 3rd 336). ML analyses of the dataset resulted in a tree of -78070.62 log-likelihood. ML tree with BS values is presented in Fig 2. Comparison of likelihood values from the Bayesian analyses revealed that all three analyses converged on a stationary phase and the three 50% majority rule consensus trees were topologically identical (data not shown). The PPs from one of the Bayesian analyses were calculated and were provided as supplementary measures of nodal support on the RAxML tree (Fig 2). Nodes were considered strongly supported when supported by both BS proportions (BP: equal to or greater than 70%) and PPs (equal to or greater than 0.95).

Phylogenetic relationships of *Torrubiella*

ML analyses of the five-gene dataset inferred three well-supported, monophyletic clades of clavicipitaceous fungi to

representative three families (*Clavicipitaceae*, *Cordycipitaceae*, and *Ophiocordycipitaceae*; Fig 1). Addition of new taxa presented here maintained the structure and support found by Sung et al. (2007) and provided additional BP and PPs support for the families *Clavicipitaceae* (BP = 100, PP = 1), *Cordycipitaceae* (BP = 100, PP = 1), and *Ophiocordycipitaceae* (BP = 100, PP = 1). Species of *Torrubiella* were found in all three families and were members of the clades or lineages designated as A–E (Fig 2).

Cordycipitaceae included species of *Torrubiella* fungi or their anamorphs, within two well-supported clades A (BP = 100, PP = 1) and B (BP = 100, PP = 1), and the isolated species *T. wallacei* (lineage C; Fig 2). Clade A represented *Cordyceps* s. str. based on the placement of the type *C. militaris* (Sung et al. 2007). This clade contained several *Cordyceps* species with torrubielloid morphologies, including *C. tuberculata*, *C. coccidioperitheciata*, *T. confragosa*, which was recently reclassified as *C. confragosa* (Sung et al. 2007), and *T. piperis* (Fig 1). Clade B was biphyletic with an *Akanthomyces* subclade (BP = 89, PP = 1) and a *Gibellula* subclade (BP = 100, PP = 1), the latter of which contained three *Torrubiella* species. *T. wallacei* (lineage C) is an isolated branch and placed in one of the earliest diverging lineages of *Cordycipitaceae*.

Clavicipitaceae s. str. contained two statistically well-supported clades of *Torrubiella* fungi; these are labelled in Fig 2 as clade D (BP = 100, PP = 1) and clade E (BP = 100, PP = 1). Clade D included species *T. luteorostrata* and *T. tenuis* (Fig 2); clade E included isolates of *T. petchii*. The internal relationships among the subclades of *Clavicipitaceae* s. str., including the two *Torrubiella* clades, were not strongly supported in these analyses. Therefore, the relationships of these two *Torrubiella* clades with other members of *Clavicipitaceae* s. str. cannot be confidently addressed based on the analyses in this study.

Ophiocordycipitaceae contained a single *Torrubiella* species (F in Fig 2), *T. pruinosa*, which was well-supported as a member of *Ophiocordyceps* (BP = 91, PP = 1) and was closely related to several species with *Hirsutella* anamorphs.

Taxonomy

Cordyceps piperis (J.F. Bisch. & J.F. White) D. Johnson, G.-H. Sung, J.F. Bisch. & Spatafora, **comb. nov.**
Mycobank no.: MB 512027.
Basionym: *Torrubiella piperis* J.F. Bisch. & J.F. White, *Stud. Mycol.* 50: 91 (2004).

Additional *Torrubiella* species and associated anamorphic genera were members of *Cordycipitaceae*, but are placed outside of the *Cordyceps* s. str. clade. The majority of these species formed a well-supported biphyletic clade (clade B) that contained species of *Torrubiella* on spiders and *Gibellula* or *Akanthomyces* anamorphs (Figs 1 and 2). *Gibellula* is specific to spiders and is restricted to this clade with species forming a well-supported, monophyletic clade. Two *Torrubiella* isolates unidentified to species, but also on spiders, grouped with *Gibellula* confirming the teleomorph–anamorph link. The spider pathogens of *Akanthomyces* formed a subclade, although no *Torrubiella* isolates grouped with these isolates and our sampling does not reflect the host diversity known for the

genus (Fig 2). Though morphological species concepts of *Akanthomyces* may vary among mycologists (e.g., *A. arachnophilus*, *A. novoguineensis*), the spider-associated taxa form a well-supported clade (clade B) and the anamorph morphology in conjunction with host affinity is a good predictor of phylogeny.

The type species of *Torrubiella* is *T. arancida*, a pathogen of salticid spiders (Boudier 1885). Boudier (1887) described an anamorph, which grew from a specimen of *T. arancida* placed in an incubating chamber as *Isaria cuneispora*. The original

drawings (Boudier 1887) depict a simple morphology similar to species of *Simplicillium* with fusiform-falcate conidia (Zare & Gams 2001). As previously shown (Sung et al. 2007; Zare & Gams 2001), the *Simplicillium* anamorph is among the first diverging lineages of *Cordycipitaceae* (Fig 1). *Simplicillium* or *Simplicillium*-like anamorphs with links to *Torrubiella* include *S. lanosoniveum* (Zare & Gams 2001) and *L. wallacei*, which is 'an anamorph of *T. wallacei*, a pathogen of *Lepidoptera* (Zare & Gams in press). Both are placed outside of the

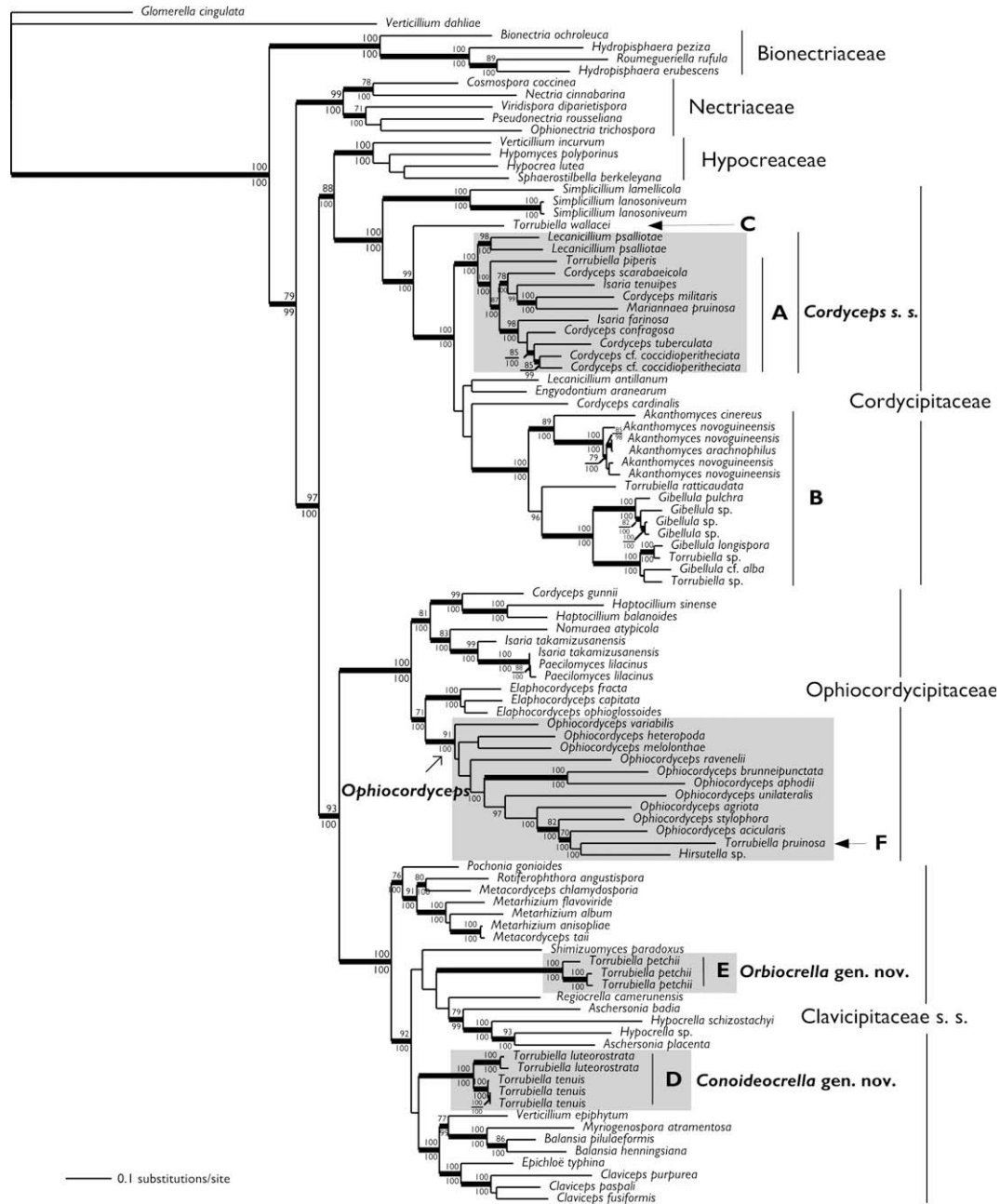


Fig 2 – Phylogeny of Hypocreales from ML analyses showing the relationships of *Torrubiella* species with other genera (e.g. *Cordyceps*, *Ophiocordyceps*). Clades that include species of *Torrubiella* and its associated genera (e.g. *Akanthomyces*, *Gibellula*) are labelled A–F. Numbers above internal branches are BS proportions (BP ≥ 70) from RAxML analyses and numbers below internal branches are PPs (PP ≥ 0.95) in percentage from Bayesian analyses. Grey rectangular boxes emphasize genera that include scientific name changes in this study.

Gibellula/Akanthomyces clade and *Cordyceps* s. s. *Simplicillium lanosoniveum* is typically found as a hyperparasite of fungi, but has been recorded on various arthropods and has been observed to have a *Torrubiella*-like teleomorph in culture (Zare & Gams 2001). The perithecia of teleomorphs with *Simplicillium* anamorphs differ in being thin-walled, delicate, and hyaline or lightly pigmented, whereas species with *C. confragosa*-type teleomorphs are thick-walled, robust, and strongly pigmented (Zare & Gams 2001). We can not discount that previous observations of incubated material were of hyperparasites of *Torrubiella*, a relatively common occurrence among arthropod-pathogenic fungi (Hywel-Jones & Spatafora pers. obs.). Although we have not sampled *T. aranicida*, its morphological description and host affiliation (spider) support its placement in *Cordycipitaceae*. The perithecia of *T. aranicida* (Fig 1) are pallid in colour, flask-shaped without elongated perithecial necks, and produced in a nonaggregated manner on a subiculum of loosely woven hyphae over the surface of the host, similar to *C. tuberculata*. This morphology is similar to the morphologies of *Torrubiella* of *Cordycipitaceae* and dissimilar from the colour and morphology of perithecia and stromata produced by *Torrubiella* species found in *Clavicipitaceae* and *Ophiocordycipitaceae* (Fig 2; discussed below). The classification of these fungi, which we treat as *Torrubiella* s. lat., awaits further investigation involving definitive *T. aranicida* material.

Torrubiella of *Clavicipitaceae* s. str

Clavicipitaceae s. str. is perhaps best known for its grass-associated genera (e.g., *Balansia*, *Claviceps*, *Epichloë*), but it also includes numerous arthropod-pathogenic genera (Sung et al. 2007). Arthropod-pathogenic genera include those of scale insects (e.g., *Hypocrella*, *Regiocrella*, *Torrubiella*), *Lepidoptera*, and *Coleoptera* (e.g., *Metacordyceps*), and generalists used in biocontrol (e.g., *Metarhizium*). Pathogens of scale insects do not form a monophyletic group, rather they are members of three unique genera. Furthermore, current phylogenetic analyses have not placed any *Torrubiella* pathogens of spiders in this family.

T. tenuis and *T. luteoestrata* (Fig 2), pathogens of scale insects, represent a unique, well-supported lineage (clade D) in *Clavicipitaceae* s. str. These two species share similar morphological characters including elongated, conical-shaped perithecia, and planar stromata (Hywel-Jones 1993). The anamorphic state of *T. luteoestrata* is reported as *P. cinnamomeus* while no anamorph has been observed in *T. tenuis* (Hywel-Jones 1993). Because we treat *Torrubiella* s. str. in *Cordycipitaceae*, a new genus is proposed to accommodate *T. tenuis* and *T. luteoestrata*.

Conoideocrella D. Johnson, G.-H. Sung, Hywel-Jones & Spatafora **gen. nov.**

Mycobank no.: MB 512028.

Etym.: In reference to conical shape of perithecium in the genus similar to *Torrubiella*.

Stromata pulvinata vel plana, haud stipitata, varie colorata. Hypothallus hyalinus. Perithecia in stromate vel in hypothallo formata, elongate lageniformia vel conica, luteo-brunnea vel

purpureo-rubida. Asci cylindrici, in summo inspissati. Ascosporae filiformes, multiseptatae, in cellulas dilabentes an non.

Typus: *Torrubiella luteoestrata* Zimm., 1901.

Stromata pulvinate to planar, not stipitate, variously coloured. Hypothallus hyaline. Perithecia produced on the stroma or hypothallus, elongated flask-shaped to conical, yellow-brown to purple-red. Asci cylindrical with thickened ascus apex. Ascospores filiform, multiseptate, disarticulating or non-disarticulating into part-spores.

Conoideocrella tenuis (Petch) D. Johnson, G.-H. Sung, Hywel-Jones & Spatafora, **comb. nov.**

Mycobank no.: MB 512029.

Basionym: *Torrubiella tenuis* Petch, *Ann. Perad.* 7: 323 (1923).

Conoideocrella luteoestrata (Zimm.) D. Johnson, G.-H. Sung, Hywel-Jones & Spatafora **comb. nov.**

Mycobank no.: MB 512030.

Basionym: *Torrubiella luteoestrata* Zimm., *Centralbl. Bakteriolog. Parasitenk., Abt. II* 7:872 (1901).

The genus *Conoideocrella* is proposed for species of *Torrubiella* s. lat. in *Clavicipitaceae* s. str. based on the phylogenetic placement of *T. luteoestrata* and *T. tenuis* (clade D, BP = 100, PP = 1; Fig 2). The morphological characters shared by these two species are elongated, conical-shaped perithecia, and planar stromata (Hywel-Jones 1993). They have been reported as pathogens of both soft (*Coccidae*) and armoured (*Diaspididae*) scale insects (Hywel-Jones 1993; Evans & Prior 1990), but definitive identification of hosts is often not possible due to the high degree of damage done to the host corpse. The genus is named based on the morphology of forming perithecia resembling conical (Latin = conoideus) structures.

T. petchii is a pathogen of scale insects found on bamboo (Hywel-Jones 1997a). The anamorph has been reported as *Hirsutella*, but the morphology is atypical of *Hirsutella* species of the *Ophiocordyceps* clade (see below). Isolates of this species (clade D) are distinct from all other generic lineages of the family, including the genera *Hypocrella* and *Regiocrella*. *Hypocrella* is a monophyletic genus with most species producing an *Aschersonia* anamorph that is characterized by producing pycnidia or acervuli, filled with often brightly coloured, slimy conidia, on a stroma (Hodge 2003). *Regiocrella* is morphologically and ecologically similar to *Hypocrella* in producing immersed perithecia in a subiculum and parasitizing scale insects. However, it produces a pycnidial non-*Aschersonia* anamorph (Chaverri et al. 2005). To reflect this newly recognized lineage of pathogens of scale insect in *Clavicipitaceae* s. s., a new genus is proposed to accommodate *T. petchii*.

Orbiocrella D. Johnson, G.-H. Sung, Hywel-Jones & Spatafora **gen. nov.**

Mycobank no.: MB 512031.

Etym.: In reference to circular shape of stromata in the genus similar to *Torrubiella*.

Stromata hospitem obtegentia, farma annuli, ochracea, stipite carentia. Perithecia dense aggregate, superficialia, lageniformia.

Asci cylindrical, in summo inspissati. Ascospores filiformes, multiseptatae, non dilabentes in cellulas.

Typus: *Torrubiella patchii* Hywel-Jones, 1997.

Stroma covering the host body, ring-like, colour ochraceous, stipe lacking. Perithecia crowded, superficial, flask-shaped. Asci cylindrical with thickened ascus apex. Ascospores filiform, multiseptate, not disarticulating into part-spores.

Orbiocrella patchii (Hywel-Jones) D. Johnson, G.-H. Sung, Hywel-Jones & Spatafora, **comb. nov.**

Mycobank no.: MB 512032.

Basionym: *Torrubiella patchii* Hywel-Jones, *Mycol. Res.* **101**: 143 (1997).

The genus *Orbiocrella* is proposed for *T. patchii* (clade E, BP = 100, PP = 1) of *Clavicipitaceae* s. str. (Fig 2). *Orbiocrella* is based on the production of perithecia and reduced stromatic tissue in a ring (Latin = orbis) around the perimeter of the host. Ecologically, this species is unique, in that it is only found on bamboo and may be specific to a bamboo scale insect (Hywel-Jones 1997a).

Ophiocordyceps pruinosa (Petch) D. Johnson, G.-H. Sung, Hywel-Jones & Spatafora, **comb. nov.**

Mycobank no.: MB 512033.

Basionym: *Torrubiella pruinosa* (Petch) Minter & B.L. Brady, *Trans. Brit. Mycol. Soc.* **74**: 278 (1980).

Calonectria pruinosa Petch, *Trans. Brit. Mycol. Soc.* **16**: 226 (1932).

Ophiocordyceps truncata (Petch) D. Johnson, G.-H. Sung, Hywel-Jones & Spatafora, **comb. nov.**

Mycobank no.: MB 512034.

Basionym: *Calonectria truncata* Petch, *Trans. Brit. Mycol. Soc.* **25**: 257 (1942).

Ophiocordyceps hirsutellae (Petch) D. Johnson, G.-H. Sung, Hywel-Jones & Spatafora, **comb. nov.**

Mycobank no.: MB 512036.

Basionym: *Torrubiella hirsutellae* (Petch) Rossman, *Mycol. Papers* **150**: 100 (1983).

Calonectria hirsutellae Petch, *Trans. Brit. Mycol. Soc.* **21**: 49 (1937).

Hirsutella was originally described as synnematus and characterized by basally inflated phialides in a discontinuous hymenial layer with conidia embedded in mucous sheaths (Hodge 2003; Speare 1920). More recently the concept of *Hirsutella* was expanded to include mononematous species and those with polyphialides, phialides without a significant basal inflation, capitata synnemata, conidia without a mucous coat, and didymoconidia (Hodge 2003). *Clavicipitaceae* s. str. and *Cordycipitaceae* also contain species of *Torrubiella* s. lat. and *Cordyceps* s. lat. that have been assigned *Hirsutella* anamorphs (e.g. *C. pseudomilitaris*) but upon further investigation were more accurately characterized in other anamorph genera (e.g. *Simplicillium*) (Sung et al. 2007). The anamorphs of *T. iriomoteara* (Hywel-Jones 1995), *T. patchii* (Hywel-Jones 1997a), and *T. siamensis* (Hywel-Jones 1995) have been described as *Hirsutella*,

but their morphologies were recognized as atypical of the genus (Hywel-Jones 1997b). Consistent with this morphological observation is the placement of *T. patchii* (Fig 2), which does not place in the *Hirsutella* s. str. clade and does not have ascospores characteristic of the *Ophiocordyceps*–*Hirsutella* clade. The morphology of the anamorph of *T. patchii* is more similar to verticillate anamorphs (*Simplicillium*-like), than *Hirsutella*. Although a more thorough investigation of *Torrubiella*-associated anamorphs of *Clavicipitaceae* s. lat. is necessary, these results along with those of Sung et al. (2007) support a narrow definition of *Hirsutella* as being phylogenetically informative.

Discussion

Previous studies have shown that *Torrubiella* is not monophyletic, but lack of sampling made drawing any definitive conclusions about the genus difficult (Artjariyasriping et al. 2001; Sung et al. 2007). Although substantial progress has been made in the systematics of *Clavicipitaceae* s. lat. and *Cordyceps* s. lat. has been segregated into four genera (*Cordyceps* s. str., *Elaphocordyceps*, *Metacordyceps*, *Ophiocordyceps*), the placement of *Torrubiella* remained largely unknown (Sung et al. 2007). Our results show that species of *Torrubiella* are members of each of the three families of clavicipitaceous fungi (*Clavicipitaceae*, *Cordycipitaceae*, *Ophiocordycipitaceae*), representing multiple derivations of the combined morphology of superficial perithecia produced on a highly reduced stroma or subiculum (Fig 2).

The morphological characters most consistent with the classification of the three families of clavicipitaceous fungi are texture, pigmentation, and morphology of the stromata (Sung et al. 2007). In addition, anamorph genera (e.g. *Aschersonia*, *Beauveria*, *Hirsutella*, *Hymenostilbe*, *Metarhizium*) were restricted in their phylogenetic distribution and were phylogenetically informative in characterizing species of *Cordyceps* s. lat. (Sung et al. 2007). As stromata are typically lacking in *Torrubiella* species, these morphological characters, which proved useful in *Cordyceps* s. lat., are limited in their utility in the systematics of *Torrubiella*. In addition to the taxonomic importance of anamorphic genera in the study of Sung et al. (2007), we find that *Gibellula* is restricted to *Cordycipitaceae* and is phylogenetically informative. *Akanthomyces* is also restricted to *Cordycipitaceae*, but our sampling does not reflect its known host diversity. In our study, new genera and combinations were made to reflect the phylogeny for the species that were previously classified in *Torrubiella*. We listed the new combinations that were confidently assigned based on these analyses or supported by morphologies reassessed as synapomorphies. The remaining species of *Torrubiella* are retained within *Torrubiella sensu* Kobayasi & Shimizu until further analyses clarify their phylogenetic placement.

Torrubielloid fungi of *Cordycipitaceae*

Cordycipitaceae contains the type species of *Cordyceps*, *C. militaris*, which parasitizes various *Lepidoptera* pupae and has a *Lecanicillium* anamorph (Zare & Gams 2001). The family is characterized as containing species that produce pallid to brightly coloured, superficial to completely immersed

perithecia on similarly coloured stromata. Most species typically attack lepidopteran or coleopteran hosts on exposed substrates or shallowly buried in leaf litter (e.g. *C. militaris*, *C. tuberculata*, *C. scarabaeicola*) and rarely occur on hosts deeply buried in soil or wood (Sung et al. 2007). *Cordyceps* s. str. was circumscribed to create a monophyletic *Cordyceps* with the remaining species of the genus reclassified as *Elaphocordyceps*, *Metacordyceps*, or *Ophiocordyceps* according to the phylogeny of *Hypocreales* (Sung et al. 2007). In addition to previously reported anamorph genera restricted in this family (e.g. *Beauveria*, *Isaria*, *Lecanicillium*, *Simplicillium*), all *Gibellula* and *Akanthomyces* isolates that we sampled were placed in *Cordycipitaceae* in clade B (Fig 2).

Clade A (*Cordyceps* s. str.) includes two scale insect torrubelloid pathogens with *Lecanicillium* anamorphs, *T. confragosa* and *T. piperis*. *T. confragosa* was recently reclassified as *C. confragosa* based on its phylogenetic placement (Sung et al. 2007). Preliminary analyses of *T. alba*, which has also been linked to a *Lecanicillium* anamorph (e.g. *L. aranearum*) (Zare & Gams 2001), suggest that it is also likely a member of *Cordycipitaceae* (data not shown). However, this placement was based on incomplete data, thus it is not synonymized in *Cordyceps* at this time and awaits further analyses. The placement of all these species is consistent with the hypothesis of a close relationship between some *Torrubiella* species and species of *Cordyceps* with similarly reduced stromata, such as *C. tuberculata* and *C. coccidioperitheciata*, the latter of which is a pathogen of spiders. As in the case of *C. confragosa*, our results support that *T. piperis* is a member of *Cordyceps* s. str. and that it should be reclassified as *Cordyceps*. Here, we provide a new combination of *T. piperis* to reflect its phylogenetic placement as a member of *Cordyceps* s. str. (Fig 2).

Torrubiella of *Ophiocordycipitaceae*

Ophiocordycipitaceae contains species previously classified as *Cordyceps* s. lat. that are pathogens of arthropods (e.g. *Ophiocordyceps unilateralis*, *O. sinensis*), as well as species that parasitize truffles (e.g. *Elaphocordyceps ophioglossoides*) (Sung et al. 2007). The truffle parasites are part of a monophyletic clade that also includes pathogens of cicada nymphs (e.g. *E. paradoxa*) and represent an interkingdom host shift between fungi and animals facilitated by co-occurrence deep in the soil (Nikoh & Fukatsu 2000). Of particular importance here, the anamorph genus *Hirsutella* s. str. is restricted to *Ophiocordyceps* and is one of the more phylogenetically informative anamorphs of *Ophiocordycipitaceae*.

Hirsutella has been documented and described as the anamorph for five species of *Torrubiella*: *T. hirsutellae* (Petch 1937), *T. iriomotiana* (Hywel-Jones 1995), *T. petchii* (Hywel-Jones 1997a), *T. pruinosa* (Petch 1932), *T. siamensis* (Hywel-Jones 1995). In our phylogenetic analyses, *T. pruinosa* (designated as lineage F in Fig 2) is a member of *Ophiocordyceps* (Fig 2). It was originally described by Petch in the genus *Calonectria* with an anamorph as *H. versicolor*, which is often found accompanying teleomorphic specimens (Petch 1932; Hywel-Jones 1997b). Whereas other species of the clavicipitaceous fungi, including *Torrubiella*, have filiform, thin-walled, hyaline ascospores that usually break into part-spores (Kobayasi & Shimizu 1982), the ascospores of *T. pruinosa* are fusiform,

distoseptate, and faintly pigmented (Petch 1932; Hywel-Jones 1997b). This apomorphic ascospore morphology relative to other species of the genus *Torrubiella* was previously recognized and the relationship of *T. pruinosa* to the genus *Cordycepioideus* (recently synonymized with *Ophiocordyceps*), which also has distoseptate, pigmented ascospores and a *Hirsutella* anamorph (Stifler 1941), was suggested (Hywel-Jones 1997b). Based on their ascospore morphology and *Hirsutella* anamorphs, it is predicted that *Calonectria truncata* and *T. hirsutellae* are closely related to *T. pruinosa*, and these *Torrubiella* species are transferred to *Ophiocordyceps*.

Evolution of astipitate stromata

Species of *Torrubiella* s. lat. do not produce perithecia on a stipitate stroma. This morphology has been derived multiple times among these fungi, presumably from stipitate ancestors. As shown in the species of *Cordyceps* s. lat., species of *Torrubiella* s. lat. do not represent a monophyletic group, but instead reflect artificial groupings of similar homoplastic morphologies and ecologies. Most species of *Torrubiella* s. lat. are pathogens of spiders and scale insects, whereas most frequent hosts of *Cordyceps* s. lat. include *Coleoptera*, *Hemiptera*, *Hymenoptera*, and *Lepidoptera* with rare occurrences on scale insects and spiders. There are numerous examples in fungal evolution of the derivation of simple morphologies from more complex fruiting bodies or vice-versa (e.g. evolution of resupinate and mushroom sporocarps in the *Basidiomycota*) (Hibbett & Binder 2002). We propose that *Cordyceps* s. lat. and *Torrubiella* s. lat. represent another example of this general phenomenon of convergent and dynamic stromatal evolution, and furthermore, that the derivation of the more simple morphology is strongly correlated with host affiliation, (spiders and scale insects), and habitat (occurring in exposed habitats).

Hosts of *Torrubiella* s. lat. are generally small and one explanation for the lack of a stroma may be that they do not provide enough nutrition to produce a stipitate stroma in addition to perithecia. Salticid spiders (the major spider host of *Torrubiella*) and scale insects are also found freely exposed on leaf surfaces. As such there is also arguably less need for the fungus to elevate the propagules for dispersal. Similarly, species of *Cordyceps* s. lat. on spiders (e.g. *C. coccidioperitheciata*) and scale insects *Ophiocordyceps clavulata* produce diminutive, stipitate stromata, and occur in exposed niches, such as the underside of leaf surfaces and elevated plant stems. The counter example is also true in the case of *C. cylindrica* and *C. caloceroides*, which occur on large subterranean spiders and produce substantial stipitate stromata (Hywel-Jones & Sivichai 1995). In addition, large species of *Hypocrella* produce stromata 1 K times the weight of their host scale insect (Hywel-Jones & Samuels 1998), but these likely represent a *Septobasidium*-like association, where the insect is a means of deriving nutrition from the plant. Species of *Hyperdermium* also produce stromata much larger than their hosts and are suggested to be epibionts of plants that derive their nutrition from the plant via the stylet of the scale insect after it is killed (Sullivan et al. 2000). *Cordyceps piperis* (syn. *T. piperis*) also produces stromata that are much larger than its scale insect host (Bischoff & White Jr 2004). The production of astipitate stromata, therefore, likely stems from a mix of ecological and

biochemical characteristics of some hosts that result in the homoplastic trait of astipitate or subiculate stromata among the clavicipitaceous fungi. Thus, historical taxonomic treatments that have classified the majority of astromatic scale insect or spider-infecting species in *Torrubiella* rely on both a phenotype and host association that have repeatedly occurred, and do not accurately reflect evolutionary history.

Acknowledgements

We thank Walter Gams for assistance with Latin diagnoses and for his constructive comments to this manuscript. This research was supported by grants from the National Science Foundation (DEB-0129212 and DEB-0_297_2 to J.W.S.).

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