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The phylogenetic placement of hypocrealean insect pathogens in the genus *Polycephalomyces*: An application of One Fungus One Name

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ABSTRACT

Understanding the systematics and evolution of clavicipitoid fungi has been greatly aided by the application of molecular phylogenetics. They are now classified in three families, largely driven by reevaluation of the morphologically and ecologically diverse genus *Cordyceps*. Although reevaluation of morphological features of both sexual and asexual states were often found to reflect the structure of phylogenies based on molecular data, many species remain of uncertain placement due to a lack of reliable data or conflicting morphological characters. A rigid, darkly pigmented stipe and the production of a *Hirsutella*-like anamorph in culture were taken as evidence for the transfer of the species *Cordyceps cuboidea*, *Cordyceps prolifica*, and *Cordyceps ryogamiensis* to the genus *Ophiocordyceps*. Data from ribosomal DNA supported these species as a single group, but were unable to infer deeper relationships in Hypocreales. Here, molecular data for ribosomal and protein coding DNA from specimens of *Ophiocordyceps cuboidea*, *Ophiocordyceps ryogamiensis*, *Ophiocordyceps paracuboidea*, *Ophiocordyceps prolifica*, *Cordyceps ramosopulvinata*, *Cordyceps nipponica*, and isolates of *Polycephalomyces* were combined with a broadly sampled dataset of Hypocreales. Phylogenetic analyses of these data revealed that these species represent a clade distinct from the other clavicipitoid genera. Applying the recently adopted single system of nomenclature, new taxonomic combinations are proposed for these species in the genus *Polycephalomyces*, which has been historically reserved for asexual or anamorphic taxa.

Introduction

Molecular phylogenetic investigation of the family Clavicipitaceae sensu Rogerson, particularly for the genus *Cordyceps*, has revealed significant phylogenetic diversity best represented by unique family level taxa (Sung *et al.* 2007a). The reevaluation of *Cordyceps* showed that characters historically used to define genera and subgenera did not corroborate with results from

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molecular data. Current taxonomic concepts for the clavicipitoid fungi now recognize three families: Clavicipitaceae sensu stricto, Cordycipitaceae and Ophiocordycipitaceae. Traits such as orientation of perithecia and morphology of ascospores, which previously were used to define subgeneric boundaries, were distributed across all three families. Morphological characters that were most consistent with the resulting molecular phylogeny and that correlated with generic boundaries included texture and colour of the stroma as well as ecological niche (Sung *et al.* 2007a). The genus *Cordyceps* s.s. in Cordycipitaceae contains species with a fleshy texture and brightly coloured stromata and tends to attack hosts in leaf litter or shallow soil. The genus *Metacordyceps* in Clavicipitaceae contains species producing a stroma with a firm, fibrous texture and predominantly green, pallid or lilac coloration, which darkens to a purple or black upon bruising or drying (Sung *et al.* 2007a; Kepler *et al.* 2012a). *Ophiocordyceps* in Ophiocordycipitaceae comprises fungi producing a rigid, pliant or wiry stipe that is darkly coloured and are typically found on hosts buried in soil or in rotting wood. Although these characters correspond well to clades, exceptions occur due to the homoplasious distributions of several character states (e.g., brightly pigmented clava of *Ophiocordyceps nutans* attacking adult hemipterans).

Anamorph morphologies were also demonstrated to have varying degrees of phylogenetic informativeness (Sung *et al.* 2007a). Species in the genus *Metacordyceps* produce anamorphs in the genera *Pochonia* and *Metarhizium*, as well as green-spored forms of *Nomuraea*, whereas pink or lilac forms of *Nomuraea* can be found in Ophiocordycipitaceae (Sung *et al.* 2007a; Kepler *et al.* 2012a). Ophiocordycipitaceae are also associated with *Hymenostilbe* and *Hirsutella* anamorphs, which are produced on stromata that often concurrently or subsequently give rise to perithecia. Anamorphic forms restricted to Cordycipitaceae include *Lecanicillium*, *Isaria* and *Beauveria* associated with *Cordyceps* s.s. and *Gibellula*, associated with *Torrubiella* (Sung *et al.* 2007a). Although these anamorphic forms are fairly indicative of family and genus level associations, examples do exist of broadly distributed anamorph genera (e.g., residual *Verticillium*, Zare *et al.* 2000; *Paecilomyces*, Luangsa-ard *et al.* 2004), which can complicate the placement of asexually reproductive taxa in the modern phylogenetic classification.

Recent hypotheses concerning evolution of host affiliation support arthropod pathogens as being an ancestral ecology for many lineages of clavicipitoid fungi with dynamic host shifts among diverse insect groups and repeated jumping onto plants and other fungi (Spatafora *et al.* 2007; Sung *et al.* 2008; Kepler *et al.* 2012b). For example, although pathogens of other fungi can be found throughout clavicipitoid fungi, those attacking false truffles in the genus *Elaphomyces* are restricted to *Elaphocordyceps* of Ophiocordycipitaceae (Sung *et al.* 2007a) and species infecting the sclerotia of *Claviceps* are restricted to *Tyrannicordyceps* (Kepler *et al.* 2012b) of Clavicipitaceae. Pathogens of spiders are most commonly encountered in Cordycipitaceae in the genera *Torrubiella* and *Cordyceps*, but insect hosts tend be more broadly distributed with Coleoptera, Lepidoptera and Hemiptera found in all three families.

Polycephalomyces Kobayasi is an anamorph genus with an unconfirmed phylogenetic placement and teleomorph affinity that has proven difficult to incorporate into evolutionary hypotheses of clavicipitoid fungi. This confusion stems from a long history of conflicting hypotheses regarding host substrate and teleomorph affinities. Species of *Polycephalomyces* have often been found associated with the stromata of entomopathogenic *Cordyceps* (Massee 1895; Kobayasi 1941). However, it has remained unclear whether *Polycephalomyces* spp. represent anamorphic expressions of *Cordyceps* spp. or are hyperparasites of the latter (Seifert 1985). The type species, *Polycephalomyces formosus*, is synnematous, determinate, and produces small obovoid to ellipsoidal conidia (A-conidia) in a mucous-like matrix (Seifert 1985).

The convergent nature of some characters across all three families of clavicipitoid fungi leaves a considerable number of taxa of uncertain placement, resulting in the residual *Cordyceps* s.l. of Sung *et al.* (2007a). Morphological and ecological character states for these taxa were either lacking or inconclusive and no molecular sequence data were available to test character state homologies. Ban *et al.* (2009) used the large subunit of nuclear ribosomal RNA (LSU) and the complete span of the internal transcribed spacer region (ITS) to address the phylogenetic placement of four species of residual *Cordyceps* s.l., including *Cordyceps alboperitheciata* Kobayasi & Shimizu, *Ophiocordyceps cuboidea* (Kobayasi & Shimizu) S. Ban, Sakane & Nakagiri, *Ophiocordyceps ryogamiensis* (Kobayasi & Shimizu) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, and *Oryogamiensis prolifica* (Kobayasi) S. Ban, Sakane & Nakagiri. They also successfully cultured the anamorphic forms from fresh material. The molecular data showed these species formed a well-supported clade sister to the species *Cordyceps ramosopulvinata* Kobayasi & Shimizu and *Cordyceps kanzashiana* Kobayasi & Shimizu. Furthermore, a cryptic species was uncovered (*Ophiocordyceps paracuboidea* S. Ban, Sakane & Nakagiri) and the name *C. alboperitheciata* was found to be synonymous with *O. cuboidea*. However, genus and family level relationships for this group remained unsupported and classification of these species within the phylogenetic framework for clavicipitoid fungi was not possible using molecular data alone. The anamorphic forms were described as *Hirsutella*-like and this was used as justification to move these taxa into the genus *Ophiocordyceps*.

In this paper we expand sampling of molecular data for of *O. cuboidea*, *O. ryogamiensis*, *O. paracuboidea* and *O. prolifica* sampled by Ban *et al.* (2009). We also expand the sampling to include *Cordyceps nipponica* Kobayasi, *Cordyceps pleuricapitata* Kobayasi and Shimizu, and the anamorph species *P. formosus* and *Blistum tomentosum*, a species previously included in *Polycephalomyces*. When incorporated into a multigene dataset including representatives from six hypocrealean families, including all clavicipitoid lineages (Sung *et al.* 2007a), we find these taxa are not supported as members of *Ophiocordyceps*, but rather represent a unique taxon that is not placed in any existing genus or family of teleomorphs. Consistent with the application of a single system of nomenclature to a clade of fungi regardless of life history states (Taylor 2011; Hawksworth *et al.* 2011), we emend the genus *Polycephalomyces* to include teleomorphs *C. kanzashiana*, *C. ramosopulvinata*, *C. nipponica*, *O. cuboidea*, *O. ryogamiensis*, *O. paracuboidea* and *O. prolifica*, and discuss its phylogenetic relationship to other hypocrealean fungi. We also conclude *B. tomentosum* belongs in *Polycephalomyces*.

Materials and methods

Specimen collection

Tissue from cultures of *Ophiocordyceps cuboidea*, *Ophiocordyceps ryogamiensis*, *Ophiocordyceps paracuboidea* and *Oryogamiensis prolifica* sampled in the Ban *et al.* (2009) paper were resampled from stocks maintained at the National Institute of Technology and Evaluation Biological Resource Center (NBRC). Material from cultures of *Cordyceps pleuricaptiata*, also maintained at NBRC, was included. In addition fresh material was collected from field sites in Japan during the months of June–Aug. in the years 2007 and 2008. Upon collection, specimens were cleaned of dirt and placed in a wax paper bag, a piece of tissue was removed and placed in CTAB buffer for DNA extraction and later air dried for herbarium storage.

Ascospores of Thai material were discharged onto PDA and germinated in 24–36 h at ambient field temperatures (20–32 °C). Part-spores of *Cordyceps ramosopulvinata* were discharged onto Sabouraud Dextrose Agar and germinated at ~25 °C. These isolations were then sent to Rutgers University where they were subcultured onto PDA and kept at 24–27 °C in continuous light (fluorescent lights). (See Table 1 for further details on isolates used in this study.)

DNA extraction, PCR and sequencing

Tissue received an initial grinding by power drill with an Eppendorf pestle in 50 μl CLS-VF buffer from the FastDNA Spin Kit (MP Biomedicals, Salon, OH). Four hundred microlitres of CLS-VF was then added to the ground tissue in a FastDNA lysing matrix A tube and ground further with the fast prep machine for two cycles, 20 s each. Cell lysis was enhanced by soaking for 20 min in a water bath at 60 °C. Tissue was then separated from the supernatant by centrifugation for 10 min at 14 000 rpm. Four hundred microlitres of supernatant was then removed for further cleaning with centrifugation at 14 000 rpm for 20 min in 500 μl chloroform:isoamyl alcohol (24:1). Cleaned and concentrated DNA was then obtained from 300 μl of the top layer of liquid after chloroform:isoamyl centrifugation with the GeneCleanIII Kit following the manufacturers protocol and eluting from glass milk in the final step with 30 μl of water.

PCR methods were used to amplify a total of six nuclear loci for each specimen. As an initial quality control step, and to serve as a voucher for barcoding efforts, the complete span of the internal transcribed spacer region of ribosomal DNA (ITS1-5.8s-ITS2) was amplified and sequenced. A BLAST search of the GenBank database was performed to ensure that DNAs obtained were not from contaminants outside of Hypocreales. After passing quality control measures, five nuclear loci were amplified and sequenced for phylogenetic analysis: SSU and LSU, elongation factor 1α (TEF), and the largest and second largest subunits of RNA polymerase II (RPB1 and RPB2, respectively). Primer information and PCR cycle parameters are described in Kepler *et al.* (2012a) unless otherwise noted. PCR reactions were performed in a MyCycler thermocycler (BioRad, Hercules, CA) using either MasterAmp 2X PCR premix E (Epicenter, Madison WI) and GenScript Taq polymerase or PuReTaq Ready-To-Go PCR Beads (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Sequencing reactions were performed at University of Washington High-Throughput Sequencing Solutions (Seattle, WA) with the primers used for the initial amplifications.

Phylogenetic analyses

Processing of raw sequence reads and construction of contigs was performed using CodonCode Aligner, version 2.0.6 (Dedham, MA). A dataset was then assembled with representative species throughout Hypocreales in the families Hypocreaceae, Nectriaceae and Bionectriaceae in Hypocreales, as well as the outgroup taxa *Glomerella cingulata* and *Verticillium dahliae*. For nearly all specimens examined, at least three of the five genes sought were obtained. Data for *Cordyceps kanzashiana* included only SSU and LSU. GenBank and specimen voucher information is provided in Table 1. MAFFT version 6 (Katoh *et al.* 2002; Katoh & Toh 2008) was used to obtain an initial alignment that was then improved by visual examination with the program BioEdit version 7.05 (Hall 1999). Ambiguously aligned regions were identified with the default settings of Gblocks server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) (Castresana 2000; Talavera & Castresana 2007) and excluded from phylogenetic analyses and gaps were treated as missing data. The final dataset contained sequences from 153 specimens. After exclusion of ambiguously aligned sites there were 1021 nucleotides for SSU, 814 for LSU, 920 for TEF, 641 for RPB1 and 1111 for RPB2. The total length of aligned sequences was 4507 bp. Conflict between loci was examined with the program compat.py (Kauff & Lutzoni 2002).

Maximum Likelihood (ML) estimation of phylogeny was performed with RAxML version 7.0.4 (Stamatakis 2006) with 500 rapid bootstrap replicates on a concatenated dataset containing all five genes. Eleven data partitions were defined for the final combined dataset, one each for SSU and LSU plus nine for each of the three codon positions for the protein coding genes TEF, RPB1, and RPB2. The CAT-GAMMA model of nucleotide substitution was applied to each partition during the rapid bootstrapping phase and the GTR-GAMMA model of nucleotide substitution was specified for the final likelihood tree as suggested by the program manual for large datasets. Bayesian estimation of phylogenetic relationships was conducted with the program Mr. Bayes v3.1 (Ronquist & Huelsenbeck 2003). The same eleven data partitions were applied with the GTR + I + G nucleotide substitution model used for all gene partitions. Two runs were conducted simultaneously, each with four chains for ten million generations. Each chain was sampled every 100 generations, and trees saved with branch length information every 500 generations. After the analysis finished, each run was examined with the program Tracer v1.5 (Drummond & Rambaut 2007) to determine burn-in and confirm that both runs had converged. Summary of the model parameters was determined with the sump command. A strict consensus tree with branch lengths and posterior probabilities was then obtained with the sumt command. The same burnin value was used for both sump and sumt commands.

Results

ML and Bayesian analyses confirmed *Cordyceps ramosopulvinata*, *Cordyceps nipponica*, *Cordyceps kanzashiana Ophiocordyceps*

Table 1 – Specimen information for materials used in this study.

Taxon	Host	Voucher#	GenBank accession numbers					
			ITS	SSU	LSU	TEF	RPB1	RPB2
Akanthomyces novoguineensis	Araneae	NHJ 11923		EU369095	EU369032	EU369013	EU369052	EU369072
Aphysiostroma stercorarium	Cow dung	ATCC 62321		AF543769	AF543792	AF543782	AY489633	EF469103
Aschersonia cf. badia	Hemiptera	BCC 7016	JN049839	DQ372091	DQ384941	DQ384969	DQ385009	DQ452460
Aschersonia confluence	Hemiptera	BCC 7961	JN049841	DQ372100	DQ384947	DQ384976	DQ384998	DQ452465
Aschersonia placenta	Hemiptera	BCC 7869	JN049842	EF469121	EF469074	EF469056	EF469085	EF469104
Balansia epichloë	Poaceae	AEG 96-15a	JN049848	EF468949		EF468743	EF468851	EF468908
Balansia henningsiana	Poaceae	GAM 16112	JN049815	AY545723	AY545727	AY489610	AY489643	DQ522413
Balansia pilulaeformis	Poaceae	AEG 94-2	JN049816	AF543764	AF543788	DQ522319	DQ522365	DQ522414
Bionectria ochroleuca	Bark	CBS 114056		AY489684	AY489716	AY489611	DQ842031	DQ522415
Claviceps fusiformis	Poaceae	ATCC 26019	JN049817	DQ522539	U17402	DQ522320	DQ522366	
Claviceps paspali	Poaceae	ATCC 13892	JN049818	U32401	U47826	DQ522321	DQ522367	DQ522416
Claviceps purpurea	Poaceae	GAM 12885	U57669	AF543765	AF543789	AF543778	AY489648	DQ522417
Claviceps purpurea	Poaceae	SA cp11		EF469122	EF469075	EF469058	EF469087	EF469105
Conoideocrella luteorostrata	Hemiptera	NHJ 11343	JN049859	EF468995	EF468850	EF468801	EF468906	
Conoideocrella luteorostrata	Hemiptera	NHJ 12516	JN049860	EF468994	EF468849	EF468800	EF468905	EF468946
Conoideocrella tenuis	Hemiptera	NHJ 345.01		EU369111	EU369045	EU369030		EU369088
Conoideocrella tenuis	Hemiptera	NHJ 6293	JN049862	EU369112	EU369044	EU369029	EU369068	EU369087
Conoideocrella tenuis	Hemiptera	NHJ 6791	JN049863	EU369113	EU369046	EU369028	EU369069	EU369089
Cordyceps bifusispora	Lepidoptera	EFCC 5690		EF468952	EF468806	EF468746	EF468854	EF468909
Cordyceps brongniartii	Lepidoptera	BCC 16585	JN049867	JF415951	JF415967	JF416009	JN049885	JF415991
Cordyceps cardinalis	Lepidoptera	OSC 93610	JN049843	AY184974	AY184963	EF469059	EF469088	EF469106
Cordyceps coccidioperitheciata	Araneae	NHJ 6709	JN049865	EU369110	EU369042	EU369025	EU369067	EU369086
Cordyceps confragosa	Hemiptera	CBS 101247	JN049836	AF339604	AF339555	DQ522359	DQ522407	DQ522466
Cordyceps gunnii	Lepidoptera	OSC 76404	JN049822	AF339572	AF339522	AY489616	AY489650	DQ522426
Cordyceps kyusyuënsis	Lepidoptera	EFCC 5886		EF468960	EF468813	EF468754	EF468863	EF468917
Cordyceps militaris	Lepidoptera	OSC 93623	JN049825	AY184977	AY184966	DQ522332	DQ522377	AY545732
Cordyceps cf. ochraceostromata	Lepidoptera	ARSEF 5691	JN049849	EF468964	EF468819	EF468759	EF468867	EF468921
Cordyceps pluricapitata	Hemiptera	NBRC 100745		KF049606	KF049624	KF049679	KF049642	KF049667
Cordyceps pluricapitata	Hemiptera	NBRC 100746		KF049607	KF049625	KF049680	KF049643	KF049668
Cordyceps scarabaeicola	Coleoptera	ARSEF 5689	JN049827	AF339574	AF339524	DQ522335	DQ522380	DQ522431
Cordyceps tuberculata	Lepidoptera	OSC 111002	JN049830	DQ522553	DQ518767	DQ522338	DQ522384	DQ522435
Cosmospora coccinea	Hymenochaetales	CBS 114050	JN049831	AY489702	AY489734	AY489629	AY489667	DQ522438
Elaphocordyceps japonica	Eurotiales	OSC 110991	JN049824	DQ522547	DQ518761	DQ522330	DQ522375	DQ522428
Elaphocordyceps ophioglossoides	Eurotiales	OSC 106405		AY489691	AY489723	AY489618	AY489652	DQ522429
Elaphocordyceps subsessilis	Eurotiales	OSC 71235	JN049844	EF469124	EF469077	EF469061	EF469090	EF469108
Engyodontium aranearum	Araneae	CBS 309.85	AJ292391	AF339576	AF339526	DQ522341	DQ522387	DQ522439
Epichloë typhina	Poaceae	ATCC 56429	JN049832	U32405	U17396	AF543777	AY489653	DQ522440
Gibellula sp.	Araneae	NHJ 13158	JN049864	EU369100	EU369037	EU369020	EU369057	EU369077
Glomerella cingulata	Rosaceae	FAU 513		U48427	U48428	AF543772	DQ858454	DQ858455
Glomerella cingulata	Rosaceae	CBS 114054	DQ286202	AF543762	AF543786	AF543773	AY489659	DQ522441
Haptocillium sinense	Nematoda	CBS 567.95	AJ292417	AF339594	AF339545	DQ522343	DQ522389	DQ522443
Hirsutella sp.	Hemiptera	OSC 128575	JN049845	EF469126	EF469079	EF469064	EF469093	EF469110
Hirsutella sp.	Hemiptera	NHJ 12525		EF469125	EF469078	EF469063	EF469092	EF469111
Hydropisphaera peziza	Bark	CBS 102038		AY489698	AY489730	AY489625	AY489661	DQ522444
Hypocrella discoidea	Hemiptera	BCC 8237	JN049840		DQ384937	DQ384977	DQ385000	DQ452461
Hypocrea lutea	Wood	ATCC 208838		AF543768	AF543791	AF543781	AY489662	DQ522446
Hypocrea rufa	Bark	CBS 114374		AY489694	AY489726	AY489621	AY489656	EF692510
Isaria coleopterorum	Coleoptera	CBS 110.73	AY624177	JF415965	JF415988	JF416028	JN049903	JF416006
Isaria farinosa	Lepidoptera	CBS 240.32	AY624178	JF415958	JF415979	JF416019	JN049895	JF415999
Isaria tenuipes	Lepidoptera	ARSEF 5135	AY624196		JF415980	JF416020	JN049896	JF416000
Lecanicillium attenuatum	Leaf litter	CBS 402.78	AJ292434	AF339614	AF339565	EF468782	EF468888	EF468935
Lecanicillium psalliotae	Soil	CBS 532.81	JN049846	AF339609	AF339560	EF469067	EF469096	EF469112
Mariannaea elegans var. punicea	Soil	CBS 239.56	AY624201	AY526489	JF415981	JF416021	JN049897	JF416001
Mariannaea pruinosa	Lepidoptera	ARSEF 5413	JN049826	AY184979	AY184968	DQ522351	DQ522397	DQ522451
Metacordyceps atrovirens	Coleoptera	TNM F10184	JN049882	JF415950	JF415966		JN049884	
Metacordyceps chlamydosporia	Nematoda	CBS 101244	JN049821	DQ522544	DQ518758	DQ522327	DQ522372	DQ522424
Metacordyceps indigotica	Lepidoptera	TNS F18553	JN049874	JF415953	JF415968	JF416010	JN049886	JF415992
Metacordyceps indigotica	Lepidoptera	TNS F18554	JN049875	JF415952	JF415969	JF416011	JN049887	JF415993
Metacordyceps khaoyaiensis	Lepidoptera	BCC 12687	JN049868		JF415970	JF416012	JN049888	
Metacordyceps khaoyaiensis	Lepidoptera	BCC 14290	JN049869		JF415971	JF416013	JN049889	
Metacordyceps kusanagiensis	Coleoptera	TNS F18494	JN049873	JF415954	JF415972	JF416014	JN049890	
Metacordyceps liangshanensis	Lepidoptera	EFCC 1523		EF468961	EF468814	EF468755		EF468918

Table 1 – (*continued*)

Taxon	Host	Voucher#	GenBank accession numbers					
			ITS	SSU	LSU	TEF	RPB1	RPB2
Metacordyceps liangshanensis	Lepidoptera	EFCC 1452		EF468962	EF468815	EF468756		
Metacordyceps martialis	Lepidoptera	TTZ070716-04	JN049871	JF415955	JF415973		JN049891	
Metacordyceps martialis	Lepidoptera	EFCC 6863			JF415974	JF416015		JF415994
Metacordyceps martialis	Lepidoptera	HMAS 197472(S)	JN049881	JF415956	JF415975	JF416016	JN049892	JF415995
Metacordyceps owariensis	Hemiptera	NBRC 33258	JN049883		JF415976	JF416017		JF415996
Metacordyceps pseudoatrovirens	Coleoptera	TNSF 16380	JN049870		JF415977		JN049893	JF415997
Metacordyceps sp.	Coleoptera	HMAS 199601	JN049879	JF415957	JF415978	JF416018	JN049894	JF415998
Metacordyceps taii	Lepidoptera	ARSEF 5714	JN049829	AF543763	AF543787	AF543775	DQ522383	DQ522434
Metacordyceps yongmunensis	Lepidoptera	EFCC 2131	JN049856	EF468977	EF468833	EF468770	EF468876	
Metacordyceps yongmunensis	Lepidoptera	EFCC 2135		EF468979	EF468834	EF468769	EF468877	
Metarhizium album	Hemiptera	ARSEF 2082	AY375446	DQ522560	DQ518775	DQ522352	DQ522398	DQ522452
Metarhizium anisopliae	Coleoptera	ARSEF 3145	JN049834	AF339579	AF339530	AF543774	DQ522399	DQ522453
Metarhizium flavoviride	Hemiptera	ARSEF 2037	AF138271	AF339580	AF339531	DQ522353	DQ522400	DQ522454
Metarhizium sp.	Coleoptera	HMAS 199590	JN049876	JF415960	JF415983	JF416023	JN049898	JF416002
Metarhizium sp.	Coleoptera	HMAS 199592	JN049877	JF415961	JF415984	JF416024	JN049899	JF416003
Metarhizium sp.	Coleoptera	HMAS 199596	JN049878	JF415962	JF415985	JF416025	JN049900	JF416004
Metarhizium sp.	Coleoptera	HMAS 199603	JN049880	JF415963	JF415986	JF416026	JN049901	JF416005
Moelleriella mollii	Hemiptera	BCC 7963		DQ372087		DQ384964	DQ385004	DQ452466
Moelleriella schizostachyi	Hemiptera	BCC 1985		DQ372105	DQ384939	DQ384959	DQ385012	DQ452471
Myriogenospora atramentosa	Poaceae	AEG 96-32	JN049835	AY489701	AY489733	AY489628	AY489665	DQ522455
Nectria cinnabarina	Betulaceae	CBS 114055		U32412	U00748	AF543785	AY489666	DQ522456
Nectria sp.	Plant	CBS 478.75		U47842	U17404	EF469068	EF469097	EF469115
Nomuraea cylindrosporae	Hemiptera	RCEF 3632	JN049872	JF415959	JF415982	JF416022		
Nomuraea cylindrosporae	Hemiptera	TNS 16371		JF415964	JF415987	JF416027	JN049902	
Nomuraea rileyi	Lepidoptera	CBS 806.71	AY624205	AY624205	AY624250	EF468787	EF468893	EF468937
Ophiocordyceps acicularis	Coleoptera	OSC 128580	JN049820	DQ522543	DQ518757	DQ522326	DQ522371	DQ522423
Ophiocordyceps agriotidis	Coleoptera	ARSEF 5692	JN049819	DQ522540	DQ518754	DQ522322	DQ522368	DQ522418
Ophiocordyceps aphodii	Coleoptera	ARSEF 5498		DQ522541	DQ518755	DQ522323		DQ522419
Ophiocordyceps brunneipunctata	Coleoptera	OSC 128576		DQ522542	DQ518756	DQ522324	DQ522369	DQ522420
Ophiocordyceps entomorrhiza	Coleoptera	KEW 53484	JN049850	EF468954	EF468809	EF468749	EF468857	EF468911
Ophiocordyceps gracilis	Lepidoptera	EFCC 8572	JN049851	EF468956	EF468811	EF468751	EF468859	EF468912
Ophiocordyceps heteropoda	Hemiptera	EFCC 10125	JN049852	EF468957	EF468812	EF468752	EF468860	EF468914
Ophiocordyceps longissima	Hemiptera	EFCC 6814			EF468817	EF468757	EF468865	
Ophiocordyceps nigrella	Lepidoptera	EFCC 9247	JN049853	EF468963	EF468818	EF468758	EF468866	EF468920
Ophiocordyceps ravenelii	Lepidoptera	OSC 110995		DQ522550	DQ518764	DQ522334	DQ522379	DQ522430
Ophiocordyceps rhizoidea	Isoptera	NHJ 12522	JN049857	EF468970	EF468825	EF468764	EF468873	EF468923
Ophiocordyceps sinensis	Lepidoptera	EFCC 7287	JN049854	EF468971	EF468827	EF468767	EF468874	EF468924
Ophiocordyceps sobolifera	Hemiptera	KEW 78842	JN049855	EF468972	EF468828		EF468875	EF468925
Ophiocordyceps longissima	Hemiptera	EFCC 6814			EF468817	EF468757	EF468865	
Ophiocordyceps stylophora	Coleoptera	OSC 111000	JN049828	DQ522552	DQ518766	DQ522337	DQ522382	DQ522433
Ophiocordyceps unilateralis	Hymenoptera	OSC 128574		DQ522554	DQ518768	DQ522339	DQ522385	DQ522436
Ophiocordyceps variabilis	Diptera	ARSEF 5365		DQ522555	DQ518769	DQ522340	DQ522386	DQ522437
Ophionectria trichospora	Plant	CBS 109876		AF543766	AF543790	AF543779	AY489669	DQ522457
Orbiocrella petchii	Hemiptera	NHJ 5318		EU369105	EU369040	EU369021	EU369062	EU369080
Orbiocrella petchii	Hemiptera	NHJ 6209	JN049861	EU369104	EU369039	EU369023	EU369061	EU369081
Paecilomyces carneus	Sand dune	CBS 239.32	AY624171	EF468988	EF468843	EF468789	EF468894	EF468938
Paecilomyces lilacinus	Nematoda	CBS 431.87	AY624188	AY624188	EF468844	EF468791	EF468897	EF468940
Paecilomyces marquandii	Soil	CBS 182.27	AY624193	EF468990	EF468845	EF468793	EF468899	EF468942
Paecilomyces carneus	Soil	CBS 239.32	AY624171	EF468988	EF468843	EF468789	EF468894	EF468938
Pochonia bulbillosa	Plant	CBS 145.70	AJ292410	AF339591	AF339542	EF468796	EF468902	EF468943
Pochonia chlamydosporia	Nematoda	CBS 504.66	AJ292398	AF339593	AF339544	EF469069	EF469098	EF469120
Pochonia gonioides	Nematoda	CBS 891.72	AJ292409	AF339599	AF339550	DQ522354	DQ522401	DQ522458
Pochonia parasiticum	Rotifera	ARSEF 3436	FJ973068	EF468993	EF468848	EF468799	EF468904	EF468945
Pochonia rubescens	Nematoda	CBS 464.88	AJ292400	AF339615	AF339566	EF468797	EF468903	EF468944
Polycephalomyces cuboidea	Coleoptera	TNS-F-18487		KF049609	KF049628	KF049683		
Polycephalomyces cuboidea	Coleoptera	NBRC 101740		KF049610	KF049629	KF049684	KF049646	
Polycephalomyces formosus	Coleoptera	ARSEF 1424	KF049661	KF049615	AY259544	DQ118754	DQ127245	KF049671
Polycephalomyces kanzashiana	Hemiptera			AB027326	AB027372			
Polycephalomyces nipponica	Neuroptera	BCC 18108	KF049657	KF049608	KF049626	KF049681	KF049644	
Polycephalomyces nipponica	Neuroptera	BCC 1881		KF049618	KF049636	KF049692		KF049674
Polycephalomyces nipponica	Neuroptera	BCC 1682	KF049664	KF049620	KF049638	KF049694		

(*continued on next page*)

Table 1 – (continued)

Taxon	Host	Voucher#	GenBank accession numbers					
			ITS	SSU	LSU	TEF	RPB1	RPB2
Polycephalomyces nipponica	Neuroptera	NHJ4286		KF049621	KF049639	KF049695	KF049654	KF049676
Polycephalomyces nipponica	Neuroptera	BCC 2325	KF049665	KF049622	KF049640	KF049696	KF049655	KF049677
Polycephalomyces paracuboidea	Coleoptera	NBRC 101742		KF049611	KF049630	KF049685	KF049647	KF049669
Polycephalomyces prolifica	Hemiptera	TNS-F-18481	KF049659	KF049612	KF049631	KF049686	KF049648	
Polycephalomyces prolifica	Hemiptera	TNS-F-18547	KF049660	KF049613	KF049632	KF049687	KF049649	KF049670
Polycephalomyces ramosopulvinata	Hemiptera	SU-65			DQ118742	DQ118753	DQ127244	
Polycephalomyces ramosopulvinata	Hemiptera	EFCC 5566	KF049658		KF049627	KF049682	KF049645	
Polycephalomyces ryogamiensis	Coleoptera	NBRC 101751		KF049614	KF049633	KF049688	KF049650	
Polycephalomyces tomentosus	Trichiales	BL4	KF049666	KF049623	AY259545	KF049697	KF049656	KF049678
Polycephalomyces sp. 1	Neuroptera	BCC 2637	KF049663	KF049619	KF049637	KF049693		KF049675
Polycephalomyces sp. 2	Unknown	JB07.08.16_08	KF049662	KF049616	KF049635	KF049690	KF049652	KF049672
Polycephalomyces sp. 2	Unknown	JB07.08.17_07b		KF049617		KF049691	KF049653	KF049673
Pseudonectria rousseliana	Buxaceae	CBS 114049		AF543767	U17416	AF543780	AY489670	DQ522459
Regiocrella camerunensis	Hemiptera	ARSEF 7682			DQ118735	DQ118743	DQ127234	
Rotiferophthora angustispora	Rotifera	CBS 101437	AJ292412	AF339584	AF339535	AF543776	DQ522402	DQ522460
Roumegueriella rufula	Nematoda	CBS 346.85		DQ522561	DQ518776	DQ522355	DQ522403	DQ522461
Samulesia rufobrunnea	Hemiptera	P.C. 613			AY986918	AY986944	DQ000345	
Septofusidium herbarum	Plant root	CBS 265.58	JN049866		JF415990	JF416030	JN049905	JF416008
Shimizuomyces paradoxus	Smilacaceae	EFCC 6279	JN049847	EF469131	EF469084	EF469071	EF469100	EF469117
Shimizuomyces paradoxus	Smilacaceae	EFCC 6564		EF469130	EF469083	EF469072	EF469101	EF469118
Simplicillium lamellicola	Agaricales	CBS 116.25	AJ292393	AF339601	AF339552	DQ522356	DQ522404	DQ522462
Simplicillium lanosoniveum	Uredinales	CBS 101267	AJ292395	AF339603	AF339554	DQ522357	DQ522405	DQ522463
Sphaerostilbella berkeleyana	Polyporales	CBS 102308		AF543770	U00756	AF543783	AY489671	DQ522465
Torrubiella ratticaudata	Araneae	ARSEF 1915	JN049837	DQ522562	DQ518777	DQ522360	DQ522408	DQ522467
Torrubiella wallacei	Araneae	CBS 101237	EF513022	AY184978	AY184967	EF469073	EF469102	EF469119
Verticillium dahliae	Solanaceae	ATCC 16535		AY489705	AY489737	AY489632	AY489673	DQ522468
Verticillium epiphytum	Uredinales	CBS 384.81		AF339596	AF339547	DQ522361	DQ522409	DQ522469
Verticillium epiphytum	Uredinales	CBS 154.61	AJ292404	AF339596	AF339547	EF468802		EF468947
Verticillium incurvum	Polyporales	CBS 460.88		AF339600	AF339551	DQ522362	DQ522410	DQ522470
Verticillium sp.	Araneae	CBS 101284	JN049858	AF339613	AF339564	EF468803	EF468907	EF468948
Viridispora diparietispora	Galls on *Crataegus*	CBS 102797	JN049838	AY489703	AY489735	AY489630	AY489668	DQ522471

Herbarium Codes: AEG, A. E. Glenn personal collection; ARSEF, USDA-ARS Collection of Entomopathogenic Fungal cultures, Ithaca, NY; ATCC, American Type Culture Collection, Manassa, VA; BCC, BIOTEC Culture Collection, Klong Luang, Thailand; BCC, BIOTEC Culture Collection, Pathum Thani, Thailand; CBS, Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; CUP, Cornell University Plant Pathology Herbarium; EFCC, Entomopathogenic Fungal Culture Collection, Chuncheon, Korea; FAU, F. A. Uecker personal collection; GAM, Julian H. Miller Mycological Herbarium Athens, GA; HMAS, Chinese Academy of Sciences, Beijing, China; JB, Joseph Bischoff, personal collection; KEW, mycology collection of Royal Botanical Garden, KEW, Surrey, UK; NBRC, National Institute of Technology and Evaluation, Chiba, Japan; NHJ, Nigel Hywel-Jones personal collection; OSC, Oregon State University Herbarium, Corvallis, OR; SA, S. Alderman personal collection; TNS, National Museum of Science and Nature, Tsukuba, Japan.

cuboidea, *O. ryogamiensis*, *Ophiocordyceps paracuboidea*, *Ophiocordyceps prolifica* and *Polycephalomyces formosus* form a well-supported clade not associated with any existing teleomorph genus of clavicipitoid fungi. We refer to this clade as the *Polycephalomyces* clade. The placement of *C. kanzashiana* as monophyletic with *C. nipponica* is suspect, likely resulting from only having nuclear ribosomal gene data available. This relationship should be investigated further with more appropriate data before making a definitive statement of relatedness.

Cordyceps pleuricaptiata was recovered as sister to the *Polycephalomyces* clade, but it was not well supported in these analyses (bootstrap proportions < 75 %; Fig 1). We therefore continue to regard *Cordyceps pleuricapitata* as residual *Cordyceps sensu lato* (i.e., incertae sedis) until further work is able to clarify its relationship with statistical confidence. Bayesian analyses and ML resolve placement of *C. pleuricapitata* plus the *Polycephalomyces* clade as sister to Ophiocordycipitaceae, although this placement was not well supported by bootstrap proportions (<75 %, Fig 1). In addition the placement of the *Polycephalomyces* clade was found to be sensitive to taxon sampling. For example, when *C. pleuricapitata* was excluded from the analyses the *Polycephalomyces* clade was inferred as sister group to Clavicipitaceae in RAxML analyses but sister to Ophiocordycipitaceae in Bayesian analyses. At no time, however, was a topology recovered supporting an association within *Ophiocordyceps*. The topology recovered here for relationships between families of Hypocreales is consistent with the results of previous analyses (Sung *et al.* 2007a; Johnson

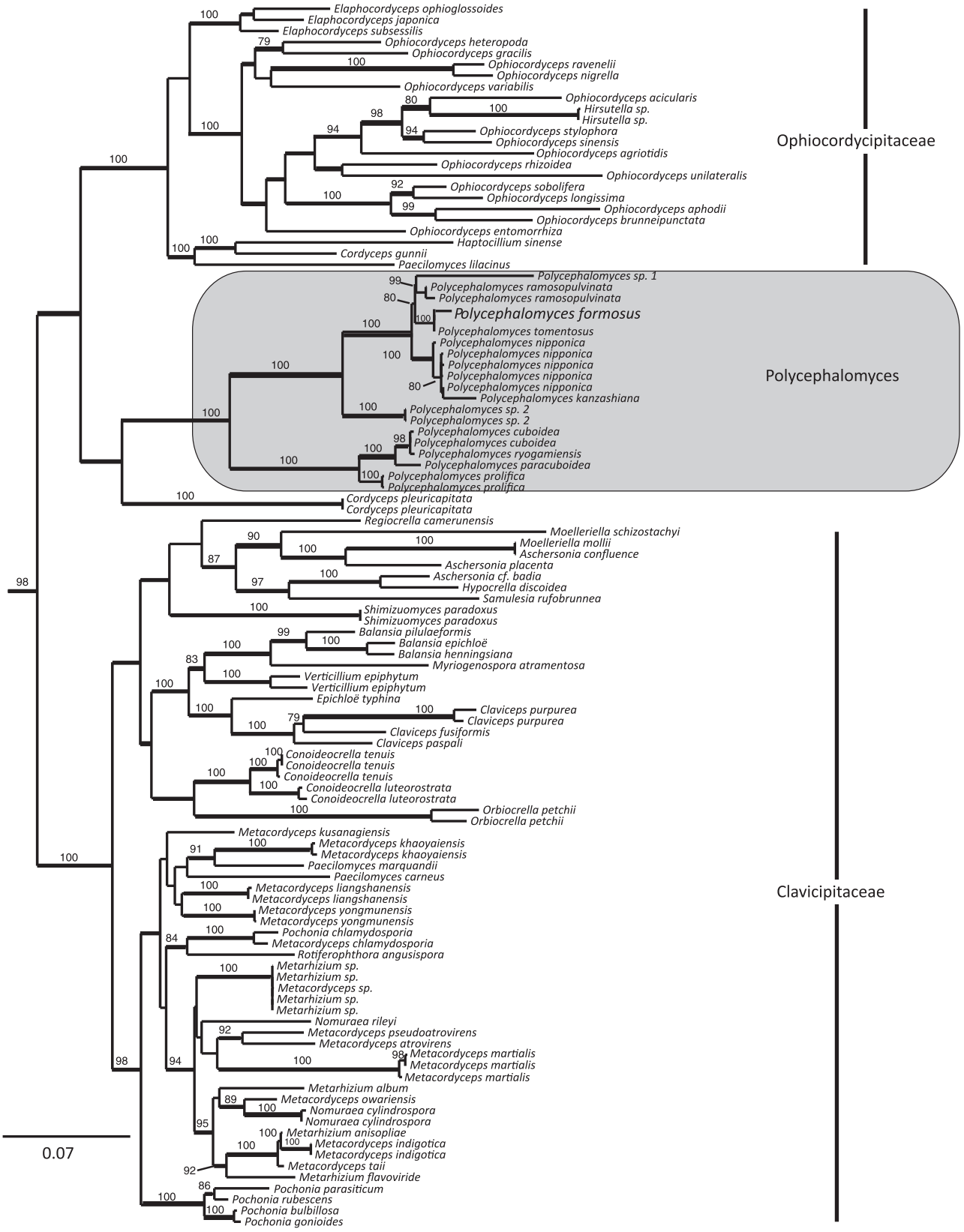

Fig 1 – ML tree obtained from analysis in RAxML of a concatenated five gene dataset (SSU, LSU, TEF, RPB1, RPB2) showing placement of *Polycephalomyces*. Values above branches represent ML bootstrap proportions greater than 70% from 500 replicates. Branches in bold denote strongly supported nodes in Bayesian analyses (≥0.95 posterior probability). Type species of *Polycephalomyces* is shown in larger font.

et al. 2009), however, support values for the relationship between Hypocreaceae and Cordycipitaceae were weakened, as was the branch subtending Nectriaceae and the rest of Hypocreales (Supplementary Fig S1).

We apply the name *Polycephalomyces* to both the anamorph and teleomorph forms present in this clade based on the inclusion of the type species *P. formosus* (Kobayasi 1941) and emend the definition accordingly. Although authenticated ex-type material is unavailable for *P. formosus*, the isolate examined in this study (ARSEF 1424, collected in Poland) has undergone extensive morphological and molecular investigation in previous work (Bischoff et al. 2003; Chaverri et al. 2005). We also transfer *Blistum tomentosum* back into *Polycephalomyces*.

Taxonomy

Polycephalomyces *Kobayasi emend. Kepler and Spatafora*

Genus accepted as circumscribed by Seifert (1985), but including teleomorphic taxa with the following general characteristics: Stromata firm but pliant, white to yellow or brown, often multifurcating or with several stipes representing several cycles of growth; rhizomorphs present or absent. Perithecia immersed in an apical or subapical pulvinate cushion, or superficial, scattered to gregarious, often concentrated on the upper stroma but the stroma tip bare. Asci long, ascospores forming many small partspores of nearly equal length. Hosts include nymphs of Cicadidae (Hemiptera), Neuroptera and larvae of Coleoptera.

Type: *Polycephalomyces formosus* Kobayasi

MB#9494

Anamorphic states: *Acremonium*-like, *Hirsutella*-like, *Polycephalomyces*

A full list of species in *Polycephalomyces* is given below, with new combinations made as appropriate. We continue to recognize *P. cuboidea* and *P. ryogamiensis* as separate taxa based on ITS data presented in Ban et al. (2009).

Polycephalomyces cylindrosporus Samson & H.C. Evans, Proceedings van de Koninklijke Nederlandse Akademie van Wetenschappen Section C, **84**(3): 297 (1981).

MB#111844

Polycephalomyces cuboideus (Kobayasi & Shimizu) Kepler & J.W. Spatafora comb. nov.

≡*Cordyceps cuboidea* Kobayasi & Shimizu. Bull. natn. Sci. Mus., Tokyo, Bot. **6**(4): 131 (1980).

≡*Ophiocordyceps cuboidea* (Kobayasi & Shimizu) S. Ban, Sakane & Nakagiri. Mycoscience **50**(4): 268 (2009).

Host: Larvae of Coleoptera; Habitat: Rotten wood; Anamorph: *Hirsutella*-like.

MB#804387

Polycephalomyces ditmarii Van Vooren & Audibert, Bulletin Mensuel de la Société Linnéenne de Lyon, **74**(7–8): 231 (2005).

MB#511222

Polycephalomyces formosus Kobayasi, Science Reports of the Tokyo Bunrika Daigaku, **5**: 245 (1941).

MB#289806

Polycephalomyces kanzashianus (Kobayasi & Shimizu) Kepler & Spatafora comb. nov.

≡*Cordyceps kanzashiana* Kobayasi & Shimizu. Bull. nat. Sci. Mus., Tokyo, Bot. **8**(3): 86 (1982).

Host: Nymph of Cicadidae (Hemiptera); Habitat: Buried in soil; Anamorph: unknown.

MB#804388

Polycephalomyces nipponicus (Kobayasi) Kepler & J.W. Spatafora comb. nov.

≡*Cordyceps nipponica* Kobayasi. Bull. of the Biogeogr. Soc. Jap. **9**: 151 (1939).

Host: Nymph of Cicadidae (Hemiptera), Neuroptera; Habitat: Buried in soil; Anamorph: unknown.

MB#804389

Polycephalomyces paracuboideus (S. Ban, Sakane & Nakagiri) Kepler & J.W. Spatafora comb. nov.

≡*Ophiocordyceps paracuboidea* S. Ban, Sakane & Nakagiri. Mycoscience **50**(4): 268 (2009)

Host: Larvae of Coleoptera; Habitat: Rotten wood; Anamorph: *Hirsutella*-like.

MB#804390

Polycephalomyces prolificus (Kobayasi) Kepler & J.W. Spatafora comb. nov.

≡*Cordyceps prolifica* Kobayasi in Kobayasi, Y.; Shimizu, D., Bull. nat. Sci. Mus., Tokyo, Bot. **6**: 289 (1963).

≡*Ophiocordyceps prolifica* (Kobayasi & Shimizu) S. Ban, Sakane & Nakagiri. Mycoscience **50**(4): 270 (2009).

Host: Nymph of Cicadidae (Hemiptera); Habitat: Buried in soil; Anamorph: Unknown.

MB#804391

Polycephalomyces ramosopulvinatus (Kobayasi & Shimizu) Kepler & J.W. Spatafora comb. nov.

≡*Cordyceps ramosopulvinata* Kobayasi & Shimizu. Bull. natn. Sci. Mus., Tokyo, Bot. **9**(1): 2 (1983).

Host: Nymph of Cicadidae (Hemiptera); Habitat: Buried in soil; Anamorph: Unknown.

MB#804392

Polycephalomyces ramosus (Peck) Mains, Mycologia, **40** (4): 414 (1948).

≡Stilbum ramosum Peck, Bulletin of the Buffalo Society of Natural Sciences, 1: 69, 1872 ≡Botryonipha ramosa (Pers.) Kuntze, Revisio generum plantarum, 2: 845, 1891

≡Stilbella ramosa (Peck) Petch, Transactions of the British Mycological Society, **21** (1-2): 53, 1938

MB#289808

Polycephalomyces ryogamiensis (Kobayasi & Shimizu) Kepler & J.W. Spatafora comb. nov.

≡*Cordyceps ryogamiensis* Kobayasi & Shimizu. Bull. natn. Sci. Mus., Tokyo, Bot. **9**(1): 4 (1983).

≡*Ophiocordyceps ryogamiensis* (Kobayasi & Shimizu) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora in Sung, Hywel-Jones, Sung, Luangsa-ard, Shrestha & Spatafora, Stud. Mycol. **57**: 45 (2007).

Host: Larvae of Coleoptera; Habitat: Rotten wood; Anamorph: Hirsutella-like.

MB#804393

Polycephalomyces tomentosus (Schrad.) Seifert, Studies in Mycology, **27**: 175, 1985

≡Stilbum tomentosum Schrad., Journal für die Botanik, 2: 65, t. 3:1, 1799

≡Stilbella tomentosa (Schrad.) Bres., Annales Mycologici, **1** (2): 129, 1903

≡Tilachlidium tomentosum (Schrad.) Lindau, Rabenhorst's Kryptogamen-Flora, Pilze – Fungi imperfecti, **1**(9): 306, 1908
≡*Blistum tomentosum* (Schrad.) B. Sutton, Mycological Papers, **132**: 19, 1973
MB#104650

Discussion

Article 59 of the International Code of Botanical Nomenclature allowed the naming of teleomorph and anamorph states of nonlichenized species of Ascomycota and Basidiomycota with unique Latin binomials, a practice referred to as the dual system of fungal nomenclature. At the 2011 meeting of the Nomenclature Session of the **XVIII** Botanical Congress (Melbourne, Australia), Article 59 was abandoned, effective Jan. 1, 2013, in favour of a single system of nomenclature, or One Fungus One Name (1F1N; Hawksworth *et al.* 2011; Taylor 2011). This change in the code allows for teleomorph and anamorph names to be considered equally for the purposes of naming clades (e.g., genera) of fungi. The recognition that several teleomorphs of *Cordyceps s.l.* are phylogenetically distinct from all other clades of cordycipioid fungi (e.g., *Cordyceps*, *Ophiocordyceps*, etc.), and are members of a clade containing the type species of *Polycephalomyces*, provides an example of applying 1F1N to a pleomorphic taxon regardless of the known reproductive life history stages of the species that are members of the clade.

The phylogenetic classification of Hypocreales is developing rapidly with the application of multigene phylogenies (Castlebury *et al.* 2004; Sung *et al.* 2007a; Chaverri *et al.* 2008). The recognition of *Polycephalomyces* as separate from the known genera of clavicipitoid fungi continues this work and provides additional support for phylogenetic associations among teleomorphs and anamorphs, which were noted previously by Ban *et al.* (2009). However, neither the depth of taxon sampling nor the nature of the molecular data was sufficient to support placement in relation to established genera. Ban *et al.* (2009) used a 504 base-pair fragment of large subunit nuclear ribosomal RNA (LSU), which did not adequately resolve deep fungal nodes (Hofstetter *et al.* 2007). Previously, Chaverri *et al.* (2005) analyzed LSU, TEF and RBP1 data for *Polycephalomyces ramosopulvinatus* and *Polycephalomyces formosus*, however taxon sampling in this analysis was focused on scale insect pathogens in Clavicipitaceae s.s. and did not include other members of *Polycephalomyces*. The dataset examined here includes additional ribosomal data, as well as protein coding genes, which have shown to be better suited for addressing divergences of genera and families of Hypocreales (Zhang *et al.* 2006; Hofstetter *et al.* 2007; Sung *et al.* 2007b; Schoch *et al.* 2009), and we also greatly expand the taxonomic scope by including species throughout the order. These data and analyses supported the *Polycephalomyces* clade as being a unique genus among hypocrealean fungi. Although the *Polycephalomyces* clade may represent a family level clade, due to conflicting support values between RAxML and Bayesian analyses, and different resolutions of this relationship to other families of Hypocreales based on different taxon sampling, we refrain from describing a new family at this time. Bayesian analyses place *Polycephalomyces* sister to Ophiocordycipitaceae, however this placement was unsupported in ML analyses (Fig 1). Therefore, we feel it is prudent to consider *Polycephalomyces* as an incertae sedis member of Hypocreales until taxonomic and phylogenetic concepts of clavicipitoid fungi are resolved further.

Polycephalomyces is morphologically distinct from the other animal pathogens of Clavicipitaceae. Species in the core *Metacordyceps* are typically pigmented green to yellow or red, and perithecia are usually oblique and embedded in a fibrous stroma. Species outside of the core clade are typically pallid with variable presentation of perithecia (Kepler *et al.* 2012a). Teleomorphs of *Polycephalomyces* produce tough, wiry, long-lasting stromata, usually with a darkened colour, which are common character traits among *Ophiocordyceps* (Fig 2). Sung *et al.* (2007) included *P. ryogamiensis* in *Ophiocordyceps* based on morphological characters found to be consistent with the relationships inferred from molecular data. Ban *et al.* (2009) presented additional data on the anamorph morphology for *P. ryogamiensis*, as well as other closely related species. All species produce *Hirsutella*-like anamorphic forms in culture, with *Acremonium*-like morphologies developing near the edge of the colony. *Hirsutella* s.s. was identified by Sung *et al.* (2007a) as being associated only with the species of *Ophiocordyceps* and is distinguished from other anamorphs of clavicipitoid fungi by having phialides swollen at the base then tapering to a tip where conidia are produced in a slimy mass. When naturally occurring on a host, hirsutelloid-anamorphs of *Ophiocordyceps* produce grey or brown stromata that often give rise to perithecia. Phialides are typically solitary, although they may be whorled or verticillate. *Acremonium* is a simple or reduced form genus associated with Hypocreales and several species associated with grass-endophytic species in Clavicipitaceae were moved from *Acremonium* to *Neotyphodium* (Glenn *et al.* 1996). Species of *Acremonium* also produce conidia in slimy heads, however the phialides are awl shaped, tapering at the tip without swollen bases. Phialides of both *Acremonium* and *Hirsutella* share a similarity to *Verticillium*, a polyphyletic form genus with multiple occurrences throughout Hypocreales (Zare *et al.* 2000; Gams & Zare 2001; Sung *et al.* 2001; Zare & Gams 2001; Zare *et al.* 2001). The range of anamorphic form genera reported by Ban *et al.* (2009) for the species of *Polycephalomyces* is therefore polyphyletic. Historical uses of *Hirsutella* have been broad with the name applied to species occurring outside of *Ophiocordyceps* that were later reclassified, e.g., *Simplicillium* (Hywel-Jones 1994; Zare & Gams 2001). It is therefore possible that the *Hirsutella*-like anamorph types produced in culture by *Polycephalomyces* reflect variation on an *Acremonium* or *Verticillium*-like anamorph commonly produced by other clavicipitoid fungi in culture.

Anamorphic forms of *Polycephalomyces* produce extremely small conidia (often 2 μm or smaller in width) in a slimy mass at the tip of a prominent synnema. These anamorphic forms are similar to *Hirsutella*, which also produces conidia in slime at the tips of phialides, however phialides lack the swollen base and are concentrated at the tips of synnemata. Seifert (1985) reported the teleomorphic state for *Polycephalomyces tomentosus* as *Byssostilbe stilbigera* (Berk. & Br.) Petch and was later found to be phylogenetically distinct from other species of *Polycephalomyces* (Bischoff *et al.* 2003), a distinction

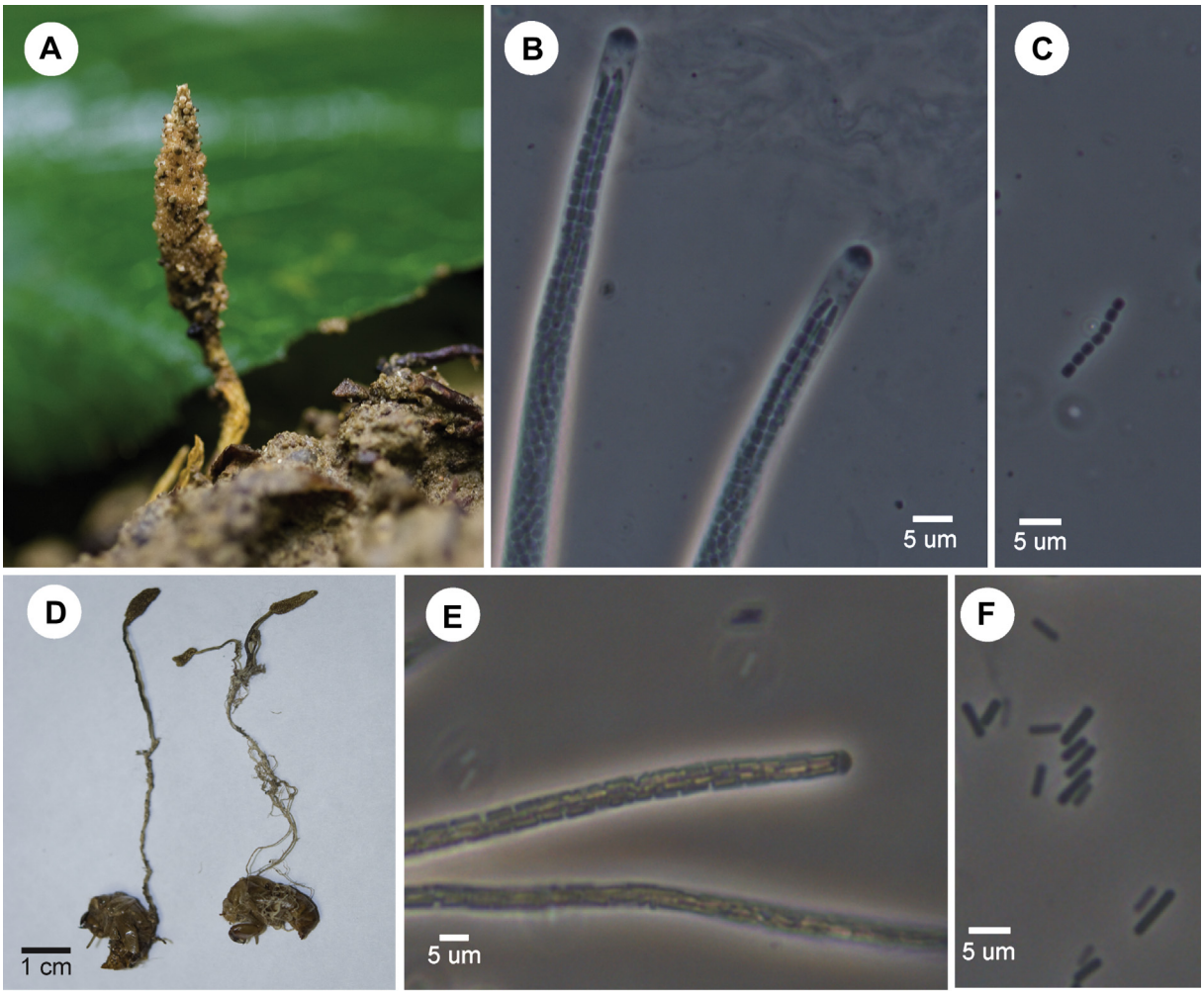

Fig 2 – *Polycephalomyces prolifica* and *Cordyceps pleuricapitata*. (A–C) *Polycephalomyces prolifica*. A. Stroma emerging from soil. B. Ascus. C. Partspores. D-F *Cordyceps pleuricapitata*. D. Dried specimens. E. Ascus. F. Partspore.

not supported by these analyses. A phylogenetic connection between *P. formosus* and *P. ramosopulvinatus* was recovered by Chaverri *et al.* (2005), however the taxon sampling was not sufficient for further inferences. Many different names have been proposed for anamorphic forms associated with this genus, and the taxonomic status has been somewhat unstable. A thorough evaluation of material from anamorphic states is necessary to fully determine relationships within this genus, including material from type localities.

The host associations for *Polycephalomyces* sensu Kobaysi are complicated and specimens are reported as hyperparasites of other clavicipitoid insect pathogens and myxomycetes, as well as insect cadavers (Seifert 1985). The invertebrate host associations for the emended *Polycephalomyces* include cicada nymphs (Hemiptera: Cicadidae), as well as larvae of Coleoptera and Lepidoptera. Specimens of *Polycephalomyces nipponicus* have also been collected from larvae of Neuroptera (Isaka & Tanticharoen 2001), which share a similar below-ground habitat that could facilitate host switching. Pathogens of cicada nymphs have evolved multiple times in Ophiocordycipitaceae, as well as in Clavicipitaceae (Sung *et al.* 2007a; Kepler *et al.* 2012a). These hosts occur buried in the soil, whereas the beetle pathogens in *Polycephalomyces* were excavated from decaying wood. This pairing of habitats is possibly another convergent character between fungi of *Ophiocordyceps* and *Polycephalomyces* (Sung *et al.* 2007a), or symplesiomorphic with respect to the most recent common ancestor of *Polycephalomyces* and Ophiocordycipitaceae.

Conclusions

Introduction of molecular phylogenetic methods to the study of clavicipitoid fungi has enabled major advances in understanding the evolution of species relationships, key life-history traits, morphologies and ecologies. Morphological characteristics such as texture and colour of teleomorphs and anamorph morphologies are phylogenetically informative for many taxa (e.g., *Cordyceps* s.s. and *Beauveria*, *Ophiocordyceps* and *Hymenostilbe*), but exceptions and problematic taxa do exist and *Polycephalomyces* is indicative of such a taxon. The character states associated with teleomorphs are either products of convergent evolution or they are symplesiomorphic for the genus and Ophiocordycipitaceae. This research

also highlights the need for additional work to refine character state homologies between *Hirsutella* synnemata of *Ophiocordyceps* and the *Hirsutella*-like anamorphs of *Polycephalomyces*. To date no *Hirsutella* anamorph typical of the brown or grey synnematous forms encountered in *Ophiocordyceps* have been recovered for *Polycephalomyces*, although *Hirsutella*-like anamorphs are observed in other species outside of Ophiocordycipitaceae when growing in culture (Hywel-Jones 1994). Regardless, we provide strong evidence for the placement of teleomorphs with isolates of *Polycephalomyces formosus* and we apply the name *Polycephalomyces* to all life history states of the clade based on priority as allowed by the recently adopted single system of nomenclature for fungi.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.funbio.2013.06.002.

REFERENCES

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.

Bischoff JF, Sullivan RF, Hywel-Jones NL, White JF, 2003. Resurrection of *Blistum tomentosum* and its exclusion from *Polycephalomyces* (Hyphomycetes, Deuteromycota) based on 28S rDNA sequence data. *Mycotaxon* **86**: 433–444.

Castlebury LA, Rossman AY, Sung G-H, Hyten AS, Spatafora JW, 2004. Multigene phylogeny reveals new lineage for *Stachybotrys chartarum*, the indoor air fungus. *Mycological Research* **108**: 864–872.

Castresana J, 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**: 540–552.

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**: 1225–1237.

Chaverri P, Liu M, Hodge KT, 2008. A monograph of the entomopathogenic genera Hypocrella, Moelleriella, and Samuelsia gen. nov. (Ascomycota, Hypocreales, Clavicipitaceae), and their aschersonia-like anamorphs in the Neotropics. *Studies in Mycology* **60**: 1–66.

Drummond A, Rambaut A, 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**: 214.

Gams W, Zare R, 2001. A revision of *Verticillium* sect. *Prostrata*. III. Generic classification. *Nova Hedwigia* **72**: 329–337.

Glenn AE, Bacon CW, Price R, Hanlin RT, 1996. Molecular phylogeny of *Acremonium* and its taxonomic implications. *Mycologia* **88**: 369–383.

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.

Hawksworth DL, Crous PW, Redhead SA, Reynolds DR, Samson RA, Seifert KA, Taylor JW, Wingfield MJ, 2011. The Amsterdam Declaration on Fungal Nomenclature. *IMA Fungus* **2**: 105–112.

Hofstetter V, Miadlikowska J, Kauff F, Lutzoni F, 2007. Phylogenetic comparison of protein-coding versus ribosomal RNA-coding sequence data: a case study of the Lecanoromycetes (Ascomycota). *Molecular Phylogenetics and Evolution* **44**: 412–426.

Hywel-Jones NL, 1994. *Cordyceps khaoyaiensis* and *C. pseudomilitaris*, two new pathogens of lepidopteran larvae from Thailand. *Mycological Research* **98**: 939–942.

Isaka M, Tanticharoen M, 2001. Structures of Cordypyridones A–D, antimalarial N-hydroxy- and N-methoxy-2-pyridones from the insect pathogenic fungus *Cordyceps nipponica*. *Journal of Organic Chemistry* **66**: 4803–4808.

Johnson D, Sung G-H, Hywel-Jones NL, Luangsa-Ard JJ, Bischoff JF, Kepler RM, Spatafora JW, 2009. Systematics and evolution of the genus *Torrubiella* (Hypocreales, Ascomycota). *Mycological Research* **113**: 279–289.

Katoh K, Misawa K, Kuma K, Miyata T, 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059–3066.

Katoh K, Toh H, 2008. Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* **9**: 286–298.

Kauff F, Lutzoni F, 2002. Phylogeny of the Gyalectales and Ostropales (Ascomycota, Fungi): among and within order relationships based on nuclear ribosomal RNA small and large subunits. *Molecular Phylogenetics and Evolution* **25**: 138–156.

Kepler RM, Sung G-H, Ban S, Nakagiri A, Chen M-J, Huang B, Li Z, Spatafora JW, 2012a. New teleomorph combinations in the entomopathogenic genus *Metacordyceps*. *Mycologia* **104**: 182–197.

Kepler RM, Sung G-H, Harada Y, Tanaka K, Tanaka E, Hosoya T, Bischoff JF, Spatafora JW, 2012b. Host jumping onto close relatives and across kingdoms by Tyrannicordyceps (Clavicipitaceae) gen. nov. and Ustilaginoidea (Clavicipitaceae). *American Journal of Botany* **99**: 552–561.

Kobayasi Y, 1941. The genus Cordyceps and its allies. *Science Reports of the Tokyo Bunrika Daigaku* **84**: 53–260.

Luangsa-ard JJ, Hywel-Jones NL, Samson RA, 2004. The polyphyletic nature of Paecilomyces sensu lato based on 18S-generated rDNA phylogeny. *Mycologia* **96**: 773–780.

Massee G, 1895. A revision of the genus Cordyceps. *Annals of Botany* **9**: 1–44.

Ronquist F, Huelsenbeck JP, 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Seifert K, 1985. A monograph of *Stilbella* and some allied Hyphomycetes. *Studies in Mycology* **27**: 1–224.

Schoch CL, Sung G-H, Lopez-Giraldez F, Townsend JP, Miadlikowska J, *et al.*, 2009. The Ascomycota tree of life: A phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. *Systematic Biology* **58**: 224–239.

Spatafora JW, Sung G-H, Sung J-M, Hywel-Jones NL, White JR, 2007. Phylogenetic evidence for an animal pathogen origin for ergot and the grass endophytes. *Molecular Ecology* **16**: 1701–1711.

Stamatakis A, 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.

Sung G-H, Hywel-Jones NL, Sung J-M, Luangsa-ard JJ, Shrestha B, Spatafora JW, 2007a. Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. *Studies in Mycology* **57**: 5–59.

Sung G-H, Poinar Jr GO, Spatafora JW, 2008. The oldest fossil evidence of animal parasitism by fungi supports a Cretaceous diversification of fungal-arthropod symbioses. *Molecular Phylogenetics and Evolution* **49**: 495–502.

Sung G-H, Spatafora J, Zare R, Hodge K, Gams W, 2001. A revision of *Verticillium* sect. *Prostrata*. II. Phylogenetic analyses of SSU and LSU nuclear rDNA sequences from anamorphs and teleomorphs of the Clavicipitaceae. *Nova Hedwigia* **72**: 311–328.

Sung G-H, Sung J-M, Hywel-Jones NL, Spatafora JW, 2007b. A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): Identification of localized incongruence using a combinational bootstrap approach. *Molecular Phylogenetics and Evolution* **44**: 1204–1223.

Talavera G, Castresana J, 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* **56**: 564–577.

Taylor JW, 2011. One Fungus=One Name: DNA and fungal nomenclature twenty years after PCR. *IMA Fungus* **2**: 113–120.

Zare R, Gams W, 2001. A revision of *Verticillium* section *Prostrata*. IV. The genera *Lecanicillium* and *Simplicillium* gen. nov. *Nova Hedwigia* **73**: 1–50.

Zare R, Gams W, Culham A, 2000. A revision of *Verticillium* sect. *Prostrata* – I. Phylogenetic studies using ITS sequences. *Nova Hedwigia* **71**: 465–480.

Zare R, Gams W, Evans H, 2001. A revision of *Verticillium* section *Prostrata*. V. The genus *Pochonia*, with notes on *Rotiferophthora*. *Nova Hedwigia* **73**: 51–86.

Zhang N, Castlebury LA, Miller AN, Huhndorf SM, Schoch CL, Seifert KA, Rossman AY, Rogers JD, Kohlmeyer J, Volkmann-Kohlmeyer B, Sung G- H, 2006. An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. *Mycologia* **98**: 1076–1087.