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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.

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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*.