Multigene phylogenetics of *Polycephalomyces* (Ophiocordycipitaceae, Hypocreales), with two new species from Thailand

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*Polycephalomyces* (Ophiocordycipitaceae) species are found in subtropical regions and are parasitic or hyperparasitic on insects. Two new species, *P. aurantiacus* and *P. marginaliradians*, parasitic on *Ophiocordyceps barnesii* and larva of Cossidae respectively, are introduced in this paper. Morphological comparison with extant species and DNA based phylogenies from analyses of a multigene dataset support the establishment of the new taxa. *Polycephalomyces aurantiacus*, exhibiting a hyperparasitic lifestyle on *Ophiocordyceps barnesii*, differs from other species in producing orange conidia in mass and have longer β-phialides in culture. *Polycephalomyces marginaliradians* differs from other *Ophiocordyces* species by producing single stromata with a stipe, smaller perithecia and branched α-phialides and catenate α-conidia and is parasitic on Cossidae. A combined nrSSU, nrLSU, ITS, tef-1a, rpb1 and rpb2 sequence data was analysed phylogenetically including *Ophiocordyceps* and *Polycephalomyces* taxa. The new species described herein are clearly distinct from other species in *Polycephalomyces*. We provide a key to the species of *Polycephalomyces* and discuss relevant interspecies relationships.

The genus *Polycephalomyces* was introduced by Kobayasi¹ to accommodate *P. formosus* Kobayasi (1941), based on its asexual characteristics² and it is presently accommodated in Ophiocordycipitaceae³. Phylogenetic placement of *Polycephalomyces* has always been a debate within the clavicipitoid fungi as the taxonomic hypotheses based on host substrate and sexual morph affinities were controversial¹⁴,⁵. Kepler et al.⁵ amended the taxonomic circumscription of *Polycephalomyces* and accepted twelve species (i.e. *P. cuboideus*, *P. cylindrosporus*, *P. ditmarii*, *P. formosus*, *P. kanzashiznus*, *P. nipponicus*, *P. paracubiodeus*, *P. prolificus*, *P. ramosopulvinatus*, *P. ramosus*, *P. ryogamiensis* and *P. tomentosus*) in Ophiocordycipitaceae based on phylogenetic analyses. Later, *P. sinensis*, *P. lianzhouensis*, *P. yunnanensis*, *P. agaricus* and *P. onorei* were introduced as new species within *Polycephalomyces* based on morphology and DNA sequence data. Then, Liang et al.¹¹ introduced a new species *P. ponerae* in the genus *Polycephalomyces*. Based on recent morphological studies and DNA based phylogenetic analyses, *Polycephalomyces* taxa have been segregated in two sister clades within Ophiocordycipitaceae⁵,¹². Matočec et al.¹² considered those two clades as two different genera, viz. *Perennicordyceps* Matočec & I. Kušan and *Polycephalomyces*. *Perennicordyceps* comprises four species (i.e. *Pe. cuboidea*, *Pe. paracuboida*, *Pe. proliferca*, and *Pe. ryogamiensis*), which are characterized by superficial perithecia, and hirsutella-like or acremonium-like

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Results

Molecular phylogeny. Table S1 comprises 39 taxa (including the seven newly collected taxa) analysed herein and their accession numbers. DNA sequence data of the new species have been submitted to GenBank. A concatenated sequence data-set was analyzed comprising 5033 characters with gaps (SSU: 971, LSU: 813, ITS: 667; TEF: 875, RPBI: 659, RPB2: 1018).

The RAxML analysis of the combined dataset yielded a best scoring tree (Fig. 1) with a final ML optimization likelihood value of $-23008.064357$. The matrix had 1750 distinct alignment patterns, with 39.76% of undetermined characters or gaps. Parameters for the GTR model of the concatenated data set were as follows: Estimated base frequencies; $A = 0.236157, C = 0.276387, G = 0.278052, T = 0.209403$; substitution rates $AC = 1.217462, AG = 3.345152, AT = 0.776221, CG = 1.574418, CT = 6.177076, GT = 1.5000$; gamma distribution shape parameter $\alpha = 0.251124$. The Bayesian analysis resulted in 200000 generations. The first 4000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 16001 trees were used for calculating posterior probabilities in the majority rule consensus tree.

The genus Polycephalomyces currently includes 15 species and only 11 species have available DNA sequence data in GenBank (Table S1), excluding the new taxa described in this study. Our multigene phylogenetic analyses herein reveal that our new taxa constitute a strongly supported monophyletic subclade and nested in between other Polycephalomyces species (Fig. 1). In particular it is noted that Polycephalomyces aurantiusc and P. margin-aliradians share a close phylogenetic affinity to P. nipponicus and P. kanzashianus (Fig. 1).

In this paper, we illustrate a collection of Ophiocordycipes barnesi, which was parasitized by a Polycephalomyces species. Two new species of Polycephalomyces, one from Ophiocordycipes barnesi, and one from a Cissidiae host are also introduced. A phylogenetic tree based on multigene sequence analyses for Ophiocordycipes (11 species) and Polycephalomyces (13 species), is also provided.

Description of Ophiocordycipes barnesi (Thwaites) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, in Sung, Hywel-Jones, Sung, Luangs-a-ard, Shrestha & Spatafora, Stud. Mycol. 57: 40 (2007).

Index Fungorum number: 504230; Facesoffungi number: FoF 03811 (Fig. 2).

Parasitic on larvae (Coleopteran), buried in the soil. Sexual morph: Host 2–2.5 long × 0.5–1 cm wide, brown to dark brown without hyphae on the surface. Stromata 13–20 long × 0.5–1 cm diam., mostly single, stipitate, unbranched or branched into 2 or 3 fertile head, arising from between the head and thorax of larva (fusiformis), dark-brown, fleshy, cylindrical, often illexous or angularly crooked. Stipe 1–2 cm long, 2–5 mm diam., brown, with a fertile apex. Fertile head 2–5 cm long, 1.5–3 cm diam., single or branched more than 2, cylindrical, apically tapered, brown with orange mycelium cover on the surface. Perithecia 380–389 × 98–132 µm ($\bar{X} = 349 × 115\mu m, n = 60$), immersed, brown, elongated pyriform or flask-shaped, thick-walled. Peridium 12–21 µm ($\bar{X} = 16\mu m, n = 60$) wide, brown, textura angularis to textura globulosa to textura prismatica. Ascii 195–229 × 6–9 µm ($\bar{X} = 212 × 7.5\mu m, n = 90$), 8-spored, hyaline, filiform, with a thin apex. Apical cap 3.1–4.2 × 4.1–5.4 µm ($\bar{X} = 3.6 × 4.7\mu m, n = 60$), with a small channel in the center. Ascosporae 155–200 × 2.2–2.7 µm ($\bar{X} = 178 × 2.5\mu m, n = 60$), 3-septate, easily breaking into 4 part-spores, filiform, tapered at each end. Secondary ascosporae 31.6–41.6 × 2.2–2.7 µm ($\bar{X} = 36.6 × 2.5\mu m, n = 90$) cylindrical, thickening at each end or tapered at one end, straight, hyaline, smooth-walled. Asexual morph: undetermined.

Notes: We collected Ophiocordycipes barnesi in this study which was colonized by an orange hyperparasite which we introduce below as Polycephalomyces aurantiusc. This species may be important in future industrial production of Cordycipes species, which are increasingly being produced because of their medicinal properties and biopesticides potential[15–18]. The specimen was deposited in MFLU Herbarium (MFLU 17-1393).

Description of Polycephalomyces aurantiusc Y.P. Xiao, T.C. Wen & K.D. Hyde, sp. nov. Index Fungorum number: IF553936; Facesoffungi number: FoF 03811 (Figs 3, 4).

Etymology: The specific epithet refers to the color of conidia in mass in the specimen and colony.

Holotype: MFLU 17-1393

Hyperparasite on Ophiocordycipes barnesi (Ophiocordycipitaceae), buried in the soil. Sexual morph: undetermined. Asexual morph: Synnemata solitary or not solitary, arising from the fertile head of the stromata, flat-shaped, orange color. Phialides 9.9–14.3 × 0.7–1.4 µm ($\bar{X} = 12.1 × 1.1\mu m, n = 90$) hyaline. Conidia 2.2–6 × 1.4–2.1 µm ($\bar{X} = 2.3 × 1.8\mu m, n = 60$), oval to globose shape, hyaline, one-celled, smooth-walled, orange in mass.

Colonies on PDA medium, growing slowly, attaining 4 cm in 17 days at 25°C, white, reverse yellow. Synnemata emerging after 30 days, solitary or not solitary, branched or unbranched, 1.3–2.2 µm long ($\bar{X} = 60$), showing 1–2
Radiating ring-like distributions. Conidial masses generating from the apex of the synnemata or covering the surface of the colony (Fig. 4). Hyphae hyaline, branched, smooth-walled, 0.3–50 mm ($\tau = 20$) wide. Conidiophores undetermined, not clear. Phialides existing in two types: $\alpha$- and $\beta$-phialides. $\alpha$-phialides 10.4–18.3 $\times$ 0.8–1.8 $\mu$m ($\tau = 14.4 \times 1.3 \mu$m, n = 90) hyaline, narrow slender, smooth. $\beta$-phialides 22.9–64.2 $\times$ 1.0–1.5 $\mu$m ($\tau = 43.6 \times 1.3 \mu$m, n = 90) solitary, growing from hyphae, narrow slender, catenate blasto conidia, smooth. $\alpha$-conidia 1.8–2.2 $\times$ 1.4–1.9 $\mu$m ($\tau = 2 \times 1.7 \mu$m, n = 90) globose to subglobose, occurring in the conidial mass on the agar or on the final portion of synnemata, one-celled, smooth-walled, yellow slimy in mass. $\beta$-conidia 3.2–3.9 $\times$ 1.4–1.8 $\mu$m ($\tau = 3.5 \times 1.6 \mu$m, n = 90) fusiform, and produced on the surface mycelium of colony or on the top of the synnema, one-celled, smooth-walled, hyaline, usually in chains on a phialide.

Figure 1. Phylogram of Polycephalomyces aurantiacus, Polycephalomyces marginaliradians and Ophiocordyceps barnesi generated from maximum likelihood analysis of ITS, SSU, LSU, RPB1, RPB2 and TEF1α sequence data. Purpureocillium lilacinum CBS 284.36 and Purpureocillium lilacinum CBS 431.87 were used as outgroup taxon. Maximum likelihood bootstrap values greater than 70% and Bayesian posterior probabilities over 0.9 are indicated above the nodes. The new species were indicated in blue. The host of Polycephalomyces aurantiacus is indicated in bold.
Material examined: THAILAND, Prachuap Khiri Khan. On dead larvae (Coleopteran), 29 July 2015, YuanPin Xiao, BK15072907 (MFLU 17-1393, holotype); BK15072902, BK15072906 (MFLU 17-1394, HKAS100693, para-types); ex-type living cultures, MFLUCC 17-2113, MFLUCC 17-2114, MFLUCC 17-2115, KUMCC 17-0256, KUMCC 17-0257.

Description of Polycephalomyces marginaliradians Y.P. Xiao, T.C. Wen & K.D. Hyde, sp. nov. Index Fungorum number: IF553937; Facesoffungi number: FoF 03812 (Figs 5, 6).

Etymology: The specific epithet refers to the feature of the colonies on the culture.

Holotype: MFLU 17-1582
Parasitic on a Cossidae larva (Lepidoptera), buried in the soil. Sexual morph: Thallus within host. Host 3.2–3.5 long × 0.4–0.6 cm wide, yellow to brown, without hyphae on the surface. Stromata 3–3.5 long × 0.2–0.45 cm diam., mostly single, stipitate, cylindrical, unbranched or branched, arising from the head of larva, brown to yellow. Stipe 1–2 cm long, 2–3 mm diam., cylindrical, yellow to brown, with one or two lateral fertile head. Fertile head 0.40–0.42 cm long, 0.3–0.45 mm diam., capitulate, lateral, globose to subglobose, pale yellow to yellow, with protruding ostiolar necks. Ascomata 676–803 × 246–328 μm (x = 739 × 287μm, n = 60), immersed, yellow,
flask-shaped, thick-walled. Peridium 11–19 µm (\( \bar{x} = 15 \) µm, n = 60) wide, brown, textura angularis to textura globulosa to textura prismatica. Asci 459–556 × 3.1–4.3 µm (\( \bar{x} = 508 \times 3.7 \) µm, n = 90), 8-spored, hyaline, filiform, with a thin apex. Apical cap 1.4–2.5 × 2.2–3.2 µm (\( \bar{x} = 2 \times 2.7 \) µm, n = 60), with a small channel in the center. Ascospores as long as the asci, easily breaking into part-spores, filiform. Secondary ascospores 3.2–4.2 × 1.3–1.7 µm (\( \bar{x} = 3.8 \times 1.5 \) µm, n = 90) cylindrical, straight, hyaline, smooth. Asexual morph: Synnemata solitary or not solitary, arising from the fertile head of the host, cylindrical, pale yellow. Phialides 11–14.4 × 1.2–1.8 µm (\( \bar{x} = 12.7 \times 1.5 \) µm, n = 90) hyaline. Conidia 3.6–4.9 × 1.8–2.5 µm (\( \bar{x} = 4.2 \times 2.1 \) µm, n = 90), fusiform, hyaline, one-celled, smooth-walled.

Colonies on PDA medium, circular, attaining 4 cm in 10 days at 25 °C, white, reverse yellow. Synnemata emerging after 14 days in the margin of the colony, single or branched into 2 or 3 branched, 3200.8–4566.3 × 142.9–661.8 µm (\( \bar{x} = 3883.5 \times 402.3 \) µm, n = 30), showing 1–2 radiating ring-like distributions. Conidial masses generating from the middle of the synnemata or covering the surface of the colony, pale yellow to yellow, with hyaline to pale yellow exu cate. Hyphae hyaline, branched, smooth-walled, 1.8–2.7 µm (\( \bar{x} = 2.2 \) wide.

Figure 3. Polyccephalomyces aurantiacus MFLU 17-1393. (a) Mycelium on the surface of stroma. (b,c) Conidiomata. (d) Section of conidiuma. (e–g) Phialides. (h) Conidia. Scale Bars: a = 1000 µm, b = 500 µm, c = 200 µm, d = 50 µm, e = 20 µm, f–g = 10 µm, h = 5 µm.
Conidiophores undetermined, not clear. Phialides existing in two types: α- and β-phialides. α-phialides
11–14.4 × 1.2–1.8 μm (x = 12.7 × 1.5 μm, n = 90), hyaline, smooth, elongated lageniform, caespitose, palisade-like,
crowed, monoverticillate, mostly branched into 2 phialides, 3 branched on one metula. β-phialides 12.8–
23.9 × 1.8–2.7 μm (x = 18.3 × 2.2 μm, n = 90), hyaline, smooth, solitary, growing from hyphae, narrow slender to
narrow lageniform, with or without metula at the base. α-conidia 1.9–2.6 μm (x = 2.3 μm, n = 90) diam, globose,
catenate, occurring in the conidial mass on the middle of synnemata, one-celled, smooth-walled, pale yellow

Figure 4. Polycephalomyces aurantiacus MFLUCC 17-2113. (a–c) Upper side of the culture. (d–f) Reverse
side of the culture. (g,o) β-phialides. (h–l) Synnemata growing on PDA medium. (m) β-phialides with hyphae.
(n) α-conidia. (p) β-conidia. Scale Bars: h = 1000 μm, i = 2000 μm, l = 500 μm, g, k, m = 50 μm,
α = 20 μm, n, p = 5 μm.
slimy in mass. β-conidia 3.1–3.9 × 1.6–2.1 μm (μ = 3.5 × 1.8 μm, n = 90) fusiform, and produced on the surface mycelium of colony or on the branch of the synnemata, one-celled, smooth-walled, hyaline.

**Material examined:** THAILAND, Chiang Mai, The Mushroom Research Center. On dead Cossidae larvae (Lepidoptera), 11 June 2017, Yuan Pin Xiao, MRC170611 (MFLU 17-1582, holotype); CM48 (MFLU 17-1583

**Figure 5.** *Polycephalomyces marginaliradians* MFLU 17-1582. (a) Habitat. (b,d) Overview of the host and stromata. (c) Part of the stroma. (e,f) Stroma. (g) Cross sections showing the immersed perithecia. (h) Perithecia. (j–l) Asci. (m) Part of the ascospores. (n) Apical cap. (o) Secondary ascospores. (p,q) Synnemata. (r) Phialide. (s) Conidia. Scale Bars: b–d = 1 cm, e, f = 2000 μm, g = 1000 μm, h = 500 μm, p, q = 200 μm, j–l = 100 μm, i = 50 μm, m, r = 10 μm, n, o, s = 5 μm.
MFLU 17-1584, HKAS100694, paratypes); ex-type living cultures, MFLUCC 17-2276, MFLUCC 17-2277, MFLUCC 17-2278, KUMCC 17-0258, KUMCC 17-0259.

Discussion
Studies based on morphology and DNA sequence analyses have provided insights into the phylogeny of *Polycephalomyces* to resolve generic delimitation. Species of this genus are commonly known to exhibit a parasitic mode of life on insects and other fungi. Our fungal diversity studies on entomophagous fungi have led to the discovery of two species, new to science, which we accommodate in *Polycephalomyces*. Molecular data also reveals that our new genus belongs to the family Ophiocordycipitaceae as circumscribed by Matočec et al.12. Species which exist in their sexual state display morphs such as fertile, capitulate, globose, tuberiform to pulvinate stromata and immersed, elongated pyriform perithecia while the asexual morphs occur as branched or unbranched.
synnemata, ending up with clavate to spherically flared, hymeniform aggregations of conidiophores, and produce large masses of conidia united in collective globular mucus. To date, six species, including *P. ramosus*, *P. sinensis*, and *P. agaricus* are considered as parasites of entomogenous fungi, while six species are recorded as entomogenous. Some species such as *P. lianzhouensis* and *P. yunnanensis* colonise both entomogenous fungi and insects. Because of their economic importance, species of this genus have been the subject for various research. The most recent new species introduced is *P. yunnanensis* and multigene phylogeny reveals a close relationship to *P. formosus*, *P. ramosopulvinatus* and *P. sinensis* based on 5-loci (nrSSU, nrLSU, tef-1α, rpb1 and rpb2) phylogenetic analyses.

Our taxonomic investigations herein reveal two new species of *Polycephalomyces*, *P. aurantiacus* and *P. marginaliradians*. Our morphological examination suggests that both species fit clearly within the generic concept of *Polycephalomyces* and both produce two types of conidia. However, they exhibit different mode of life and there are sufficient morphological differences that can justify their segregation into two species. These two new species similar to *P. agaricus*, *P. formosus*, *P. ponerae*, *P. sinensis*, *P. ramosus*, and *P. yunnanensis* have produce two types of conidia, while *P. ditmarrii*, *P. lianzhouensis*, *P. paludosus* and *P. tomentosus* have only one type of conidia. *Polycephalomyces aurantiacus* and *P. marginaliradians* have two types of phialides, while *P. formosus* and *P. sinensis* have only one type of phialide. *Polycephalomyces ponerae* also differs from *P. aurantiacus* and *P. marginaliradians* by producing *Akanthomyces*-like β-phialides and parasitic on ant (*Ponera Latreille*). *Polycephalomyces aurantiacus* differs from *P. aurantiacus* and *P. marginaliradians* by producing *Akanthomyces*-like β-phialides and parasitic on ant (*Ponera Latreille*).

Phylogeny based on our concatenated dataset recovered also support that our two new species belong to *Polycephalomyces* and are distinct from each other (Fig. 1). A close relationship is observed between the two species, but both constitute independent and strongly supported monophyletic subclades indicative of two phylogenetically distinct species. To further compare our two species, we delved in pairwise nucleotide sequence comparison and noted sufficient differences to justify them as independent taxa. ITS pairwise nucleotide sequence comparison between *P. aurantiacus* and *P. marginaliradians* revealed striking differences in 15 base pairs that justifies that both are different from each other and hence can be considered as two distinct species. There are also 6, 21, 4, 15 and 13 differences in the nrSSU, nrLSU, tef-1α, rpb1, and rpb2 DNA sequence data respectively. Two species not considered in our phylogenetic sampling are *P. ditmarrii* and *P. paludosus* due to the unavailability of sequence data. However these two are different from our new species with respect to one type of conidia occurring on its natural substrate and under cultural conditions. The hosts from which our new species have been recovered are also different. *Polycephalomyces ponerae* was not considered in our phylogenetic sampling as the DNA (ITS) sequence is too short, ambiguous and did not align well with other species. However, *Polycephalomyces ponerae* is morphologically different from our new species with respect to *Akanthomyces*-like β-phialides and parasitic on ant (*Ponera Latreille*). Further morphological differences among species are detailed in Tables S2 and S3.

Our multigene phylogeny derived herein also provides robust and well-resolved intergeneric relationships between *Polycephalomyces* and *Ophiocordycipes*. Members of both genera are clearly distinct from each other and we managed to successfully identify and sequence *Ophiocordycipes barnesi*, the host from which *P. aurantiacus* was isolated. Further interspecies taxonomic relationships are also elucidated in our molecular phylogeny. All *Polycephalomyces* species currently analysed constitute a strongly supported monophyletic lineage (Fig. 1), which corroborates with previous taxonomic schemes. In particular, a robust relationship in observed between *P. onorei* and *P. agaricus* sharing *P. yunnanensis* as sister taxa. These three species are also markedly different in terms of morphological characters. *Polycephalomyces yunnanensis* is clearly distinct from *P. onorei* and *P. agaricus* in terms of being parasitic on *Ophiocordycipes matsut* (*Pat.*). G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, longer synnemata, cylindrical to subulate α-phialides and subglobe or ellipsoidal α-conidia. *Polycephalomyces ramosopulvinatus* is closely related to *P. lianzhouensis*, but each species is positioned in different well-supported subclades and hence merit species status. *Polycephalomyces ramosopulvinatus* is also different from *P. lianzhouensis* with respect to being parasitic on *Ophiocordycipes matsut*. This species is positioned in different well-supported subclades and hence merit species status. *Polycephalomyces ramosopulvinatus* is also different from *P. lianzhouensis* with respect to being parasitic on nymph of Cicada and characterised by a long stipe and pseudo-immersed, pyriform perithecia. While phylogeny resolves our new species into well-segregated subclades, we note that relationships of *P. formosus*, *P. tomentosus*, *P. ramosus* and *P. sinensis* are still obscure and the concatenated dataset used herein did not provide adequate species resolution. A similar phylogenetic scenario is observed for *P. nipponicus* and *P. kanshiansus*. Whether these species are conspecific warrants further taxonomic investigations. The latter two species do share some morphological resemblances to *P. marginaliradians* especially with respect to the yellow cylindrical stipe with capitate lateral fertile part (known from their sexual morph). However, *P. marginaliradians* differs in having a capitate stromata with stipe, smaller perithecia and parasitic on Cossidae, while *P. nipponicus* and *P. kanshiansus* have polycephalous stromata and parasitic on Cicadidae. Meanwhile, *P. onorei* and *P. ramosopulvinatus* are distinct from *P. marginaliradians* by producing bigger perithecia and parasitic on caterpillar (*Arctinae*) and nymph of Cicada respectively. Phylogenies retrieved herein also support them as separate taxonomic entities.

Key to the species of the genus *Polycephalomyces*
1. Synnemata arising from fungi or insect or culture. 2

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1. Stromata arising from insect……………………………13
2. Two types of conidia absent in nature or culture……………………………3
3. One type of conidia absent in nature and culture……………………………10
4. Synnemata agaric-shaped……………………………..P. aromaticus
5. Synnemata other shaped……………………………..
3. Stromata other shaped…………………..
4. Two types of phialides absent……………………………..5
5. Only one type of phialides absent……………………………..9
6. α-conidia globose to subglobose (1.4–3.2 × 1.2–2.2) μm……………………………..6
7. β-phialides Akanthomyces-like, inflated at base, slenderneck at top……………………………..P. ponerae
8. β-phialides lanceolate or narrowly lageniform or subulate……………………………..7
9. β-phialides lanceolate or narrowly lageniform, 22.9–64.2 μm length……………………………..P. aurantiacus
10. β-phialides narrowly lageniform or subulate, 7–30 μm length……………………………..8
11. α-conidia subglobose, not catenate, β-conidia fusiform, catenate……………………………..P. marginaliradians
12. α-conidia globose, catenate, β-conidia fusiform, not catenate……………………………..P. yunnanensis
13. Phialides lanceolate or narrowly lageniform, 12.5–66 μm length……………………………..P. sinensis
14. Phialides cylindrical, subulate, 10–15 μm length……………………………..P. formosus
15. Host is insect……………………………..11
16. Host is myxomycetes……………………………..P. tomentosus
17. Conidia globose to subglobose or cylindrical……………………………..12
18. Conidia globose, covered by a mucus, agglutinating……………………………..P. paludosus
19. Conidia globose to subglobose, 2.2–3.4 × 1.3–1.6 μm……………………………..P. ditmarii
20. Conidia subglobose to cylindrical, 5–7 × 1.3–1.6 μm……………………………..P. lianzhouensis
21. Host is Cicada or nymph of Cicada……………………………..14
22. Host is neither Cicada nor nymph of Cicada……………………………..16
23. Stipe less than 80 mm……………………………..15
24. Stipe more than 90 mm……………………………..P. ramosopolvinatus
25. Perithecia flask-shaped, 900–1050 × 270–300 μm…………………………….P. kanzashianus
26. Perithecia flask-shaped or ovoid, 800–950 × 300–370 μm…………………………….P. nipponicus
27. Stromata numerous……………………………..17
28. Stromata single……………………………..P. marginaliradians
29. Fertile part narrowly ovoid 355–473 × 158–197 μm……………………………..P. lianzhouensis
30. Fertile part pyriform, 854–950 × 330–395 μm……………………………..P. onorei

Materials and Methods
Collection, isolation, and morphology study. Four fresh specimens were collected from southern Thailand (Prachuap Khiri Khan Province), and two from northern Thailand in the soil. The specimens were noted and photographed in the field and transported to the laboratory individually in plastic boxes and stored at 4 °C until examined. Strains were isolated from single spore isolation from both stomata and synnemata following the protocol described in Chomnunti et al.36,37. PCR amplifications were conducted as outlined by Jeewon et al.30. Cultures were incubated at 18 °C for 14–25 days on potato extract agar (PDA) as outlined by Vijaykrishna et al.49 and Jeewon & Hyde44. Herbarium material is deposited at MFLU herbarium and Facesoffungi numbers and Index Fungorum numbers are provided as in Jayasiri and RPB1. BioEdit 41 was used to check alignment manually. Gaps were treated as missings.

DNA extraction, PCR amplification and determination of DNA sequences. DNA was extracted from both dried specimens and cultures by using E.Z.N.A.TM Fungal DNA MiniKit (Omega Biotech, CA, USA) according to the manufacturer’s protocols. The primers used in PCR amplification were (Table S4); ITS4/ITSS for internal transcribed spacer gene region (ITS)44, NS1/NS4 for partial small subunit ribosomal RNA gene region (SSU)45, LROR/LR5 for partial large subunit rDNA gene region (LSU)46. 983 F/2218 R for partial translation elongation factor 1-alpha gene region (TEF-1α)48, CRPB1A/RPB1Cr for partial RNA polymerase II largest subunit gene region (RPB1)49, RPB2-5F/RPB2-5R for partial RNA polymerase II second largest subunit gene region (RPB2)50. PCR amplifications were conducted as outlined by Jeewon et al.56,57 and PCR products were sequenced by GenScript Biotechnology Co., Nanjing, China.

Phylogenetic analyses. All reference sequences were obtained from GenBank based on previously published data (Table S1). MAFFT v.7.130 (http://mafft.cbrc.jp/alignment/server/) was used to align combined datasets of ITS, SSU, LSU, TEF-1α and RPB1. BioEdit 41 was used to check alignment manually. Gaps were treated as missing data. Two strains of Perenmicordyceps prolifica (Kobayasi) Matočec & I. Kušan, in Matočec et al.52 were selected as outgroup taxa.
ML trees were estimated by using the software RAxML 7.2.8 Black Box12,43 in the CIPRES Science Gateway platform44. MrModeltest v.2.3.45 was used to determine the best-fit model of evolution for Bayesian analyses. MrBayes v.3.1.246 was used to evaluate posterior probabilities (PP)47,48 by Markov Chain Monte Carlo sampling (BMCMC). Six simultaneous Markov chains were run for 2,000,000 generations and trees were sampled every 100th generation and 20,000 trees were obtained. The first 20% of trees were discarded, which representing the burn-in phase of the analyses, while the remaining trees were used for calculation posterior probabilities in the majority rule consensus tree (critical values for the topological convergence diagnostic is 0.01). Phylogenetic trees were also constructed based on parsimony analyses as detailed by Cai et al.49 and Jeewon et al.50,51.
figured in FigTree v1.4.0 program. Bayesian Posterior Probabilities (BYP) equal to or greater than 0.95 were given below each node (Fig. 1).

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Author Contributions
Y.P.X. and T.C.W. designed the study. Y.P.X. and F.Y.L. conducted all the experiments. Y.P.X., S.H., J.J.L., S.B., D.N., R.J. and K.D.H. analysed the result. Y.P.X., S.H., R.J., J.J.L., S.B., D.N., T.C.W. and K.D.H. edited the manuscript. All authors reviewed the manuscript and approved the manuscript for publication.

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