Comprehensive Review of *Tolypocladium* and Description of a Novel Lineage from Southwest China

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Abstract: *Tolypocladium*, a diverse genus of fungicolous fungi belonging to Ophiocordycipitaceae, includes saprotrophic soil inhabitants, plant endophytes and pathogens of insects, nematodes, rotifers, and parasites of truffle-like fungi. Here, we review the research progress achieved for *Tolypocladium* regarding its taxonomy, species diversity, geographic distribution, host affiliations and ecological diversity. Furthermore, an undescribed taxon from China was established using morphology and multi-gene phylogeny. *Tolypocladium inusitaticapitatum* is introduced as a new species parasitizing ectomycorrhizal *Elaphomyces* species. It is diagnosed by its irregularly enlarged fertile heads and lemon, yellow-to-dark-brown, smooth and nearly cylindrical stipe. Phylogenetic analyses based on SSU, LSU, ITS, TEFα and RPB2 sequence data showed *T. inusitaticapitatum* to be an independent lineage separated from *T. flavonigrum* in the clade comprising *T. capitatum*, *T. fractum* and *T. longisegmentatum*. A key for identifying the sexual *Tolypocladium* species is also provided.

Keywords: new taxon; diversity; ecology; host shift; multi-gene; mycoparasite; taxonomic key

1. Introduction

Fungal species establish antagonistic to mutualistic associations with numerous prokaryotes and eukaryotes, including bacteria, algae, animals, plants and other fungi [1]. More than 1500 fungicolous taxa are widely distributed in aquatic and terrestrial ecosystems from tropical to polar regions [1]. Their hosts are ecologically diverse across the fungal kingdom. Truffle-like fungi are hypogeous and taxonomically distributed in Ascomycota and Basidiomycota [2]. Some truffle-like fungi were reported to be hosts of fungicolous species belonging to *Absidia* Tiegh., *Battarrina* (Sacc.) Clem. and Shear, *Entoloma* P. Kumm., *Hypocrea* Fr., *Hypomyces* (Fr.) Tul. and C. Tul., *Hypoxylon* Bull., *Melanospora* Corda, *Sporothrix* Hektoen and C.F. Perkins, and *Tolypocladium* W. Gams [1,3].

*Tolypocladium* W. Gams was established based on three soil-inhabiting asexual species: *Tolypocladium cylindrosporum* W. Gams, *T. geodes* W. Gams and *T. inflatum* W. Gams (the type species) [4]. Hodge and colleagues linked the asexual *T. inflatum* to the sexual species...
Cordyceps subsessilis Petch [5]. Subsequently, Sung and colleagues introduced the sexual genus Elaphocordyceps G.H. Sung and Spatafora and linked it to the asexual Tolypocladium and some species within Verticillium Nees based on multigene phylogeny [6]. Moreover, Sung and colleagues transferred the species of Cordyceps sensu lato that parasitize ectomycorrhizal Elaphomyces (18 species and two forma), cicada nymphs (C. inegoënsis Kobayasi, C. paradoxa Kobayasi, and C. toriharamontana Kobayasi) and beetle larvae (C. subsessilis) to Elaphocordyceps [6]. Chaunopycnis was established by Gams to accommodate Ch. alba, which resembles Tolypocladium in conidiogenesis [7]. Later, Quandt and colleagues synonymized Chaunopycnis and Elaphocordyceps under Tolypocladium, following the “One Fungus One Name” rule, as Tolypocladium is much more widely known, medicinally important and an older genus [4,6–8].

Most Tolypocladium species are Elaphomyces-attacking mycoparasites, except for few entomopathogens [9,10]. The evolution of host specificity and the dynamics of host jumping were investigated by several researchers using molecular data [6,8,11–15]. Nikoh and Fukatsu inferred that there was a shift from entomoparasitism to mycoparasitism during the evolution of the Cordyceps-like fungi [11]. However, with the addition of more gene regions and taxa, insect pathogens such as T. paradoxum and T. inflatum were found to be clustered with some parasites on truffles. The researchers explained that the ancestral ecology was a truffle parasitism, with multiple switches to insect pathogenicity [6,8,12]. Notably, the interspecific relationships of closely related Tolypocladium species are weakly supported and inconsistently resolved with different datasets [6,8,13,14]. To compensate for the shortage of limited loci, Quandt and colleagues performed genome-scale phylogenetic analyses based on two entomopathogens (T. ophioglossoides and T. capitatum) and two mycoparasites (T. inflatum and T. paradoxum) and demonstrated that truffle parasites form a monophyletic clade. They suggest that this lineage is derived as a result of a single ecological transition or host-jumping from insects to fungi [15].

A successful infection caused by fungal pathogens generally undergoes host recognition, attachment, and then infection and degradation, depending on the gene content, expression, or regulation [16]. Tolypocladium is recognized as an ideal candidate for investigating the mechanisms associated with host-jumping [15,16]. Quandt and colleagues researched the set of genes that are differentially regulated in Tolypocladium species during their first encounter with their hosts [16]. They found that PTH11-related G-protein-coupled receptors (GPCRs), predicted to be involved in host recognition, were up-regulated in T. ophioglossoides when grown on media containing insect cuticles [16]. Furthermore, a divergent chitinase and an adhesin gene, Mad1, were significantly up-regulated on media containing Elaphomyces [16]. According to the transcriptomic data, genes involved in redox reactions and transmembrane transport were the most overrepresented during T. ophioglossoides growth on Elaphomyces media. However, the genes involved in secondary metabolism may not be necessary for the parasitism of truffles as their products are only highly expressed during the growth on insect tissues [16].

To date, Tolypocladium comprises 41 species (Table 1) with a cosmopolitan distribution [2,17]. Some of them produce various secondary metabolites, such as cyclosporin, efaapeptins, ophiocordin and ophiosetin [18]. They have been widely used in biopharmaceuticals and biocontrol [18]. During an investigation of fungi in Yunnan Province, Southwest China, an undescribed Tolypocladium species was discovered on Elaphomyces sp. The present study aimed to (i) systematically review species diversity, hosts/habitat, geographical distribution and host affiliations of Tolypocladium species, (ii) broaden the knowledge of species diversity and host shifts in Tolypocladium species, (iii) refine the diagnostic characters of the interspecific classification of Tolypocladium in sexual morphs and provide a taxonomic key.
| Fungal Name | Hosts/Isolated From | Known Distribution |
|-------------|---------------------|-------------------|
| T. album    | Sapwood of Hevea brasiliensis | Colombia, France, Scotland, Sri Lanka, Sweden, The Netherlands [7], Peru [12] |
| T. amazonense | Sapwood and leaf tissue of Hevea brasiliensis and H. guianensis | Peru [12] |
| *T. capitatum | Elaphomyces granulatus, E. japonicus, Elaphomyces sp. | Asia (China (Taiwan, Yunnan), Japan), Europe (France, Holland, Hungary), North America (Canada, U.S.A.) [9,10,19–22] |
| T. cylindrosporum | Soil, sewage, peat, roots of Pinus mariana, Plecia neotricula, larvae of Aedes siarrensis, larvae of Aedes australis, larvae and pupae of Lucilia sericata, Drosophila larvae (Diptera) | Brazil, China, Czech, England, New Zealand, Nepal, The Netherlands, The North Island, U.S.A. [4,23–27] |
| *T. deliciostipitatum | E. ashtimontanus | China (Jiangsu) [28], Japan [10] |
| *T. dauxiolumiae | Cicada nymphs | China (Anhui, Fujian, Jiangsu, Jiangxi, Zhejiang) [29] |
| T. endophyticum | Living sapwood of Hevea brasiliensis and H. guianensis | Brazil, Mexico, Peru [13] |
| T. exiguus | Larvae of Arachnocampa luminosa (Diptera) | New Zealand [24] |
| *T. fractum | E. appalachiensis | U.S.A. (Tennessee) [9] |
| *T. flavovirens | Elaphomyces sp. | Thailand [30] |
| *T. fumaceum | Cocooned pupa of bagworm moth (Psychidae) buried among mosses | Poland [31] |
| T. geodes | Soil | Austria, Canada, China, Denmark, England, The Netherlands [4,23–26] |
| *T. guangdongense | Elaphomyces sp. | China (Guangdong) [32] |
| *T. inegaense | Cicada nymphs (e.g., Hyalosa musulaticollis) | China (Fujian, Taiwan) [33], Japan [34], Korea [6] |
| *T. inflatum | Larvae of Scaranicidae (e.g., Aphidionide, Rutelinae) (sexual morph); soil, humus, Picea glauca, roots of Pop. mariana, surface of Mycobates sp. (Acar, Murcotidae), sclerotium of Opophorus granulis (sexual morph) | Sexual morph: Japan, U.S.A. (Tennessee, North Carolina, Michigan, New York, Washington) [5]; asexual morph: Austria, Canada, China, Nepal, Germany, U.S.A. [4,23–26,35] |
| *T. intermedium | E. grunulatus, E. subarachigatus | Japan, U.S.A. (New York) [10,36] |
| *T. japonicum | E. granulatus, E. japonicus, E. neasperulus | Austria, Japan [10], China (Guizhou, Taiwan) [28,37] |
| *T. jezoense | E. anatrichinus, E. miyabeanus, E. nopporensis | Japan [10] |
| T. ligificola | Rotting wood (parasitic in bdelloid rotifers) | Canada (Ontario) [38] |
| *T. longisegmentatum | E. granulatus, E. japonicus, E. muricatus, Elaphomyces sp. | Asia (China (Jilin), Japan), Europe (England, Germany, Holland), North America (Canada, Mexico, U.S.A.) [9,10,20,21,39] |
| T. microsporum | Soil | Canada, Germany, The Netherlands, U.S.A. [23] |
| *T. minacukusense | Elaphomyces sp. | Japan [40] |
| *T. mimotanum | Elaphomyces sp. | Japan [40] |
| T. nubicola | Soil | Canada (Alberta), China (Guizhou) [23,41] |
| *T. ophioglossoides | E. grunulatus, E. japonicus, E. muricatus, E. shimizuisinis, E. tibitissinis, and Elaphomyces sp. | Commonly in Asia (e.g., China (Guangxi, Jiangsu, Jiangxi, Jilin, Shandong, Sichuan, Taiwan, Yunnan), Japan, Korea), Europe and North America [9,10,42–44] |
| T. ovuliporum | Lichen Polycauliona regulis | Antarctica (King George Island) [45] |
| *T. paradoxum | Cicada nymphs (e.g., Platypus leaeyfeneri, Cryptopinula nigrorosula) | China (Hainan, Yunnan) [46], Japan, Korea [34,47] |
| T. pustulatum | Soil, twigs in oak forest, and living leaf of Kalma latifolia | Mexico (Nuevo Leon), Spain (Cádiz), U.S.A. (New Jersey) [48] |
| *T. ramulorum | Elaphomyces sp. | China (Anhui, Fujian, Gansu, Guangdong) [44,49,50] |
| *T. rautii | E. variegatus | France [51] |
| T. sinense | Stroma and sclerotium of Opophorus sinensis | China (Yunnan) [52] |
| *T. sarmoense | E. granulatus | China (Yunnan) [53] |
| *T. tenuisporum | Host not found (probably Elaphomyces sp.) | U.S.A. (Pennsylvania) [9] |
| T. terricola | Soil | Finland [54] |
| *T. teretihumatum | Cicada nymph (Auritibicen thoracatus) | Japan [34] |
| T. trigonosporum | Rotting stump (parasitic on bdelloid rotifers) | Canada (Nova Scotia) [55] |
| T. tropicale | Sapwood and leaf tissue of Hevea brasiliensis | Mexico, Peru [12] |
| T. tundrense | Soil | Canada (Northwest Territories) [23] |
| *T. valleformae | E. grunulatus, Elaphomyces sp. | Canada (Ontario), U.S.A. (Carolina, New York, Virginia) [9] |
| *T. valdiviostipitatum | E. granulatus, E. neasperulus | Japan [10] |
| *T. virices | Elaphomyces sp. | Japan [56] |

* indicates sexual morphs (25 species).

Table 1. Species diversity, hosts/habitats and geographic distribution of Tolypocladium species.

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2. Results

2.1. Phylogenetic Placement

The combined SSU, LSU, ITS, TEF1-α and RPB2 sequence dataset comprised 35 species, containing 5384 nt (SSU: 1–1536, LSU: 1537–2441, ITS: 2442–3306, TEF1-α: 3307–4264, RPB2: 4265–5384) after the alignment (including gaps). Among them, 3731 bp (base pairs) were conserved, 378 variable, parsimony-uninformative, and 1275 parsimony-informative. The ML and BI analyses resulted in phylogenetic trees with a similar topology. The ML tree with a final log-likelihood of $-27186.604$ is shown in Figure 1. Specimens HKAS 112152 and HKAS 112153 clustered together and formed a distinct clade with strong support values (SH-aLRT = 100, UFB = 100 and BIPP = 1), indicating a conspecific relationship. These two specimens separated from other Tolypocladium species with SH-aLRT = 90.2 and BIPP = 0.98 support values. However, their LSU sequences showed an 11 bp difference (1.28%) across the 862 bp region, contributing to the different branch lengths in the phylogenetic tree. Based on the available molecular data for Tolypocladium species, some differences are known to occur due to intraspecific variations in the LSU sequences, ranging from 0.25 to 1.28% (Table 2).

![Figure 1](image-url)

Table 2. Intraspecific base-pair differences in LSU genes among Tolypocladium species.

| Species                  | Locus     | 522 | 532 | 855 | Ratio        |
|--------------------------|-----------|-----|-----|-----|--------------|
| T. album                 | CBS 39349 | C   | C   | C   | 0.35% (3/870 bp) |
| T. inflatum              | OSC 71235 | A   | G   | A   | 0.76% (6/794 bp) |
| T. ophioglossoides       | CBS 100239| C   | T   | C   | 0.25% (2/816 bp) |
| T. paradoxum             | NBRC 100958| T   | C   | G   | 0.90% (8/891 bp) |
| T. inusitaticapitatum    | HKAS 112152| T   | A   | A   | 1.28% (11/862 bp) |

The locus numbers refer to the base-pair positions of the gene sequences, and “#” represents the reference sequences. Gaps are indicated with “-”.

Specimens Tolypocladium inusitaticapitatum (China), together with four Tolypocladium species occurring on Elaphomyces spp., i.e., T. capitatum (intercontinental distribution), T. flavonigrum (Thailand), T. fractum (USA) and T. longisegmentatum (intercontinental distribution), formed a monophyletic clade with weak support (SH-aLRT = 81.1, UFB = 82 and BIPP = 0.90. UFB values not shown in the ML tree). Tolypocladium inusitaticapitatum formed a separate clade sister to T. flavonigrum. However, the nucleotide comparison between T. inusitaticapitatum (holotype: HKAS 112152) and T. flavonigrum (holotype: BCC 66576) showed 154 bp (26.78%) differences across 575 bp ITS, 87 bp (9.83%) differences across 885 bp LSU, and 47 bp (4.99%) differences across 942 bp TEF1-α (including gaps), respectively. The phylogenetic evidence suggested that these two specimens represent new species.

2.2. Taxonomy

Tolypocladium W. Gams, Persoonia 6: 185 (1971); emended by Quandt and colleagues, IMA Fungus 5: 125 (2014).

Index Fungorum number: IF10242; Facesoffungi number: FoF 10425.

Synonyms: Chaunopycnis W. Gams, Persoonia 11: 75 (1980).

Elaphocordyceps G.H. Sung and Spatafora, Stud. Mycol. 57: 36 (2007).

Type species: Tolypocladium inflatum W. Gams 1971.
Figure 1. Maximum likelihood (ML) tree of *Tolypocladium inusitaticapitatum* and its allies within *Ophiocordycipitaceae* inferred from combined SSU, LSU, ITS, TEF-1-α and RPB2 dataset. Bootstrap support values for ML ≥ 80 of SH-aLRT or 95 of UFB and posterior probability for BI ≥ 0.90 are indicated above the nodes and separated by ‘/-/-/’ (SH-aLRT/UFB/BIPP). Specimens of the current study are given in red. Type specimens are in bold and the superscript ‘ex’ indicates ex-type.

**Morphological characterization:** Sexual morph: Stromata arise directly from the host and are sometimes indirectly connected to the host through rhizomorph-like structures. They range from solitary to several and can be simple or branched. Stipe is fibrous to tough, rarely fleshy, dark-brownish to greenish with an olivaceous tint, rarely whitish, cylindrical and enlarges near the fertile part. The fertile part is clavate- to capitulate-shaped.
and varies in color. *Perithecia* are partially to completely immersed, or superficial, or produced on a highly reduced stromatic pad, and ostiolate. *Asci* are unitunicate and long cylindrical with a thickened apical cap. *Ascospores* are filiform, approximately as long as asci, multi-septate, typically disarticulate into part-spores, and are occasionally non-disarticulating when mature (e.g., *T. ramosum*). *Part-spores* are hyaline, fusiform to cylindrical with round to truncate ends [6,8]. *Asexual morph*: They are *Tolypocladium-, Chaunopycnis-, or Verticillium-like*. Colonies are white, cottony and grow slowly on artificial media (e.g., potato dextrose agar, Czapek-Dox agar, malt extract agar, Sabouraud Glucose agar and water agar). *Conidiophores* usually are short and bear lateral or terminal phialides whorls. *Phialides* usually are swollen at the base and thin, often with bent necks. *Conidia* are globose to oval, one-celled, hyaline, smooth, and aggregative in small heads at the tips of the phialides [4,23].

**Hosts and habits**: Found in terrestrial and humid environments. Species of *Tolypocladium* parasitize hypogeous *Elaphomyces* (20 species including the novel species described in this study), cicada nymphs (4 species), beetle larvae (*T. inflatum*), pupa of the bagworm moth (*T. fumosum*), mosquito larvae (*T. extinguens*), and even bdelloid rotifers exposed to air (*T. lignicola* and *T. trigonosporum*). Their ascospores/conidia and mycelia survive in soil, or on various humus, rotting wood, plant tissues and surfaces, body surfaces of insects and mites, tissues of *Cordyceps* and lichens (Table 1).

**Species diversity and distribution**: *Tolypocladium* currently consists of 42 species (including the novel species described in this study) distributed worldwide [2,3,17]. Seventeen species were recorded from China (Table 1).

### 2.3. Description of the Novel Species

*Tolypocladium inusitaticapitatum* F.M. Yu, Q. Zhao and K.D. Hyde, sp. nov. Figure 2. Index Fungorum: IF558123; Facesoffungi number: FoF 10407.

**Typification**: China, Yunnan Province, Lijiang City, Lijiang Alpine Botanic Garden, E100°10′58.07, N26°59′58.35, alt. 3338 m, 5 Oct 2019, Jian-Wei Liu (HKAS 112152, holotype).

**Etymology**: The specific epithet ‘inusitaticapitatum’ is derived from the combination of two Latin words, 1) adjective inusitata (strange, odd) and 2) noun capitatum (head), pointing to the fertile head, which is irregularly expanded.

**GenBank accession numbers**: ITS = MW 537735, LSU = MW 537718, SSU = MW 537733, TEF1-α = MW 507527, RPB2 = MW 507529.

**Description**: Asexual morph *Stromata* 9–11.5 cm high, solitary and simple, arising directly from the fruiting bodies of *Elaphomyces* sp. *Stipe* yellow at base, olive-brown to dark brown at the middle part, and yellowish brown at the terminal part. They are 7.5–11.5 cm long and 7–8.5 mm thick in the widest parts and nearly cylindrical, but the middle part is slightly thicker than the basal and upper parts. The *fertile part* developed from the terminal of the stipe, and is somewhat ellipsoidal, irregularly barrel-shaped, and sometimes slightly compressed, 1.5–2.0 cm × 1.5–2.0 cm. The surface is decorated with white ascospores released from the mature perithecia, which is olive yellow when immature, and olive to dark brown when mature. The outer layer becomes cracked and the olive internal texture is exposed. Structure of cortex of *fertile part*: composed of olive brown pseudoparenchymatous tissue and an ectal layer. *Perithecia* 580–720 µm × 180–270 µm (x = 650 µm × 220 µm, n = 10), crowded, entirely immersed, obovoid, ellipsoidal to pyriform. *Ostioles* papillate, and are visible (protruding up to 55 µm in high) or invisible, lined with periphyses. *Asci* is 410–510 µm × 10–15 µm (x = 461 µm × 13 µm, n = 20), hyaline, and long cylindrical, with a conspicuously thickened cap (measuring 6.5–7.5 µm × 6.0–7.0 µm). *Ascospores* are approximately as long as ascii, and extremely easy to break into part-spores. *Part-spores* 20–32 µm × 3.0–4.5 µm (x = 25 µm × 3.6 µm, n = 20), hyaline, cylindrical with rounded ends. Asexual morph: Unknown.
Host and habitat: Directly arising from the fruiting bodies of hypogeous Elaphomyces sp. (Elaphomycetaceae, Eurotiales), in a humid and evergreen broad-leaved rainforest (Lijiang Alpine Botanic Garden), Lijiang, Yunnan Province, P.R. China. As serious degradation has occurred, truffle-like Elaphomyces sp. could not show any morphological evidence of taxonomic significance. Based on the ITS sequence dataset, the phylogenetic analyses showed that the host of T. inusitaticapitatum clustered together with Elaphomyces fuscus M. Shirakawa (Japan) and formed a sister group. However, there are sufficient molecular differences between the host from HKAS 112152 (ITS = MW 513695) and E. fuscus F-a170629 (ITS = LC 500967) to consider them as distinct species.

Known distribution: P.R. China (Yunnan).

Other specimen examined: CHINA, Yunnan, Lijiang, Lijiang Alpine Botanic Garden, alt. 3338 m, 5 October 2019, Jian-Wei Liu (HKAS 112153).
Notes: Based on the multi-gene phylogeny results, our specimens are closely related to *Tolypocladium flavonigrum*, known only from Thailand. Both species have stromata directly emerging from the surface of *Elaphomyces* sp., and capitulate fertile heads with the perithecia entirely immersed in a well-differentiated valliform-like structure [30]. However, *T. inusitaticapitatum* considerably differs from *T. flavonigrum* for the olive, yellowish-brown to dark brown fertile part, and is yellow to yellowish-brown at both ends of the stipe compared to the yellow–black to black stromata in *T. flavonigrum*. *Tolypocladium inusitaticapitatum* produces obovoid, ellipsoidal to pyriform perithecia, which are markedly distinguished from the elongate-ovoid perithecium produced by *T. flavonigrum*. Asci and part-spores of *T. inusitaticapitatum* (410–510 µm × 10–15 µm, 20–32 µm × 3.0–4.5 µm) are larger than those of *T. flavonigrum* (318–330–416(–482) µm × 7–8 µm, 2–5 µm × 1.5–2 µm) [30].

When comparing *Tolypocladium inusitaticapitatum* with its other phylogenetic relatives (*T. capitatum*, *T. fractum* and *T. longisegmentatum*), differences were found. *Tolypocladium capitatum* differs from *T. inusitaticapitatum* mainly due to its larger perithecia (900–1100 µm × 340–430 µm) and slimmer part-spores (2.5–3 µm wide) [10]. *Tolypocladium fractum* differs from *T. inusitaticapitatum* by having smaller stromata (1.5–2.5 cm long) and asci (300–480 µm × 5–6 µm) [10]. *Tolypocladium longisegmentatum* is distinguished from *T. inusitaticapitatum* by its longer stipe (13 cm long when fresh and up to 11 cm long when dried) and longer part-spores ((12–)40–65 µm) [20]. Morphologically, *T. inusitaticapitatum* is similar to *T. intermedium* for the yellow to dark brown stipe but differs in its smaller asci and shorter part-spores (main differences are outlined in Table 3). Regrettably, the molecular data of *T. intermedium* is not available in GenBank.

Table 3. Main differences between *T. intermedium* and *T. inusitaticapitatum.*

|                | *T. intermedium* [10]                                      | *T. inusitaticapitatum* (This Study)                                                        |
|----------------|-------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| Fertile part   | Dark reddish brown                                          | Olive brown, yellowish-brown to dark brown                                                 |
| Stipe          | Slender, 6–8.5 cm long and 2–4 mm thick, middle part clearly expanded, surface with many longitudinal grooves, upper part squamulose | Thicker, 7.5–11.5 cm long and 7–8.5 mm thick, middle part indistinctly expanded, surface smooth |
| Asci           | 240–300 µm × 7–8 µm, caps about 5 µm in diameter            | 410–510 µm × 10–15 µm, caps 6.5–7.5 µm × 6.2–7.0 µm                                        |
| Part-spores    | Short, 3–6 (commonly 4.5) µm × 1.5–2 µm, truncated at two ends (shape) | Long, 20–32 µm × 3.0–4.5 µm, cylindrical with rounded ends                                |
| Distribution   | Japan, USA                                                  | P.R. China (Yunnan)                                                                        |

3. Discussion

*Tolypocladium*, a generalist genus, has been reported to have diverse lifestyles on a wide range of hosts and environments, including soil, insects, plants, lichens and hypogaeal fungi [6,8]. The current pattern of host affiliation of *Tolypocladium* fungi is inferred to be an evolutionary product of intra- and inter-kingdom host shifts [57]. In the last two decades, researchers aimed to infer the evolution of host affiliation within the *Tolypocladium*, either using a handful of gene loci from dozens to hundreds of taxa, or using genome-scale data from fewer taxa [11,12,15,58]. To date, the studies on the host-jumping of *Tolypocladium* have been performed with multigene phylogeny (seven genes from 202 taxa of *Hypocreales*) [12] and genome-scale phylogeny (1350 genes from 20 taxa of *Hypocreales*) [15]. The multigene phylogenies supported three hypotheses for *Tolypocladium*, as follows: (1) the ancestral hosts were fungi (false truffles) [11,12,57,58]; (2) there were multiple switches to insect pathogenesis from a mycorrhizal ancestor [8,12,13]; (3) the endophytic lineage has arisen with the contact of plant hosts via mycorrhizal associations or plant-associated insects [12]. However, these conclusions, made from multigene phylogenies, conflict with those made from genome-scale phylogenies, which suggested a single ecological transition from insects to fungi within *Tolypocladium* [15]. Our phylogenetic tree, inferred from five genes of 35 species (Figure 1), resulted in consistent conclusions, similar to those from
previous multigene phylogenies. Similarly, we encountered several problems, such as phylogenetic conflicts among genetic data partitions and moderate to low support values for some important nodes [8,12,13]. Although whole-genome data provide insights that can further resolve the phylogenetic relationships of *Tolypocladium* [15,59,60], it is still unknown whether those conclusions will be limited by the few available species.

In this study, a novel *Tolypocladium* species occurring on *Elaphomyces* sp. is known from its sexual morph. A taxonomic key is also provided for 26 *Tolypocladium* species. The shape of the fertile part, the connection between the stipe and host, the structure of the cortex of the fertile part, size of part-spores and host affiliation are thought to be characteristic of taxonomic significance for interspecific identification [8–10]. However, there are 16 species whose sexual morphs are still unknown. In addition, the phylogenetic relationships among *Tolypocladium* species are very sensitive to taxa sampling and loci information [8,15]. Further studies should focus on obtaining more samples from different geographic regions and/or ecological niches, sequencing more markers and even genomic data, building a more robust phylogenetic relationship, and establishing their sexual-axenial morph connections. (Table 4).

### Table 4. Key to Sexual Morphs of *Tolypocladium* species.

| Key | Description | *Tolypocladium* species |
|-----|-------------|-------------------------|
| 1.  | Host insects | 2                        |
| 1'. | Host hypogeous *Elaphomyces* spp. | 7                       |
| 2.  | Host beetle or moth larvae | 3                       |
| 2'. | Host cicada nymphs | 4                       |
| 3.  | Fertile part capitata, with stellate appearance; perithecia ovoid to pear-shaped, 740–760 × 444–558 µm | *T. fumosum* |
| 4.  | Fertile part, strap-shaped pseudostalk; perithecia superficial, narrow flask-shaped, 1000–1500 × 330–440 µm | *T. inflatum* |
| 5.  | Stromata arising from underground mycelial membrane or strand; part-spores 3–5 × 1.5–2 µm | *T. paradoxon* |
| 6.  | Stromata arising directly from host | 5                       |
| 7.  | Fertile part elongated, obpyriform; part-spores 1.5–2.5 × 1.5–1.7 µm wide | *T. toriharamontanum* |
| 8.  | Fertile part oblong or clavate | 6                       |
| 9.  | Perithecia superficial or apparently half-immersed, pyriform, 520–550 × 260–280 µm; part-spores 2.5–3 × 2 µm | *T. inegoense* |
| 10. | Perithecia wholly immersed, ampullaceous, (233–)520–740 (–780) × (250–)300–330 (–360) µm; part-spores 3–5 (–7.0) × 2–3 µm | *T. dajiaolongae* |
| 11. | Stromata attached to host by rhizomorphs | 7                       |
| 12. | Stromata arising directly from the host | 8                       |
| 13. | Part-spores articulate, moniliform, 3–3.5 × 2–2.5 µm | 9                       |
| 14. | Part-spores with truncate or rounded ends | 10                      |
| 15. | Stromata capitate | 11                      |
| 16. | Stromata solitary or rarely caespitose | 12                      |
| 17. | Perithecia small, 480–580 × 225–255 µm; part-spores large-sized, 18–28 × 3–5 µm | *T. deliastistipitatum* |
| 18. | Perithecia 770–800 × 350–430 µm; part-spores medium-sized, 8–11 × 1.5–2 µm | *T. miomotecianum* |
| 19. | Perithecia oblong with long neck, 700–720 × 200–250 µm; part-spores long, 20–30(50) × 3–4.5 µm | *T. jzoneae* |
| 20. | Perithecia ovoid, 550–600 × 200–300 µm; part-spores small short rod-shaped, 2.5–5 × 1.5–2 µm | *T. ophioglossoides* |
| 21. | Perithecia superficial, ascospores nonfructified | 14                      |
| 22. | Perithecia entirely embedded or ostiole slightly projecting | 19                      |
| 23. | Fertile part, cortex composed of pseudoparenchymatous peridial layer, and with an ectal layer | 24                      |
| 24. | Fertile part, composed of pseudoparenchymatous peridial layer, but without ectal layer | 25                      |
| 25. | Stromata clavate; perithecia narrowly ovoid, 750–1000 × 250–300 µm; part-spores cylindric, 6–8 × 1–1.5 µm | *T. tenaisporum* |
| 26. | Stromata capitata | 15                      |
| 27. | Part-spores, larger-sized, more than 20 µm long | 16                      |
| 28. | Part-spores, less than 20 µm long | 17                      |
| 29. | Part-spores (12–40)–65 × (3–14–5 µm | 18                      |
| 30. | Part-spores 20–32 × 3.0–4.5 µm | 19                      |
| 31. | Part-spores, medium-sized, (13–)16(–21) × 2.5–3 µm | 20                      |
| 32. | Part-spores, small-sized, 2.5–6 µm long | 21                      |
| 33. | Perithecia elongate-ovoid, (560–590–697–750) × (200–206–248–250) µm; part-spores 2.5–1.5 × 2 µm | *T. flavonigrum* |
| 34. | Perithecia ovoid, 450–540 µm × 230–260 µm; part-spores 3–6 (commonly 4.5) × 1.5–2 µm | *T. intermedium* |
| 35. | Stromata clavate | 22                      |
| 36. | Stromata capitata | 23                      |
| 37. | Part-spores, 245–495 µm long, deeply embedded; asci short, 195–270 µm long | 24                      |
| 38. | Perithecia 500–550 µm long, ostiola slightly projecting; asci 330–370 µm long | 25                      |
| 39. | Perithecia large, more than 900 µm long | 26                      |
| 40. | Perithecia medium-sized, 400–700 µm long | 27                      |
| 41. | Perithecia ovoid, 900–1100 × 340–430 µm; part-spores cylindric or somewhat fusoid, 18–27 (commonly 24) × 2.5–3 µm | *T. capitatum* |
| 42. | Perithecia ampullaceous, 900–930 × 220–250 µm; part-spores fusoid, 16–18 × 3 µm | *T. minazukiense* |
| 43. | Stipe slender, less than 1.0 mm thick | 28                      |
| 44. | Stipe thick, columnar, 1.0–6.0 mm thick | 29                      |
| 45. | Perithecia 500–600 × 250–350 µm; part-spores 2.5–1.5 × 2 µm | *T. tenuisporum* |
| 46. | Perithecia 400 × 250 µm; part-spores 6 × 1.5 µm | *T. fumosum* |
| 47. | Asci 10–12 µm wide; part-spores medium-sized, 7.5–16 × 2.5–3 µm | *T. inegoense* |
| 48. | Asci slender, 6–8 µm wide; part-spores small-sized, 3–8 × 2 µm | *T. vulgariforme* |
4. Materials and Methods

4.1. Collections and Morphology

*Tolypocladium* specimens, including their underground host *Elaphomyces* sp., were collected in an evergreen broad-leaved forest in Lijiang Alpine Botanic Garden, Lijiang City, Yunnan Province, China. The specimens were examined as described in Senanayake and colleagues with the following modifications [61]. Colour codes were recorded following those of Kornerup and Wanscher [62]. Specimens were deposited at the Herbarium of Cryptogams Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China (HKAS, KUN).

4.2. DNA Extraction, PCR Amplification and Sequencing

The genomic DNA was extracted from the dried materials following the method described by Dissanayake and colleagues [63]. Fertile tissues from the parasitic fungi and the peridium of the host mushroom were used to extract DNA. Primer pairs ITS1F/ITS4 [64], LR0R/LR5 [65,66], PNS1/NS8 [64], TEF1-α 983F/TEF1-α 2218R [67] and iRPB2-5F/iRPB2-7R [68] were used for the amplification of the internal transcribed spacer region ITS1-5.8S-ITS2 (ITS), the large subunit rDNA (LSU), the small subunit rDNA (SSU), the translation elongation factor 1-α (TEF1-α) gene and RNA polymerase II second-largest subunit (*RPB2*), respectively. PCR reaction was performed in a 25 µL reaction volume, comprising 12.5 µL Taq PCR Master Mix (Abmgood, Richmond, BC, Canada), 1 µL forward primer, 1 µL reverse primer, 2 µL DNA template and 8.5 µL ddH2O. For ITS, LSU, SSU and *RPB2*, PCR reaction conditions were as follows: 5 min at 94 ºC, followed by 35 cycles of 40 s at 94 ºC, 40 s at 53 ºC and 1 min at 72 ºC, and a final extension of 10 min at 72 ºC. PCR reaction condition of TEF1-α was as follows: 5 min at 94 ºC, followed by 35 cycles of 50 s at 94 ºC, 40 s at 64 ºC and 1 min at 72 ºC, and a final extension of 10 min at 72 ºC. The PCR products were visualized using agarose gel electrophoresis after staining with dyes (TS-GelRed Ver.2, Tsingke Biotechnology Co., Ltd., Beijing, China). Then, the products were sent for sequencing at Sangon Biotech Co. Ltd., Shanghai, China.

4.3. Sequence Alignment and Phylogenetic Analyses

Phylogenetic trees were constructed using the sequencing data of *T. inusitaticapitatum* and the allied reference sequences of closely related *Ophiocordycipitaceae* species obtained from the GenBank (Table 5). *Aschersonia confluens* (BCC 7961) and *A. paraphysata* (BCC 1467) of *Clavicipitaceae* were used as outgroup taxa. All sequences were assembled and aligned using MAFFT v 6.8 [69] and manually edited where necessary in BioEdit version 7.0.9 [70]. Individual alignments were compiled for SSU, LSU, ITS, TEF1-α and *RPB2* genes. The optimal substitution model for each gene dataset was determined using MrModeltest 2.3 [71] under the Akaike information criterion (AIC). The results indicated that the GTR+I+G model was optimal for all the gene regions. Individual datasets were combined to assemble the combined dataset (gene order: SSU, LSU, ITS, TEF1-α and *RPB2*). The resulted combined dataset was deposited in the TreeBASE database (http://purl.org/phylo/treebase/phylows/study/TB2:S27887?x-access-code=746eddc746009259527edd3d4c69526b&format=html, accessed on 10 March 2021).
### Table 5. Voucher information and GenBank accession numbers for samples appearing in the *Tolypocladium* phylogenetic tree.

| Taxon                          | Strain/Specimen Voucher | ITS 28S | 18S | TEF1-α | RPB2 |
|-------------------------------|-------------------------|--------|-----|--------|------|
| **Aschersonia confluens**     | BCC 7961                | JN049841 | DQ384947 | DQ372100 | DQ384976 | DQ452645 |
| **A. paraphysata**            | BCC 1467                | JN049822 | AF393522 | AF395727 | AF490616 | DQ452463 |
| **Drechmeria gunnii**         | OSC 76404               | MH866230 | AF393945 | AF395994 | DQ522243 | DQ452246 |
| **D. sinensis**               | CBS 567.95              | MH866129 | AF393840 | AF395890 | AF490602 | DQ490109 |
| **D. zeospora**               | CBS 582.80              | MH865280 | AF394920 | AF490287 | EF468571 | EF468912 |
| **Ophiocordyceps gracilis**  | EFCC 8572               | EF468811 | EF469569 | EF468751 | EF468972 | EF468914 |
| **O. heteropoda**             | EFCC 10125              | EF468812 | EF469572 | EF468752 | EF468971 | EF468940 |
| **Paecilomyces lilacinus**    | CBS 831.87              | AY624188 | EF468944 | EF468791 | EF468972 | EF468941 |
| **Pa. paecilimyces**          | CBS 284.36              | AY624189 | FR775484 | EF468792 | EF468941 |
| **Perennicordyceps cuboidea**| NBRC 10174              | JN943331 | JN941417 | JN941724 | KF049684 |
| **Pe. confluens**             | NBRC 100941             | JN943329 | JN941416 | JN941725 | KF049684 |
| **Pe. paraphysata**           | NBRC 10174              | JN943338 | JN941431 | JN941710 | KF049685 | KF049669 |
| **Pe. paracordyceps**         | NBRC 100942             | JN943337 | JN941430 | JN941711 | AB972954 | AB972958 |
| **Poecilomyces lateritius**   | CBS 1682                | KF049660 | KF049632 | KF049612 | KF049687 | KF049670 |
| **Polycephalomyces aurantiacus** | MFLUCC 17-2113       | MG136919 | MG136913 | MG136907 | MG136877 | MG136875 |
| **Po. marginaliradians**      | MFLUCC 17-1582          | MG136916 | MG136910 | MG136904 | MG136875 | MG136870 |
| **Po. margaritilimyces**      | MFLUCC 17-2276          | MG136920 | MG136914 | MG136908 | MG136875 | MG271931 |
| **Po. nigromargaritilimyces** | NBRC 101406             | JN943301 | JN941388 | JN941735 | KF049684 | KF049694 |
| **Po. yunnanensis**           | OSC 76404               | JN943338 | JN941430 | JN941711 | AB972954 | AB972958 |
| **Tolypocladium amazonense**  | TNS-F-18481             | KF779849 | KF779849 | KF779875 | KF778750 | KF778784 |
| **T. capitatum**              | CBS 106325              | KF747267 | KF747329 |
| **T. cylindrosporum**         | NBRC 10097              | MG228381 |
| **T. endophyticum**           | MM385 | KF747260 | KF747153 | KF747322 |
| **T. flavonigrum**            | BCC 50942 | MN338090 | MN337228 |
| **T. fumosum**                | YHCC16005               | KF779874 | KF779849 | KF779875 | KF778750 | KF778784 |
| **T. geodes**                 | CBS 126045              | KF747329 |
| **T. inflatum**               | CBS 127302              | MG228387 |
| **T. jezoense**               | MX535 | KF747260 | KF747153 | KF747322 |
| **T. ophioglossoides**        | BCC 567.84              | KF747260 | KF747153 | KF747322 |
| **T. pustulatum**             | CBS 568.84              | KF747260 | KF747153 | KF747322 |
| **T. tropicale**              | BCC 66578               | KF747260 | KF747153 | KF747322 |
| **T. valliforme**             | DAOM 196368             | KF747260 | KF747153 | KF747322 |

New sequencing data are displayed in bold. Specimens of the current study are given in red. Type specimens are in bold; superscript 'ex' indicates ex-type.

Maximum likelihood (ML) analysis was performed using IQ-Tree (http://iqtree.cibiv.univie.ac.at/, accessed on 20 May 2021) [72,73]. The substitution model options for each gene were auto-evaluated according to the provided partition file. Clade support for the ML analysis was assessed using an SH-aLRT test with 1000 replicates [74] and the ultrafast bootstrap (UFB) [75]. In the ML analyses, nodes with support values of SH-aLRT ≥ 80 and UFB ≥ 95 were considered well-supported, those with either SH-aLRT < 80 or UFB < 95 were considered weakly supported, and nodes with SH-aLRT < 80 and UFB < 95 were considered unsupported.

Bayesian Inference (BI) analysis was carried out in MrBayes v3.2.6 [76]. Gaps were treated as missing data. Four simultaneous Markov Chain Monte Carlo (MCMC) chains were run for 10,000,000 generations and were sampled at every 100th generation until the standard deviation of the split frequencies fell below 0.01 and ESS values > 200. Subsequently, phylogenetic trees were summarized and posterior probabilities (PP) were
calculated using MCMC by discarding the first 25% generations as the burn-in phase [77]. Phylogenetic trees were viewed in FigTree v.1.4.4. Nodes with BI posterior probability (BIPP) > 0.90 were considered to be well supported.

Author Contributions: This study was initiated by F.-M.Y. and K.D.H. Samples were collected by J.-W.L. Morphological observation and description were done by F.-M.Y., K.D.H., K.W.T.C., D.-P.W., S.-M.T., J.-W.L. and L.L., and phylogeny analyses were done by F.-M.Y., K.W.T.C. and Q.Z. The manuscript was mainly drafted by F.-M.Y. with contributions from all other authors. All authors have read and agreed to the published version of the manuscript.

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References
1. Sun, J.-Z.; Liu, X.-Z.; McKenzie, E.H.; Jeewon, R.; Liu, J.-K.; Zhang, X.-L.; Zhao, Q.; Hyde, K.D. Fungicolous fungi: Terminology, diversity, distribution, evolution, and species checklist. Fungal Divers. 2019, 95, 337–430. [CrossRef]
2. Wijayawardene, N.N.; Hyde, K.D.; Al-Ani, L.K.T.; Tedersoo, L.; Haelewaters, D.; Rajeshkumar, K.C.; Zhao, R.L.; Aptroot, A.; Leontyev, D.V.; Saxena, R.K.; et al. Outline of Fungi and fungus-like taxa. Mycosphere 2020, 11, 1060–1456. [CrossRef]
3. Hyde, K.D.; Norphanphoun, C.; Maharachchikumbura, S.S.N.; Bhat, D.J.; Jones, E.B.G.; Bundhun, D.; Chen, Y.J.; Bao, D.F.; Boonmee, S.; Calabon, M.S.; et al. Refined families of Sordariomycetes. Mycosphere 2020, 11, 305–1059. [CrossRef]
4. Gams, W. Tolypocladium, eine Hyphomycetengattung mit geschwollenen Phialiden. Persoonia 1971, 6, 185–191.
5. Hodge, K.T.; Krasnoff, S.B.; Humber, R.A. Tolypocladium inflatum is the anamorph of Cordyceps subsessilis. Mycologia 1996, 88, 715–719. [CrossRef]
6. Sung, G.H.; Hywel-Jones, N.L.; Sung, J.M.; Luangsa-ard, J.J.; Shrestha, B.; Spatafora, J.W. Phylogenetic classification of Cordyceps and the clavicipitaceous fungi. Stud. Mycol. 2007, 57, 5–59. [CrossRef] [PubMed]
7. Gams, W. Chaunopycnis alba, gen. et sp. nov., a soil fungus intermediate between Moniliaceae and Sphaeropsidales. Persoonia 1980, 11, 75–79.
8. Quandt, C.A.; Kepler, R.M.; Gams, W.; Araújo, J.P.M.; Ban, S.; Evans, H.C.; Hughes, D.; Humber, R.; Hywel-Jones, N.; Li, Z.; et al. Phylogenetic-based nomenclatural proposals for Ophiocordycipitaceae (Hypocreales) with new combinations in Tolypocladium. IMA Fungus 2014, 5, 121–134. [CrossRef]
9. Mains, E.B. Species of Cordyceps parasitic on Elaphomyces. Bull. Torrey Bot. Club 1957, 84, 243–251. [CrossRef]
10. Kobayasi, Y.; Shimizu, D. Monographic studies of Cordyceps 1, group parasitic on Elaphomyces. Bull. Natl. Sci. Mus. Tokyo 1960, 5, 69–85.
11. Nikoh, N.; Fukatsu, T. Interkingdom host jumping underground: Phylogenetic analysis of entomoparasitic fungi of the genus Cordyceps. Mol. Biol. Evol. 2000, 17, 629–638. [CrossRef]
12. Gazis, R.; Skaltsas, D.; Chaverri, P. Novel endophytic lineages of Tolypocladium provide new insights into the ecology and evolution of Cordyceps-like fungi. Mycologia 2014, 106, 1090–1105. [CrossRef] [PubMed]
13. Sung, G.H.; Sung, J.M.; Hywel-Jones, N.L.; Spatafora, J.W. A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): Identification of localized incongruence using a combinational bootstrap approach. *Mol. Phylogen. Evol.* 2007, 44, 1204–1223. [CrossRef] [PubMed]

14. Kepler, R.; Ban, S.; Nakagiri, A.; Bischoff, J.; Hywel-Jones, N.; Owensby, C.A.; Spatafora, J.W. The phylogenetic placement of hypocrealean insect pathogens in the genus *Polycephalomyces*: An application of One Fungus One Name. *Fungal Biol.* 2013, 117, 611–622. [CrossRef]

15. Quandt, C.A.; Patterson, W.; Spatafora, J.W. Harnessing the power of phylogenomics to disentangle the directionality and signatures of interkingdom host jumping in the parasitic fungal genus *Tolypocladium*. *Mycologia* 2018, 110, 104–117. [CrossRef]

16. Wijayawardene, N.N.; Hyde, K.D.; Rajeshkumar, K.C.; Hawksworth, D.L.; Madrid, H.; Kirk, P.M.; Braun, U.; Singh, R.V.; Crous, P.W.; Kukwa, M.; et al. Notes for genera: Ascomycota. *Fungal Divers.* 2017, 86, 1–594. [CrossRef]

17. Wang, J.-C.; Zhang, Z.-Z.; Li, C.-L.; Wang, Y. Research Progress of *Tolypocladium* in Ophiocordycipitaceae. *J. Fungal Res.* 2019, 1–10. [CrossRef]

18. Maas Geesteranus, R.A. On ‘Cordyceps capitata’. *Persoonia* 1963, 2, 477–482.

19. Ginz, J. Typification of *Cordyceps canadensis* and *C. capitata*, and a new species, *C. longisegmentis*. *Mycologia* 1988, 80, 217–222. [CrossRef]

20. Zeng, X.-L.; Yang, W.-S. *Cordyceps canadensis*. *Bot. Stud.* 2015, 240–384. [CrossRef] [PubMed]

21. Wright, D.A.; Cummings, N.J.; Haack, N.A.; Jackson, T.A. *Tolypocladium cylindrosporum*, a novel pathogen for sheep blowflies. *New Zeal. J. Agric. Res.* 2009, 52, 315–321. [CrossRef]

22. Xu, W.-S.; LYU, G.-Z.; Jiang, H.; Zhao, Z.-H.; Sun, X.-D.; LYU, S.-Y. Three species of *Tolypocladium* isolated from forest soil of Changbai mountain. *J. Fungal Res.* 2012, 10, 143–146.

23. Montalva, C.; Silva, J.J.; Rocha, L.F.N.; Luz, C.; Humber, R.A. Characterization of *Tolypocladium cylindrosporum* (Hypocreales, Ophiocordycipitaceae) isolates from Brazil and their efficacy against *Aedes aegypti* (Diptera, Culicidae). *J. Appl. Microbiol.* 2019, 126, 266–276. [CrossRef] [PubMed]

24. Liang, Z.-Q.; Liu, A.-Y.; Liu, M.-H. Two new records of mycogenous *Cordyceps* in China. *Mycosystema* 2003, 22, 159–160.

25. Li, C.; Hywel-jones, N.; Cao, Y.; Nam, S.; Li, Z. *Tolypocladium dujaoalonge* sp. nov. and its allies. *Mycotaxon* 2018, 133, 229–241. [CrossRef]

26. Crous, P.W.; Cowan, D.A.; Yilmaz, N.; Larsson, E.; Angelini, C.; Brandrud, T.E.; Dearnaley, J.D.W.; Dima, B.; Dovana, F.; Fechner, N.; et al. Fungal Planet description sheets: 1112–1181. [CrossRef] [PubMed]

27. Xu, W.-S.; LYU, G.-Z.; Jiang, H.; Zhao, Z.-H.; Sun, X.-D.; LYU, S.-Y. Three species of *Tolypocladium* isolated from forest soil of Changbai mountain. *J. Fungal Res.* 2012, 10, 143–146.

28. Liang, Z.-Q. *Flora Fungorum Sinicorum*. Cordyceps; Science Press: Beijing, China, 2007; Volume 32.

29. Kobayasi, Y.; Shimizu, D. Monographic studies of *Cordyceps* 2, group parasitic on cicadae. *Bull. Natl. Sci. Mus. Tokyo* 1963, 6, 286–314.

30. Suo, F.-Y.; Huang, L.-D.; Yu, H. Identification and antibacterial effect research of a *Tolypocladium* strain isolated from sclerotium of *Ophiocordyceps gracilis* in Xinjiang. *China J. Chin. Mater. Med.* 2014, 39, 965–971.

31. Imai, S. On a new species of *Cordyceps* parasitic on Elaphomyces in Japan. *Proc. Imp. Acad.* 1934, 10, 677–679. [CrossRef]

32. Ke, Y.-H.; Ju, Y.-M. Two rare ophiocordycipitaceous fungi newly recorded in Taiwan. *Bot. Stud.* 2015, 56. [CrossRef]

33. Barron, G.L. Structure and biology of a new *Tolypocladium* attacking bdelloid rotifers. *Can. J. Bot.* 1983, 61, 2566–2569. [CrossRef]

34. Zeng, X.-L.; Yang, W.-S. *Cordyceps canadensis* a new record in China. *Edible Fungi China* 1990, 9, 27.

35. Kobayasi, Y.; Shimizu, D. *Cordyceps* species from Japan 5. *Bull. Natl. Sci. Mus. Tokyo Ser. B Bot.* 1982, 8, 111–123.

36. Han, Y.-F.; Liang, Z.-Q.; Chu, H.-L. *Tolypocladium nubicola*, a new record of *Tolypocladium* in China. *J. Fungal Res.* 2004, 2, 50–52.

37. Sung, J.M.; Choi, Y.S.; Kim, Y.O.; Kim, S.H.; Sung, G.H. Cordyceps species collected by Korean entomopathogenic fungal collection. In Proceedings of the Third Korean-China Joint Symposium for Mycology (The Korean Society of Mycology and Chinese Academy of Sciences), Seoul, Korea, December 1997; The Korean Society of Mycology: Seoul, Korea, 1997; pp. 49–60.

38. Lee, T.S.; Yoon, K.H. *The Index of Korea-Japan Mushroom Names in Korea*; Personal Printing: Seoul, Korea, 2002.

39. Song, B.; Lin, Q.-Y.; Li, T.-H.; Shen, Y.-H.; Li, J.-J.; Luo, D.-X. Known species of *Cordyceps* from China and their distribution. *J. Fungal Res.* 2006, 4, 10–26.

40. Möller, C.; Gams, W. Two new hynomycetes isolated from Antarctic lichens. *Mycotaxon* 1993, 48, 441–450.

41. Zha, L.-S.; Xiao, Y.-P.; Jeeow, R.; Zou, X.; Wang, X.; Boonme, S.; Eungwanichayapant, P.D.; McKenzie, E.H.C.; Hyde, K.D.; Wen, T.-C. Notes on the medicinal mushroom chanhua (*Cordyceps cicadae* (Miq.) Massee). *Chiang Mai J. Sci.* 2019, 46, 1023–1035.
