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Contributed Paper

Notes on the Medicinal Mushroom Chanhua (*Cordyceps cicadae* (Miq.) Massee)

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Abstract

Chanhua fungus is an important Traditional Chinese Medicine and has a history of use for about 1,500 years. Chanhua has commonly been referred to *Cordyceps cicadae* S.Z. Shing, *Isaria cicadae*, *I. sinclairii*, *Ophiocordyceps sobolifera* and by other names. This has resulted in taxonomic confusion and nomenclatural problems. The biology, ecology and host affiliation of Chanhua are also poorly understood, and these restrict further development and application in various areas, especially in traditional medicine. In this paper, we reviewed the medicinal history and distribution of Chanhua and reinvestigated its phylogenetic relationships with allied species. Based on results obtained we clarified its taxonomy and reviewed its host and its biological and ecological aspects. The phylogeny based on ITS sequence data indicates that Chanhua is an independent species of the genus *Cordyceps*. Although Chanhua, *I. cicadae* and *I. sinclairii* have generally been accepted as the same species, the latter two lack DNA sequence data from their type localities. *C. cicadae* S.Z. Shing (syn. *C. zhejiangensis*), which has been treated as the sexual morph of Chanhua for over 40 years, has been reported to be *Tolypocladium paradoxum*, and the sexual morph of Chanhua is considered to be *C. kobayasii* (syn. *C. cicadae-sm*). We propose to use the scientific name *C. cicadae* (Miq.) Massee, together with the Chinese common name Chanhua, to describe or record this important medicinal mushroom.

Keywords: biology, distribution, *Isaria cicadae*, *Isaria sinclairii*, phylogeny, taxonomy

1. INTRODUCTION

Chanhua fungus like other cordyceps (*Cordyceps* sensu lato) species [1] are used in Traditional Chinese Medicines as they are believed to have special pharmaceutical efficacy and high nutritional value [2-4]. As an important member of cordyceps, Chanhua has been extensively studied, second only to *Ophiocordyceps sinensis* (Berk.) G.H. Sung et al. and *Cordyceps militaris* (L.) Fr.. Because of its high demand, Chanhua has become scarce in nature, and this demand is being supplemented by artificial culture.

In the pharmaceutical industry and Traditional Chinese Medicine fields, a correct identification for source material is essential, as an incorrect scientific name may lead to legal disputes and ineffective conservation [5]. Because of historical reasons and taxonomic problems, the use of the scientific name of Chanhua has been controversial [4]. *C. cicadae* S.Z. Shing and *Isaria cicadae* Miq. have been the most frequently used, other names viz. *C. cicadae* (Miq.) Massee, *I. sinclairii* (Berk.) Lloyd, *Paecilomyces cicadae* (Miq.) Samson and *O. sobolifera* (Hill ex Watson) G.H. Sung et al. are also used [1]. Besides this, host affiliation, ecological and biological aspects of Chanhua are poorly understood and there is a lack of documentation on these topics.

In this paper, we reviewed the medicinal history and distribution of Chanhua, reinvestigated its phylogenetic relationships with related species, clarified its taxonomic placement and provided insights into its host affiliation, ecological and biological aspects. Based on results obtained, existing published information and our practical investigations, we clarified some important aspects of this economically important medicinal mushroom.

2. MATERIALS AND METHODS

2.1 Specimens

Specimen collections and biological and ecological investigations were made in southern China. A Nikon Coolpix P520 camera, a Nikon Eclipse 80i compound microscope connected with a Cannon EOS 600D camera and an Optec SZ660 stereo dissecting microscope were used for morphologic observing and photographing. Measurements were made using Tarosoft (R) Image Frame Work software. Specimens and strains are deposited in the Herbarium of Guizhou Agricultural College (= Guizhou University, GACP), Institute of Fungus Resources, Guizhou University (GZUIFR) and the Herbarium of Cryptogams of Kunming Institute of Botany, Chinese Academy of Sciences (HKAS).

2.2 DNA Extraction and Sequencing, and Construction of Phylogenetic Tree

Total genomic DNA were extracted from specimens to confirm fungus identification, using E.Z.N.A.TM Fungal DNA MiniKit (Omega Biotech, CA, USA). Primers ITS4 and ITS5 were used to amplify and sequence the internal transcribed spacer (ITS) gene of ribosomal DNA (rDNA) [6]. PCR programs followed those of Agrawal et al. [7] and amplification reactions were performed in an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR products were purified using the Bioteke's Purification Kit (Bioteke Corporation, Beijing, China), and were sequenced using an ABI 3730 DNA analyzer and an ABI BigDye 3.1 terminator cycle sequencing kit (Sangon Co., Ltd., Shanghai, China). Blast searches were performed to reveal the closest matches in GenBank database for selection of taxa in the phylogenetic analysis. Clustalx1.81 [8], ContigExpress (Invitrogen, Carlsbad, CA, USA), Chromas230 (http://www.technelysium.com.au/chromas.html) and Mega6.06 [9] were used for sequence assembly and alignment, and alignments were checked visually and improved manually. Molecular phylogeny was constructed using ITS sequence dataset. Maximum likelihood (ML) analysis was performed using RAxML v. 8.2.8 employing a GTRGAMMA model of nucleotide substitution, other details are described in Jeewon *et al.* [10] and Hongsanan *et al.* [11].

3. RESULTS AND DISCUSSION

3.1 The Common Name and the Medicinal History

The word Chanhua (Figure 1) came from ancient Chinese culture. As a Traditional Chinese Medicine, Chanhua has a long history. The earliest record is in 'Lei Gong Pao Zhi Lun' (Lei's Treatise on Preparing Drugs) written by Xiao Lei (the Southern and Northern Dynasties, 5th century AD) who recorded it as a medicine, that is 300 years earlier than the first record of *O. sinensis* ('Worm in winter, herb in summer', 'Dongchong Xiacao') [1, 4]. Other early records include 'Yao Xing Lun' (Theory of Drugs) of Quan Zeng in the Sui and Tang Dynasties (7th century AD); 'Ben Cao Tu Jing' (Illustrated Pharmacopoeia) of Song Su, 'Xi Xi Zong Yu' (Yao's Comments on Everything) of Kuan Yao, 'Yi Bu Fang Wu Lüe Ji' (Notes on Useful Organisms) of Qi Song, 'Shen Ji Zong Lu' (General Records of Holy Universal Relief) of Ji Zhao, 'Xiao Er Yao Zheng Zhi Jue' (Craft of Medicinal Treatment for Children) of Xiao-Zhong Yan and 'Jing Shi Zheng Lei Bei Ji Ben Cao' (Classic Classified Materia Medica for Emergency) of Shen-Wei Tang in the Song Dynasty (960-1279 AD); 'Ben Cao Gang Mu' (Compendium of Materia Medica) of Shi-Zhen Li in the Ming Dynasty (1,596 AD) [4, 12, 13]. All these monographs recorded the medicinal value of Chanhua; among them Chanhua was described as 'flower of cicada', 'corolla cicada', 'with a horn on the head of a cicada shell, which looks like corollas', etc. [4, 13].

Chanhua means 'flower of cicada', because it grows on cicada nymphs and resembles flowers. According to the literal meaning and the Chinese ancient records above, Chanhua should only be *Isaria*-like fungi that grow on cicada nymphs. But in Japanese culture, Chanhua (Chen hua, Semihana) is a general name for cicadicolous fungi and occasionally used for *O. sobolifera* (≡ *C. sobolifera* (Hill ex Watson) Berk. & Broome); later, another two names 'Tsunozemi (horned cicada)' and 'Hanazemi (flowering cicada)' were applied to *O. sobolifera* and *I. sinclairii* respectively [14].

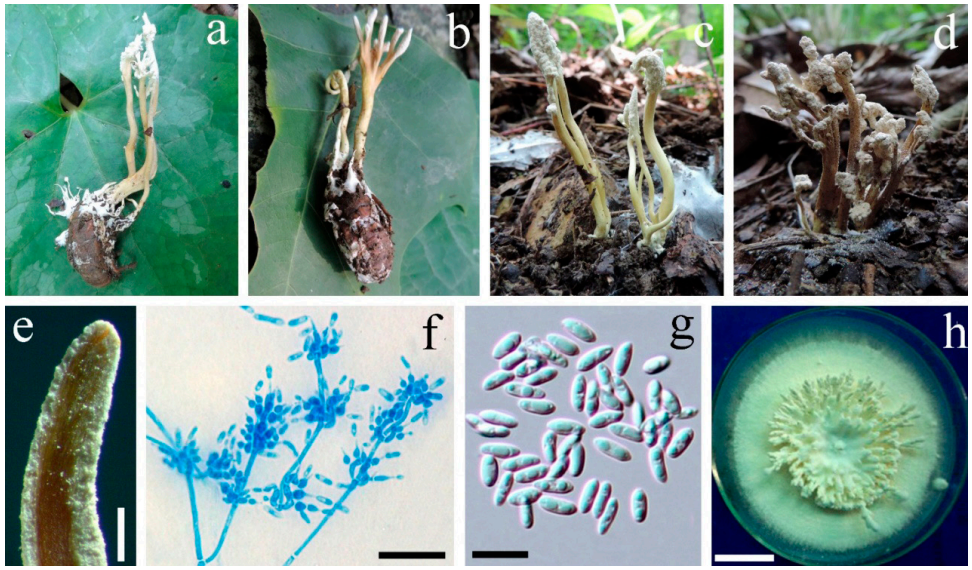

Figure 1. Chanhua (*Cordyceps cicadae* (Miq.) Massee). a-d Immature and mature synnemata (collected by Yuanpin Xiao and Chuangen Lin in June 2014 from Jiangxi, China; c, GACP 14061604). e Sectional view of the apex of a synnema. f Conidiophores and conidia cultured on PDA. g Conidia. h Culture on PDA at 25°C for one month (Gzuifr-06722). Scale bars: e = 0.5 mm, f = 20 μm, g = 10 μm, h = 2 cm.

Obviously, the understandings of Chanhua from Chinese and Japanese cultures are not the same, presumably because of the different interpretations of languages. For example, the 'horn' in the sentence 'with a horn on the head of a cicada shell, which looks like corollas' may easily be misunderstood as a fruiting body.

3.2 Phylogeny

The ITS sequences of suspected species of Chanhua available in GenBank database have been checked and analyzed, which comprises eighty-six sequences that include *I. cicadae*, *I. sinclairii*, *C. cidadae*, *P. cicadae* and our sequencing results for Chanhua (KX017276 (GACP 14061604 from Jiangxi) and KX017277 (GACP 07071701 from Sichuan, China)). Apart from two suspicious sequences (AJ536573 and AJ536574 from China, use names as *C. cidadae* but can not match any known species), the rest eighty-four ones, together with their closest matches, were used for sequence alignment and phylogenetic analysis. The ITS gene dataset comprised 680 characters (including gaps), of which 307 were variable and 230 were parsimony-informative. Using *Colletotrichum gloeosporioides* (Glomerellaceae, Glomerellales) as outgroup taxon, the ML tree was constructed (Figure 2).

Among the eighty-four suspected sequences of Chanhua: 1) seventy-eight from Asia (including sixty-three from China, five from Japan, two from Korea, six from Thailand and two from Taiwan) are identical (of them, only five have minor base insertions or gaps, which we think are errors during the process of sequence assembly), and in phylogenetic tree they form an sister group also have the same branch length; 2) HQ880826 from Koera and LT220698, LT220699 and LT220700 from Portugal are identical and represent a *C. cateniannulata*-like species; 3) KU933437 from USA represents an *O. sobolifera*-like species; and 4) KF696557 (*C. cicadae* S.Z. Shing) from China is actually *Tolypocladium paradoxum* (Kobayasi) Quandt et al. (Figure 2).

Undoubtedly, the seventy-eight ITS sequences from southern Asia represent Chanhua, and the molecular phylogenetic analysis strongly supports that Chanhua is one independent species of the genus *Cordyceps*. Chanhua is widely distributed in southern Asia (Figures 2, 3). *I. cicadae* was originally described from Brazil [15] and *I. sinclairii* from New Zealand [16], unfortunately there are no DNA sequence data from their type localities. Considering the different geographical locations (South America, New Zealand and Asia, Figure 3), there is insufficient evidence that Chanhua is *I. cicadae* and *I. sinclairii* is a synonym of *I. cicadae*.

3.3 Distribution

Chanhua, including *I. cicadae* and *I. sinclairii*, are distributed in southern Asia (China including Taiwan, India [17], Indonesia, Japan, Nepal [18], South Korea, Sri Lanka, Thailand and Vietnam [19]), Oceania (Australia and New Zealand), South America (Brazil, Guatemala and Mexico), North America (Canada) and Africa (Madagascar) [4, 14, 20, 21] (Figure 3).

In China, Chanhua is widely distributed in the Oriental Region of China (the southern regions of the Qinling Mountain and the Huaihe River), including Hainan, Guangdong, Yunnan, Guangxi, Taiwan, Fujian, Guizhou, Jiangxi, Hunan, Sichuan, Chongqing, Zhejiang, Hubei, Anhui (south of the Huaihe River), Shanghai, Shaanxi (south of the Qinling Mountain), Henan (south of the Huaihe River) and Jiangsu (south of the Huaihe River) provinces (from south to north) [4].

3.4 Taxonomy

Based on published reports, *C. cicadae* S.Z. Shing, *I. cicadae*, *I. sinclairii*, *O. sobolifera* had routinely been used as the scientific name of Chanhua. Based on a revised ITS based phylogeny done in this study and based on distribution of Chanhua above, all these suspected scientific names are reviewed and taxonomy of Chanhua is summarized as follows.

3.4.1 *Isaria cicadae* Miq.

Isaria cicadae was introduced by Miquel in 1838

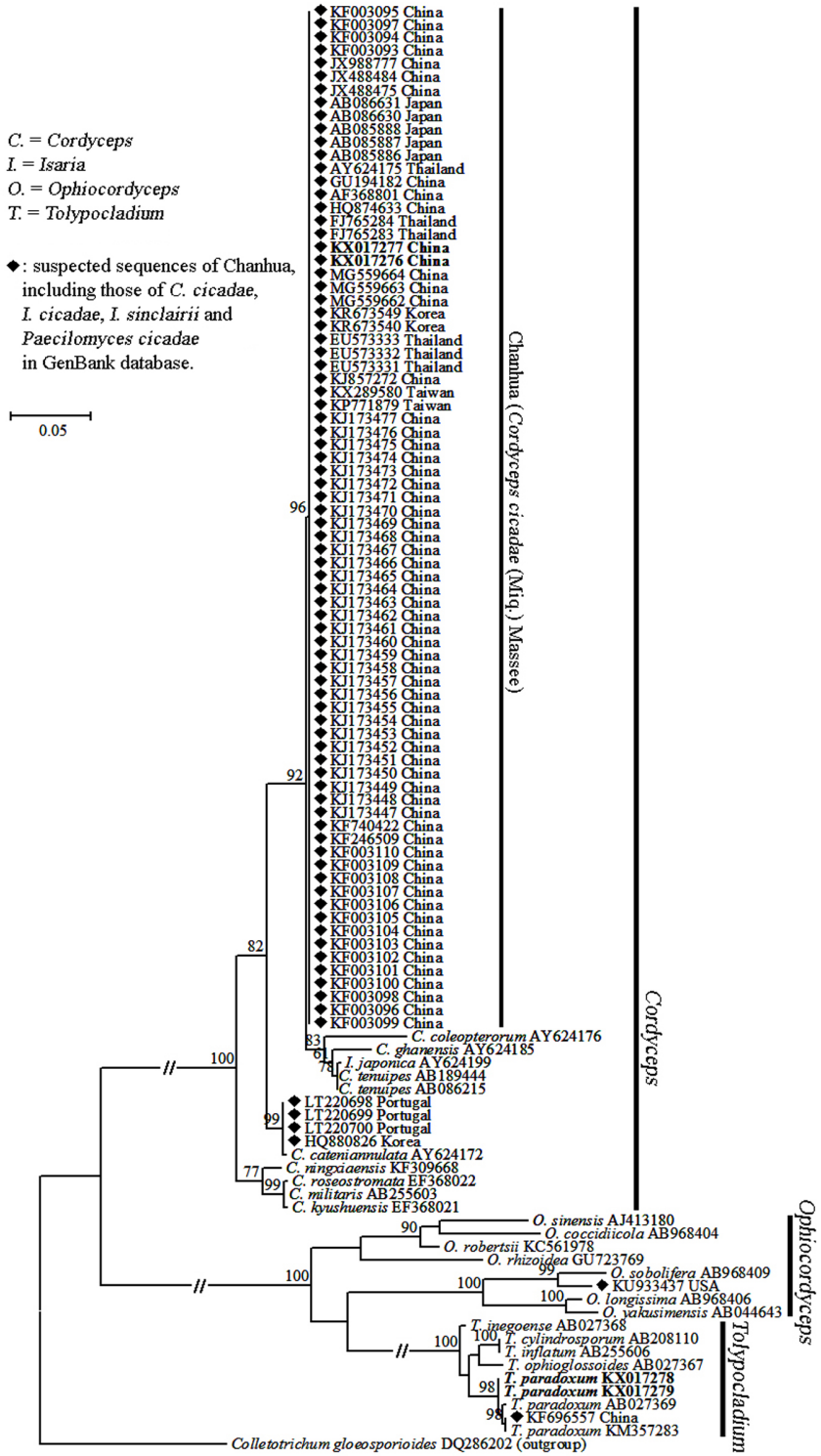

Figure 2. Maximum likelihood (ML) tree of suspected species of Chanhua (*Cordyceps cicadae* (Miq.) Massee) and their allies generated from ITS dataset. Bootstrap support values greater than 60% are indicated above the nodes. The sequences from this study are shown in blod.

Figure 3. Distribution of Chanhua (*Cordyceps cicadae* (Miq.) Massee) including *Isaria cicadae* and *I. sinclairii*. Every distributed country is marked with a red '★'. The original map was from: http://m.onegreen.net/maps/HTML/37132.html.

with type specimens from Brazil [15]. Massee synonymized it to *C. cicadae* (Miq.) Massee in 1895 [21]. Based on morphological evidence, Samson [21] recircumscribed the genus *Isaria* into the sect. *Isarioidea* of the genus *Paecilomyces* and transferred all *Isaria* species to *Paecilomyces*. *I. cicadae*/*C. cicadae* (Miq.) Massee was renamed as *P. cicadae*. Luangsa-ard et al. [22] analysed DNA sequence of *Paecilomyces* sensu lato and found the genus to be polyphyletic, which lead to the genus *Isaria* including the name *I. cicadae* being revived. Now, *I. cicadae* has generally been accepted as the scientific name of Chanhua, in spite of no DNA sequence data to support such a taxonomic arrangement.

Sexual morph: see *C. kobayasii* below.

3.4.2 *Isaria sinclairii* (Berk.) Lloyd

This species was originally described as *Sphaeria sinclairii* Berk. with types from New Zealand [16], followed by two revisions from *C. sinclairii* (Berk.) Sacc. to *I. sinclairii* [14, 21], and at last Samson [21] treated it as a synonym of *I. cicadae* based on morphological evidence. Last century in Japan, the name *I. cicadae* had never been used and Chanhua had always been recorded as *I. sinclairii* [14, 23]. Though *I. sinclairii* is commonly considered as a synonym of *I. cicadae*, they both lack DNA sequence data from their type localities.

Sexual morph: see *C. kobayasii* below.

3.4.3 *Cordyceps kobayasii* Koval and *Cordyceps cicadae-sm* A.Y. Liu & X. Zou, sexual morph of Chanhua

Kobayasi [24] described *C. sinclairii* Kobayasi and regarded it as the sexual morph of *I. sinclairii*, based on one immature specimen from Japan. Considering the same epithet with *C. sinclairii* (Berk.) Sacc., Koval [25] gave a new scientific name, *C. kobayasii*. Because *I. sinclairii* had already been treated as a synonym of *I. cicadae*, now *C. kobayasii* is generally considered as the sexual morph of *I. cicadae*.

Liu et al. [26] described the sexual morph of Chanhua, *C. cicadae-sm*, based on one specimen from Leye County, Guangxi, China (Figure 4a, b). Although being a sexual specimen, conidiophores are presented beside the perithecia, and at apices of conidiophores, numerous conidia are clustered together. Depending on microcycle sporulation of ascospores and isolated strains from conidiophores, Liu et al. confirmed that the compound specimen is Chanhua [26]. Though without molecular evidence, we believe the specimen is the sexual morph of Chanhua, which is also supported by Prof. Zongqi Liang who checked and deposited the specimen in Institute of Fungus Resources, College of Life Sciences, Guizhou University (GZUIFR 08721-6). Unfortunately, we could not extract DNA from the specimen because it has been preserved in solution. Subsequently, Liu [27] recorded the same collection in her monograph. Prof. Xingliang Wu provided a picture of a similar specimen collected from Dushan, Guizhou, China [1] (Figure 4c), but the specimen is lost.

Descriptions of *C. kobayasii* [24] and *C. cicadae-sm* [26] are consistent. Since they have been considered as the sexual morphs of *I. cicadae* and Chanhua respectively, together with the molecular phylogeny (Figure 2) and the distributions (Figure 3) of Chanhua, *C. kobayasii* and *C. cicadae-sm* (= *C. kobayasii*) should be the sexual morph of Chanhua. Unfortunately, *C. kobayasii* and *C. cicadae-sm* lack DNA sequence data.

3.4.4 *Cordyceps cicadae* S.Z. Shing, a synonym of *Tolypocladium paradoxum* (Kobayasi) Quandt et al.

Tolypocladium paradoxum (≡ *C. paradoxa* Kobayasi), as well as Chanhua, is also used as a Traditional Chinese Medicine, widely distributed in southern China and has a high yield [1]. This species produces a single black and hornlike fruiting body, so Chinese people called it 'Du-Jiao-Long' ('One-horned dragon', Figure 4d). Shing [28] introduced *C. cicadae* S.Z. Shing and treated it as the sexual morph of Chanhua, and in his report the author explicitly stated that its types are 'Du-Jiao-Long' that was bought from a pharmaceutical company in Ningbo, Zhejiang, China. Using morphological evidence and sequence (ITS) data, Chen et al. [4] clearly explained that 'Du-Jiao-Long' is *T. paradoxum* instead of the sexual morph of Chanhua. We sequenced the ITS gene of 'Du-Jiao-Long' from Yunnan (HKAS 87772, KX017279) and Hainan (GACP 14081002, KX017278), and the sequence alignment result indicates they are similar with that of *T. paradoxum* from Japan (AB027369) and Zhejiang (KM357283) and *C. cicadae* S.Z. Shing from Anhui (KF696557). Our molecular phylogeny also supports the view that they are all *T. paradoxum* (Figure 2).

As the same epithet between *C. cicadae* (Miq.) Massee and *C. cicadae* S.Z. Shing, Liang [29] gave a new scientific name *C. zhejiangensis* (Shing) Z.Y. Liu et al. for the latter. So *C. cicadae* S.Z. Shing, *C. zhejiangensis* (≡ *C. cicadae* S.Z. Shing) are both synonyms of *T. paradoxum*. *T. paradoxum* may include a complex of cryptic species. Although there are no distinct morphological differences [28, 30] (Figure 4d), the existing five ITS sequences from Japan and China have minor but distinct variations among each other (Figure 5).

3.4.5 *Ophiocordyceps sobolifera* (Hill ex Watson) G.H. Sung et al.

Ophiocordyceps sobolifera (Figure 4e, f) had previously been regarded as the sexual morph of *I. cicadae*/*I. sinclairii* [20, 31], but its asexual morph was shown to be *Beauveria sobolifera* Z.Y. Liu et al. [32, 33]. Our molecular phylogeny also proves Chanhua and *O. sobolifera* to be two distinct species (Figure 2).

3.4.6 Other synonyms of Chanhua

Petch [20], Kobayasi [34], Kobayasi and Shimizu [14] and Samson [21] listed some synonyms of *I. cicadae* and/or *I. sinclairii*, including *C. caespitosa* (Tul. & C. Tul.) Sacc. (≡ *Torrubia caespitosa* Tul. & C. Tul.) from New Zealand, *I. arbuscula* Har. from Guatemala, *I. basili* (Taylor) Kobayasi

Figure 4. Chanhua (*Cordyceps cicadae* (Miq.) Massee), *Tolypocladium paradoxum* and *Ophiocordyceps sobolifera.* a, b Sexual morph of Chanhua from Guangxi, China (collected by Xiao Zou on 21 July 2008, GZUIFR 08721-6). c Sexual morph of Chanhua from Guizhou, China (phographed by Xingliang Wu in 15 July 2012). d *T. paradoxum* from Zhejiang, China (collected by local people in July 2015 for sale). e, f *O. sobolifera* from Hunan, China (collected by Ping Zhang on 5 July 2011, GACP 110705).

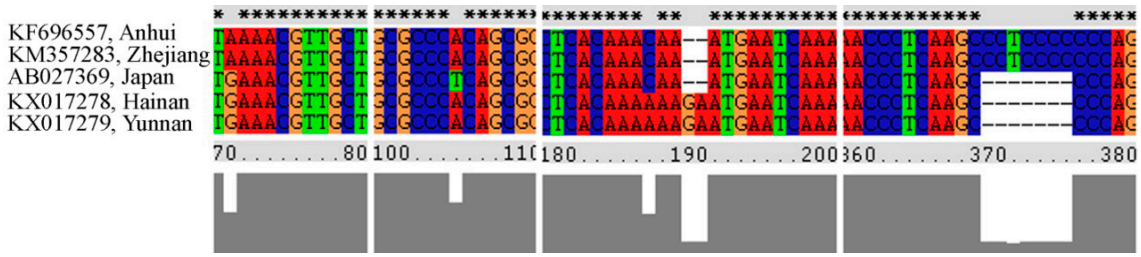

Figure 5. DNA base variations among the existing five ITS sequences of *Tolypocladium paradoxum* (total length 558 bp alignment).

(≡ *Sphaeria basili* Taylor) from New Zealand, *I. cosmopsaltriae* Yasuda from Japan, *I. cryptotympanae* Sawada from China (Taiwan), *I. hariotii* Arnaud from Madagascar and *I. mokanshawii* Lloyd from China. These species lack DNA sequence data. Together with the distributions (Figure 3) and the molecular phylogeny (Figure 2) of Chanhua, the three Asian species, *I. cosmopsaltriae*, *I. cryptotympanae* and *I. mokanshawii*, are most likely Chanhua.

3.4.7 Proposal of the scientific name for Chanhua

Recently, the asexual genus *Isaria* has been treated as a synonym of the sexual genus *Cordyceps* [35]. So *I. cicadae* Miq. should be revised to *C. cicadae* (Miq.), that is to say the old name *C. cicadae* (Miq.) Massee should be revived.

With a history of 1,500 years, the Chinese common name Chanhua has been widely used and is far more popular than *C. cicadae, I. cicadae*, etc., so

it should be protected. *I. cicadae* [15, 21], *I. sinclairii* [16, 21] and Chanhua [14, 29] are morphologically nearly identical and have generally been accepted as the same species. Considering these factors, the One Fungus One Name (1F1N) [36, 37] concept and in order not to cause taxonomic confusion, herein we propose to use the scientific name *C. cicadae* (Miq.) Massee, together with the Chinese common name Chanhua, to describe or record this important medicinal mushroom [1].

3.5 Medicinal Values

Chanhua is sweet, innoxious and cold in nature for the body; it is good for the eyes, mainly cure convulsions, palpitations, crying at night for children, and can even cure malaria [4, 13]. These medicinal values are closely similar to that of cicada sloughs which were recorded in The Chinese Pharmacopoeia [38]. Recent studies indicate, apart from being safe [39], Chanhua has a high nutritional value and contains many bioactive substances [40-42], which can enhance immunity significantly [43, 44], improve kidney function [45-47] and can be used for treating cancer [48, 49].

3.6 The Hosts

Chanhua (including *I. cicadae* and *I. sinclairii*) has been reported from insect hosts which are all cicada nymphs (Cicadidae, Hemiptera) [14, 21, 26, 50], and shows typical host specificity. Liu [27] reported that Chanhua had been isolated from a synnema growing on a Scarabaeidae (Coleoptera) larva, however, no description was provided in her report, thus it cannot be confirmed. In Japan, Chanhua is commonly found on the nymphs of *Meimuna opalifera* [14]; while in southern China, the most common host is the nymphs of *Macrosemia pieli*.

Names of all known hosts of Chanhua from China are listed and updated as follows:

Auritibicen flammata (Distant)
Cicadatra shaluensis China
Cryptotympana atrata (Fabricius)
Hyalessa ella (Lei & Chou)
Hyalessa maculaticollis (Motschulsky)
Hyalessa ronshana China
Macrosemia pieli (Kato)
Mogannia conica (Germar)
Platypleura kaempferi (Fabricius)

3.7 Ecology

Chanhua grows in warm, humid and low elevational regions. In China, these areas are covered by bamboo forests, broad-leaved forests or coniferous and broad-leaved mixed forests, at altitudes below 2,500 m, have an average annual temperature of about 15 °C, an annual rainfall between 1,000 and 1,200 mm and about 65% forest coverage. When the temperature rises to 18-24 °C and the soil humidity is above 80% from June to August in these areas, synnemata of Chanhua may be produced on cadavers of cicada nymphs in soil. Chanhua has a conspicuous vertical distribution, and most individuals grow on sunny slopes of 30-40° (summarized from Chen et al. [4] and our investigations).

In China, sexual morph of Chanhua were only reported from Guangxi (Leye) [26] and Guizhou (Dushan) [1] where the temperature difference is smaller and the humidity is higher than that of other distributed areas of Chanhua. Apart from more complicated natural and environmental factors, sexual morph of Chanhua might need smaller temperature difference and continuous high humidity than its asexual morph.

3.8 Life Cycle

Based on Chen et al. [4] and our observations on Chanhua and cicadas, herein we summarize the life cycle of Chanhua.

When temperatures rise to 18-24 °C and soil humidity reaches above 80% (before and after July in southern China, which is just the eclosion period of the cicada nymphs), old-mature cicada nymphs are easily infected by the conidia of the Chanhua fungus. Conidia attach to the body surface of a nymph at first, then germinate

and form germ tubes which may penetrate the nymph's body. After successful infection, the germ tubes will separate into numerous short parts (hyphal bodies). They use blood (hemolymph) and solid organizations of the host as nutrients and reproduce rapidly, and they can occupy the whole body cavity (haemocoel) of the host within two to three days. These hyphal bodies elongate gradually and form into mycelia, which will fill the whole haemocoel, use up water and nutrients, produce some noxious metabolites, and eventually cause the nymph to die.

After having been infected by the fungus, the nymph will accelerate to mature, which may cause it to molt early and to die easily during molting. Most nymphs are close to the ground with their head upwards when they die (killed by the fungus), only a few can get to the ground. Mycelia within the nymph form a solid and dense sclerotia which can resist harsher environments such as low temperature and drought, and mycelia also produce antibiotics and toxins that can resist attacks of other organisms, and the sclerotium will remain dormant and not rot away.

When temperature rises to 18-24 °C and soil humidity reaches above 80% again (the current or the following year), mycelia within the sclerotium will begin to grow. They gather and entwine (only one mating type) and form one or several synnemata. Gradually the synnemata become larger and form one or several light yellow 'buds' which will grow out from the molting line of the dorsal thorax of the nymph. The 'buds' grow straightly upwards, break through the soil and grow above the ground. Length and size of a synnema varies according to: 1) depth of the nymph in the soil and thickness of litter layer that covered the hole; 2) temperature, soil humidity and light; 3) the instar stage of the nymph; and 4) number of synnemata growing on the nymph. The apex of a synnema will branch repeatedly and form many conidiophores, on which numerous whorled conidiogeous cells (phialides) will be produced. Numerous conidia will be produced repeatedly from these conidiogeous cells, forming chains of conidia. While forming synnemata, mycelia can also grow out from some thin parts of the body surface (including intersegmental membranes, joints of legs etc.), cover the cadaver partially or completely.

Dispersed by air or water, the conidia eventually fall to the ground and infiltrate the soil along with water flow. They can attach to the body surface of a nymph if they make contact. Once the nymph is infected successfully, a life cycle of Chanhua is completed.

In southern China, the humidity of shallow soil layer changes continually and this interferes with the life cycle of Chanhua. Together with fungal growth and the habits of host cicada nymphs, we can divide the life cycle of Chanhua into four separate stages [50] in Table 1.

Table 1. Four stages of life cycle of Chanhua in the wild.

Stages	Occurrences	Essential factors
Stage I	In soil, conidia attaching to the body surface of a nymph	Conidia have already infiltrated into the soil
Stage II	Infected–death–a sclerotium formed	Suitable temperature and humid soil environment
Stage III	Sclerotium begins to germinate and an original synnema will be formed	Suitable temperature and humid soil environment
Stage IV	Growth of synnemata	Suitable temperature, humid soil environment and light

Under laboratory conditions, factors such as infection, temperature, humidity, light and even host nymphs can be artificially controlled but these are different from those in the wild. For the needs of both research and industrial production, we compared life cycles of Chanhua in the wild and under artificial culture (Table 2).

Table 2. A comparison of life cycle of Chanhua in the wild and under artificial culture.

Factor	In the wild	Artificial culture
Conidial contact by nymph	By attachment	Most injecting, few spraying
Death of host	Killed by Chanhua fungus, slowly	Killed by injecting or high temperature, quickly
Temperature and humidity	18-24 °C and above generally 80%	Both are controlled steadily, the infection needs a higher temperature
Light	Natural light, not steady	Artificial control, steady
Synnema	One or a few large synnemata produced from dorsal thorax of an undamaged cadaver	Many small synnemata produced from many parts of a rotten cadaver
Four stages of life cycle	Separate from one another	Connected one by one
Time span of a life cycle	Varies from 2 weeks to one year or more	About 2 weeks
The hosts	Cicada nymphs, 9 species known in China	Cicada nymphs or lepidopteran larvae, alternative

Conflict of interest

The author(s) declare(s) that there are no conflicts of interest to disclose.

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