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Morphological Description of a New Record of *Chaetotyphula columbiana* Singer from Thailand

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Abstract. Only eight taxa of Chaetotyphula have been described worldwide. In 2001, Flegel and Ruksawong introduced the genus Chaetotyphula to Thailand; however, the specimen of Chaetotyphula has not yet been formally classified as a species. The present research postulates a novel record of Chaetotyphula columbiana from Thailand. The specimen was discovered amidst leaf litter in the dipterocarp forest located in Khao Yai National Park in 1999. This mushroom is small, 10–20 mm long, 1-2 mm wide, typhuloid in shape, white with a translucent appearance, smooth on the head, but velvety from the stalk surface to the basal of the basidiocaps. The basidiocarps were raised directly from the leaf litter. The aforementioned species exhibits a notable dichotomy in the morphology of its metuloid dermato-cystidia, which can be classified into two distinct categories: encrusted and non-encrusted, yet diverticulate.

Keywords: Taxonomy, *Chaetotyphula*, Khao Yai National Park, Typhuloid fungi, Basidiomycota, Mushroom

1. Introduction

Typhuloid fungi are a taxonomic group of mushrooms that exhibit shared morphological characteristics, including the basidiocarps type of filiform to aciculate, nonpigmentation, and distinct microscopic features such as the hymenium system and dermatocystidia.

The taxonomic classification of typhuloid fungi includes the genera Chaetotyphula, Pistillaria and Typhula, which are included within the Agaricales in Basidiomycota (Corner 1967). Several of these species are acknowledged to demonstrate plant pathogenicity, particularly in the genera Typhula and Pistillaria (Singer 1975). It is crucial to recognize that, among this assemblage, Chaetotyphula represents the sole saprophytic species. The Chaetotyphula genus has a limited global distribution, comprising only eight discrete taxa (The Index Fungorum 2023). The distinguishing characteristics between Typhula and Chaetotyphula are the absence of sclerotia, non-thickening hyphae, and thick-walled cystidia in Chaetotyphula. The genus Pistillaria exhibits a close taxonomic relationship with Chaetotyphula due to the absence of sclerotium in their basidiocarps. However, it is worth noting that Pistillaria lacks cystidia (Corner 1950, 1953). Currently, the only physical similarities

between Chaetotyphula and Actiniceps are their small size, lack of pigmentation, presence of hymenial- and/or caulocystidia, hyaline appearance, and smooth-walled, globose to ellipsoid spores. The boundaries of this particular collection have not been definitively established, and these inconspicuous fungi may have an additional level of diversity than is presently acknowledged. (Dentinger and McLaughlin 2006)

Corner (1950) documented the first recorded occurrences of Chaetotyphula in Southeast Asia. The identified species were C. actiniceps and C. hyalina, originating from Java and Malaysia, respectively, and C. hyalina var. microcystis, was found in Singapore. Corner (1967, 1970) and Singer (1975) reported the discovery of five newly identified species in South America. The aforementioned taxa, namely Chaetotyphula gelatinosa and C. tetraspora, were observed in Panama, whereas C. amylochaete, C. columbiana, and C. montana were detected in the country of Ecuador (Corner 1950, 1953). Documentation of introducing of this genus to Thailand was reported by Ruksawong and Flegel in 2001. However, it has not yet received formal classification as a species up to the present time (Ruksawong and Flegel 2001). The aim of this investigation is to analyze the collection of fungal specimens in the BIOTEC Bangkok Herbarium (BBH), which is considered a key element of the Funga of Thailand Project.

2. Materials and Methods

2.1 Materials

The five samples taken between 1999 and 2008 were observed to possess solely one comprehensive field photograph, which was obtained in 2008. Two samples were contaminated by other fungi and were therefore excluded from both microscopic and rDNA examinations. Due to the small size of the sample, limitations were taken with field photography in 1999, so the photographs used were from 2008. However, the samples were acquired in limited quantities. In light of contemporary technological advancements, it is advisable to employ rDNA data analysis as a means of verifying the identified type. Consequently, the fungal BBH regulation was scrutinized using a limited number of specimens, with the only viable option being the specimen retrieved from the BIOTEC Bangkok Herbarium (BBH) in Thailand. Despite using multiple kits or the MagLED Automatic Robot extraction method, the extraction of rDNA was unsuccessful. Thus, in this categorization, reliance is placed solely on intricate morphological characteristics, ascertained both through visual inspection and camera-based analysis, and subsequently compared to determine the optimal classification.

On the day of collection, the initial author provided a description of the macroscopic characteristics of the specimen. Subsequently, the specimens were subjected to desiccation at a temperature range of 40–50 °C using a food dehydrator to extract cellular moisture for a minimum duration of 8 hours during the nocturnal period. The following day, the dried samples were promptly relocated to plastic receptacles. The container is hermetically sealed to prevent moisture entry and bears an affixed label. After the researchers returned to the laboratory, the samples underwent decontamination by being stored at a temperature of -20 °C for 24 hours (Bridson and Forman 1998). Following this, the samples were dispatched to the BIOTEC Bangkok Herbarium (BBH). Implement

quality management in accordance with the ISO 9001 framework of the BBH service provider. The five samples were gathered and looked at up close using a microscope. Due to other fungi contaminating two of the five samples, they were excluded from this investigation. All three specimens were identified as being of the same species. The best field images were chosen, with the shot from 2008 being chosen as the best. All three good-quality samples could be examined under a microscope. The first author examined and provided illustrations for the micromorphological characteristics of the first specimen that was found in 1999. For a complete description, all morphological data was gathered and documented. A single microscopic illustration demonstrated the most comprehensive morphology, and the submitted field photo had to be taken from a different specimen.

2.1 Methods

It was imperative to document the gathered specimens with regard to their morphological attributes, such as the dimensions of the flowers, their shape, color, texture of all components, and scent (Hemmes and Desjardin 2002; Largent et al. 1986). The significance of mushroom color is limited to its appearance under natural or artificial daylight illumination. The hue was delineated in accordance with the Kornerup and Wanscher color systems (Kornerup and Wanscher 1963). To document the visual characteristics of mushroom specimens before desiccation, they were either depicted through illustrations or captured via photography. Following the desiccation process, a morphological analysis of the specimens was conducted using a microscope (Olympus BX51). The text provides a comprehensive account of various cellular and tissue structures, spanning from the uppermost part to the lowermost region of the basidiocarps. The procedure involved the dissection of the sample using a thin razor blade, followed by the application of 95% ethanol. Reactive agents or dyes were then applied, depending on the intended purpose of the examination. For instance, distilled water was used to examine the primary color and overall shape of the cell. Additionally, Melzer's reagent was applied to verify the reaction of iodine. Staining substances such as Phloxine 1% and Congo Red 1% were utilized for structural examination and microscopic drawings (Largent et al. 1977).

The basidiospores and special cells from the different parts of basidiocarps, as well as the hymenium and surface layer of basidiocarps, were examined using an eye micrometer and depicted using the Camela Lucida system, which is specifically designed for rendering cellular structures under observation. The Olympus BX51 attached to the Camera Lucida was utilized for the purpose of illustration. Microscopic characteristic data, encompassing dimensions, morphology, and surface characteristics, in addition to positioning and arrangement, were depicted and quantified. Documentation of chemical reactions occurring within diverse cells and tissues was slated to be undertaken (Desjardin et al 2000).

3. Results

3.1 Macromorphological characteristics

The basidiocarps exhibited an off-white hue in their fresh state. However, upon drying in a fungarium, the color of the basidiocarps underwent a transformation to a brownish, opaque shade. The basidiocarps were cylindrical in shape and never spathuloid. As they matured, the apical

region of the basidiocarps became straight, but in their juvenile stage, they exhibited a hooklike or spherical shape. The surface of the basidiocarps was smooth, and the head measured 10–20 mm in length, respectively, with a width of 1–2 mm. The specimen exhibited a cylindrical, pubescent stipe with a brownish gray (6F8) hue that is translucent in nature. The stipe was observed to be upright from the leaves without any discernible base, which is also known as insititious. When in the growth process, primordium exhibits a spherical shape with an off-white coloration. The juvenile phase of the basidiocaps is distinctly demarcated into the capitulum and peduncle, featuring two elongated segments emanating from the nodes. The proportionality between the stem and head is frequently dissimilar from that observed in the adult population. Typically, the stem exhibits greater length than the head. The process of distinguishing between the head and stem can be accomplished by examining the transparency and opacity of the respective parts. In cases where the object in question is opaque, it can be inferred that it pertains to the head. Conversely, if the object exhibits translucency, it can be deduced that it pertains to the stem. The stem exhibits irregularities in its surface, whereas the apex typically presents a more uniform texture. The presence of sclerotium was not observed.

3.2 Micromorphological characteristics

Basidiospores exhibit a sub-globose to ellipsoid shape, with dimensions of 20–15 µm in length and 5–10 µm in width 15-20 × 5-10 µm [x = 18±1.77 × 7.88±1.67 µm, Q = 2.0–3.0, Qm = 2.36± 0,23; n = 25 spores]. They possess a hyaline coloration, a smooth surface, and a thin-walled structure. Upon treatment with Melzer's reagent, the basidiospores exhibit an inamyloid nature. Basidia were observed to have dimensions of 37.5×11.5 µm, clavate shape, and a hyaline appearance. The surface of the basidia was found to be smooth, with a thin-walled structure, and lacking in amyloid content. The basidia contained four spores, exhibiting infrequent traits such as clavate shape, hyaline color, smooth surface, thin-walled cell, and inamyloid in Melzer's reagent. The basidia measured 37.5 µm in length and 11.5 µm in width. Basidioles are a sterile cell variant with a club-shaped morphology characterized by a smooth surface and a transparent aspect. The aforementioned structures are situated in the hymenial stratum, extending from the apical region referred to as the "head" to the basal region known as the "base". The emergence of basidioles has been observed to occur either internally or near the basidia. These cells were characterized by their thin-walled structure and were inamyloid when subjected to Melzer's reagent. Their dimensions typically ranged from 25–55 µm in length and 10–11.5 µm in width. The hymenium is composed of cylindrical hyphae that exhibit two distinct types of cystidia, namely non-metuloid cystidia and metuloid cystidia. The cystidia lacking metuloids are composed of two distinct cellular structures, namely diverticulate cells and triangular cells.

A notable observation was the presence of non-metuloid cystidia in substantial quantities, characterized by a clavate morphology and a diverticulate apical extremity. The dimensions of the diverticulate cystidia were determined to be within the range of 18–25 µm in length and 5–7 µm in width. These cystidia exhibited a hyaline coloration and a smooth surface texture. It is noteworthy that the non-metuloid cystidia exhibited a lack of encrustation at the apex and possessed a

maximum thickness of 2 µm, accompanied by a thick cell wall. Additionally, it was observed that the cystidia exhibited inamyloid properties when subjected to Melzer's reagent. A triangular-shaped non-metuloid cystidium was identified among a collection of metuloid and non-metuloid cystidia, constituting the second instance of such a finding. The triangular cells situated on the head hymenium exhibit dimensions similar to those of the stipe, with measurements of around 70–100 µm in length and 50–70 µm in width, and a wall thickness of up to 10 µm. Furthermore, the cystidia were found to be inamyloid in Melzer's reagent. The metuloid cystidia comprised two discrete cellular structures, specifically ventricose-rostrate cells, and triangular cells. The ventricose-rostrate morphology is characterized by a gradually tapering towards a slender apex and an obtuse apical region. Furthermore, the dimensions were assessed at three discrete locations, specifically the apical, middle, and basal regions. The cystidia's width at the apex was measured to fall within the range of 5–10 µm, whereas the middle portion displayed a width of 25–30 µm. The cystidia's base exhibited a width ranging from 6.5 to 7.5 µm. The study determined that the cystidia exhibited a size range of 70–105 µm. Numerous cystidia were identified in the outer region of the hymenium stratum, displaying a transparent morphology and a conspicuous encrustation surrounding the apex. The cellular structures exhibited the notable feature of possessing robust cellular membranes, with a quantified dimension of approximately 6.5 µm. The cystidia that are triangular in shape and possess metuloid surfaces to exhibit comparable dimensions, and their response to Melzer's reagent is similar to that of cystidia lacking metuloid surfaces. The hymenium of the head region's tramal structure comprises parallel hyphae that displayed a sleek surface and slender walls. The presence of a clamp connection is observed, and the cells exhibit a robust dextrinoid response upon exposure to Melzer's reagent. The subhymenial trama comprises of a cylindrical arrangement of hyphae measuring 5–10 µm in width and exhibiting a smooth surface. Furthermore, the trauma exhibits a negative reaction to Melzer's reagent. Stipe trama, which consists of parallel arrangements of cylindrical hyphae, form caulocystidia. A significant quantity of triangular cells exhibit wide bases. The filament is connected to the apical tip of the stem cell at its point of origin. The dimensions of the caulocystidia, which comprise the apical, median, and basal widths, were assessed at three distinct sites, namely the preapical, medial, and basal regions, which are analogous to other cystidial cells. The apical width was found to vary between 5–7.5 µm, whereas the median width ranged from 10–12.5 µm. The dimensions of the base range between 22.5 and 77.5 µm, while the height ranges from 82.5 to 100 µm.

The hue perceived is hyaline. An observation of encapsulation from the middle to the apical cells was made. The thickness of the cell wall was observed to be 8.5 µm and its non-inamyloid nature was confirmed through testing with Melzer's reagent. Exhibits a range of amyloid reactivity from weak to moderate upon exposure to Melzer reagents. The basal level of highly productive cells is devoid of clamp connections. The hymenium trama system exhibits the existence of basidia and dermatocystidia.

Habit and Habitat: The growth habit of the species in controversy is gregarious, with a preference for both foliicolous and lignicolous habitats.

Figure 1. Photograph of *Chaetotyphula columbiana* Singer BBH24935 (2008) Scale bar = 5 mm

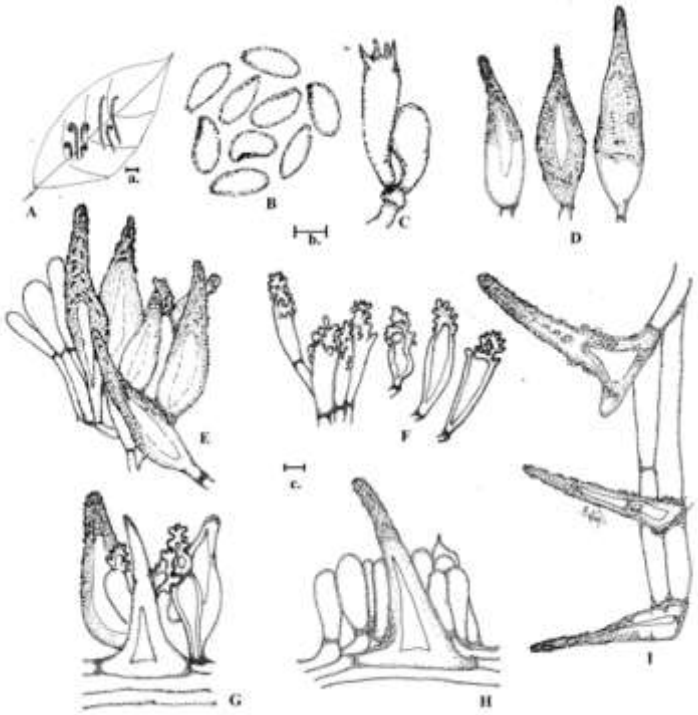

Figure 2. Illustration of *Chaetotyphula columbiana* Singer BBH01684 (1999)
A. Basidocarps on leaf litter, B. Basidiospores, C. A basidium and a basidioles, D. Head-metuloid cystidia, E. Head-metuloid cystidia with basidioles, F. Thick- and thin-walled head diverticulate, G. Three types of cystidia on head hymenial layer, H. Triangular cystidia with metuloid, I. Triangular caulocystidia with metuloid at stipe. Scale bars a. = 5 mm, b. – c. = 10 µm.; A = a., B – C = b., D – I = c.

The specimens can be found in both mountainous dry dipterocarp forests (Khao Yai National Park) and mountainous evergreen forests (Khao Sok National Park).

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3.3 Specimen Examined

Thitiya Boonpratuang procured BBH24935 on October 14, 2008, from decomposing leaf debris within a heterogeneous woodland environment situated in the Sanyang Roi Nature Trail of the Khao Sok National Park, which is located in the Phanom District of Surat Thani Province. Thitiya Boonpratuang, Timothy W. Flegel, and Pucharapa Puyngain obtained BBH22906 on June 15, 2008, from the bark of an unidentified tree in a mixed forest along the Fern Nature Trail of Khao Yai National Park, Pak Chong District, Nakhon Ratchasima Province. BBH01684 was detected on May 16, 1999, on deteriorating foliage in a heterogeneous forested region situated in Khao Yai National Park, which is positioned in Pak Chong District, Nakhon Ratchasima Province. Poramate Ruksawong and Timothy William Flegel were responsible for collecting the specimen.

4. Discussion

The limited quality of the samples and the presence of five samples, coupled with the small size and antiquity of the sample dating back to 1999, rendered the extraction of rDNA of adequate quality for phylogenetic analysis unfeasible. Consequently, the only available approach was the morphological characterization that ensued from the examination of the specimen. In 2000, Ruksawong and Flegel first introduced the genus Chaetotyphula to Thailand. However, the identification of the species was not possible due to the constraints of keys and monographs for these species (Ruksawong and Flegel 2001). The present investigation was conducted utilizing the Ruksawong and Flegel fungal assemblage, which was previously identified as Chaetotyphula sp. The observed phenomenon is attributed to the production of two distinct categories of metuloid dermatocystidia, namely incrusted and non-incrusted but diverticulate. Chaetotyphula columbiana was initially documented in Ecuador (Singer 1978), where it was observed that the tropical forest bore resemblance to that of Thailand. The observed dissimilarities between the two specimens, situated in Ecuador and Thailand, pertain to the configuration of basidiome, dimensions of basidiospores, and the presence of dermatocystidia. The morphology of the Thai specimen's basidiome is characterized by a cylindrical shape, whereas the Ecuadorian specimen exhibits a spathuloid form. The spore dimensions of the Thai sample exhibit greater magnitude (8.5–11×6.5–8.5 µm) and breadth in comparison to the Ecuadorian

counterparts. The metuloid and cystidia structures of Thai species exhibit smaller dimensions (80–155×6.5–12.5 µm) in comparison to their Ecuadorian counterparts. Since 2008, there has been a dearth of new specimens, and two of the five fungarium specimens have been rendered unusable due to contamination. As a result, only three specimens with very low quantities remain. Until such time as they are willing to employ it in molecular and other methodologies, its utility remains untapped.

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