Taiwania 67(2): 267–270, **2022**
DOI: 10.6165/tai.2022.67.267

Pyrgillus mammosus (Pyrenulaceae, lichenized Ascomycota), a new species from Taiwan

Miyu SUGIMOTO[1], Yoshihito OHMURA[2,*]

1. *Atagawa Tropical & Alligator Garden, 1253-10 Naramoto, Higashiizu-cho, Kamo-gun, Shizuoka, 413-0302, Japan.*
2. *Department of Botany, National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba, Ibaraki, 305-0005, Japan.*
[*] *Corresponding author's email: ohmura-y@kahaku.go.jp*

(Manuscript received 10 February 2022; Accepted 28 April 2022; Online published 2 May 2022)

ABSTRACT: *Pyrgillus mammosus*, a new species from Xitou, Taiwan is described and illustrated. It is similar to *P. tibellii* and *P. rufus* but differs in its potassium hydroxide reaction of the thallus (K+ red) as a main distinguishing feature, additional chemical compounds detected by thin layer chromatography, and larger conical to hemispherical ascomata. Morphological and chemical differences among the closely similar taxa are discussed.

KEY WORDS: Chemistry, eastern Asia, lichenized fungi, mazaedia, morphology, *Pyrgillus rufus*, *P. tibellii*, taxonomy, TLC.

INTRODUCTION

The genus *Pyrgillus* Nyl. belongs to the Pyrenulaceae (lichenized Ascomycota). Nine species are recognized worldwide (Aptroot *et al*., 2018). The genus is characterized by crustose, corticolous, epiphloeodal thalli with trentepohlioid photobiont; sessile, solitary, black, perithecioid, mazaediate ascomata with carbonized exciple ± lateral thalline margin; non-inspersed hamathecium, I−, with simple paraphyses, 8-spored asci without an ocular chamber, and broadly ellipsoid, brown, distoseptate to euseptate, 1–3-trans-septate ascospores (Singh and Singh, 2012a). Among the known species, *P. tibellii* Kr.P. Singh & Pushpi Singh has a distinctive morphology including a corticate thallus, rather large perithecioid ascomata (1.7–2.3 mm high) with orange-reddish pruina (UV−) at the edge of the exciple, and 3-septate, large ascospores (15.3–22.8×7.5–10.0 µm) (Singh and Singh, 2012b, 2017). The chemistry of this species is distinctive as well: the thallus is UV+ yellow indicating the presence of lichexanthone; two additional unidentified substances have been detected by thin layer chromatography (TLC) (Singh and Singh, 2012b). This species is known from only two localities in India (Arunachal Pradesh in Eastern Himalaya and Kerala in Western Ghats) (Singh and Singh, 2017). *Pyrgillus idukkiensis* Kr.P. Singh & Pushi Singh and *P. rufus* Aptroot & M. Cáceres may be confused with *P. tibellii*. *Pyrgillus idukkiensis* is distinguished by 1-septate ascospores, and *P. rufus* by the UV+ red pruina on the mazaedium.

During the examination of *Pyrgillus* specimens housed in the herbarium of the National Museum of Nature and Science (TNS), Tsukuba, Japan, several specimens collected from Taiwan in 1924 and 1933 were first identified as '*P. tibellii*'. However, the chemical characters were not consistent with the protologue of *P. tibellii*, and the ascoma size was larger. We concluded that the Taiwanese individuals differed from *P. tibellii* and all other known taxa.

The aim of this study is to describe and illustrate the new species, *P. mammosus*, and to discuss the variation within the species and the differences to the similar taxa.

MATERIALS AND METHODS

Sample collection

This study is based on eight herbarium specimens housed in the herbarium of the National Museum of Nature and Science (TNS), Tsukuba, Japan. The isotype is deposited in the herbarium of the National Taiwan University (TAI), Taipei, Taiwan.

Morphological observations were made using a dissecting microscope (Olympus SZX16) and a differential interference contrast microscope (Olympus BX51). Anatomical examinations were made on hand cut sections mounted in water. Ascospore measurements are given as (minimum–) range including mean ± standard deviation (–maximum) (n = number of measurements).

Color spot tests with K, C, KC, and Pd followed Orange *et al*. (2001). UV color reactions were tested under 365 nm wavelength.

Chemical compounds were examined using TLC (Culberson and Kristinsson, 1970). Solvent systems A (toluene: 1,4-dioxane: acetic acid = 180: 45: 5) (Culberson and Ammann, 1979), B′ (hexane: methyl tert-butyl ether: formic acid, 140: 72: 18) (Culberson and Johnson, 1982), and C (toluene: acetic acid = 170: 30) (Mietzsch *et al*., 1994) were used. The spot color was checked under 254 nm and 365 nm wavelength of UV and visible light, before and after spraying the TLC plates with 10% sulfuric acid and charring at 110°C for 10 minutes.

Table 1. Comparison of chemical features for ***Pyrgillus mammosus***, ***P. tibellii*** and ***P. rufus***. *The color of TLC spot was checked after a spray of 10% sulfuric acid and 110°C heating for 10 minutes (Fig. 2A). **The color of TLC spot was checked just after development of TLC without the procedure of sulfuric acid and heating mentioned above (Fig. 2B).

Chemical features	*P. mammosus* (this study)	*P. tibellii* (Singh & Singh, 2012b)	*P. rufus* (Aptroot *et al*., 2018)
Color sport test	Thallus K+ red (Fig. 3), C–, KC–, Pd–, UV+ yellow (Fig. 4)	Thallus K–, UV+ yellow	Thallus UV+ yellow (K reaction not indicated)
	Mazaedium pruina at ostiole rim of ascomata K+ deep red, UV–	Mazaedium pruina K+ reddish violet, UV–	Mazaedium pruina K+ orange, UV+ red
TLC solvent A	Terpenoids: Rf class 1, pink*; Rf class 3, brown*	Rf class 4, brown*	N/A
	Red pigments**: Rf class 0–1, purple*, $UV_{365\ nm}$– quench; Rf class 1, purple*, $UV_{365\ nm}$– quench	Rf class 7, orange-pink*	
solvent B′	Terpenoids: Rf class 2, pink*; Rf class 3–4, brown*	N/A	N/A
	Red pigments**: Rf class 3, purple*, $UV_{365\ nm}$– quench; Rf class 4, purple*, $UV_{365\ nm}$– quench		
solvent C	Terpenoids: Rf class 1, pink*; Rf class 4, brown*	N/A	N/A
	Red pigments**: Rf class 1–2, purple*, $UV_{365\ nm}$– quench; Rf class 3, purple*, $UV_{365\ nm}$– quench		

TAXONOMIC TREATMENT

Pyrgillus mammosus Mi. Sugim. & Y. Ohmura, ***sp. nov.***
MycoBank No. 843733

Figs. 1–3

Similar to *P. tibellii* and *P. rufus* but differs in the K+ red color reaction of the thallus, the presence of unidentified terpenoids and red pigments detected by TLC, and larger conical to hemispherical ascomata.

Type: **TAIWAN**. **Nantou Co.:** Keitau (Xitou), 24 December 1933, *Y. Asahina* (*TNS-L-31717*, holotype: TNS, isotype: TAI).

Thallus crustose, corticolous, epiphloeodal, up to 5.7 cm across (the largest fragment in the specimen), surface yellowish brown, continuous, smooth to cracked, rimose, up to 0.3 mm thick, locally with black spots indicating pycnidia, without distinct prothallus; cortex ca. 20 μm thick, without any crystals; medulla white with patchy yellow pigmentation; photobiont presumably a trentepohlioid alga (but specimens too old for proper identification). Ascomata perithecioid, mazaediate, sessile, solitary or 2–5 ascomata grouped, scattered, conical to hemispherical, often slightly constricted at the base, 1.6–3.5 mm high, 1.6–3.7 mm wide, partly immersed in substratum, laterally covered by a well-developed thalline margin (0.2–0.3 mm thick); proper excipulum strongly carbonized, without crystals, 0.5–0.8 mm thick; ostiole apical, cylindrical to obconical, with dark red pruina at the rim. Hamathecium not inspersed, I−, paraphyses simple; periphyses absent. Asci 8-spored, becoming evanescent, cylindrical, with uniseriate and periclinally arranged ascospores, 125–143 × 9.6–12 μm. Ascospores dark brown, long ellipsoid, (2–)3(–5)-septate, smooth, thick-walled, distoseptate, (12.7–)13.7–18.2(–25.8) × (7.5–)8.5–9.9(–10.5) μm (n=37) including (1.3–)1.7–2.5(–2.9) μm thick outer wall and (1.3–)1.5–2.0(–2.6) times as long as wide in the hamathecium; (11.0–)13.1–16.1(–24.8) × (5.4–)6.0–7.4(–9.1) μm (n = 403) with shrunken form, lacking a thick outer wall and (1.6–)2.0–2.4(–2.9) times as long as wide in the mazaedium; ends rounded. PYCNIDIA occasionally present, semi-immersed in the thallus, black to dark brown in the upper part, spherical, 60–110 μm diam. CONIDIA hyaline, bacilliform, 7–11 × 0.9 μm (n = 2).

Chemistry: Thallus K+ red (strong in the cortex and weak in the medulla), C–, KC–, Pd–; UV+ yellow. Pruina at the ostiolar rim of the ascomata K+ deep red and UV–. TLC: two unidentified terpenoids, and two unidentified red-pigmented spots before charring (the position of these TLC spots in different solvents is shown in Table 1 and Fig. 2).

Etymology: The epithet '*mammosus*' means having large breasts in Latin, for the shape of ascomata.

Remarks: This new species, *P. mammosus*, is similar to *P. tibellii* and *P. rufus*, but differs in the potassium hydroxide reaction of the thallus (K+ red) as a main distinguishing feature, different chemical compounds detected by TLC (see Table 1), and larger conical to hemispherical ascomata [1.6–3.5 mm high and 1.6–3.7 mm wide for *P. mammosus* vs. 1.7–2.3 mm high and 1.2–2.1 mm wide for *P. tibellii* which was measured from the figures in Singh and Singh (2012b, 2017) and 0.5–0.8 mm high and 0.5–0.8 mm wide for *P. rufus* (Aptroot *et al*., 2018)]. Although the K+ red reaction on the thallus of *P. mammosus* was very distinct (Fig. 3), we could not find the TLC spot associated with this reaction. Further chemical study is needed to identify the compound. The K reaction on the pruina of the mazaedium of *P. mammosus* is more or less the same as in *P. tibellii* but distinctly different from that of *P. rufus* (UV+ red, K+ orange; Aptroot *et al*., 2018).

Pyrgillus mammosus shows other characteristic chemical features that are yellow pigmentation in medulla (Fig. 1I) and UV+ yellow on thallus (Fig. 4). However, we also could not identify the TLC spots associated with these features. Although the presence of lichexanthone was reported in various species of *Pyrgillus* (see Singh and Singh, 2012b), the compound was not detected in our TLC analyses (Fig. 2).

The ascospore size of *P. mammosus* is in the same range as of *P. tibellii* [i.e., (12.7–)13.7–18.2(–25.8) × (7.5–)8.5–9.9(–10.5) μm for *P. mammosus* vs. (15.3–)16.0–20.0(–22.8)

Fig. 1. ***Pyrgillus mammosus***. **A**. Thallus with ascomata (holotype, TNS). The ascoma in the center is conical in shape. **B**. Hemispherical ascoma (TNS-L-31720, TNS). **C**. Paired ascomata as a variation (TNS-L-31720, TNS). **D**. Vertical section of ascoma (TNS-L-31721, TNS). Arrow indicating hamathecium. **E**. Ascospores with thick outer wall in hamathecium (TNS-L-31722, TNS). **F**. Shrunken form of ascospores lacking thick outer wall that were observed in exposed mazaedia (holotype, TNS). **G**. Ascospore with five septa as a variation (TNS-L-31722, TNS). **H**. Cylindrical ascus with eight ascospores (TNS-L-31716, TNS). **I**. Yellow medulla (TNS-L-31717, TNS). **J**. Pycnidia on thallus (TNS-L-31719, TNS). **K**. Cross section of pycnidium (holotype, TNS). Scales: A–C = 1 mm; D, I, J = 0.5 mm; E–H = 10 µm, K = 20 µm.

× 7.5–10.0 µm for *P. tibellii*] and *P. rufus* (15.0–17.5 × 5.0–6.5 µm), while the ascospores of other species in the genus are much smaller (i.e., less than 13.5 µm in length) (see Singh and Singh, 2012b; Aptroot *et al*., 2018). The ascospores of *P. mammosus* are mainly 3-septate, but ascospores with two or five septa were rarely observed (see Fig. 1G). When ascospores were present in the hamathecium, they have thick outer wall (Fig. 1E). The exposed mazaedium part was filled with shrunken ascospores lacking such thick outer wall (Fig. 1F).

Small black spots on the thallus (Fig. 1J) were confirmed as pycnidia (Fig. 1K), but they were usually immature and without conidia. Singh and Singh (2012b) described the presence of black spots on the thallus of *P. tibellii* but they did not mention pycnidia and conidia. The black spots in *P. tibellii* are here considered to be pycnidia like in *P. mammosus*.

Ecology and distribution: *P. mammosus* was found on bark of trees (species unknown) at 1000–2000 m elevation. This species is currently known only from the type locality in Taiwan. The current status of this species in Xitou is unknown. Regarding the other similar species, *P. tibellii* is known only from India and *P. rufus* only from Brazil. In spite of their conspicuous morphology, only few collections from limited localities were reported for each species, and the facts suggest that these species as well as *P. mammosus* may be threatened.

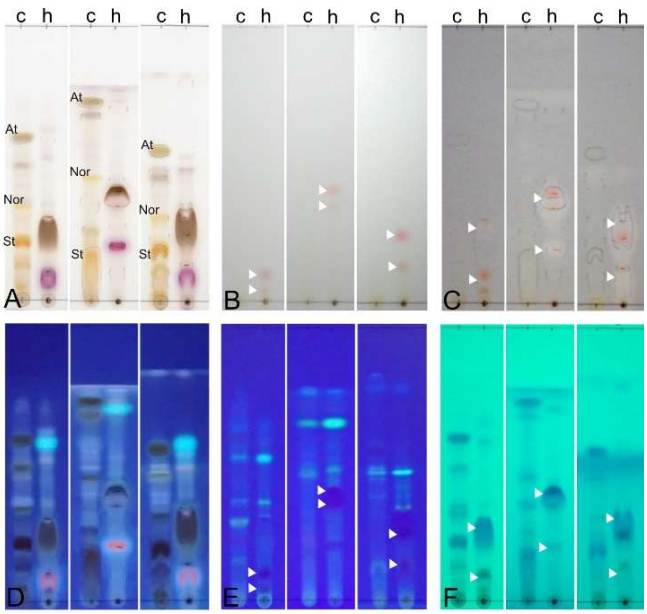

Fig. 2. TLC analyses of chemical compounds extracted from ***Pyrgillus mammosus*** (holotype, TNS). A set of three plates were developed in solvents A (left), B′ (center) and C (right). **A**. Visible light after charring with sulfuric acid spray. **B**. Visible light without sulfuric acid spray before charring. Arrows indicate unidentified red pigments. **C**. Visible light with sulfuric acid spray before charring. Arrows indicate unidentified terpenoids that repel water. **D**. $UV_{365\ nm}$ after charring with sulfuric acid spray. **E**. $UV_{365\ nm}$ before charring with sulfuric acid spray. Arrows indicate quench spots of unidentified red pigments. **F**. $UV_{254\ nm}$ before charring with sulfuric acid spray. Arrows indicate unidentified terpenoids although the lower spots are obscure. c = control that contains stictic acid (St) for R_f class 2, norstictic acid (Nor) for R_f class 4, and atranorin (At) for R_f class 7. h = holotype of ***P. mammosus*** (TNS). Details for the detected TLC spots of ***P. mammosus*** are mentioned in Table 1.

***Additional specimens examined*: TAIWAN. Nantou Co.:** Keitau (Xitou), 24 December 1924, *Y. Asahina* (*TNS-L-31720*, TNS); the same locality, 24 December 1933, *Y. Asahina* (*TNS-L-31715, 31716, 31718, 31719, 31721, 31722*, TNS).

ACKNOWLEDGMENTS

We would like to thank anonymous reviewers for their critical reading of our manuscript and constructive comments.

LITERATURE CITED

Aptroot, A., H.J.M. Sipman, J.A. Mercado Diaz, C.O. Mendonça, S.C. Feuerstein, I.P.R. Cunha-Dias, T.A. Pereira and M.E.S. Cáceres. 2018. Eight new species of Pyrenulaceae from the Neotropics, with a key to 3-septate *Pyrgillus* species. The Lichenologist **50(1)**: 77–87.

Culberson, C.F. and K. Ammann. 1979. Standardmethode zur Dünnschichtchromatographie von Flechtensubstanzen. Herzogia **5**: 1–24.

Culberson, C.F. and A. Johnson. 1982. Substitution of methyl tert.-butyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products. J. Chromatogr. **238(2)**: 483–487.

Fig. 3. K+ red reaction on thallus of ***Pyrgillus mammosus*** (TNS-L-31720, TNS).

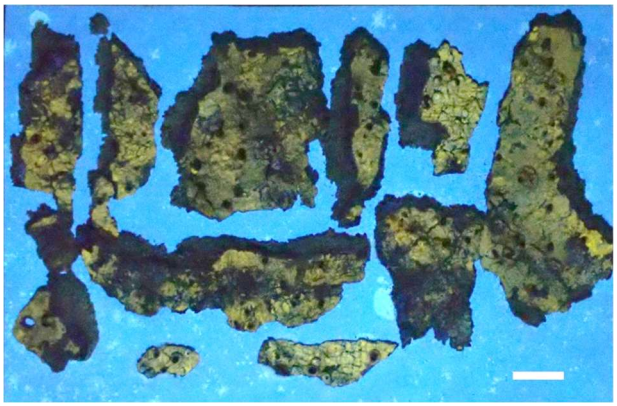

Fig. 4. UV+ yellow reaction of thallus and UV- on mazaedia and pruina of ascomata for ***Pyrgillus mammosus*** (holotype, TNS). Scale: 1 cm.

Culberson, C.F. and H.D. Kristinsson. 1970. A standardized method for the identification of lichen products. J. Chromatogr. **46**: 85–93.

Mietzsch, E., H.T. Lumbsch and J.A. Elix. 1994. *Wintabolites* (*Mactabolites for Windows*) Users Manual. 2nd ed. Universität Essen, Essen.

Orange, A., P.W. James and F.J. White. 2001. Microchemical Methods for the Identification of Lichens. British Lichen Society. 101 pp.

Singh, K.P. and P. Singh. 2012a. Genus *Pyrgillus* Nyl. (lichenized Ascomycota: Pyrenulaceae) in India. Taiwania **57(4)**: 391–395.

Singh, K.P. and P. Singh. 2012b. A new species of *Pyrgillus* from India. The Lichenologist **44(6)**: 773–776.

Singh, P. and K. P. Singh. 2017. Note on the taxonomic status of *Pyrgillus tibellii* Kr. P. Singh & Pushpi Singh with a world key to the species of *Pyrgillus* Nyl. (Pyrenulaceae: Pyrenulales). The Lichenologist **49(3)**: 287–289.