

# ***Graphostroma formosanum*, a newly described xylariaceous fungus in Taiwan**

Yu-Ming Ju\*, Huei-Mei Hsieh, Chun-Ru Lin, Chun-Yun Huang

*Institute of Plant and Microbial Biology, Academia Sinica, Nankang, Taipei 115, Taiwan*

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## **ABSTRACT**

A *Graphostroma* species, described as *G. formosanum*, was collected in Taiwan. It shares the main characteristics of the type species *G. platystoma* but differs mainly in having smaller ascospores, larger conidia, smaller ostiolar openings, and a subtropical distribution. The distinctiveness of *G. formosanum* from *G. platystoma* and other *Graphostroma* species is further supported by ITS sequence analyses.

**Key words:** Ascomycota, Graphostromataceae, taxonomy, Xylariaceae

## **Introduction**

*Graphostroma* Piroz. had remained as a monotypic genus, with *G. platystoma* (Schwein.) Piroz. as its only species (Pirozynski 1974), until Li et al. (2021) added the second species *G. guizhouense* Q. R. Li. *Graphostroma* is characterized by bipartite, applanate stromata, resembling geographical maps and thus the generic name, and short-stalked asci with a minute apical ring, and hyaline, cylindrical to suballantoid ascospores lacking a germination site. *Graphostroma platystoma* was reported to be distributed in North America east of the Rockies (Pirozynski 1974) and Europe (Stadler et al. 2014; Zhang et al. 2006).

Several molecular phylogenetic studies have placed *Graphostroma* within *Biscogniauxia*, rendering the latter a paraphyletic genus. *Graphostroma* differs primarily from *Biscogniauxia* in

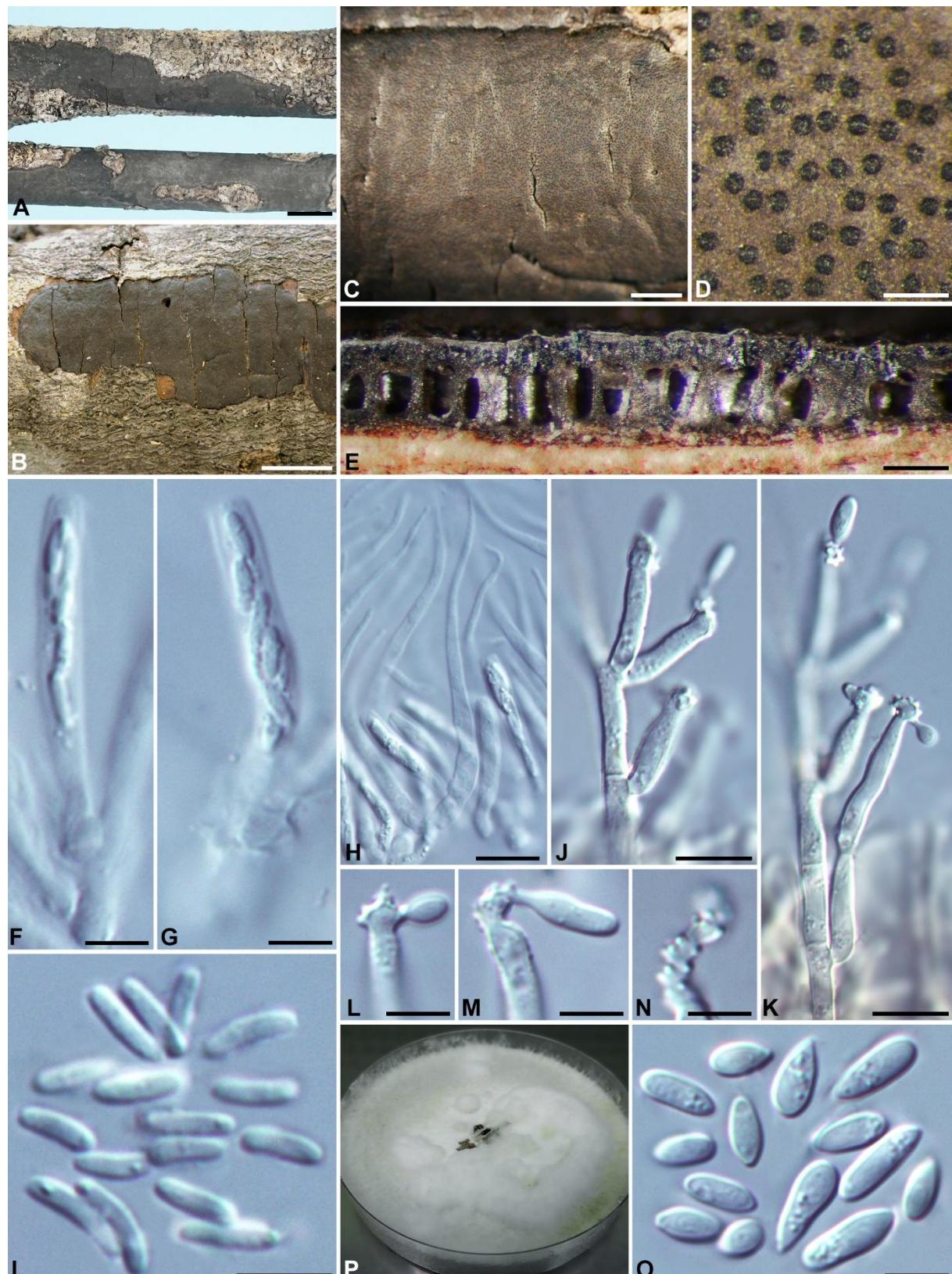
having hyaline ascospores lacking a germination site. Nevertheless, the two genera share similar macromorphological and anamorphic features, including bipartite, applanate stromata and *Nodulisporium* anamorphs.

A *Graphostroma* species, newly described here as *G. formosanum*, was collected in Taiwan, an island situated in the subtropics. Unlike most previously reported *Graphostroma* specimens, which are from temperate regions, *G. formosanum* occurs in a subtropical environment. Its distinctiveness is strongly supported by both morphological features and phylogenetic analyses.

## **Materials and Methods**

Asci, ascospores, conidiogenous cells, and conidia were examined using differential interference contrast microscopy and bright field microscopy. Material was examined in water, and Melzer's

\*Corresponding author, e-mail: [yumingju@gate.sinica.edu.tw](mailto:yumingju@gate.sinica.edu.tw)



**Fig. 1.** *Graphostroma formosanum* (from holotype). A, B. Stromata. C, D. Stromatal surface. E. Vertical section of perithecial layer overlain with a carbonaceous layer. F, G. Ascospores. H. Asci and paraphyses. I. Ascospores. J, K. Conidiophores. L–N. Conidiogenous cells. O. Conidia. P. Colony on a 9-cm Petri plate containing OA at 4 wk Bars in A, B = 1 cm; C = 2 mm; D, E = 0.2 mm; H, J, K = 10  $\mu$ m; F, G, I, L–O = 5  $\mu$ m.

iodine reagent was applied to test the amyloid reaction of ascal apical rings. The color designations follow Rayner (1970).

Cultures were obtained by placing pieces of tissue of freshly collected stromata on malt extract agar without peptone added as given in Kenerley and Rogers (1976). Resulting colonies were transferred to 9-cm plastic Petri dishes containing 2% oatmeal agar (OA), from which the culture description was made, and incubated at 20°C under 12 hours of fluorescent light. Cultures were deposited at BCRC (Bioresource Collection and Research Center, Hsinchu, Taiwan).

Total DNA was extracted from dried mycelia following Ju et al. (2022). PCR amplification of ITS was performed as described in Hsieh et al. (2009), and the resulting sequences were subjected to NCBI MEGABLAST searches. ITS sequences of *Graphostroma* species were retrieved from GenBank (Table 1) and aligned to form a dataset for Maximum-Likelihood (ML) and Bayesian Inference (BI) analyses. The ML and BI analyses followed the procedures of Ju et al. (2023). The final dataset comprised 15 taxa, with *Whalleya microplaca* (Berk. & M. A. Curtis) J. D. Rogers, Y.-M. Ju & F. San Martín as the outgroup taxon.

## Taxonomy

***Graphostroma formosanum* Y.-M. Ju & H.-M. Hsieh, sp. nov.**

Fig. 1

MycoBank MB860728

**Etymology.** Referring to Taiwan.

**Typification.** TAIWAN. I-lan County, Yuan-shan, Fu-shan, on dead corticated twigs of *Cyclobalanopsis longinu*x, 12 Nov 2002, Ju, Y.-M.

& Hsieh, H.-M. 91111201 (cultured from stroma YMJ2324) (holotype HAST 147527); ITS sequence deposited at GenBank as PX387079.

Stromata applanate, with irregular margins, up to 20 cm long × 1–3 cm broad × 0.3 mm thick; surface dull blackish brown, plane, with major cracks across the width of stromata, overlain with a brown, thin dehiscing layer on developing stromata, carbonized immediately beneath surface 70–90 µm thick and between perithecia; tissue beneath perithecia thin, dark brown. Perithecia ellipsoid to short-cylindrical, 100–150 µm diam × 150–250 µm high. Ostioles discoid, slightly raised above the stromatal surface, 40–60 µm broad at base. Asci with eight ascospores arranged in biserrate to partially biserrate manner, cylindrical to clavate, 25–35 µm total length, the spore-bearing part 15–20 µm long × 3.5–4.5 µm broad, with an apical ring staining negative or only occasionally faintly amyloid in Melzer's iodine reagent, flattened-discoid, 0.5 µm high × 1.5 µm broad. Ascospores hyaline, unicellular, cylindrical to suballantoid, with broadly rounded ends, smooth, (4.2–)4.4–5.4(–6.2) × (1.1–)1.4–1.6(–1.8) µm (4.9 ± 0.5 × 1.5 ± 0.1 µm, N = 40), lacking a germination site, lacking a hyaline sheath; episporae smooth. Paraphyses copious, thin-walled, septate, swollen at base 3.5–4 µm broad, tapering upwards.

**Cultures and anamorph.** Colonies reaching the edge of 9-cm Petri dish in 3 wk, white, becoming pale olivaceous in places, appressed, becoming cottony from center outwards, azonate, with diffuse margins. Reverse hazel (88) from center outwards. Sporulating regions close to center. Conidiogenous structure upright, mononematous, with nodulisporium-like branching pattern as de-

**Table 1.** ITS sequences of *Graphostroma formosanum* (in boldface) and those of *Graphostroma* species retrieved from GenBank.

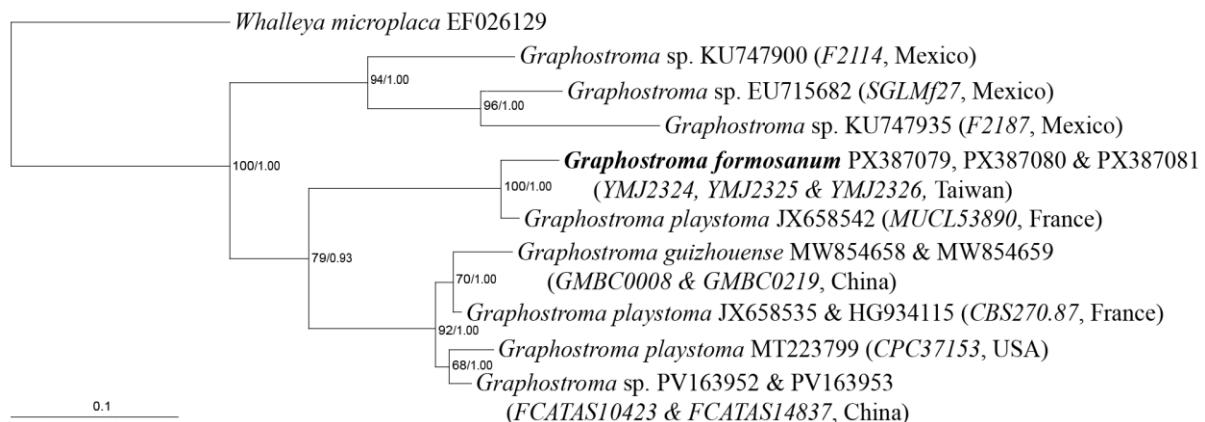
Species	Origin	Collecting data	GenBank accession number
<i>G. formosanum</i>	Taiwan	<i>YMJ2324</i> ex holotype, this study	<b>PX387079</b>
<i>G. formosanum</i>	Taiwan	<i>YMJ2325</i> , this study	<b>PX387080</b>
<i>G. formosanum</i>	Taiwan	<i>YMJ2326</i> , this study	<b>PX387081</b>
<i>G. guizhouense</i>	China	<i>GMBC0219</i> , Guizhou Province, on wood (Li et al. 2021)	MW854659
<i>G. guizhouense</i>	China	<i>GMBC0008</i> , Guizhou Province, on wood (Li et al. 2021)	MW854658
<i>G. platystoma</i>	USA	<i>CPC37153</i> , New York City, corticated branch of <i>Lindera benzoin</i> (Crous et al. 2020)	MT223799
<i>G. platystoma</i>	France	<i>CBS270.87</i> , Saône et Loire, Bissy/Uxelles, on wood of <i>Cra-taegus</i> ( <a href="https://wi.knaw.nl/page/fungal_display/32345">https://wi.knaw.nl/page/fungal_display/32345</a> )	HG934115
<i>G. platystoma</i>	France	<i>CBS270.87</i> , as above	JX658535
<i>G. platystoma</i>	France	<i>MUCL53890</i> , on wood of <i>Quercus</i> (Stadler et al. 2014)	JX658542
<i>G. sp.</i>	China	<i>FCATAS14837</i> , Jilin Province	PV163953
<i>G. sp.</i>	China	<i>FCATAS10423</i> , Jilin Province	PV163952
<i>G. sp.</i>	Mexico	<i>F2187</i> , isolated from asymptomatic surface-sterilized fern frond	KU747935
<i>G. sp.</i>	Mexico	<i>F2114</i> , isolated from asymptomatic surface-sterilized fern frond	KU747900
<i>G. sp.</i>	Mexico	<i>SGLMf27</i> , isolated from <i>Taxus globosa</i>	EU715682
<i>Whalleya micro-placa</i>	Taiwan	<i>Ju, Y.-M. &amp; Hsieh, H.-M. 91111215</i> , I-lan County, Yuan-shan, Fu-shan, on bark of <i>Elaeocarpus japonicus</i> , 12 Nov 2002 (HAST)	EF026129

fined in Ju and Rogers (1996), 3.5–4  $\mu\text{m}$  broad at main axis, smooth, hyaline to yellowish. Conidiogenous cells 2–4 arising from each terminal branch, cylindrical, 16–25  $\times$  2.5–4  $\mu\text{m}$ , smooth, bearing denticulate conidial secession scars at the apex, apex often swollen after multiple conidia produced in basipetal succession, and this process sometimes repeated several times. Conidia produced holoblastically in sympodial sequence, hyaline, smooth, obovoid, ellipsoid to short-clavate, (5.0–)6.4–8.2(–9.1)  $\times$  (2.6–)2.8–3.2(–3.5)  $\mu\text{m}$  (7.3  $\pm$  0.9  $\times$  3.0  $\pm$  0.2  $\mu\text{m}$ , N = 40), with a flattened base indicating former point of attachment to conidiogenous cell.

**Specimens examined (paratypes):** TAIWAN. Kaohsiung County, Tao-yuan, Bao-shan, Ten-chee, on dead corticated twigs, 26 Apr 2005, *Guu*,

*J.-R. 94042611* (cultured from stroma *YMJ2326*) (HAST 147528); Nan-tou County, Ren-ai, Tsui-feng, on dead corticated wood, 23 Sep 2002, *Ju, Y.-M. & Hsieh, H.-M. 91092315* (cultured from stroma *YMJ2325*) (HAST 147529).

**ITS sequence analyses.** NCBI MEGABLAST searches using ITS sequences of *G. formosanum* revealed the highest similarity, 93.33%, with GenBank accession JX658542, derived from culture *MUCL53890* isolated from a French specimen (Stadler et al. 2014). Phylogenetic analyses based on ITS sequences of *Graphostroma* species retrieved from GenBank (Table 1) likewise grouped *G. formosanum* with *MUCL53890* of *G. platystoma* (Fig. 2). Notably, specimens previously identified as *G. platystoma* appear to represent three distinct taxa based on ITS phylogeny.



**Fig. 2.** Phylogenetic tree inferred from the ITS dataset using ML analysis. *Graphostroma formosanum*, newly described in this study, is in boldface. Numbers at internodes indicate ML bootstrap support values, followed by Bayesian posterior probability values greater than 50%. *Whalleya microplaca* was as the outgroup.

*MUCL53890* from France clustered with *G. formosanum*, whereas *CBS 270.87* from France and *CPC37153* from the USA clustered within another clade but in different subclades.

**Notes.** *Graphostroma formosanum* differs from *G. platystoma* primarily by having smaller ascospores, larger conidia, smaller ostiolar openings, and a subtropical distribution, whereas *G. platystoma* is restricted to temperate regions. The ascospores of *G. platystoma* have been reported to range from  $6.2\text{--}6.6 \times 0.6\text{--}0.8$  to  $8\text{--}10 \times 1.5\text{--}1.9$   $\mu\text{m}$  by Pirozynski (1974) and  $7\text{--}10 \times 1\text{--}2$   $\mu\text{m}$  by Crous et al. (2020). *Graphostroma platystoma* also differs from *G. formosanum* by producing smaller conidia, measuring  $2.5\text{--}4 \times 1.5\text{--}3$   $\mu\text{m}$  in Pirozynski (1974) and  $4\text{--}6 \times 2.5\text{--}3$   $\mu\text{m}$  in Crous et al. (2020). The ascus apical rings of *G. platystoma* stain faintly amyloid (Crous et al. 2020; Pirozynski 1974), whereas those of *G. formosanum* stain negative or only occasionally faintly amyloid.

*Graphostroma guizhouense* from Guizhou Province, China (Li et al. 2021) is morphologically

indistinguishable from *G. platystoma* as described by Pirozynski (1974) and Crous et al. (2020). However, it does not produce an anamorph in culture, limiting the availability of additional morphological traits for comparison.

Of the three specimens of *G. formosanum*, the holotype was collected from twigs of *Cyclobalanopsis longinu*x (Fagaceae), whereas the hosts of the other two specimens remain unknown. Further collections are needed to clarify the host range of *G. formosanum*.

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## 在臺灣發現的圖座菌屬新種：臺灣圖座菌

朱宇敏\*、謝慧美、林君如、黃淳筠

中央研究院植物暨微生物學研究所，臺北市南港區研究院路二段 128 號，臺灣

### 摘要

在臺灣採集到的一種圖座菌屬真菌，被描述為新種臺灣圖座菌 (*Graphostroma formosanum*)。其主要形態特徵與該屬模式種平孔圖座菌 (*G. platystoma*)相似，但不同處在於子囊孢子較小、分生孢子較大、孔口較窄，以及分佈於亞熱帶地區。ITS 序列分析進一步支持臺灣圖座菌與平孔圖座菌及其它圖座菌屬物種之間的差異性。

**關鍵詞：**子囊菌門、圖座菌科、分類學、炭角菌科