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# Observations on Sri Lankan *Hypoxylon*: a comprehensive morphological study on *H. anthochroum, H. flavoargillaceum,* and *H. piceum*

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## Abstract

Most of the *Hypoxylon* species are saprobic and usually one of the earliest species to colonize the deteriorated wood. The species of *Hypoxylon* belonging to the family Hypoxylaceae exhibit a large diversity of fungi in tropical countries like Sri Lanka. However, research on the identification and classification of Hypoxylaceous species is scarce in Sri Lanka. The present study was aimed towards the identification of Sri Lankan Hypoxylaceous species and to produce a stable classification for species nomenclature based on a reliable approach using distinguishable morphological characteristics. An assessment of hypoxylaceous fungi was carried out in the Pilikuththuwa lowland wet zone forest area in Sri Lanka and several *Hypoxylon* species were identified morphologically, using both macroscopic and microscopic characteristics including features of stromata, ascomata, asci, ascospores, the colour of KOH extractable pigments and culture characteristics. Based on the results the identity of *H. anthochroum, H. flavoargillaceum,* and *H. piceum* were confirmed by morphology.

**Keywords:** Fungi, Morphology, Taxonomy

## Introduction

Genus *Hypoxylon* (Bull.) was described in the family Xylariaceae in previous classification systems (Ju & Rogers, 1996; Stadler, 2011; Lee & Whalley, 2000). Based on a multigene analysis of ITS and β–tubulin genes and morphology of asexual morphs, *Hypoxylon* was transferred to the family *Hypoxylaceae* (Ascomycota, Xylariales) with its other related genera (Daranagama et al., 2018; Wendt et al., 2018). Recently the genus *Hypoxylon* was divided among several other genera in previous classification systems, as well as newly erected genera, due to their stromatal anatomy, and the multi-DNA locus genealogy. For example, Lambert et al. (2019) accommodated a new genus *Hypomontagnella* following morphology and chemotaxonomic studies along with multigene phylogeny. With this addition, *Hypomontagnella* now comprises five species including *Hypomontagnella monticulosum*.

Morphological identification is the basic step of fungal species identification. Species in the genus *Hypoxylon* have a characteristic feature, which is the presence of KOH extractable pigments due to their secondary metabolites. In the precise identification and characterization of fungi, target regions of the ribosomal DNA genes subjected to amplification and sequencing have become a promising tool (Bitzer et al., 2008; Triebel et al., 2005). However, due to the slow evolution of protein-coding genes such as RNA polymerases (RPB1, RPB2) and β-tubulin, they are more effective in inferring distant phylogenetic relationships among species (Hongsanan et al., 2017; Daranagama et al., 2018; Wendt et al., 2018).

Although a small country in its landmass, Sri Lanka has a great biodiversity of fungi. However, information available on Sri Lankan fungi is scattered. In Sri Lanka, the studies on fungal diversity were initiated before the 1800s and the first Sri Lankan fungi to be recorded were *Peziza ceylonische* and *P. lembosa* (Karunarathna et al., 2012). From there, several mycologists carried out several studies on Sri Lankan fungi. According to Karunarathna et al. (2012), current information suggests only a little more than 2,000 species of Sri Lankan fungi are presently known although the diversity can be several folds higher than that. Research on identification, taxonomy, and phylogeny of Hypoxylaceous species is scarce in Sri Lanka though there are a few studies carried out to characterize species of *Hypoxylon* (Kuhnert et al., 2014, Palapathwala et al., 2019). The present study is a part of the major study conducted by Palapathwala et al. (2019). This paper will provide a major contribution towards the identification and nomenclature of Sri Lankan hypoxylaceous fungal species while producing a stable morphological classification for species.

## Methodology

Fresh specimens of species of *Hypoxylon* (3-5 specimens representing each species) were collected on the track to the caves of Pilikuththuwa lowland wet zone forest, located just 30 miles from Colombo, in Gampaha district from decaying wood material lying on the ground, based on the macro morphological features, during May-July 2018. Collected hypoxylaceous fungal species were morphologically characterized while surface colour, colony colour, and KOH extractable pigments were recorded. Ascomata of collected fungal species were observed using the stereomicroscope (Olympus SZ61 model, Philippines). Microscopic characters of asci and ascospores were observed using Phase Contrast Microscope (Olympus CX41 model, Tokyo, Japan). The apical ring of the ascus was stained using Melzer's reagent. Microphotography was taken using Olympus DP 26 Mega Pixel camera fitted to the Phase Contrast Microscope (X400 magnification) (Olympus CX41 model, Tokyo, Japan). Measurements of stromata (n=10), perithecia (n=10), asci (n=20), and ascospore (n=20) were taken from material mounted in water and the mean values were used in the description. Measurements were made with the Tarosoft (R) Image Framework Program and images used for figures were processed with Adobe Photoshop CC version 18.0 (Adobe Systems Inc., The USA). To isolate fungi, the upper surface of any fruiting bodies was excised using a sterilized scalpel blade. Pure cultures were obtained either from single spore or multispore isolation (Chomnunti et al., 2014; Daranagama et al., 2015). The cultures were maintained at 27 °C in Malt Extract Agar (MEA) in the laboratory. Morphological identification of the collected and isolated species was done using the previous literature and the morphological keys by Ju and Rogers (1999), Kuhnert et al. (2014), and Daranagama et al. (2018).

## Results and Discussion

In this section, three species of *Hypoxylon* are described with illustrations and pictorial guides for identification.

*Hypoxylon anthochroum* Berk. & Broome J. Linn. Soc., Bot. 14: 122. 1873.

Saprobic on decaying dicot bark. Stromata Superficial, black, effuse-pulvinate, plane, conspicuous (Figure 1f), KOH extractable pigments dull green (70) (Figure 1e). *Ascomata* 47.2 x 36.6 µm, roughly spherical (Figure 1d). *Asci* Cylindrical, 8-spored, 93.9 µm in total length 4.1 µm broad, spore-bearing part 58.2 µm, stipe 35.7 µm (Figure 1a, b). *Ascospores* 7.8 x 3.4 µm, dark brown to brown, ellipsoidal-inequilateral, broadly rounded ends, sigmoid germ slit, nearly spore-length (Figure 1c).

Culture characteristics - smooth, velvety, primrose (65) (Figure 1h), symmetrical edges, reverse olivaceous dull (69), with concentric rings (Figure 1g).

Material examined - 7 $^{0}$ 03' 50.2147" N and 8 $^{0}$ 03' 01.1160" E, 25 May 2018, herbarium = UKBH012, Daranagama and Palapathwala, HYXL 012, living culture = UKBC012.

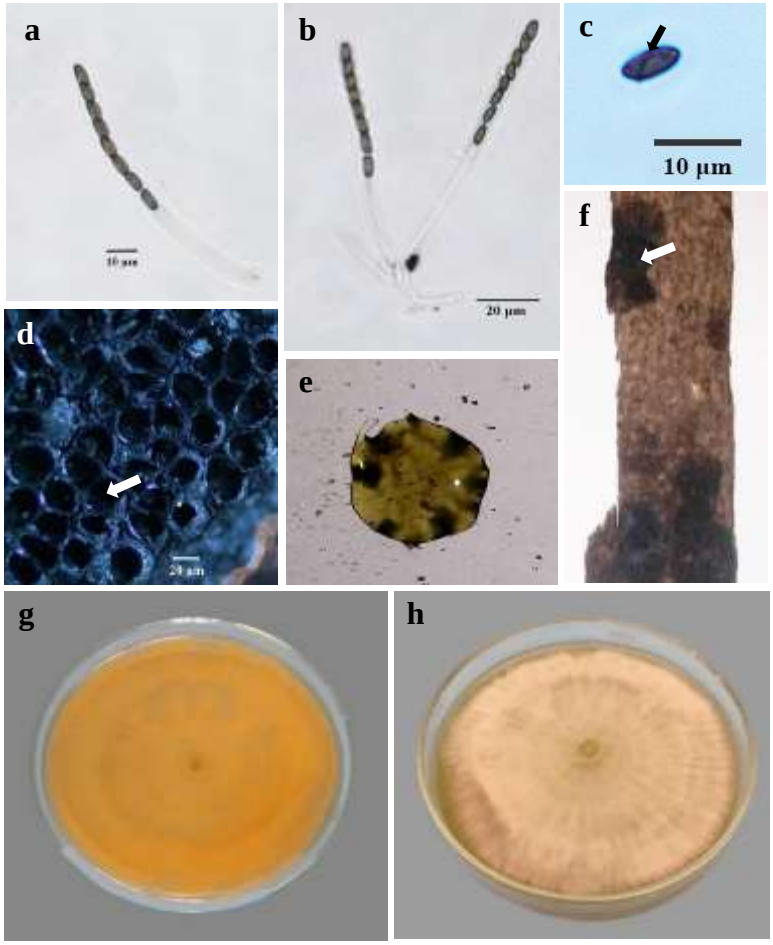


***Figure 1.*** *Morphological features of Hypoxylon anthochroum;* ***a, b*** *Asci,* ***c*** *Ascospore (germ slit shown by arrowhead),* ***d*** *Cross section of stromata showing ascomata (shown by arrowheads),* ***e*** *KOH extractable pigment,* ***f*** *Appearance of stromata on substrate (shown by arrowhead),* ***g*** *Lower surface of the culture and* ***h*** *Upper surface of the culture on MEA.*

*Hypoxylon flavoargillaceum* J. H. Mill., in Chardón & Toro, *Monograph Univ. Puerto Rico*, Series B 2: 200 (1934).

Saprobic on decaying dicot bark. *Stromata* superficial, inconspicuous, surface brown vinaceous (84) (Figure 2f), KOH extractable pigments greenish glaucous (90) (Figure 2e). *Ascomata* 185.2 x 158.2 µm, spherical to hemispherical (Figure 2c). *Asci* Cylindrical, 8-spored, 114.6 µm in total length x 4.4 µm broad, spore-bearing part 62.7 µm, stipe 51.9 µm (Figure 2a, b). *Ascospores* 8.0 x 3.8 µm, light brown to brown, ellipsoidal-equilateral, broadly rounded ends, uniseriate, unicellular, sigmoid germ slit, nearly spore-length (Figure 2d).

Culture characteristics - plane, sepia (60) to dark vinaceous (82) mycelia, pale vinaceous (85) centre, with forming concentric rings after 20-25 days (Figure 2h), reverse brown vinaceous (84) (Figure 2g).

Material examined - 7 $^{0}$ 03' 50.2126" N and 8 $^{0}$ 03' 01.1008" E, 29 June 2018, herbarium = UKBH016, Daranagama and Palapathwala, HYXL 016, living culture = UKBC016.

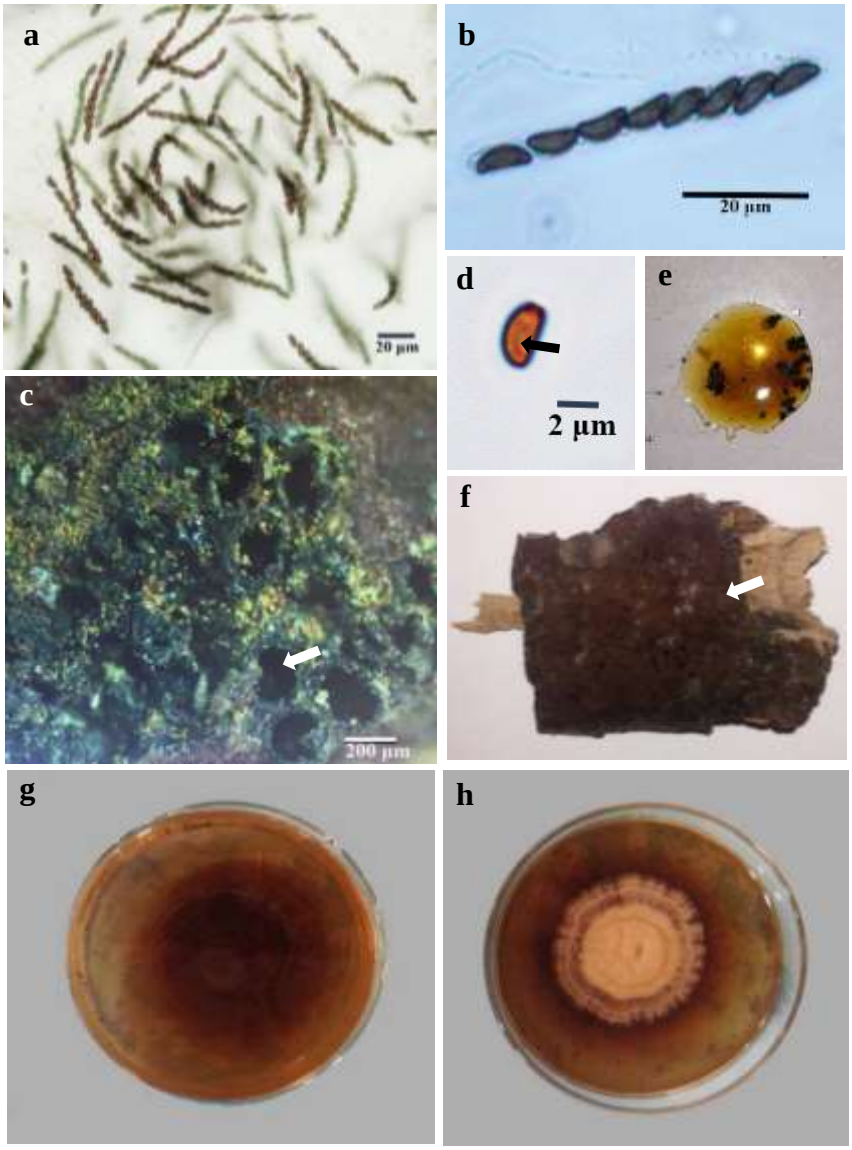


***Figure 2.*** *Morphological features of Hypoxylon flavoargillaceum;* ***a, b*** *Asci,* ***c*** *Cross section of stromata showing ascomata (shown by arrowheads),* ***d*** *Ascospore (shown by arrowhead),* ***e*** *KOH extractable pigment,* ***f*** *Appearance of stromata on substrate (shown by arrowhead),* ***g*** *Lower surface of the culture and* ***h*** *Upper surface of the culture on MEA.*

*Hypoxylon piceum* Ellis, *Am. Nat.* 17(1): 194 (1883).

Saprobic on decaying dicot branches. *Stromata* superficial, conspicuous, dense, surface dark black (Figure 3f), KOH extractable pigments dark herbage green (68), dull green (70) (Figure 3d). *Ascomata* 256.4 x 214.8 µm, spherical (Figure 3e). *Asci* Cylindrical, 8-spored, 81.2 µm in total length x 3.9 µm broad, spore-bearing part 49.5 µm, stipe 31.8 µm (Figure 3a, b). *Ascospores* 6.4 x 2.8 µm, dark brown, ellipsoidal-nearly equilateral, uniseriate, unicellular, broadly rounded ends, straight germ slit, spore-length (Figure 3c).

Culture characteristics - smooth, soft, plane, vinaceous buff (86), primrose (65), (Figure 3f), reverse vinaceous (84), sepia (63) with straw (46) margins, with symmetric edges (Figure 3g).

Material examined - 7 $^{0}$ 03’ 50.2151” N and 8 $^{0}$ 03’ 01.1068” E, 20 July 2018, herbarium = UKBH027, Daranagama and Palapathwala, HYXL 027, living culture = UKBC027.

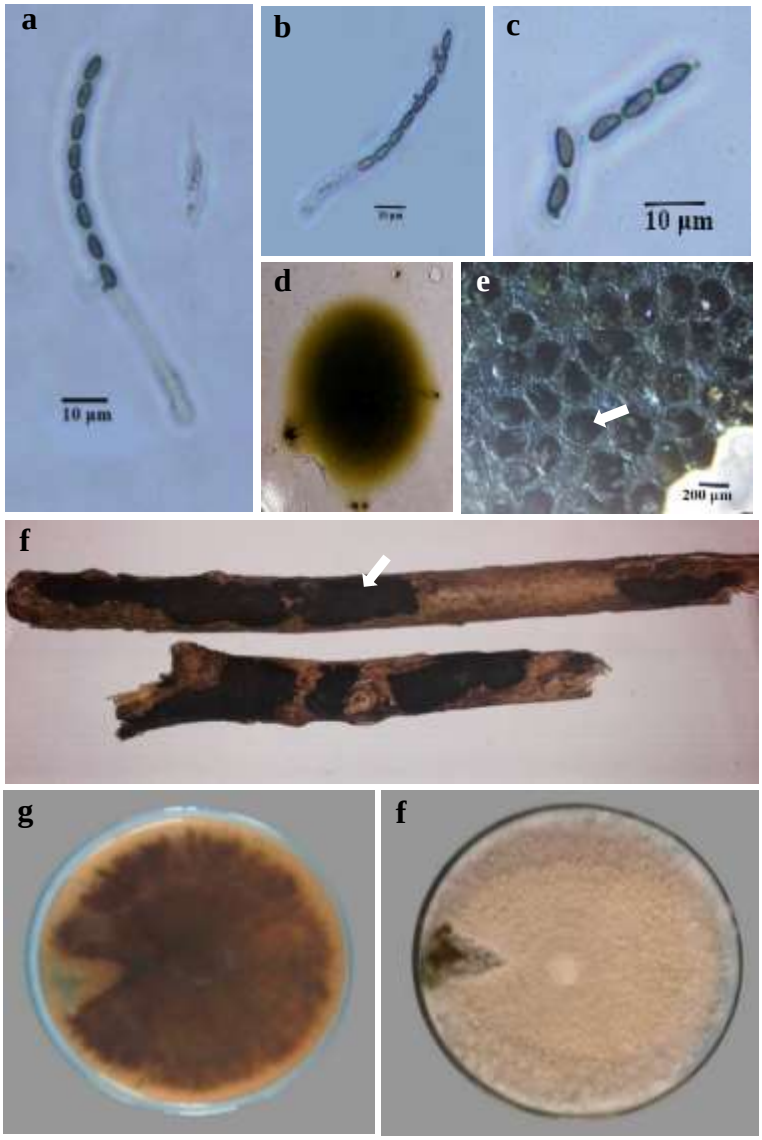


***Figure 3.*** *Morphological features of Hypoxylon piceum;* ***a, b*** *Asci,* ***c*** *Ascospore,* ***d*** *KOH extractable pigment,* ***e*** *Cross section of stromata showing ascomata (shown by arrowheads),* ***f*** *Appearance of stromata on substrate (shown by arrowhead),* ***g*** *Lower surface of the culture and* ***h*** *Upper surface of the culture on MEA.*

## Conclusion

Based on the results, Pilikuththuwa low land wet zone forest exhibits a high diversity of *Hypoxylon* species with clear morphological variations, which can be used as an identification tool in classification, during the cases where it is failed or difficult to conduct the molecular analysis. *H. anthochroum*, *H. flavoargillaceum*, and *H. piceum* are recognized by new combinations of teleomorphic morphological characters. Even though in this study the morphological characters were helpful in the identification of three described species, it is recommended to carry out a molecular analysis to find out whether the genealogy is fully in agreement with phenotype-derived traits.

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