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Phylogenetic Analysis of Polyporous Fungi Collected from Batam Botanical Garden, Riau Province, Indonesia

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Abstract

Botanical gardens are areas that provide protection for trees and other organisms like polyporous fungi. Polyporous fungi are important fungi that degrade remaining lignocellulosic in leaf litter or dead trees. These mycobiota are also noted for their vital role in biorefinery, bioremediation, medicine and phytopathogen. The knowledge of the importance of the polyporous fungi to describe polyporous fungal species is fundamental for generating data base information of their occurrence and their functions. This research's goal was to explore and characterize the polyporous fungi collected in Batam Botanical Garden in three sampling areas. Fungal samples were collected in May and July 2017. Subsequently, morphological characters were recorded, the fungal tissue was isolated to extract the DNA, then the data sequence was amplified and aligned to construct a phylogenetic tree. Five fungal families found belong to order Polyporales and were classified morphologically. They were Polyporaceae, Ganodermataceae, Fomitopsidaceae, Irpicaceae and Hymenochaetaceae. Three fungal species namely; *Pycnoporus sanguineus, Trametes ijubarskii,* and *Antrodia wangii* were identified based on phyllogenetic analysis whereas seven other fungal samples were identified as *Earliella scabrosa, Hexagonia tenuis, Polyporus tenuiculus Lenzites betulina, Lentinus concavus, Phellinus rimosus* and *Hexagonia apiaria.* This study classifies and adds fundamental databases on fungal taxonomy and diversity on the fungal organisms found in Batam Botanical Garden. This background data is vital to carry out an advance research in some areas such as bio-chemistry, bio-degradation, pharmacology and biotechnology.

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INTRODUCTION

Polyporous fungi belong to order Polyporales which was grouped into the 18 families based on morphology, biochemical, and phylogenetic observation (Justo *et al.*, 2017). Generally, polyporous fungi are further characterized from family to genera based on the characteristics of basiodiocarp and hymenophores (Ryvaden & Gilbertson, 1993). Due to the interest in studying polyporous fungi draws, especially with molecular analysis, currently more than 500 taxonomic proposals were developed from 2010-2017 and 2,183 research articles were highlighted the order Polyporales (Zhao *et al.,* 2015; Justo *et al.,* 2017).

Polyporous fungi are ubiquitous, some of the members are necrotrophs and mychorriza whereas most members are saprotrophic fungi. Most species of Polyporales are found living on dead wood or logs. Mushrooms called wood decay fungi are the major cause of wood rot and degradation of remaining lignocellulosic which are essential in the forest carbon cycle or plantation ecosystem. The ability of polyporous fungi to degrade lignocellulosic materials is due to its secretion of extra-cellular enzymes with various lignocellulose-degradation abilities (Berrin *et al.,* 2012). Action of these hydrolytic enzymes were studied in the bioconversion of cell wall polyssacharide to simple sugar for bioethanol (Margeot *et al.,* 2009). Family Polyporaceae, Ganodermataceae and Fomitopsidaceae are known and studied due to their ability to degradae lignin and cell wall (Kirk & Farrell., 1987).

Studies to explore fungi order Polyporales in Sumatera had been conducted in one sampling location as well as in several sampling sites (Wahyudi *et al.*, 2016; Afrida *et al.*, 2009). The previous studies described the fungi collected in that area but did not specifically target Polyporales fungi and did not use any phylogenetic analysis procedure. The unavailability of adequate resource for referencing the fungal species that belong to Polyporales family leads to the need of more experiments of collecting, identifying, isolating and documenting polyporous fungi in Indonesia. Furthermore, the importance of fungi and their medicinal value was documented in a previous study in Baduy, Indonesia (Khastini *et al.*, 2018). However, the previous study did not specifically mention any other genus beside Ganoderma.

In Indonesia, botanical gardens are established to preserve endangered trees which occurs due to the monoculture plantations log industries practice. Hence, all aspects of botanical garden ecosystem is essential including polyporous fungi. The classification of polyporous fungi is also fundamental to maintain their positive association with wood substrates such as the decomposition process in botanical gardens. Additionally, the study can be applied to tackle detrimental effects which arise from fungi-wood association like some fungal diseases that damage trees. This study was conducted to explore, characterize, isolate and identify polyporous fungi found in Batam Botanical garden based on its morphology and phylogenetic analysis. The isolated fungi were also described for their valuable importance economically and commercially.

This study will add valuable databases related with taxonomy and diversity of Polyporales fungi especially those which are collected in Batam Botanical garden. Additionally, this study emphasis on the roles and functions of fungi grown on wood or dead logs.

METHODS

Collection of fungi

Fungal specimens were collected from three different sampling areas in Batam Botanical Garden (Figure 1). Each plot area was explored and mushroom bodies were collected.

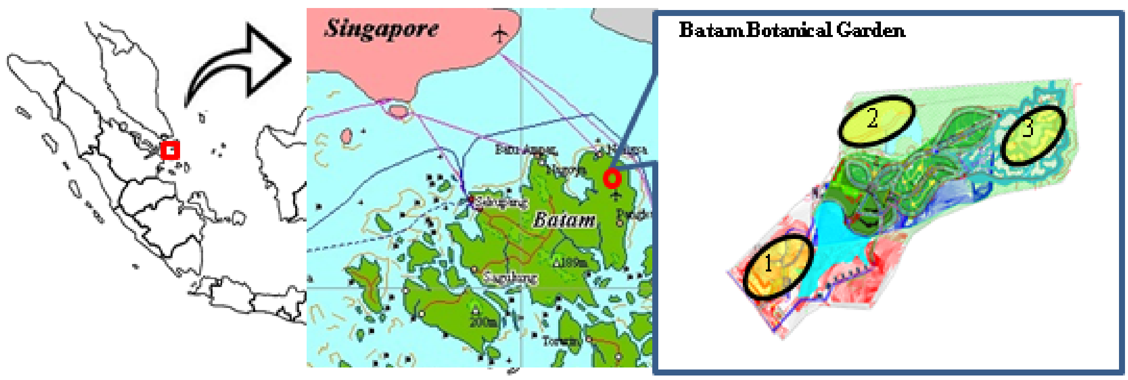

Figure 1. Batam island (left and middle): Batam Botanical Garden (right). The sampling areas: 1. Arboretum, 2. Mangrove swamp, 3. Forest. Map source: Aninomus (2012) and Aninomus (2013)

Identification of collected fungi

The collected polyporous fungi were characterized and described to family level based on their morphological characteristics such as basiodiocarp type (mushroom body), hymenophore (fungal underside appearance) and the rot type.

Some of the terms used to describe basidiocarp type are pileate, stipitate and resupinate. Pileate means a whole mushroom body with or without a stalk; Stipitate is defined as a fungus containing a mushroom body & stalk and Resupinate is a fungus type with its mushroom body or colony directly growing on substrates like wood. Hymenophore appearances on this study were also characterized for the identification depicted in Figure 2.

Isolation of fungi

The fungal tissue was sterilized in 1% of NaClO for about 3-4 minutes, rinsed in sterile distilled water before inoculated in PDA (*Potato Dextose Agar*) or MEA (*Malt Extract Agar*) media. Successfully isolated fungi were further classified based on their characteristics such as size, color, cap, gills, stalk, veil, annulus, volva and spore prints. Chemical characterization methods that were also employed such as the addition of 5% KOH as described in Justo & Hibbet (2011); Tellez-Tellez *et al.* (2016).

Cultures of isolated fungi were sent to Genetika Science lab Indonesia to isolate, amplify, purify, and sequence the isolated fungal DNA. Genomic DNA extraction was performed using Presto Mini gDNA Yeast Kit (Geneaid). The Internal Transcribed Spacer region (ITS) of rRNA was amplified using the primer pair ITS-1 (5'-TCC GTAGGT GAA CCTTGC GG- 3') and ITS-4 (5'- TCC TCC GCT TAT TGA TAT GC-3') (White *et al.* 1990), with BiolineMyTaq Red Mix (Bioline) following the manufacturer's instructions. PCR products were purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research) and sequenced using Bi-directional Sequencing. BLASTN searches were performed on fungal sequences to confirm their identification by comparing them with reference strains in NCBI GenBank to support phylogenetic analysis.

Phylogenetic Analysis

Fungal sequences of ITS-1 and ITS-4 were assembled, edited and aligned using Bioedit (Hall, 1999). The sequences were also compared with the NCBI GenBank database using BLAST searches, which confirmed that they were polyporous fungi, members of order Polyporales. Clustal W, MEGA 6 was used to group and align the sequences followed by Maximum-Likelihood Analysis (Tamura *et al.,* 2013). DNA sequences of fungal samples were aligned with ITS sequence data described in Lesage-Meessen *et al.*, 2011; Lomascolo *et al.,* 2002; Tomšovsky *et al.* 2006;

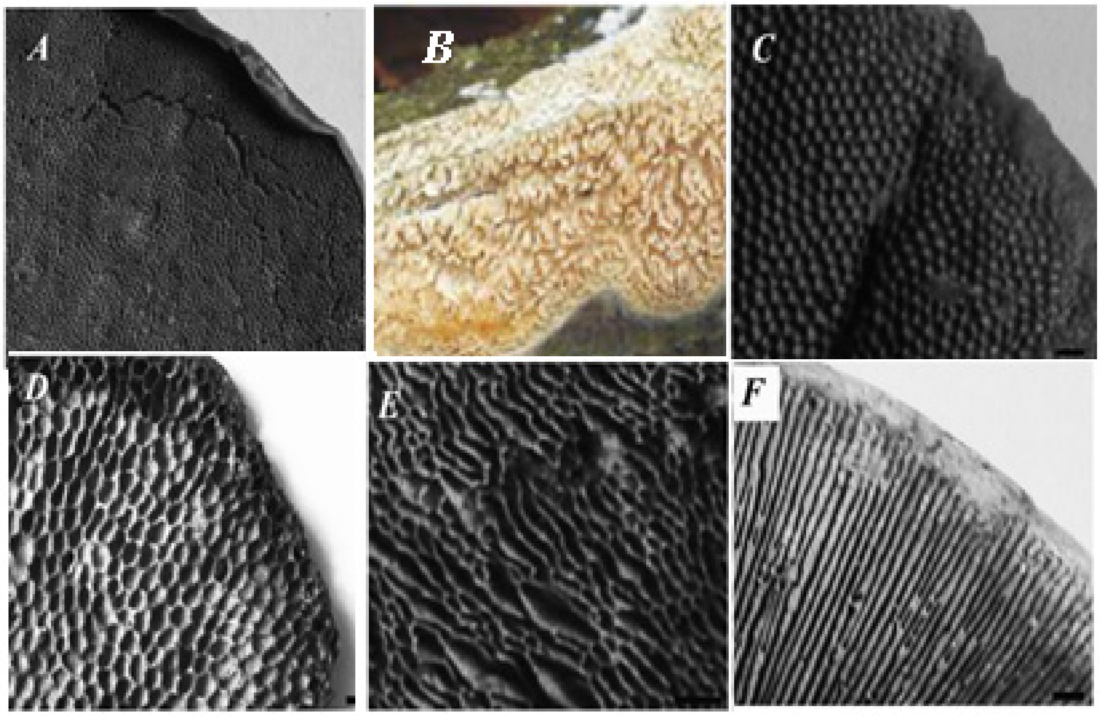

Figure 2. Hymenophore characters: A. *poroid,* B. *irpicoid,* C. *hexagonoid,* D. *favoloid, E. daedaloid,* F. *lenzitoid or lamellate.* Images taken from Zmitrovich & Ezhov (2012); Karasiński & Wolkowycki (2015).

Welti *et. al.,* 2012; Cui, 2013; Spirin *et al.,* 2013; Vlasak *et al.,* 2013. Phylogeny test was performed with the Nearest-Neighbor-Interchange as the ML-Heuristic method, and the General Reversible Time used as the Substitution Model (Felsenstein, 2004). The reliability of the phylogram was tested with bootstrap analysis with 1000 times replication.

RESULTS AND DISCUSSION

This study documented the study, classification and identification of the polyporous fungi based on the phylogenetic analysis and morphological characters observed in the Batam Botanical Garden, Riau island Province. Previous research was focused on the fungal diversity in Pekanbaru, Sumatra as documented by Wahyudi *et al.* (2016). In this study, three species of Polyporous fungi collected from Batam Botanical Garden were identified using the phylogram analysis.

Based on the fungal analysis, samples coded M3 belong to the cluster group of *Pycnoporus sanguineus* (bootstrap value: 97%), M4 belongs to the cluster group of *Trametes ijubarskii* (bootstrap value: 84%), M7 belongs to the cluster group of *Antrodia wangii* (bootstrap value: 99%) (Figure 2). However, only these aforementioned fungal specimens were successfully cultured purely. According to Berrin *et al.* (2012) due to contamination by other microorganisms, only about 30% of field collected-fungi from tropical areas.

The Polyporous fungi collected in the Batam Botanical Garden were classified into three types of basidiocarp, five types of hymenophore and two types of wood decay (Table 1). Furthermore, classification based on morphology showed that *Hexagonia apiaria*, *Hexagonia tenuis, Phellinus rimosus*, *Polyporus tenuiculus, Lenzites betulina, Earliella scabrosa* and *Lentinus concavus* were sampled in Batam Botanical Garden and their morphological characters are depicted and described on Figure 3-4. Morphology of fungal species found in Batam Botanical garden is described as *Trametes ijubarskii* (Figure 3)

This species is a white rotting fungus and is found only in the mangrove area of Batam Botanical garden. Basidiocarp: annual, fan-shaped, semicircular zone lined, smooth, tough. Size: 1.5 - 5.5 cm. Color: white. Stalk: absent, sessile. Thickness: 0.3 - 0.7 cm. Habitat: solitary or in group found on dead hardwoods causing white rot. Pores: poroid, 2 - 3 mm. Spore print: white. Chemical reaction with KOH 5%: (-) no reaction.
Earliella scabrosa (Figure 3)

Upperside basidiocarp: annual, fan-shaped semicircular zoned, smooth, tough. Underside basidiocarp: light brown Color: reddish dark brown to black with white margin. Size: 2-7 cm. Stalk: absent, sessile. Thickness: 2 - 3 mm. Habitat: solitary or in group found on dead hardwoods causing white rot. Spore print: white. Pores: 1 - 2 mm, vertically elongated (slot-like) or tube concolorous. Chemical reaction with KOH 5%: (-) no reaction.

Hexagonia tenuis

This species cause white rot on wood and logs. In Batam, this fungus was detected in the forest sample area. The description of this fungal species is further discussed in Lestari *et al.* (unpublished) (Figure 3).

Antrodia wangii

This fungal species causes brown rot in woods and is found only in the mangrove area in Batam Botanical garden. Basidiocarps: annual to perennial, resupinate fruiting body, it looks like a white or cream layer growing on dead wood or logs. Size: 5-8 cm in width. Texture: Mostly light coloured and tough to hard. Spore print: white. Chemical reaction with KOH 5%: light brown (+) (Figure 3D).

Pycnoporus sanguineus

This species is a very common white rotting fungus. This fungus is found in all sampling sites in Batam Botanical Garden. Basidiocarp: fan-shaped semicircular zoned, smooth, wrinkled, tough. Upper surface: glabrous Margin: wavy. Size: 1.5 - 5.5 cm. Color: bright orange. Stalk: absent, sessile. Thickness: 1- 5 mm. Habitat: solitary or in group found on dead hardwoods causing white rot. Spore print: white. Pores: poroid, 4-5 pores/mm. Chemical reaction with KOH 5%: (+) greenish brown. Notes: Found in forest, mangrove and arboretum areas in Batam Botanical Garden (Figure 3E).

Lenzites betulina

Upperside basidiocarp: fan-shaped, hairy, leathery. Underside basidiocarp: gill-formed, brown Color:brown. Size: 3 - 4 cm. Stalk: absent, sessile. Thickness: 1 - 2 mm. Habitat: solitary or in group found on dead hardwoods Spore print: white. Chemical reaction with KOH 5%: (-) no reaction (Figure 3).

Lentinus concavus

Upperside basidiocarp: glabrous, no hair, white. Underside basidiocarp: gill-formed, decurrent, white. Broad size: 2 - 5 cm. Stalk: present.

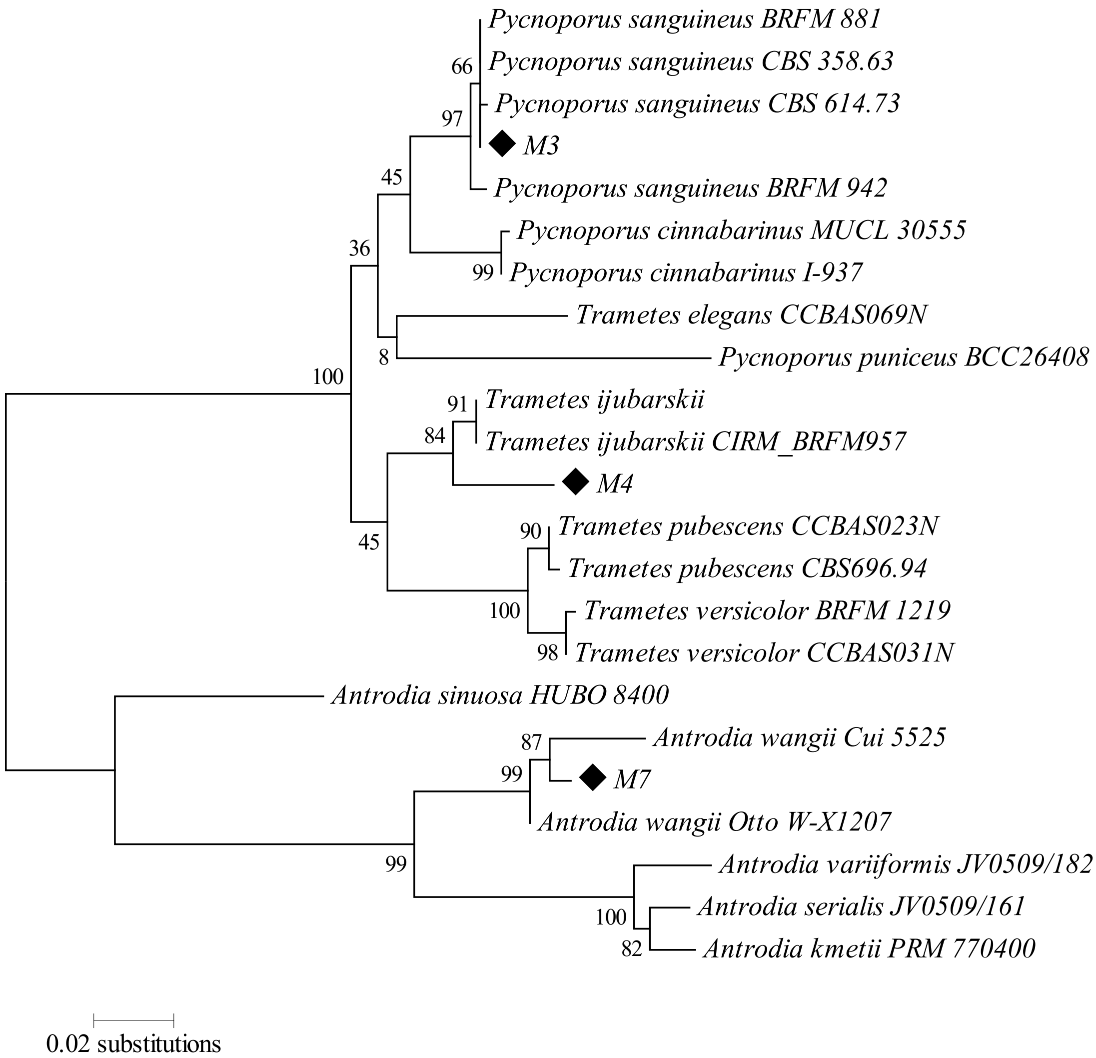

Figure 2. Maximum likelihood phylogeny of four Polyporous fungi collected from Batam Botanical Garden based on ITS data. ◆= field isolated fungus

Table 1. Broad view of morphological and rot characteristics of polyporous fungi collected in Batam Botanical Garden

Taxon	Basidiocarp			Hymenophore					Type of wood decay	
	pile-ate	stip-itate	re-supi-nate	po-roid	irpicoid or hyd-noid	hexag-onoid	lenz-itoid or lamel-late	fa-vo-loid	white rot	brown rot
Batam Botanical Garden										
Polyporaceae	20	2	1	18	-	2	2	1	21	-
Ganodermataceae	-	3	-	3	-	-	-	-	3	-
Fomitopsidaceae	-	-	1	1	-	-	-	-	-	1
Irpicaceae	-	-	1	-	1	-	-	-	1	-
Hymenochaetaceae	1	-	-	1	-	-	-	-	1	-
Total		29								

Stalk length: 1.5 - 3.5 cm. Texture: pliant. Habitat: in group found on dead hardwoods. Spore print: white. Chemical reaction with KOH 5%: (-) no reaction (Figure 3G).

Table 2. Identification of fungi collected in Batam Botanical Garden

Sampling sites and taxon	Classification
Arboretum	
Pycnoporus sanguineus	Basidiomycota, Polyporaceae
Hexagonia tenuis	Basidiomycota, Polyporaceae
Earliella scabrosa	Basidiomycota, Polyporaceae
Lentinus concavus	Basidiomycota, Polyporaceae
Mangrove	
Pycnoporus sanguineus	Basidiomycota, Polyporaceae
Trametes ijubarskii	Basidiomycota, Polyporaceae
Antrodia wangii	Basidiomycota, Fomitopsidaceae
Hexagonia apiaria	Basidiomycota, Polyporaceae
Phellinus rimosus	Basidiomycota, Polyporaceae
	Basidiomycota, Hymenochaetaceae
Forest	
Pycnoporus sanguineus	Basidiomycota, Polyporaceae
Polyporus tenuiculus	Basidiomycota, Polyporaceae
Earliella scabrosa	Basidiomycota, Polyporaceae
Lenzites betulina	Basidiomycota, Polyporaceae
Hexagonia tenuis	Basidiomycota, Polyporaceae

Hexagonia apiaria

Upperside basidiocarp: circular to semicircular body, zoned, wrinkled, tough. Underside basidiocarp: honey comb-like, poroid, Basidiocarp size: 2 - 3.5 cm broad. Color: brown. Stalk: absent, sessile. Thickness: 1 - 5 mm. Habitat: solitary or in group found on dead hardwoods causing white rot. Spore print: white. Pores: 1 - 3 mm. Chemical reaction with KOH 5%: (+) darker brown (Figure 4A, 4B). This fungal species found on dead wood and showed white rot symptom.

Polyporus tenuiculus

Basidiocarp: fan-shaped, smooth, tough. Margin: wavy, no ciliate hair. Basidiocarp size: 1.5 -5 cm. Color: white-light brown. Stalk: present. Stalk length: 4 - 9 mm. Thickness: 1 - 3 mm. Habitat: solitary or in group found on dead hardwoods. Spore print: white. Pores: 1 - 3 mm (Figure 4C, 4D). Chemical reaction with KOH 5%: (-) no reaction. Odor: mild.

Phellinus rimosus

Upperside basidiocarp: fan-shaped, annual,woody, corky, cracked, tough. Underside basidiocarp: dark brown. Basidiocarp size: 12 - 13 cm broad. Color: dark brown. Stalk: absent, sessile. Thickness: 4.5-8cm. Habitat: solitary or in group found on dead hardwoods causing white rot. Spore print: white (Figure 4E, 4F). Chemical reaction with KOH 5%: (+) black. Odor: pleasant.

Fungal species are specific to their habitat especially the fungi sampled in the mangrove area (Table 2). Fruiting bodies such as *Hexagonia apiaria*, *Phellinus rimosus, Antrodia wangii* and *Trametes ijubarskii* were only found in the mangrove area. This is supported by results of a study conducted by Gilbert *et al.,* (2002) which stated that mangrove vegetation is a habitat for distinctive fungal species such as *P. rimosus* which is often spotted in the mangrove areas in South East Asia. Majority of the polyporous fungi found in the Batam Botanical Garden were white rotting type of fungi except the *Antrodia wangii* which was a brown rotting fungus and was found only in the mangrove area. The white rotting fungi is massively dispersed round the forest ecosystem and plantation because they secrete hydrolytic enzymes capable of metabolizing lignin and cellulose whereas the brown rotting fungi typically degrades only cellulose materials. Its capability to degrade lignin is excellent characteristic as decomposers.

According to Lindsey & Gilbertson, (1978) beside their function as detritivore, polyporous fungi that are known to transfer fungal diseases to trees. In this research, there are no samples found infecting the living trees. However, it requires more extensive and periodical studies to determine thoroughly the association between fungal pathogens with tree species in the Batam Botanical garden.

In this study, *Pycnoporus sanguineus* was the dominant species since it was found in all sampling sites (Table 2). *P. sanguineus* was ubiquitous polyporous species mostly found in several regions from sub-tropic to tropical regions. Genus

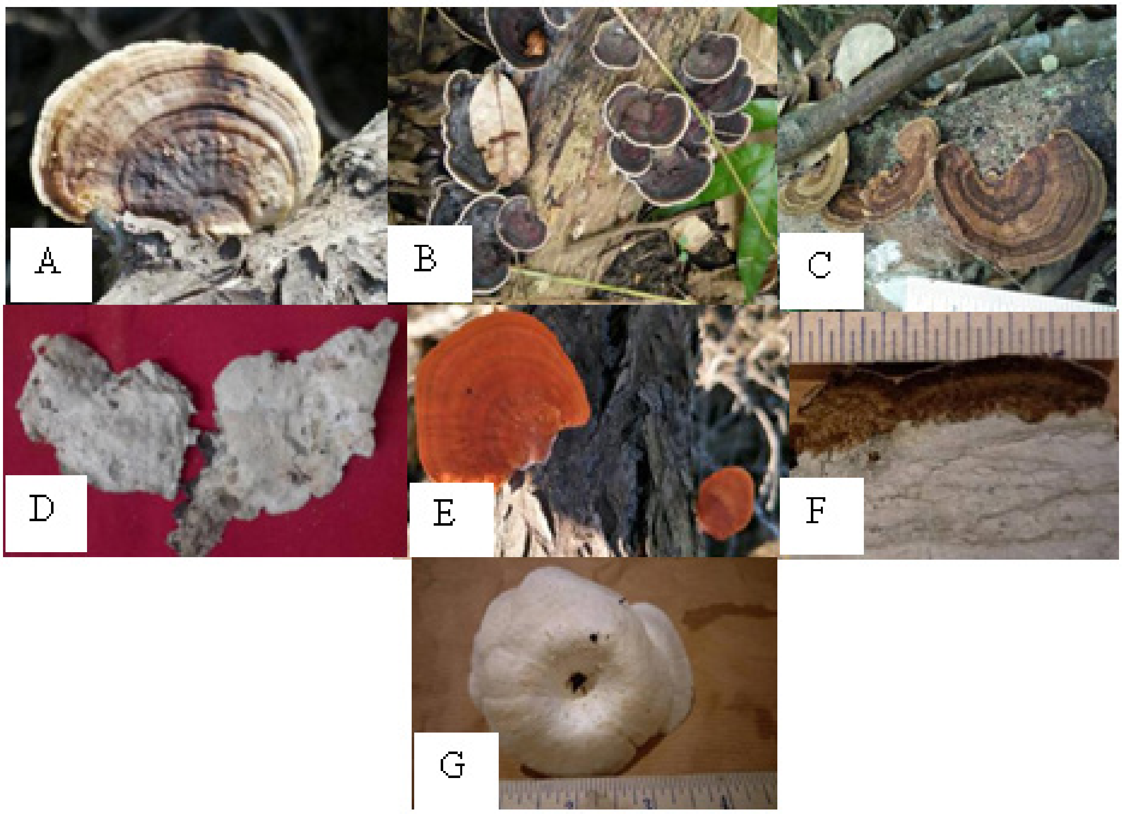

Figure 3. Fungal species found in Batam Botanical garden: A. *Trametes ijubarskii;* B. *Earliella scabrosa*; C. *Hexagonia tenuis*; D. *Antrodia wangii*; E. *Pycnoporus sanguineus*; F. *Lenzites betulina,* G. *Lentinus concavus*

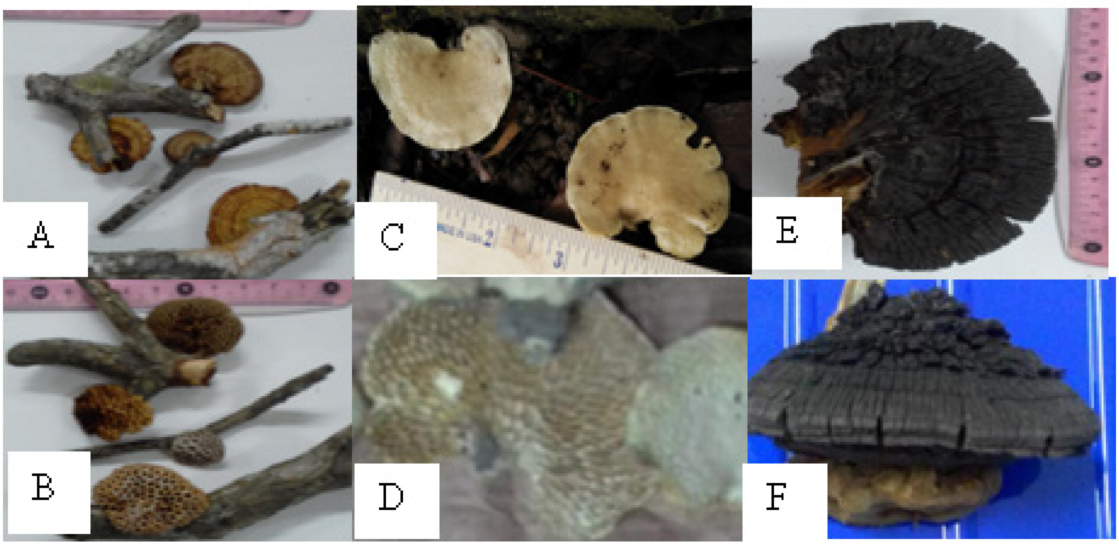

Figure 4. Fungal species found in Batam Botanical garden: upperside (A) and underside (B) of *Hexagoniaapiaria;* upperside (C) and underside (D) of *Polyporus tenuiculus;* upperside (E) and side view (F) of *Phellinus rimosus.*

Pycnoporus including *P. sanguineus* are well studied especially for their capability to synthesize several phenozone compounds such as tramesanguin, cinnabarin, cinnabarin acid, pycnoporin, etc (Diaz & Urban, 2009). In some regions such as Australia, Brazil and Africa, *P. sanguineus* is valuable for developing local medicine for toothache, arthritis, sore throat, and fever (Smania *et al.*, 1995). The presence of this fungus indicates ecological disturbance in the environment (Tellez-Tellez *et al.,* 2016).

Trametes ijubarskii and *Antrodia wangii* are categorized as the most recent known polyporous fungi and their roles has not been extensively studied. However, according to Wu *et al*. (2016) and Thiribhuvanamala *et al.* (2017) both fungi shows hemicellulotic activity under laboratory tests. Other fungal species, *Phellinus rimosus* is reported to show antitumor activity whereas *Earliella scabrosa* secretes enzymes useful in degradation of synthetic dyes (Geurra *et al*., 2008; Ajith & Janardhanan, 2003).

Botanical gardens serve as a reserve for protecting endangered tree species and they also offer refuge for polyporous fungi since they are essential as decomposer. More studies related to the diversity of polyporous fungi in the ecosystem botanical gardens are fundamental to provide a concise data on the ecological relationship. This study offers useful data on taxonomy and diversity of fungi order Polyporales which are necessary for conducting advance research closely related for bio-refineries, bioremediation and pharmaceutical industries.

CONCLUSION

Three species of polyporous fungi were identified based on phylogenetic analysis, namely: *Pycnoporus sanguineus, Trametes ijubarskii,* and *Antrodia wangii.* Other fungal species that were collected were the *Phellinus rimosus, Earliella scabrosa, Hexagonia tenuis, Hexagonia apiaria*, *Lenzites betulina, Polyporus tenuiculus,* and *Lentinus concavus*. The fungal species found in Batam were unique to their habitat. *Pycnoporus sanguineus* was discovered in all sampling sites including the mangrove area. The polyporous fungi collected and identified in this study are economically and industrially vital, therefore, the existence of botanical gardens is very important to preserve both tree and fungal species.

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