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Ecological and Taxonomical Perspective of Yeasts in Indonesia

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In the course of ecological and taxonomical study of yeasts in Indonesia, a total of 2147 yeast isolates from 315 samples in the year 2003, 2005, 2006 and 2008 had been obtained from eight locations e.g. Liwa (Sumatera), Cibinong (Java), Cibodas (Java), Kutai (Kalimantan), Enrekang (Sulawesi), Pucak (Sulawesi), Gili and Kuta (Lombok), and Kupang (Timor). Leaves, flowers, litters, soils, epiphytic soils, insects and insect's nests were collected for yeasts isolation. Our molecular identification based on D1/D2 region of nuclear large-subunit rDNA and the internal transcribed spacer (ITS) regions sequence data on 525 representative isolates revealed that 306 isolates belong to 48 described species (18 genera) and 209 strains belong to 19 undescribed species (19 genera), and 10 isolates were discarded because of contamination. Based on their substrates, litter had the highest yeasts genera (19) followed by soils (18), flowers (10), leaves (6), epiphytic soils (4), and insects and insect's nests (4). Genera found on soils were also common on litters. Yeasts genera found on flowers and epiphytic soils were common on leaves and litters. The genera *Aureobasidium, Cryptococcus, Pseudozyma, Rhodotorula* and *Sporidiobolus* were found in all substrates. Based on their locations, Kutai had the highest number of genera (15) followed by Cibodas (10), Cibinong (10), Enrekang (10), Kupang (10), Pucak (9), Liwa (7) and Lombok (7). The genus *Cryptococcus* was found in all locations. Our study shed a light to detection of many new taxa of yeasts, 41% of yeasts found in this study represented novel taxa.

Key words: yeasts, diversity, Indonesia, D1/D2 of LSU rDNA, ITS regions

Tropical fungi are a major component of biodiversity essential to the survival of other organisms, crucial in global ecological processes, a source of novel bioactive compounds, a source of biocontrol agents, a source of plant pathogens, a threat to human health, and able to contribute to sustainable development and a part of human culture (Hawksworth 2002). It is estimated that 1.5 million species of fungi exist in the world (Hawksworth 1991), however, up to now only 10% of those number have been described. More than 50% of fungal diversity is expected to be found in tropical regions. The studies from the viewpoint of biodiversity in Asia have just started and further extensive studies are required (Nakase *et al.* 2006). Indonesia as a tropical country has a large area and rich in variety of habitats to be explored for its fungal diversity. Various habitats in Indonesia are under-explored microbial ecosystems and are threatened for biodiversity loss. Alikodra and Syaukani (2004) reported that the rate of deforestation in Indonesia was the highest in the world and reached 3.8 million ha per year. Tropical deforestation causes habitat loss and its accompanying fungal loss will include some species which may have the ability to produce important but yet undiscovered chemical molecules. To date, there is very little information on species diversity of Indonesian indigenous yeasts and yeast-like fungi.

In Indonesia, like other South-East Asian countries, yeast research has been started in the early stage of microbial studies of traditional fermented products, foods, beverages and starters of fermentation (Nakase *et al.* 2006). The study of yeasts from natural environments in Indonesia started some 40 years ago when Deinema in 1961 found *Candida bogoriensis* from the surface of leaves of the flowering shrubs *Randia melleifera* (*Rubiaceae*) in Bogor. In the last decade, many studies have been conducted to investigate yeasts biodiversity from natural environment of Indonesia, however most of the identification were based on conventional methods (Sjamsuridzal and Gandjar 1994; Oetari *et al*. 1999; Sudiana and Rahmansyah 2002). Information on yeasts diversity based on molecular method from natural environment in Indonesia appeared in the year 1999 as reported by Haryono *et al.* (1999), they informed the basidiomycetous yeasts isolated from the suburb of Yogyakarta; then Sjamsuridzal *et al.* (2003) reported yeasts from Pulau Rambut, Muara Angke and marine of Teluk Jakarta. Based on their studies, yeasts biodiversity in the natural environment of Indonesia was predicted to be rich. Their report indicated that more novel yeasts species living in natural environment of Indonesia are waiting to discover.

In this study, we investigated yeasts isolated from various sources in environment in Indonesia based on the phylogenetic analysis of D1/D2 region of LSU rDNA and ITS regions.

MATERIALS AND METHODS

Sampling Locations. Samplings were conducted in the year 2003, 2005, 2006, and 2008 and we sampled several substrates e.g., living plants (leaves, flowers), litters, soils, epiphytic, insects (arthropods), and insect's nests. Collection of samples was conducted in Cibodas Botanical Garden, West Java in the year 2003; Kupang (Nusa Tenggara Timur), Enrekang (South Sulawesi) and Cibinong (West Java) in the year 2005; Kutai (East Kalimantan), Gili and Kuta (Lombok Island), and Cibinong (West Java) in the year 2006; and Pucak Botanical Garden (South Sulawesi), and Liwa

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Botanical Garden (Lampung, South Sumatera) in the year 2008 (Fig 1). The number of samples collected at eight sampling sites in Indonesia in 2003, 2005, 2006, and 2008 can be seen in Table 1.

Isolation of Yeasts. Several kinds of sample materials, e.g., leaves, flowers, litters, epiphytic soils, insects, and insect's nests, were collected from various parts of Indonesia. Each of sample materials was used for the isolation of yeasts and yeast-like fungi. Methods for isolation, maintenance, and preservation were carried out based on Yarrow (1998). Yeasts were isolated by dilution, direct inoculation, membrane filtration, particle filtration, walk over plate (for insects), ballistospore fall-method, UV treatment and heat shock treatment.

DNA Preparation. The cultures were cultivated in YM broth (3 g yeasts extract, 3 g malt extract, 5 g polypeptone, 10 g glucose, 1L distilled water), and the cells were harvested during the logarithmic phase of growth and collected by centrifugation. The DNA was extracted and purified by using the method of Sjamsuridzal and Oetari (2003), or by using DNeasy Plant Tissue Kit (QIAGEN).

Amplification of D1/D2 of LSU rDNA and ITS Regions by PCR. The D1/D2 region of nuclear large subunit ribosomal DNA and the ITS regions were amplified and sequenced using the primer sets, NL1 (GCATATCAATAA GCGGAGGAAAAG) and NL 4 (GGTCCGTGTTCAAG ACGG), and ITS4 (TCCTCCGCTTATTGATATGC) and ITS5 (GGAAGTAAAAGTCGTAACAAGG), respectively, as described by White *et al.* (1990).

Sequencing of D1/D2 of LSU rDNA and ITS Regions. The nucleotide sequences were determined with Big Dye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems) following the manufacturer's instructions. The gel electrophoresis and data collection were performed on ABI Prism Genetic Analyzer (Applied Biosystems). The sequence of D1/D2 region of LSU rDNA and ITS regions of the strains were aligned with other LSU rDNA sequences on the basis of similarity of the sequences. Yeast isolates were identified by the 99% similarity criteria of D1/D2 region of LSU rDNA (Kurtzman and Robnett 1998) or by at least 1% sequence diversity of ITS regions (Sugita *et al*. 1999; Caligiorne *et al*. 2005).

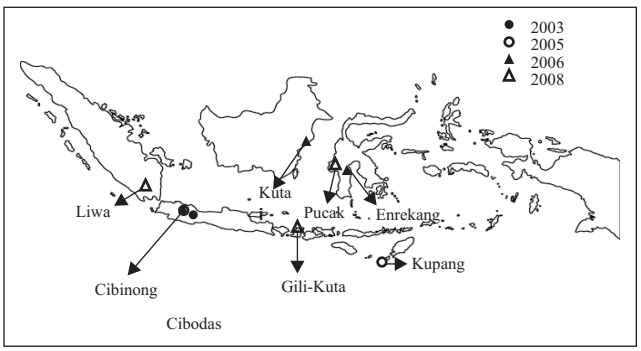

Fig 1 Map of sampling sites in Indonesia.

Table 1 Number of samples and isolates obtained from each year collection

Year	Number of samples	Number of isolated yeasts	Number of representative isolates
2003	41	1029	75
2005	144	549	250
2006	77	344	100
2008	53	225	100
Total	315	2147	525

Phylogenetic Analysis. The sequences data were sent to online international DNA database for homology search by Basic Local Algorithm Search Tools (BLAST) (Altschul *et al.* 1997). Sequences were aligned using CLUSTALX (Thompson *et al.* 1994) and were adjusted manually. The gaps were not included in our phylogenetic analyses. The distance matrix for the aligned sequences was calculated using the two-parameter method of Kimura (1980). The neighbor-joining (NJ) method (Saitou and Nei 1987) was used to construct all phylogenetic trees. The robustness for individual branches was estimated by bootstrapping (Felsenstein 1985) with 1 000 resamplings.

Preservation of Yeast Cultures. The preservation of yeasts isolates were conducted by two methods, e.g., preservation in 10% glycerol solution (in -80°C), each isolates preserved in 3 cryogenic tubes; and preservation by liquid drying lyophilization, five ampoules were prepared for each isolates. Yeasts were preserved in University of Indonesia Culture Collection (UICC) and LIPI Microbial Culture Collection (LIPIMCC), Indonesia, and one copy of representative isolates were deposited at the Department of Biotechnology National Institute of Technology Evaluation (DOB NITE), Japan.

RESULTS

Isolation of Yeasts and Yeast-Like Fungi in Indonesia. We sampled various natural habitats including leaves, flowers, litter, soil, epiphytic soil, and insects and insect's nests. As a total of 2147 isolates (Table 1) were obtained from 315 of samples in four years from eight sampling locations in Java, Kalimantan, Lombok, Sulawesi, Sumatera, and Timor (Fig 1), we selected 525 representative isolates for molecular identification (Tables 1 and 2). Table 3 showed that a total of 133 representative yeasts isolates were selected from 115 soil samples. Table 4 showed that a total of 276 isolates were selected from 95 litter samples. Table 5 showed that a total of 52 isolates were selected from 21 flower samples; 12 isolates were selected from 12 pollen samples; 22 isolates were selected from 20 leaves samples; 21 isolates from 11 epiphytic soil; and 9 isolates from 41 insects and insect's nest samples.

Yeasts and Yeast-Like Fungi Found from Natural Environment of Indonesia. We selected 525 from a total 2147 isolates as representative isolates for molecular identification. From those selected isolates, we had sequenced 515 isolates and 10 isolates were discarded because of contamination. Our molecular identification based on D1/D2 region of nuclear large-subunit rDNA and the internal transcribed spacer (ITS) regions sequence data revealed that 306 isolates belong to 48 described species (18 genera) and 209 strains belong to 19 undescribed species

Table 2 Number of samples collected at nine sampling sites in Indonesia

Year	Sampling site	Location	Number of samples
2003	Cibodas	West Java	41
	Kupang	West Timor	53
2005	Enrekang	South Sulawesi	67
	Cibinong	West Java	24
	Kutai	East Kalimantan	35
2006	Gili and Kuta	Lombok Island	15
	Cibinong	West Java	27
2008	Pucak	South Sulawesi	29
	Liwa	South Sumatera	24
Total			315

Table 3 Number of yeasts and yeast-like fungi cultures isolated from soil samples collected at seven sampling sites in Indonesia

Sampling Site	Location	Number of samples	Number of cultures
Kupang	West Timor	20	7
Enrekang	South Sulawesi	24	16
Cibinong	West Java	6	7
Kutai	East Kalimantan	15	24
Gili and Kuta	Lombok Island	14	15
Cibinong	West Java	7	9
Pucak	South Sulawesi	16	25
Liwa	South Sumatera	13	30
Total		115	133

Table 4 Number of yeasts and yeast-like fungi cultures isolated from litter samples collected at several sampling sites in Indonesia

Year	Sampling Site	Location	Number of Samples	Number of Cultures
2005	Kupang	West Timor	20	116
	Enrekang	South Sulawesi	25	74
	Kutai	East Kalimantan	20	31
2006	Gili-Kuta	Lombok	1	10
	Cibinong	West Java	5	0
2008	Pucak	South Sulawesi	13	25
	Liwa	South Sumatera	11	20
Total			95	276

Table 5 Number of yeasts and yeast-like fungi cultures isolated from other samples collected at several sampling sites in Indonesia

Year	Sampling Site, Location	Sample	Number of Samples	Number of Cultures
2003	Cibodas	Flower	17	41
		Pollen	12	12
		leaves	12	22
2005	Kupang, West Timor	Insects (arthropods),	11	4
		Epiphytic soil	2	4
	Enrekang, South Sulawesi	Insects (arthropods)	15	3
		Epiphytic soil	3	4
	Cibinong West Java	Insects (arthropods),	15	2
		Epiphytic soil	3	13
2006	Cibinong West Java	Flower	4	11
		Leaves	8	0
		Epiphytes	3	0
Total			105	116

(19 genera). The yeasts species found in this study were shown in Table 6.

Diversity and Distribution of Yeasts and Yeast-Like Fungi at Different Sampling Locations. Table 6 showed the occurrence of the species of yeasts and yeast-like fungi at different sampling sites. Based on their locations, Kutai (Kalimantan) had the highest number of genera (15) followed by Cibodas (10), Cibinong (10), Enrekang (10), Kupang (10), Pucak (9), Liwa (7), and Lombok (7). The genus *Cryptococcus* was found in all locations.

Diversity and Distribution of Yeasts and Yeast-Like Fungi on Various Substrates. As shown in Table 7, in this study, we found 10 genera of yeasts from 21 flower samples, 6 genera of yeasts from 12 pollen samples, 5 genera from 11 epiphytic soil samples, and 4 genera from 41 insects and insect's nest samples. We found 19 genera of yeasts isolated from 95 litter samples, 18 genera of yeasts from 115 soil samples, and 6 genera from 20 leaf samples (Table 7).

Phylogenetic Placements of Yeasts from Indonesia and Detection of Novel Taxa. Phylogenetic analysis based on D1/D2 of rDNA sequence data showed the phylogenetic placements of interesting yeast isolates within the major classes of the phyla *Ascomycota* and *Basidiomycota*. The phylogenetic placements of new taxa within the class *Euascomycetes* which were obtained in Indonesia can be seen in Fig 2; some novel yeasts species within *Metschnikowiaceae* lineage in the class *Hemiascomycetes* can be seen in Fig 3; other novel yeast taxa within other lineages in the class *Hemiascomycetes* can be seen in Fig 4; novel yeasts taxa within the class *Urediniomycetes* can be seen in Fig 5; novel yeasts taxa in the class *Ustilaginomycetes* can be seen in Fig 6; and some undescribed *Cystofilobasidium* species in the class *Hymenomycetes* which were obtained in Indonesia can be seen in Fig 7.

DISCUSSION

Our molecular identification based on D1/D2 region of nuclear large-subunit rDNA and the internal transcribed spacer (ITS) regions sequence data on 525 representative isolates revealed that 306 isolates belong to 48 described species (18 genera) and 209 isolates belong to 19 undescribed species (19 genera). Our molecular analysis revealed that those yeast isolates are phylogenetically diverse and distributed in the two major classes of the phylum *Ascomycota* (i.e. *Hemiascomycetes* and *Euascomycetes*) and in three major classes of the phylum *Basidiomycota* (i.e. *Urediniomycetes*, *Hymenomycetes*, and *Ustilaginomycetes*) (Figs 2-7). Many genera and species found in this study, such as *Anomalomyces, Cystofilobasidium bisporidii*, *C. capitatum, C. ferigula, Dothichiza, Kabatiella, Kodamaea ohmeri, Lecythophora, Spathaspora*, *and Tetrapisispora* had never been reported from Indonesia.

In this study, the yeast *Sporidiobolus ruineniae* var. *ruineniae* was found in all sampling locations except in Kutai (Kalimantan) and Lombok (Table 6). This species seems to be a tropical yeast because only found in tropical

Table 6 Occurrence of the species of yeasts and yeast-like fungi at different sampling sites. Location: A= Cibodas (West Java); B= Cibinong (West Java); C= Kupang (West Timor); D= Enrekang (South Sulawesi); E= Kutai (East Kalimantan); F= Lombok (Lombok Island); G= Pucak (South Sulawesi); H= Liwa (Lampung).

Species	A	B	C	D	E	F	G	H	
Anomalomyces sp.	√								
Aureobasidium pullulans	√	√	√	√					
Aureobasidium sp.	√	√	√	√					
Bullera sinensis	√								
Bullera sp.							√		
Candida chrysomelidarum	√								
C. etchellsii			√					√	
C. friedrichii							√	√	
C. hawaiiana	√								
C. insectorum					√				
C. intermedia								√	
C. parapsilosis			√						
C. rancensis	√								
C. silvae								√	
Candida sp.	√		√	√	√		√	√	
C. tropicalis			√						
Cryptococcus flavescens									
C. heveanensis	√	√	√	√	√	√	√		
C. humicola					√		√	√	
C. laurentii									
C. liquefaciens				√				√	
C. cf. luteolus								√	
C. rajasthanensis									
Cryptococcus sp.	√	√	√	√	√	√	√	√	
Cystofilobasidium sp.			√			√			
Debaryomyces castellii									
D. hansenii					√				
Dipodascus sp.		√		√					
Dothichiza sp.			√		√				
Kabatiella sp.							√		
Kluyveromyces africanus					√				
K. hubeiensis								√	
Kodamaea ohmeri			√						
Lecythophora sp.				√					
Metschnikowia sp.			√			√		√	
Myxozyma geophila						√			
Pichia hawaiiensis						√			
P. ohmeri					√				
P. pijperi									√
Pichia sp.					√	√			√
P. stipitis					√		√		
P. sydowiarum						√			
Pseudozyma hubeiensis								√	
P. paraantarctica			√	√					
P. prolifica						√			
Pseudozyma sp.						√			
Rhodosporidium fluviale						√		√	
R. kratochvilovae					√	√	√		
R. paludigenum				√	√	√	√		
R. toruloides					√	√			
Rhodotorula glutinis						√			
R. mucilaginosa						√	√		
Rhodotorula sp.			√	√		√	√	√	√
Spathaspora sp.									
Sporidiobolus ruineniae var. *ruineniae*	√		√	√	√	√		√	√
Sporidiobolus sp.				√			√		
Sporisorium loudetiae-pedicellatae				√					
Sporobolomyces nylandii					√				
S. poonsookiae				√		√			
Sporobolomyces sp.				√	√		√		
Tetrapisispora sp.						√			
Trichosporon sp.						√			√
T. sporotrichoides									√
Ustilago alcornii				√					
U. esculenta				√		√			
Williopsis saturnus						√			√

Table 7 Occurrence of the genera of yeasts and yeast-like fungi on different substrates. Substrates: 1, soil; 2, litter; 3, flower; 4, pollen; 5, leaves; 6, epiphytic soil; 7, insect

Genus	1	2	3	4	5	6	7
Anomalomyces			√				
Aureobasidium	√	√	√	√	√	√	
Bullera	√		√				
Candida	√	√	√	√			√
Cryptococcus	√	√	√	√	√		√
Cystofilobasidium	√	√					
Debaryomyces		√				√	
Dipodascus	√						
Dothichiza		√					
Kabatiella		√					
Kluyveromyces	√						
Kodamaea	√						
Lecythophora		√				√	
Metschnikowia		√	√				
Myxozyma	√						
Pichia	√	√				√	√
Pseudozyma	√	√	√	√	√		
Rhodosporidium	√	√	√				
Rhodotorula	√	√	√	√	√		
Spathaspora		√					
Sporidiobolus	√	√		√	√	√	√
Sporisorium	√						
Sporobolomyces	√	√					
Tetrapisispora		√					
Trichosporon	√						
Ustilago	√	√			√		
Williopsis		√					

region. It was first found in Indonesia by Ruinen in (1963), then it was also isolated in Thailand but not in the temperate zone (Nakase *et al*. 2006).

It seems that the distribution of yeasts was effected by the local climate and geography of the location. The occurrence of the genera of yeasts and yeast-like fungi at different sampling sites is shown in Table 6. Kutai (Kalimantan) which has rain forest and high humidity harbors the highest number of genera (15 genera). We found that the yeasts diversity is high in the rain forests (such as Kutai, Cibodas, Enrekang, and Pucak) or the locations with high humidity (Cibinong) and tends to decrease in the forests with low humidity such as in Lombok (7 genera). Our results as shown in Table 6 indicated that there is some degree of similarity on yeasts diversity of two locations which are geographically close, for example between Cibodas and Cibinong; between Enrekang and Pucak.

Kupang which is located in Timor Island covered by savanna and dry forest harbors a unique diversity of yeasts which are differ from those of yeasts found from the rain forests in Kutai, Cibodas, Enrekang, and Pucak. Lombok which has dry climate showed some degree similarity on its yeasts diversity to those yeasts found in Kupang (Timor). The following species e.g., *Cryptococcus flavescens, Cryptococcus* sp., *Cystofilobasidium* spp., *Pichia stipitis, Rhodosporidium kratochvilovae, R. paludigenum,* and *Sporobolomyces* sp. were found both in Kupang and Lombok. We suggest the similarity on yeasts diversity between Kupang and Lombok because these two locations have dry climate. The presence or absence of capsules on

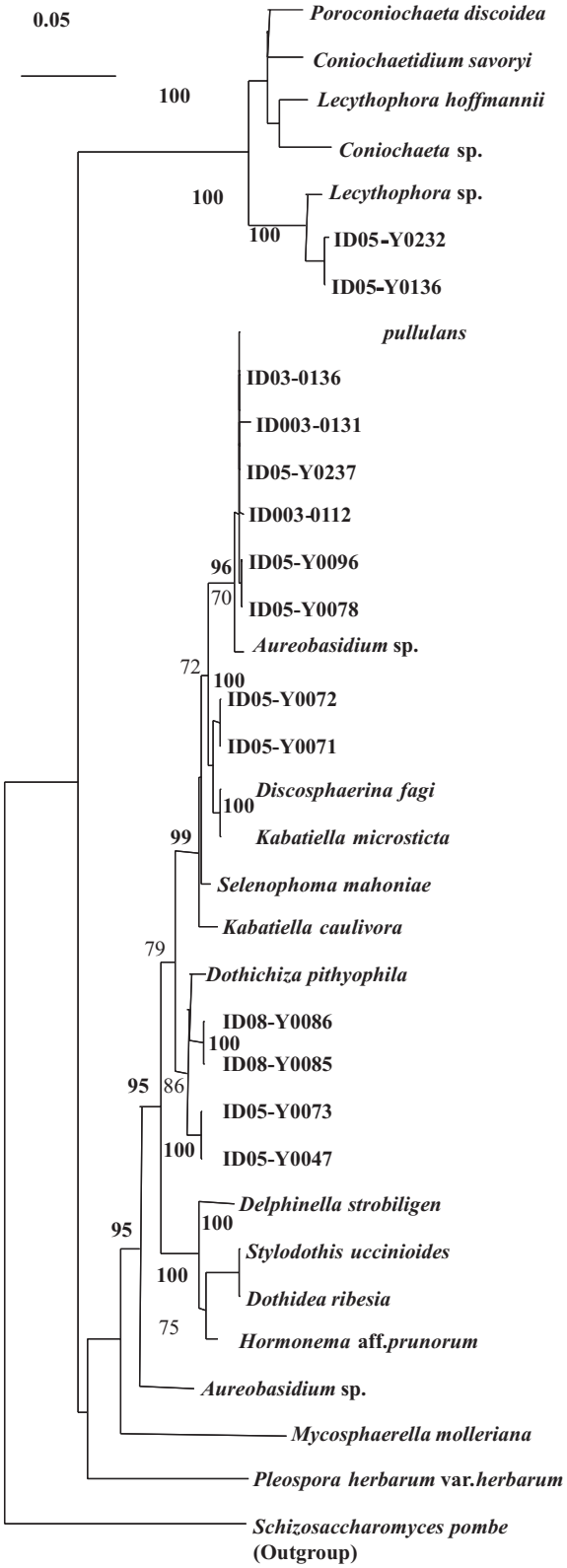

Fig 2 Phylogenetic placement of Indonesian isolates within the *Euascomycetes* lineage based on D1/D2 of LSU rDNA sequences.

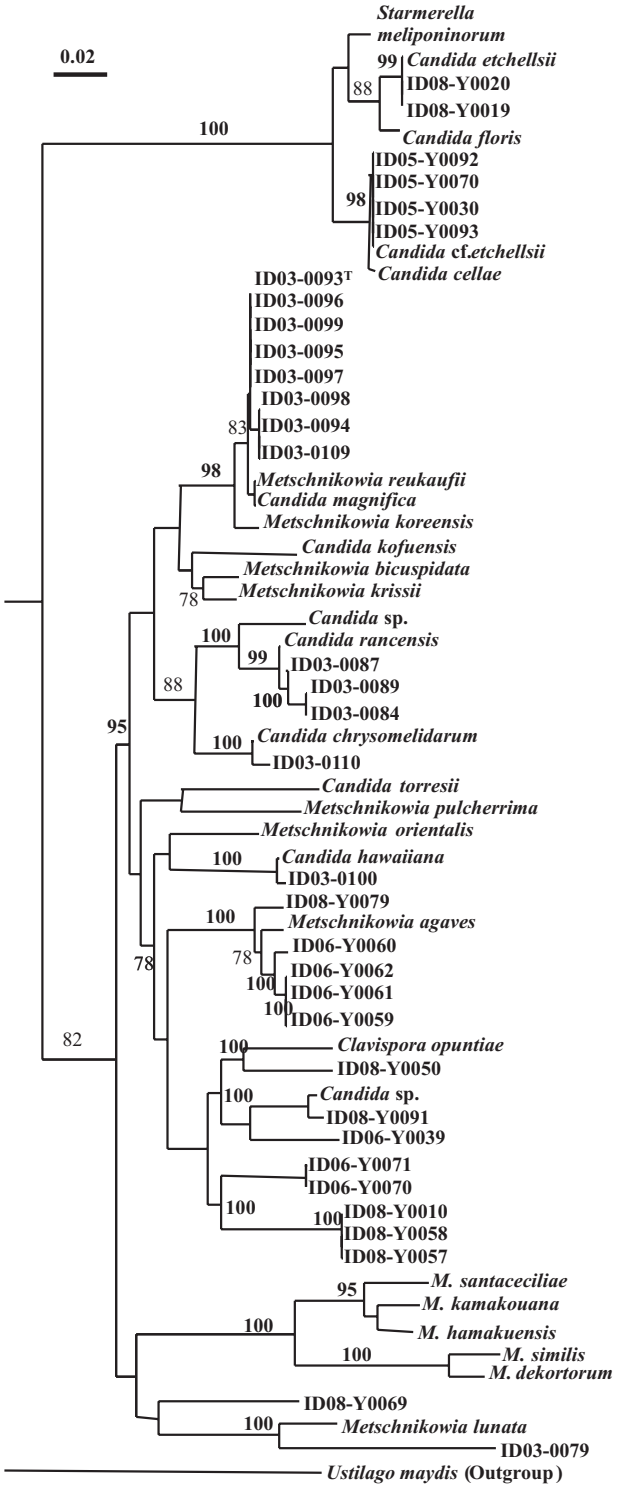

Fig 3 The phylogenetic placement of Indonesian isolates within *Metschnikowiaceae* lineage in the class *Hemiascomycetes* based on D1/D2 of LSU rDNA sequences.

yeast species, especially in the arid and semiarid types, may influence the ability of the yeast cells to survive in low moisture conditions (Spencer and Spencer 1997). *Cryptococcus, Rhodosporidium*, and *Sporobolomyces* are among the yeasts genera which have multi layer of cell wall and cells with capsules. Among those yeasts found in this study, *Cystofilobasidium* was only found and common in Kupang and Lombok. The genus *Cystofilobasidium* has a thick-wall of teliospores (teleomorphic state) and multi layered of cell wall, these are suitable characters for yeasts to survive in dry forests.

The characteristics of substrate (physico-chemical properties) as a habitat, the intrinsic abilities (physiology) of yeasts, and the interactions within the substrate's microbial community will determine the population density and the

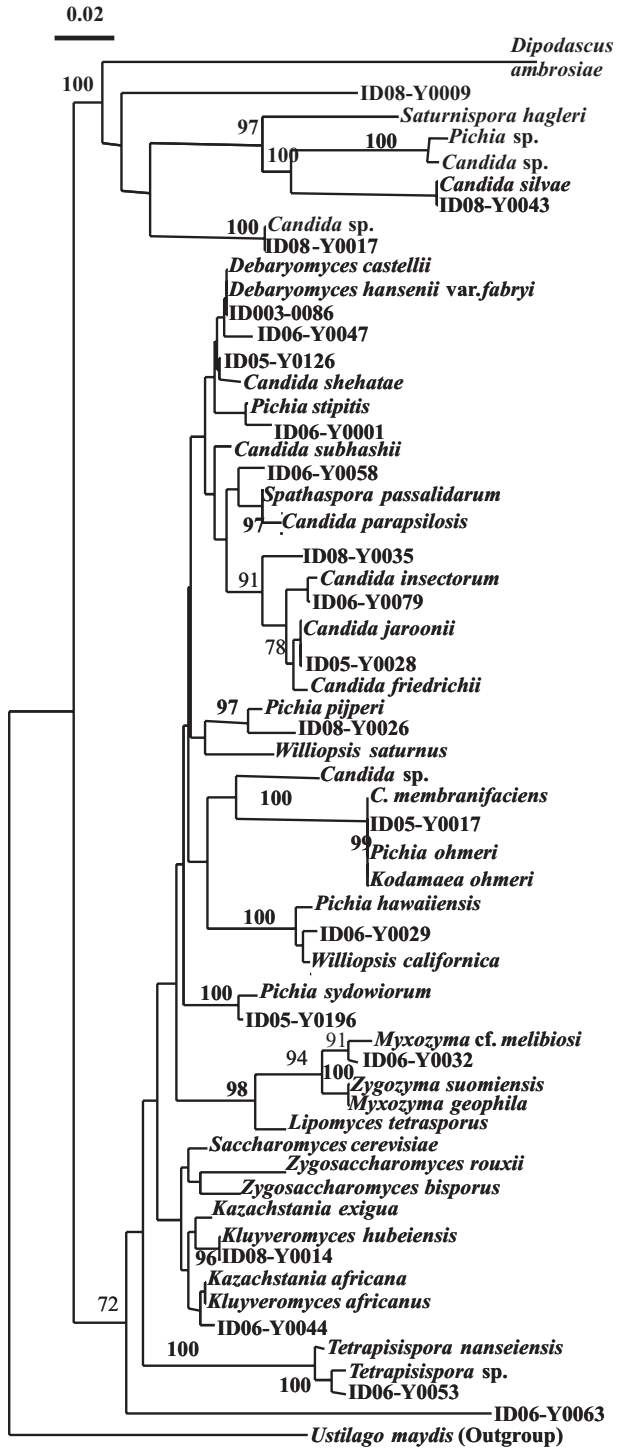

Fig 4 The phylogenetic placement of Indonesian isolates within other lineages in the class *Hemiascomycetes* based on D1/D2 of LSU rDNA sequences.

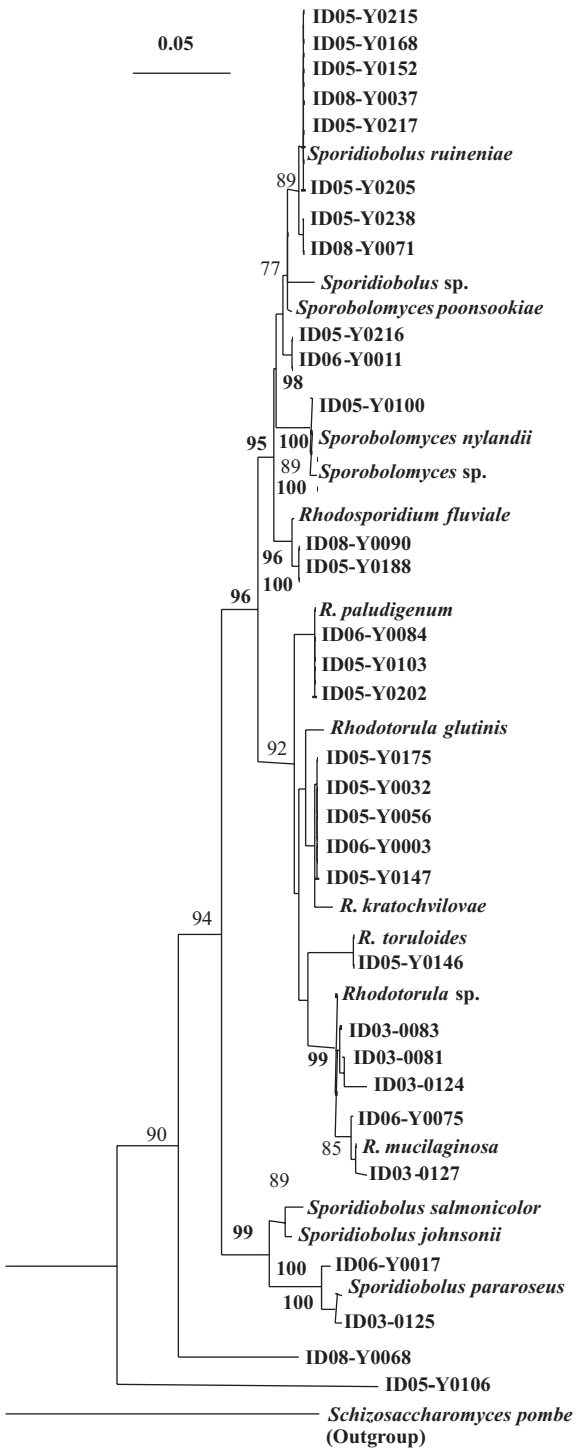

Fig 5 The phylogenetic placement of Indonesian isolates within the class *Urediniomycetes* as inferred from D1/D2 of LSU rDNA sequences.

diversity of yeasts live in that substrate. The yeasts microflora of soils depends on the type of nutrients reaching them. Some yeasts are permanent residents in the soil and some are transients, residing temporarily in the soil. Based on the substrates, litter had the highest yeasts genera (19) followed by soil (18), flower (10), pollen (6), leaves (6), epiphytic soil (4), and insect and insect's nests (4). Genera found on soil were also common on litter. Our study showed that yeasts genera found on flower and epiphytic soil were common on leaves and litter. The genera *Aureobasidium, Cryptococcus, Pseudozyma, Rhodotorula*, and *Sporidiobolus* were found in all kind of substrates.

Plant surfaces have been recognized as an important habitat for yeasts. Jager *et al.* (2001) reported that saprophytic yeasts are common on the surfaces of leaves with the most common genera being *Candida, Cryptococcus, Pichia, Rhodotorula*, and *Trichosporon*. The yeast species *Aureobasidium pullulans* is common in senescent leaves (litter) and in soil (Sampaio *et al.* 2004).

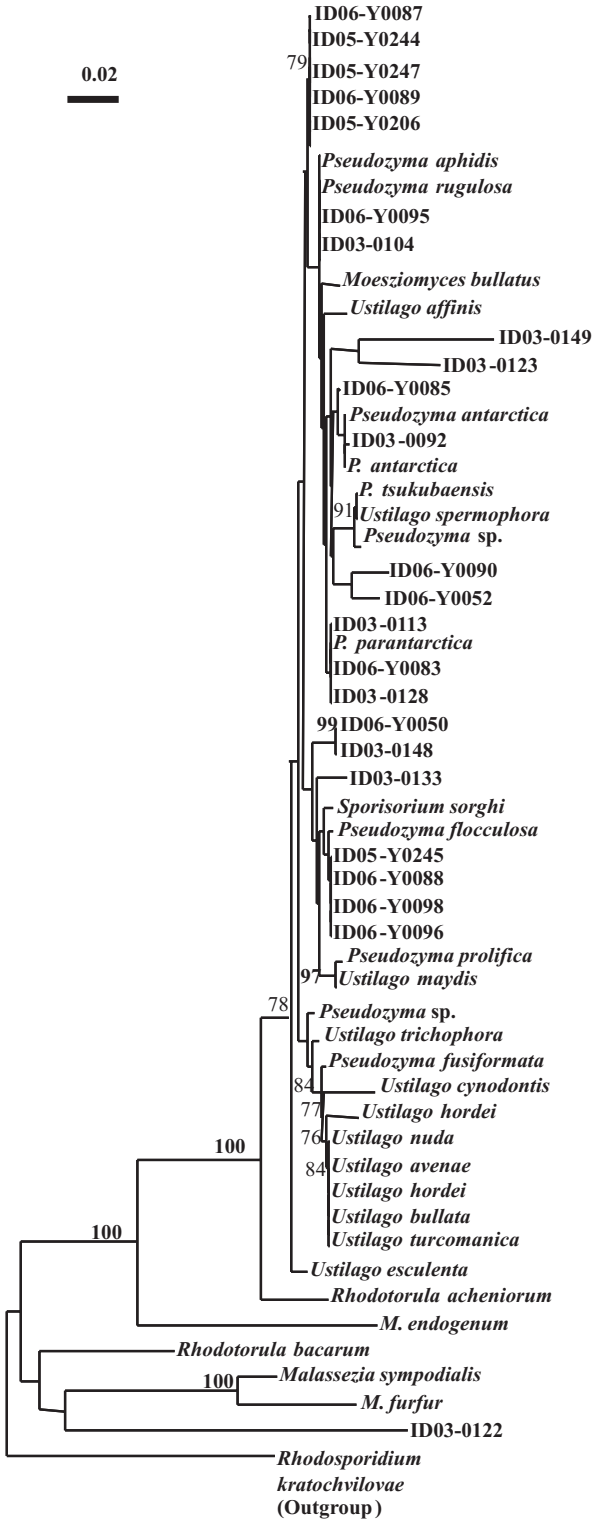

Fig 6 The phylogenetic placement of Indonesian isolates within the class *Ustilaginomycetes* as inferred from D1/D2 of LSU rDNA sequences.

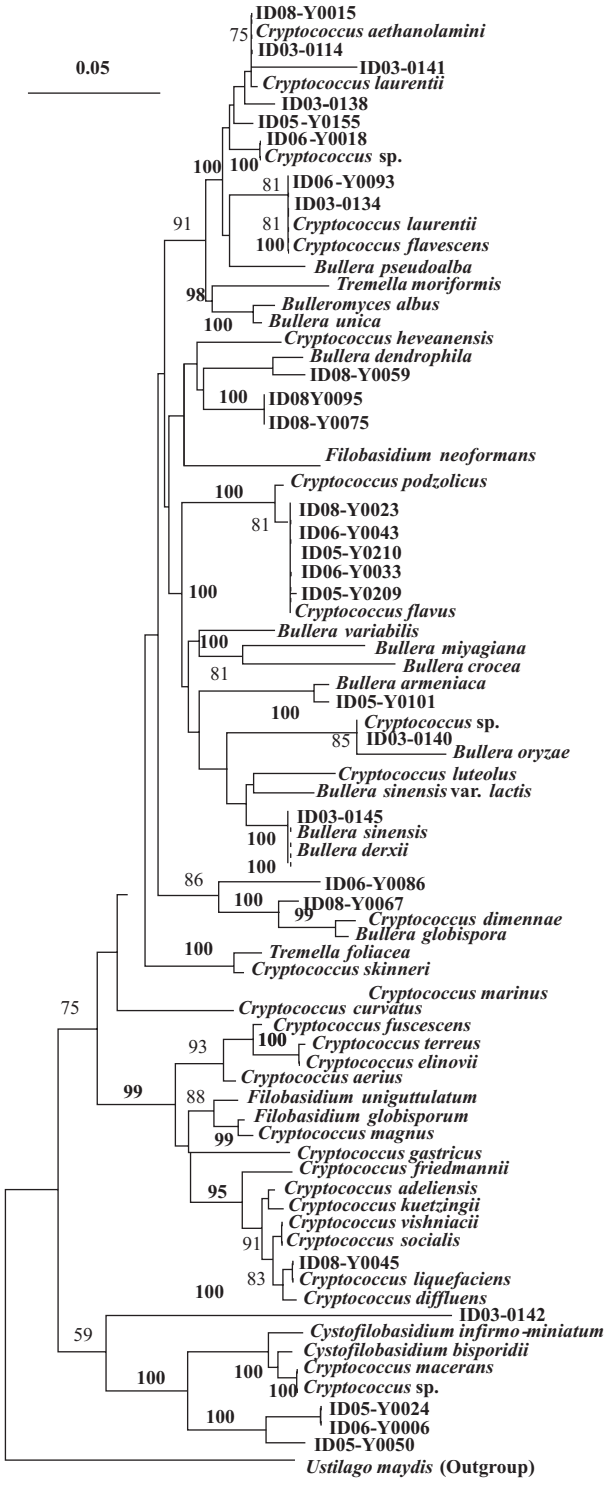

Fig 7 The phylogenetic placement of Indonesian isolates within the class *Hymenomycetes*, as inferred from D1/D2 of LSU rDNA sequences.

Most flowers provide two distinct habitats for yeast. The outer surfaces and the inside of the flower near the opening harbor a yeast population similar to that of leaf and stem surfaces (i.e., *Sporobolomyces, Rhodotorula, Cryptococcus*, *Trichosporon*, and *Aureobasidium*). The nectaries, containing nectar of a high sugar content but low in nitrogen, have a different yeast population such as *Candida* and *Metschnikowia,* which are fermentative yeasts (Spencer and Spencer 1997; Lachance *et al.* 2001a).

The relationships between yeasts and insects are well established (Lachance *et al.* 2001a, b). Yeasts have been isolated frequently from the gut or surface of insects that feed on a variety of materials, including basidiomycete fruiting bodies, woody substrates, ephemeral flowers and nectar exudates (Lachance *et al.* 2001a, b; Suh and Blackwell 2004; Nguyen *et al.* 2007; Nakase *et al.* 2009). Yeasts have

an important role in the food chain in insects. Insects not only feed on the substrates known to serve as yeast habitats, but use yeast as food source (Lachance *et al.* 2001b; Lachance and Bowles 2002). Yeasts convert simple nitrogenous compounds into proteins and other nutrients beneficial to insects (Sampaio *et al.* 2004). Suh *et al.* (2005) found host specialization among some of the associated insects and yeasts indicated a significant interaction between the organisms.

Epiphytic soil is an interesting habitat which has specific yeast diversity and the information of yeast diversity on this type of habitat is still limited. This study is the first contribution to the knowledge of diversity of yeasts from arthropods and epiphytic soils in Indonesia. We found some interesting taxa from insects and insect's nests (arthropods), and epiphytic soils from Kupang. We need further studies for exploration more samples of arthropods and epiphytic soils, and further study to elucidate the identity of new taxa.

Our study shed a light to detection of many undescribed yeasts from Indonesia. Molecular identification was conducted for those 515 isolates. The results based on D1/D2 region of LSU rDNA showed that 209 isolates with D1/D2 homology values less than 99%, and the results based on sequence of ITS regions of those 209 isolates confirmed that they are separate species. Sequence analysis based on D1/D2 of LSU and ITS regions data detected that 209 strains from the total of 515 strains (41%) represented new taxa. Further studies are needed to elucidate the identity of novel taxa found in this study. Yeasts have always been blind spot for biodiversity studies in Indonesia. Our study has broadened our knowledge on Indonesian biodiversity. This study has very important implication on our knowledge of estimates of yeasts diversity in tropical forests in Indonesia and provides some insights into the distribution of yeasts on different substrates and ecosystems in Indonesia.

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