

The number of nucleotide differences of 283 strains of Thai yeasts from their respective nearest species are shown in Table 15. One hundred and sixteen strains of unknown yeasts, which were classified into 101 species, showed nucleotide substitutions of 4 or more (0.7% or more) in the D1/D2 domains of 26S rDNA from their respective nearest known species including 4-6 nucleotides (0.7-1%) in 35 strains of 26 species, 7-12 nucleotides (>1-2%) in 28 strains of 24 species, 13-24 nucleotides (>2-4%) in 20 strains of 17 species, 25-60 nucleotides (>4-10%) in 19 strains of 19 species and 60-110 nucleotides (>10-18%) in 14 strains of 14 species. It is very interesting that 12.1% (14 species) of new species demonstrated great nucleotide substitutions in D1/D2 domain from respective nearest known yeasts. Apparently, these 14 strains that had nucleotide substitutions of 10% or more belong to new groups of yeasts and are considered to differ at the level of higher taxonomic ranks such as genus and family. At present, the numbers of strains belong to these groups are not enough to define the concept of genus or family. However, further extensive isolation studies will provide data to define these new yeast groups.

As clearly shown in Table 15, the number of isolated strains per species is different in respective categories of species. In the known species, the average of 2.3 strains were isolated. On the other hand, the average of 1.1 strains were recovered in new species and the average of 1.3 strains, the intermediate number between known species and new species, were isolated in not identified species (species considered conspecific or sister species with respective known species). Generally speaking, among new species, the average number of isolated strains decreased accompanied by the increase of nucleotide differences from 1.3 strains in species with 4-6 nucleotide differences to 1 strain in species with 61-110 nucleotide differences. The number of strains in species showing 4-6 nucleotide substitution is the same as not yet identified species which showed 2-3 nucleotide substitutions (0.3-0.6%) and only 1 strain was isolated in the species showing more than 4% substitutions.

The above mentioned tendency is clearer when we look at the number of strains of respective species. The isolation data for respective species showed that, among 101 new species found in the present study, 93 species (92.1%) comprised one strain and the remaining 8 species (7.9%) comprised 2-5 strains (Table 13). In 28 not yet identified species, 24 species (85.7%) comprised one strain and the remaining 1 species (14.3%) comprised 4 strains (Table 14). In contrast, 26 known species (42.9%) comprised one strain and 32 known species (57.1%) comprised 2-9 strains (Table 11). This is quite different from yeasts associated with fermented foods where 2 or more strains were isolated in the most of species (Saito *et al.*, 1983; Suzuki *et al.*, 1987; Jindamorakot, 2000). Based on the fact mentioned above, it is assumed that, in the natural environment, the number of yeast cells of known species is bigger than those of new species (unknown species) so that yeast researchers could find and described these species in early stages of yeast taxonomy. Meanwhile, species with small cell numbers in the environment still remain “unknown” to mankind.

## 2.1 Diversity of yeasts from flowers

Twenty-four yeast strains isolated from flowers were identified as 17 species comprised of 8 known species (13 strains; 52%) and 9 new species (11 strains; 48%). Species found from flowers are shown in Table 16. Among new species, 9 strains (8 species) related to *Candida* and the remaining 2 strains related to *Hanseniaspora* and *Trichosporonoides*.

The frequency of yeasts isolated from flowers were shown that 6 species contained only 1 strain, 1 species contained 2 strains and 1 species contained 5 strains. *Metchnikowia koreensis* is the dominant species (5 strains) and occupied 20.8% of the strains and followed by *Sporidiobolus ruineniae* var. *ruineniae* (2 strains; 8.3%).

Table 16 List of species found from flowers collected in Thailand.

Species	No. of Strains	Species	No. of Strains
<b>Ascomycetous yeasts</b>		<b>Basidiomycetous yeasts</b>	
<b>Known species</b>		<b>Known species</b>	
<i>Aureobasidium pullulans</i>	1	<i>Sporidiobolus ruineniae</i>	2
<i>Candida leandrae</i>	1	var. <i>ruineniae</i>	
<i>Candida parapsilosis</i>	1	<b>Total 1 species</b>	<b>2</b>
<i>Debaryomyces</i> sp. NRRL Y-7804	1		
<i>Hanseniaspora</i> sp. CBS 8772	1		
<i>Kodamaea ohmeri</i>	1		
<i>Metschnikowia koreensis</i>	5		
<b>Total 7 species</b>	<b>11</b>		
<b>New species</b>		<b>New species</b>	
ST-22, ST-26, ST-29, ST-32, ST-387, ST-388, ST-390, ST-391, ST-490, ST-533 and ST-536		-	
<b>Total 9 species</b>	<b>11</b>	<b>Total 0 species</b>	<b>0</b>
<b>Not yet identified species</b>		<b>Not yet identified species</b>	
-		-	
<b>Total 0 species</b>	<b>0</b>	<b>Total 0 species</b>	<b>0</b>

## 2.2 Diversity of yeasts from insect frass

Based on the sequences of D1/D2 of 26S rDNA, 134 strains out of 144 from insect frass were identified as 84 species comprised of 36 known species (78 strains; 58.2%) of 12 genera of ascomycetous yeasts (26 species, 57 strains) and 7 genera of basidiomycetous yeasts (10 species, 21 strains), 48 new species (56 strains; 41.8%) of ascomycetous (44 strains) and basidiomycetous yeasts (4 strains). Species found from insect frass are shown in Table 17. The remaining 10 strains (8%) are not yet identified, they were differed in 2-3 nucleotides from nearest species. These strains were considered to belong to known species or sister species of nearest

known species. Probably, some of them represent new species closely related to respective known species.

**Table 17** List of species found from insect frass collected in Thailand.

<b>Species</b>	<b>No. of Strains</b>	<b>Species</b>	<b>No. of Strains</b>
<b>Ascomycetous yeasts</b>		<b>Basidiomycetous yeasts</b>	
<b>Known species</b>		<b>Known species</b>	
<i>Ambrosiozyma monospora</i>	2	<i>Bullera dendrophila</i>	1
<i>Aureobasidium pullulans</i>	1	<i>Cryptococcus heveanensis</i>	8
<i>Blastobotrys capitulata</i>	1	<i>Cryptococcus laurentii</i>	3
<i>Candida diversa</i>	1	<i>Cryptococcus</i> sp. CBS 8372	1
<i>C. fukuyamaensis</i>	3	<i>Rhodospordium paludigenum</i>	1
<i>C. gotoi</i>	2	<i>Rhodospordium toruloides</i>	1
<i>C. parapsilosis</i>	1	<i>Rhodotorula nothofagi</i>	1
<i>C. rancensis</i>	1	<i>Sporidiobolus ruineniae</i>	1
<i>C. sihepensis</i>	1	<i>Sporobolomyces poonsookiae</i>	1
<i>C. tropicalis</i>	8	<i>Trichosporon asahii</i>	3
<i>Candida</i> sp. NRRL Y-17456	3	<b>Total 10 species</b>	<b>21</b>
<i>Candida</i> sp. UWO(PS)00-147.3	1		
<i>Debaryomyces nepalensis</i>	4		
<i>D. polymorphus</i>	3		
<i>D. polymorphus</i> var. <i>africanus</i>	1		
<i>D. vanrijiiae</i> var. <i>yarrowii</i>	3		
<i>Geotrichum fragrans</i>	2		
<i>Kluyveromyces lactis</i>	2		
<i>Kodamaea (Pichia) ohmeri</i>	2		
<i>P. stipitis</i>	2		
<i>P. sydowiorum</i>	1		
<i>Pichia</i> sp. UWO(PS)99-305.1	1		
<i>Saccharomyces cerevisiae</i>	2		
<i>S. kluyveri</i>	5		
<i>S. unisporus</i>	1		
<i>Stephanoascus smithiae</i>	2		
<i>Williopsis saturnus</i> var. <i>subsufficiens</i>	1		
<b>Total 26 species</b>	<b>57</b>		

Table 17 (Continued)

Species	No. of Strains	Species	No. of Strains
<b>Ascomycetous yeasts</b>		<b>Basidiomycetous yeasts</b>	
<b>New species</b>		<b>New species</b>	
ST-17, ST-33, ST-37, ST-43, ST-49, ST-50, ST-57, ST-60, ST-78, ST-79, ST-84, ST-95, ST-96, ST-112, ST-164, ST-211, ST-224, ST-225, ST-228, ST-229, ST-233, ST-234, ST-236, ST-237, ST-238, ST-239, ST-240, ST-249, ST-250, ST-297, ST-300, ST-306, ST-309, ST-310, ST-311, ST-314, ST-315, ST-320, ST-328, ST-329, ST-330, ST-331, ST-333, ST-334, ST-335, ST-337, ST-338, ST-339, ST-394, ST-431, ST-433 and ST-493		ST-59, ST-71, ST-73 and ST-318	
<b>Total 44 species</b>	<b>52</b>	<b>Total 4 species</b>	<b>4</b>
<b>Not yet identified species</b>		<b>Not yet identified species</b>	
ST-38, ST-39, ST-108, ST-116, ST-127, ST-235 and ST-248		ST-52, ST-121 and ST-299	
<b>Total</b>	<b>7</b>	<b>Total</b>	<b>3</b>

Based on the sequences of D1/D2 domain of new species, 52 strains of them belong to ascomycetous yeasts and were classified into 44 species. They are related to species of *Candida* (24 species; 26 strains), *Debaryomyces* (1 species; 2 strains), *Dipodascus* (1 species; 1 strain), *Hanseniaspora* (2 species; 3 strains), *Issatchenkia* (1 species; 1 strain), *Metschnikowia* (3 species; 3 strains), *Pichia* (11 species; 14 strains), *Saccharomyces* (1 species; 1 strain) and *Wickerhamia* (1 species; 1 strain). The remaining 4 strains belong to basidiomycetous genera *Cryptococcus* (2 species; 2 strains) and *Trichosporon* (2 species; 2 strains). Therefore, it is considered that about a half of yeasts isolated from insect frass represent new species.

The results of D1/D2 sequences showed the diversity of yeasts from insect frass in natural environment of Thailand. *Candida tropicalis* and *Cryptococcus heveanensis* are the dominant species (8 strains of each species) and occupied 5.8% of the strains from insect frass followed by *Saccharomyces kluyveri* (5 strains; 3.6%) and *Debaryomyces nepalensis* (4 strains; 2.9%). Thirty-nine percent (56 strains) of yeasts isolated from insect frass were found to represent new species. Twenty-three species contained only 1 strain, 8 species contained 2 strains, 1-2 species contained 3-5 strains, and only 2 species contained 8 strains.

### 2.3 Diversity of yeasts from mosses

Twenty-two strains out of 27 isolated from mosses belonged to 18 species; 12 known species (17 strains; 63.0%) of 7 genera of ascomycetous yeasts (11 species, 16 strains) and a genus of basidiomycetous yeasts (1 species, 1 strain) and 6 new species (6 strains; 22.2%). The remaining 4 strains (14.8%) are not yet identified. Species found from mosses are shown in Table 18. Eighteen species contained only 1 strain, 2 species contained 2 strains and only 1 species contained 3 strains. *Saccharomyces cerevisiae* (3 strains; 13.6%) is the dominant species and followed by *Candida diversa* (2 strains; 9.1%) and *Saccharomyces kluyveri* (2 strains; 9.1%). Six strains of new species were related to *Candida* (2 strains), *Pichia* (2 strains), *Galactomyces* (1 strain) and *Schizoblastosporion* (1 strain).

### 2.4 Diversity of yeasts from mushrooms

Twenty- six strains out of 28 isolated from mushrooms belonged to 22 species; 10 known species (12 strains; 43%) of 5 genera of ascomycetous yeasts (9 species, 10 strains) and a genus of basidiomycetous yeasts (1 species, 1 strain), 12 new species (13 strains; 46.4%) of ascomycetous yeasts. The species are related to genus *Candida* (8 species; 9 strains), *Endomyces* (1 species; 1 strain), *Hanseniaspora* (1 species; 1 strain), *Kluyveromyces* (1 species; 1 strain), and *Pichia* (1 species; 2 strains). The remaining 2 strains (7%) are not yet identified. The most of species

found from mushrooms contained only 1 strain and 3 species contained 2 strains.  
Species found from mosses are shown in Table 19.

**Table 18** List of species found from mosses collected in Thailand.

<b>Species</b>	<b>No. of Strains</b>	<b>Species</b>	<b>No. of Strains</b>
<b>Ascomycetous yeasts</b>		<b>Basidiomycetous yeasts</b>	
<b>Known species</b>		<b>Known species</b>	
<i>Candida diversa</i>	2	<i>Trichosporon asahii</i>	1
<i>Candida palmae</i>	1	<b>Total 1 species</b>	<b>1</b>
<i>Hanseniaspora opuntiae</i>	2		
<i>Hanseniaspora vineae</i>	1		
<i>Pichia sydowiorum</i>	1		
<i>Saccharomyces cerevisiae</i>	3		
<i>Saccharomyces kluyveri</i>	2		
<i>Saccharomyces unisporus</i>	1		
<i>Torulaspora</i> sp. IFO 11061	1		
<i>Williopsis saturnus</i> var. <i>markii</i>	1		
<i>Zygosaccharomyces</i> sp. IFO 11070	1		
<b>Total 11 species</b>	<b>16</b>		
<b>New species</b>		<b>New species</b>	
ST-30, ST-36, ST-269, ST-445, ST-449 and ST-451	-		
<b>Total 6 species</b>	<b>6</b>	<b>Total 0 species</b>	<b>0</b>
<b>Not yet identified species</b>		<b>Not yet identified species</b>	
ST-14, ST- 385 and ST-441and ST-446	-		
<b>Total</b>	<b>4</b>	<b>Total</b>	<b>0</b>

Table 19 List of species found from mushrooms collected in Thailand.

Species	No. of Strains	Species	No. of Strains
<b>Ascomycetous yeasts</b>		<b>Basidiomycetous yeasts</b>	
<b>Known species</b>		<b>Known species</b>	
<i>Candida natalensis</i>	1	<i>Cryptococcus humicola</i>	2
<i>Candida parapsilosis</i>	1	<b>Total 1 species</b>	<b>2</b>
<i>Candida</i> sp. NRRL Y-17456	1		
<i>Candida tropicalis</i>	1		
<i>Hanseniaspora opuntiae</i>	2		
<i>Pichia nakazawae</i> var. <i>akitaensis</i>	2		
<i>Stephanoascus smithiae</i>	1		
<i>Torulasporea delbrueckii</i>	1		
<b>Total 9 species</b>	<b>10</b>		
<b>New species</b>		<b>New species</b>	
ST-2, ST-3, ST-4, ST-18, ST-19, ST-246, ST-253, ST-343, ST-358, ST-365, ST-366, ST-370 and ST-377		-	
<b>Total 12 species</b>	<b>14</b>	<b>Total 0 species</b>	<b>0</b>
<b>Not yet identified species</b>			
ST-380		ST-323	
<b>Total</b>	<b>1</b>	<b>Total</b>	<b>1</b>

## 2.5 Diversity of yeasts from leaves

Thirty strains out of 53 from leaves belonged to 7 known species (15 strains; 28.3%) of 4 genera of basidiomycetous yeasts, 18 new species (26 strains; 47.2%) and 13 (24.5%) not yet identified strains. Species found from leaves are shown in Table 20. New species are related with the genera *Bullera* (2 species; 2 strains), *Cryptococcus* (1 species; 2 strains), *Cryptotrichosporon* (1 species; 1 strains), *Sporidiobolus* (3 or 4 species; 11 strains), *Sporobolomyces* (7 species; 8 strains) and *Tilletiopsis* (1 species; 1 strains). The remaining 13 strains (24.5%) are not yet



identified. The dominant genus of yeast isolated from leave is *Sporobolomyces* (17 species, 21 strains; 67.7%). From the phylogenetic analysis based on the D1/D2 domain sequences of 26S rDNA, 18 species contained only 1 strain, 5 species contained 2 strains, 5 species contained 4 strains and only 1 species contained 5 strains.

**Table 20** List of species found from leaves collected in Thailand.

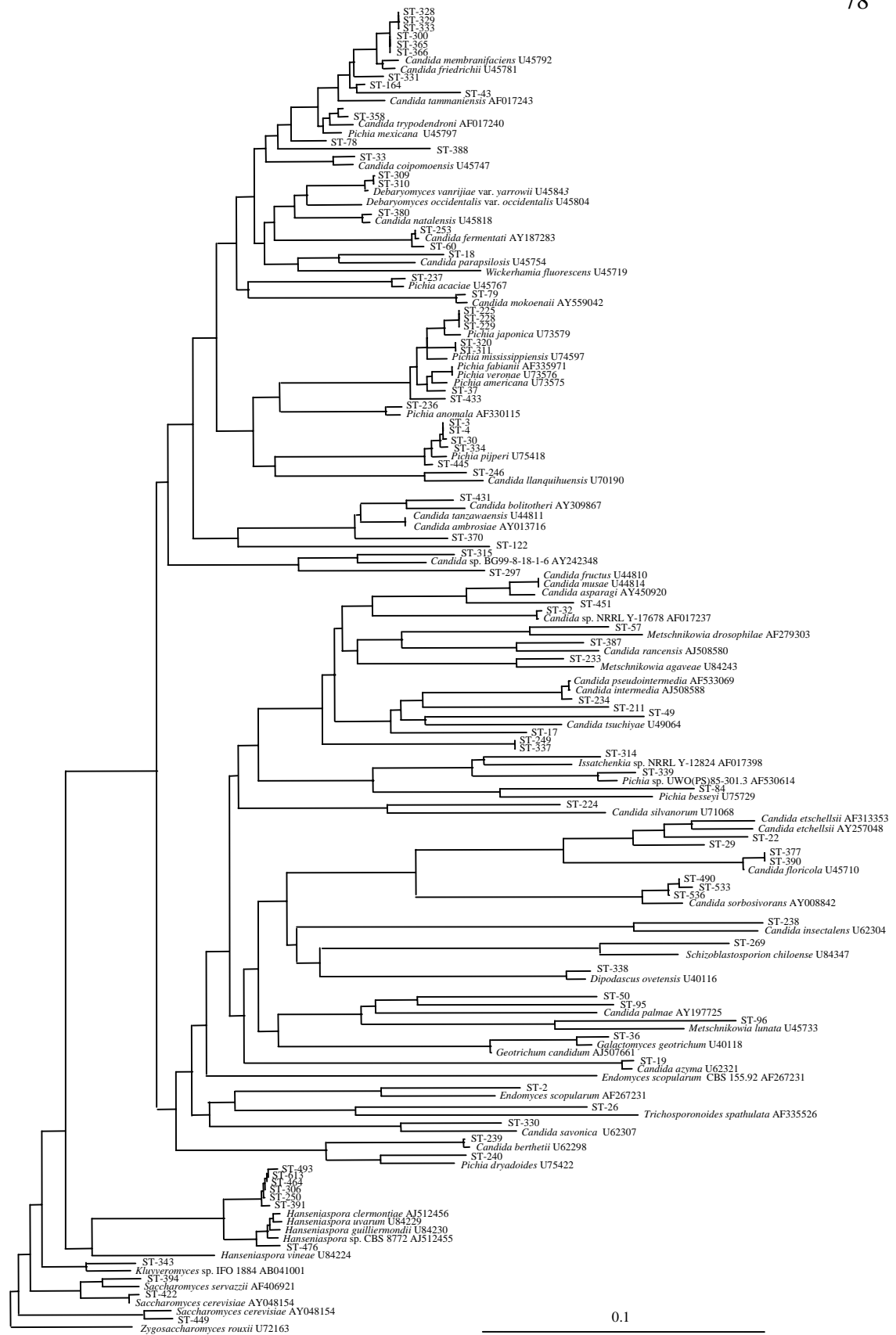
<b>Species</b>	<b>No. of Strains</b>
<b>Ascomycetous yeasts</b>	0
<b>Basidiomycetous yeasts</b>	
<b>Known species</b>	
<i>Bullera sinensis</i>	2
<i>Exobasidium vexans</i>	4
<i>Sporobolomyces bannaensis</i>	1
<i>Sporobolomyces odoratus</i>	3
<i>Sporobolomyces</i> sp. TY-241	1
<i>Sporobolomyces</i> sp. TY-257	3
<i>Tellectiopsis</i> sp. TY 235	1
<b>Total 7 species</b>	<b>15</b>
<b>New species</b>	
ST-87, ST-88, ST-90, ST-91, ST-92, ST-98, ST-102, ST-105, ST-111, ST-115, ST-119, ST-128, ST-144, ST-145, ST-151, ST-153, ST-156, ST-173, ST-184, ST-186, ST-195, ST-198, ST-201, ST-202, ST-206 and ST-226	
<b>Total</b>	<b>26</b>
<b>Not yet identified species</b>	
ST-94, ST-100, ST-123, ST-159, ST-170, ST-172, ST-175, ST-178, ST-181, ST-183, ST-192, ST-197 and ST-213	
<b>Total</b>	<b>13</b>

### 3. **Taxonomic and Phylogenetic Positions of New Yeasts from Thailand**

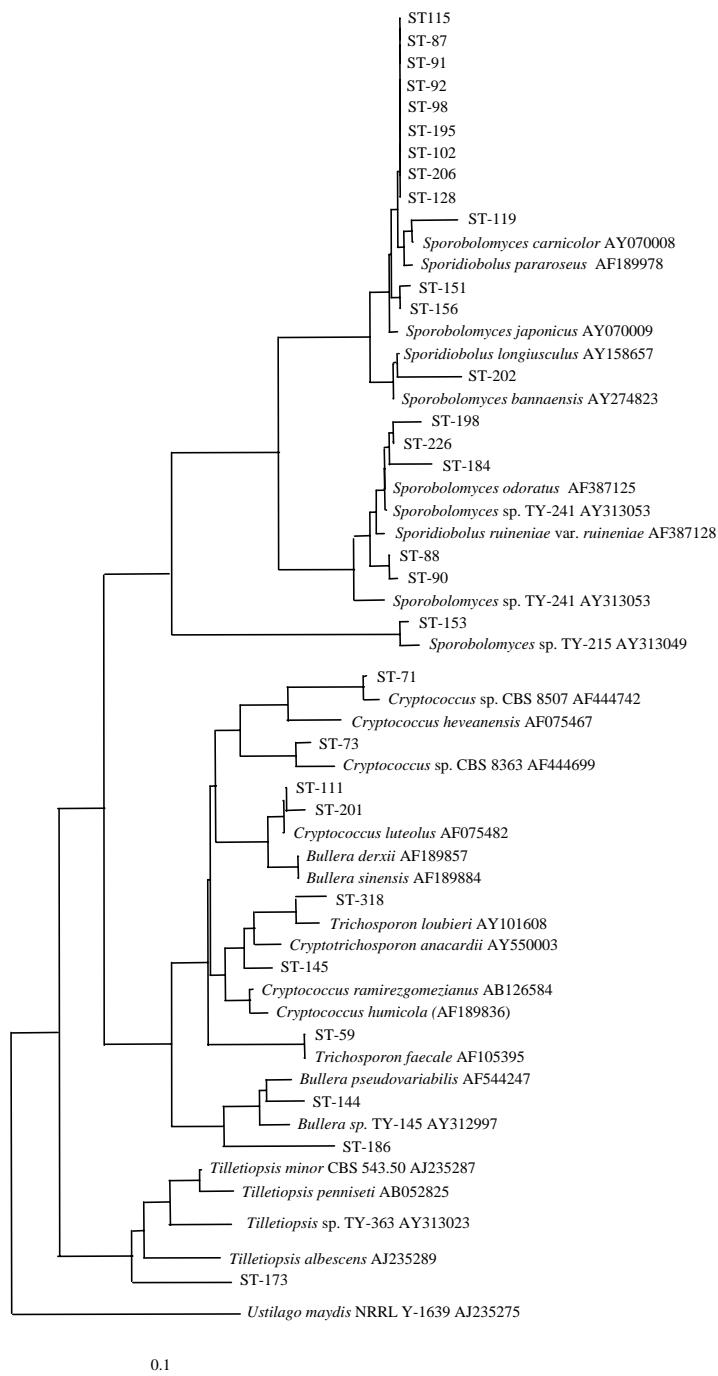
The phylogenetic trees for new species isolated in the natural environment of Thailand were constructed based on the D1/D2 sequences together with their closest known species. A tree for new ascomycetous yeasts comprised 86 strains of 75 species and their closest known species (Fig. 4) and that for new basidiomycetous yeasts comprised 30 strains (26 species) and their closest known species (Fig.5). The new yeasts isolated from natural habitat of Thailand were widely distributed in the phylogenetic trees but not concentrated in certain groups of yeasts.

Eighty-six strains of new ascomycetous yeasts were classified into 14 genera of 6 families (Order Saccharomycetales, Class Hemiascomycetes) and represented 76 species (Table 21). Thirty strains of new basidiomycetous yeasts were distributed in 7 genera of 3 classes, Uredinomycetes, Hymenomycetes and Ustilaginomycetes (Table 21). Urediniomycetes included 19 strains which were classified into the genera *Sporidiobolus* and *Sporobolomyces* representing 11 or 12 species. Hymenomycetes comprised 9 strains of the genera *Bullera*, *Cryptococcus*, *Trichosporon* and *Cryptotrichosporon*. Ustilaginomycetes comprised 2 strains of the genus *Tilletiopsis*.

As already mentioned, most of new ascomycetous species contained only 1 strain. Some species have more than 1 strains but less than 4. In the case of new basidiomycetous yeasts, most species have 1 strain same as ascomycetous yeasts. Although 9 strains (ST-87,ST-91,ST-92,ST-98,ST-102,ST-115,ST-128,ST-195 and ST-206) were very close to *Sporidiobolus pararoseus* (Fig. 5), they may be separated into 2 or 3 species because 0-4 nucleotides (including gaps) differences were observed among them (Table 22).



**Figure 4** The phylogenetics tree of the new species of ascomycetous yeasts and their closest species based on the D1/D2 domain sequences of 26S rDNA.



**Figure 5** The phylogenetics tree of the new species of basidiomycetous yeasts and their closest species based on the D1/D2 domain sequences of 26S rDNA.

Table 21 Taxonomic position of new yeast species found in this study.

Classification	No. of species (No. of strains)
<b>Phylum Ascomycota</b>	
Class Hemiascomycetes	
Order Saccharomycetales	
Family Dipodascaceae	
Genus <i>Dipodascus</i>	1 (1)
Genus <i>Galactomyces</i>	1 (1)
Genus <i>Trichosporonoides</i>	1 (1)
Family Endomycetaceae	
Genus <i>Endomyces</i>	1 (1)
Family Metschnikowiaceae	
Genus <i>Metschnikowia</i>	3 (3)
Family Saccharomycetaceae	
Genus <i>Debaryomyces</i>	1 (2)
Genus <i>Issatchenkia</i>	1 (1)
Genus <i>Kluyveromyces</i>	1 (1)
Genus <i>Pichia</i>	14 (18)
Genus <i>Saccharomyces</i>	2 (2)
Family Saccharomycodaceae	
Genus <i>Hanseniaspora/Kloeckera</i>	6 (7)
Genus <i>Wickerhamia</i>	1 (1)
Family Candidaceae	
Genus <i>Candida</i>	41 (47)
Genus <i>Schizoblastosporion</i>	1 (1)
<b>Total 14 genera</b>	<b>76 (87)</b>
<b>Phylum Basidiomycota</b>	
Class Urediniomycetes	
Order Sporidiobolales	
Family Sporidiobolaceae	
Genus <i>Sporidiobolus</i>	7 (11)
Family Sporobolomycetaceae	
Genus <i>Sporobolomyces</i>	8 (8)
Class Hymenomycetes	
Genus “ <i>Cryptotrichosporon</i> ”	1 (1)
Family Cryptococcaceae	
Genus <i>Bullera</i>	2 (2)
Genus <i>Cryptococcus</i>	4 (4)
Genus <i>Trichosporon</i>	2 (2)
Class Ustilaginomycetes	
Order Entylomatales	
Family Entylomataceae	
Genus <i>Telletiopsis</i>	1 (1)
<b>Total 7 genera</b>	<b>25 (29)</b>

**Table 22** Number of nucleotide differences in new species of genus *Sporidiobolus* found in this study.

	ST-87	ST-91	ST-92	ST-98	ST-115	ST-128	ST-195	ST-206	ST-102
ST-87	0								
ST-91	4(2)	0							
ST-92	4(1)	4(1)	0						
ST-98	2(1)	3(1)	2	0					
ST-115	2(1)	3(1)	2	0	0				
ST-128	2(1)	2(1)	2	0	0	0			
ST-195	2(1)	2(1)	2	0	0	0	0		
ST-206	2(1)	2(1)	2	0	0	0	0	0	
ST-102	3(2)	2(1)	2	1(1)	1(1)	1(1)	1(1)	1(1)	0

Remark: Numerals in parentheses indicate the number of gaps.

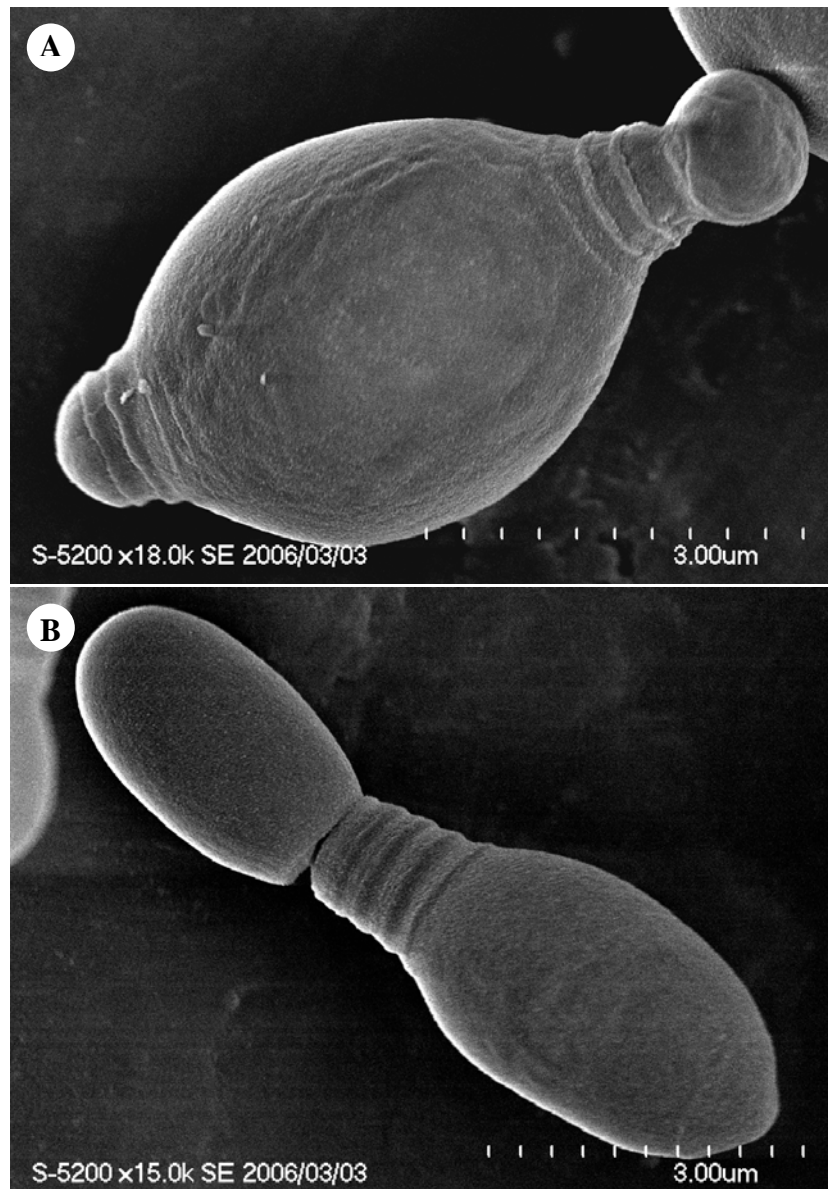
#### **4. Taxonomic Study on Selected Groups**

Twenty-one new species were selected for precise taxonomic studies by considering phylogenetic clusters and nucleotide differences. These yeasts were studied by polyphasic approaches, conventional taxonomy, chemotaxonomic study and molecular taxonomy. These strains belong to the genus *Candida* (14 species), *Hanseniaspora/Kloeckera* (4 species), *Pichia* (2 species), and *Trichosporon* (1 species). Their conventional taxonomic characteristics are shown in Appendix B (Appendix Table B2 and B3). Their taxonomy and descriptions are shown below.

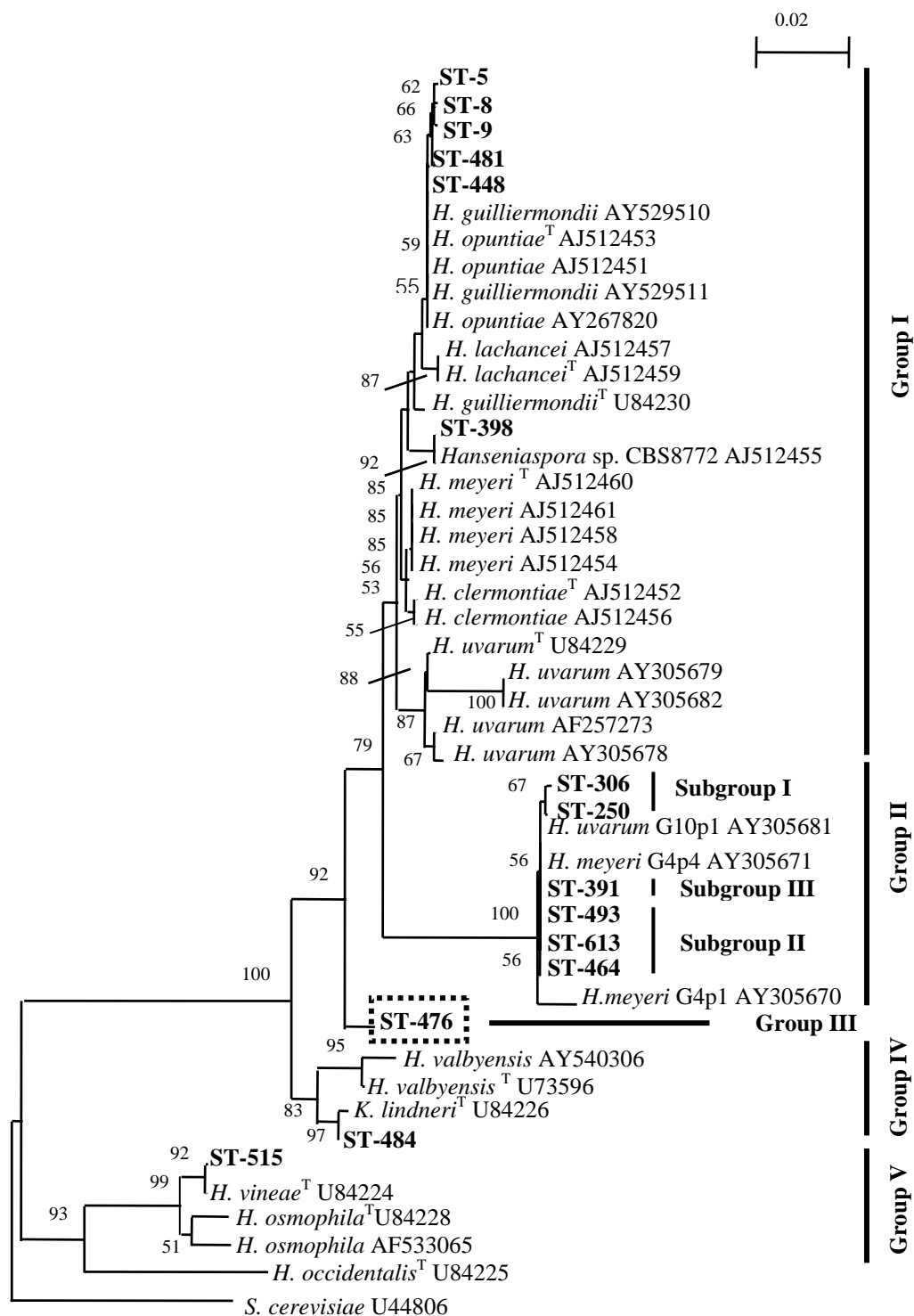
##### **4.1 Taxonomic studies on *Hanseniaspora/Kloeckera* (anamorph)**

Fifteen strains isolated in the present study proliferated by bipolar budding, buds are produced repeatedly from the same position (anneallation) only at the pole of the cell in both sides (Fig. 6). In the phylogenetic tree based on the D1/D2 domain sequences, they were located in the cluster where species of the genus *Hanseniaspora* and its anamorph counterpart *Kloeckera* were located. They were separated into 5 groups, Group I, Group II, Group III, Group IV and Group V as shown in Fig. 7.

Nucleotide similarities and number of nucleotide differences from closest species were shown in Table 23.



**Figure 6** Cell morphology of *Hanseniaspora thailandica* (strain ST-250), after 2 days at 25°C in YPD broth, (A) typical apiculate cell (B) matured new bud releasing from mother cell.



**Figure 7** Phylogenetic tree showing the positions of *Hanseniaspora*/*Kloeckera* isolated in Thailand and their related species based on the sequences of the D1/D2 domain of 26S rDNA with bootstrap values by 1000 re-samplings (< 50% is not shown).



**Table 23** Nucleotide similarity in D1/D2 domain sequence of 26S rDNA of Thai strains and their closest species (Type strains).

Group	Strains	Closest Species with GeneBank Accession No.	Nucleotide Identity (%)	No. of Nucleotide Difference	Remark
I	ST-5	<i>H. opuntiae</i> AY267820	571/571 (99.8)	1	Known species
	ST-8	<i>H. opuntiae</i> AY267820	560/561 (98.9)	1	Known species
	ST-9	<i>H. opuntiae</i> AY267820	559/560 (99.8)	1	Known species
	ST-448	<i>H. opuntiae</i> AY267820	572/572 (100)	0	Known species
	ST-481	<i>H. opuntiae</i> AY267820	571/572 (99.8)	1	Known species
	ST-398	<i>H. pseudoguilliermondii</i> AJ512455 (in press)	570/570 (100)	0	Known species
II	ST-250	<i>H. meyeri</i> AJ 512460	548/568 (96.3)	20	New species
	ST-306	<i>H. meyeri</i> AJ 512460	548/569 (96.3)	21	New species
	ST-493	<i>H. meyeri</i> AJ 512460	558/577 (96.5)	19	New species
	ST-613	<i>H. meyeri</i> AJ 512460	479/498 (96.2)	19	New species
	ST-464	<i>H. meyeri</i> AJ 512460	563/582 (96.6)	19	New species
	ST-391	<i>H. meyeri</i> AJ 512460	542/566 (95.9)	24	New species
III	ST-476	<i>H. clermontiae</i> AJ512456	568/578 (98.6)	10	New species
IV	ST-484	<i>Kloeckera lindneri</i> U84226	571/572 (99.8)	1	Known species
V	ST-515	<i>H. vineae</i> U84224	518/519 (99.8)	1	Known species

#### **Group I (ST-5, ST-8, ST-9, ST-398, ST-448 and ST-481)**

Six strains of group I had similar mol% G+C of 34.1-36.1 with those (33.6-35.3) of the nearest species (Table 24). They constituted a cluster with known species of *Hanseniaspora*, *H. guilliermondii*, *H. opuntiae*, *H. lachancei*, *H. meyeri*, *H. clermontiae* and *H. uvarum* (Fig. 7). Among 6 strains, 5 strains, ST-5, ST-8, ST-9, ST-448 and ST-481, were identified as *Hanseniaspora opuntiae*. ST-448 showed the identical sequence with the type strain of *H. opuntiae* and 4 strains, ST-5, ST-8, ST-9, ST-448, differed in 1 nucleotide from the type strain (Table 23). In the DNA-DNA hybridization experiment among these 5 strains showed the similarities more than 95% with the type strain of *H. opuntiae* (Table 24). In the conventional taxonomy, these strains had the same morphological and physiological characteristics with *H.*

*opuntiae*. Apparently, these 5 strains are conspecific with *H. opuntiae*. In the D1/D2 domain, ST-398 showed the identical sequence with *Hanseniaspora* sp. CBS 8772 and constituted a cluster with this strain. These two strains differed in 4-5 nucleotides from other strains of Group I (Table 25). In the DNA-DNA hybridization experiment, ST-398 was differentiated from other strains of Group I though the value to *H. opuntiae* was fairly high, 55.0-74.2% (Table 24). ST-398 and CBS 8772 are considered to represent a single new species different from *H. opuntiae*. According to Smith, CBS 8722 will be described soon as a new species *Hanseniaspora pseudoguilliermondii* (personal communication to Y. Imanishi, NBRC). So, ST-398 is the second strain of *H. pseudoguilliermondii*.

#### **Group II (ST-250, ST-306, ST-391, ST-464, ST-493 and ST-613)**

Six strains of Group II constituted a cluster with known species of *Hanseniaspora*, *H. guilliermondii*, *H. opuntiae*, *H. lachancei*, *H. meyeri*, *H. clermontiae* and *H. uvarum* (Fig. 7). They had mol%G+C lower than *H. meyeri*, the nearest species but the differences are not significant (Table 26). In the D1/D2 domain sequence, they differed in 19-22 nucleotides from the type strain of nearest species (Table 27). Based on the DNA-DNA hybridization and nucleotide differences in D1/D2 domain, 6 strains of Group II were suggested to be separated to 3 subgroups, Subgroup I, Subgroup II and Subgroup III (Table 26 and Table 27). In the phylogenetic tree of *Hanseniaspora*, strains of Group II constituted a cluster with *H. uvarum* G10p1, *H. meyeri* G4p4 and *H. meyeri* G4p1, which were registered to DNA databank however they do not belong to *H. uvarum* and *H. meyeri* because their D1/D2 sequence much differ from respective type strains. (Fig. 7). *H. uvarum* G10p1 and *H. meyeri* G4p4 differed from Group II of Thai strains in 3 and 4 nucleotides, respectively. So that these strains are conspecific or different species of Group II. *H. meyeri* G4p1 differed from Group II in 20 nucleotides. In the taxonomic criteria commonly employed, strains of subgroup I are differentiated from Group II by ability to assimilate trehalose and requirement of thiamine. Subgroup III differed from subgroup I and subgroup II by weak assimilation of 2-ketogluconic acid, inability to assimilate trehalose, glucono- $\delta$ -lactone, and no growth at 36°C.