Candida andamanensis sp. nov., *Candida laemsonensis* sp. nov. and *Candida ranongensis* sp. nov., anamorphic yeast species isolated from estuarine waters in a Thai mangrove forest

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Five strains (RV5^T, RV140, R31^T, RS17 and RS28^T) representing three novel anamorphic ascomycetous yeast species were isolated by membrane filtration from estuarine waters collected from a mangrove forest in Laem Son National Park, Ranong Province, Thailand, on different occasions. On the basis of morphological, biochemical, physiological and chemotaxonomic characteristics, sequence analysis of the D1/D2 domain of the large-subunit rRNA gene and the internal transcribed spacer region and phylogenetic analysis, three strains were found to represent two novel *Candida* species. Two strains (RV5^T and RV140) represented a single novel species, for which the name *Candida laemsonensis* sp. nov. is proposed. The type strain is RV5^T (=BCC 35154^T =NBRC 105873^T =CBS 11419^T). Strain R31^T was assigned to a novel species that was named *Candida andamanensis* sp. nov. (type strain R31^T =BCC 25965^T =NBRC 103862^T =CBS 10859^T). On the basis of morphological, biochemical, physiological and chemotaxonomic characteristics, sequence analysis of the D1/D2 domain of the large-subunit rRNA gene and phylogenetic analysis, strains RS17 and RS28^T represented another novel species of *Candida*, for which the name *Candida ranongensis* sp. nov. is proposed. The type strain is RS28^T (=BCC 25964^T =NBRC 103861^T =CBS 10861^T).

Yeasts in the genus *Candida* are distributed widely in both terrestrial and aquatic habitats. In aquatic habitats, *Candida* strains have been found in fresh water, estuarine water and sea water (Cooke *et al.*, 1960; Hagler & Ahearn, 1981, 1987; Spencer & Spencer, 1997; de Almeida, 2005; Butinar *et al.*, 2005; Nagahama, 2006; de García *et al.*, 2007; Limtong *et al.*, 2007, 2008; Boonmak *et al.*, 2009; Limtong *et al.*, 2010). Mangrove forests, or intertidal forests, are plant communities in saline coastal habitats distributed over a wide range of geographical zones (Aksornkoae, 1999). Yeasts and other fungi are prevalent in mangrove ecosystems, where they play an important role in the detrital food web and may be a food source for some marine invertebrates and zooplankton (Nagahama, 2006). Yeasts are generally abundant in water adjacent to and within mangrove swamps (Fell *et al.*, 2004). Recently, we

reported two novel yeast species from estuarine waters collected from mangrove forests in a coastal area of the Andaman Sea in Khao Lumpee-Haad Thaimueang National Park and Mu Ko Ra-Ko Prathong National Park, Phang-Nga Province, as *Candida thaimueangensis* (Limtong *et al.*, 2007) and *Candida phangngensis* (Limtong *et al.*, 2008). Two more novel species, *Candida siamensis*, obtained from sediment (Boonmak *et al.*, 2009), and *Candida suwanaritii* (Limtong *et al.*, 2010), obtained from water in a mangrove forest in Pred Nai village, Trat Province, in coastal areas of the Gulf of Thailand have also been reported.

In the course of investigations of yeasts in waters of a mangrove forest in Laem Son National Park, Ranong Province, Thailand (9° 13–32' N 98° 16–27' E), various yeast strains were isolated by the membrane filtration technique. *Kluyveromyces siamensis* (Am-In *et al.*, 2008) and *Candida sanitii* (Limtong *et al.*, 2010) were described for yeasts isolated from this mangrove forest, and five other strains which were found to represent three novel species of the genus *Candida* are described in this study.

Abbreviation: LSU, large subunit.

The GenBank/EMBL/DDBJ accession numbers for the 26S rRNA gene D1/D2 and ITS region sequences determined in this study are AB438205, AB438206, AB334210–AB334213, AB534173, AB534174 and AB525239.

Yeast isolation

Yeasts were isolated from estuarine waters collected in 1998–1999, 2005 and 2006 by membrane filtration following the method of Limtong *et al.* (2007). Purified yeast strains were suspended in YM broth (0.3 % yeast extract, 0.3 % malt extract, 0.5 % peptone and 1 % glucose) supplemented with 10 % glycerol and maintained at -80°C .

DNA sequencing and phylogenetic analysis

Methods for DNA isolation, amplification of the D1/D2 domain of the large subunit (LSU) rRNA gene by PCR and sequencing with the ABI BigDye Terminator cycle sequencing kit, version 3.1 (Applied Biosystems), using an ABI PRISM 3100 automated DNA sequencer (Applied Biosystems), were described previously (Am-In *et al.*, 2008). The sequences were compared pairwise using the BLASTN sequence similarity search program (Altschul *et al.*, 1997) and were aligned with sequences of related species retrieved from GenBank using the multiple alignment program CLUSTAL_X version 1.83 (Thompson *et al.*, 1997). A phylogenetic tree was constructed from the evolutionary distance data with Kimura's two-parameter correction (Kimura, 1980) using the neighbour-joining method (Saitou & Nei, 1987). Confidence levels of clades were estimated from bootstrap analysis (1000 replicates) (Felsenstein, 1985).

Yeast characterization

The strains were characterized morphologically, biochemically and physiologically according to the standard methods described by Yarrow (1998). Assimilation of nitrogen compounds was investigated on solid media with starved inocula following the method of Nakase & Suzuki (1986a). Vitamin requirements were determined according to the method of Komagata & Nakase (1967). Growth at various temperatures was determined by cultivation in YM broth. Ubiquinones were extracted from cells cultivated in 500 ml Erlenmeyer flasks containing 250 ml YPD broth (1 % yeast extract, 2 % peptone and 2 % glucose) on a rotary shaker at 28°C for 24–48 h and harvested by centrifugation. The cells were washed three times with distilled water. The isolation, purification and identification of ubiquinones were carried out according to Nakase & Suzuki (1986b).

Yeast isolation and identification

A total of 149 yeast strains were obtained from the estuarine water samples. Identification on the basis of comparison of sequences of the D1/D2 domain of the LSU rRNA gene revealed that 94 of the strains were found to belong to 25 ascomycetous species (*Candida berthetii*, *C. boidinii*, *C. butyri*, *C. glabrata*, *C. parapsilosis*, *C. phangngensis*, *C. picinguabensis*, *C. pseudolambica*, *C. rugosa*, *C. silvae*, *C. thaimueangensis*, *C. tropicalis*, *Debaryomyces*

nepalensis, *Galactomyces geotrichum*, *Linera saturnus*, *Kodamaea ohmeri*, *Pichia burtonii*, *P. caribbica*, *P. galeiformis*, *P. kluyveri*, *P. kudriavzevii*, *P. occidentalis*, *P. sporocuriosa*, *P. terricola* and *Torulaspora maleeae*) and four basidiomycetous species (*Rhodotorula mucilaginosa*, *Trichosporon asahii*, *Trichosporon coremiiforme* and *Trichosporon japonicum*). The remaining strains were found to represent undescribed and/or novel yeast species. Among the strains of novel species, strains RV5^T and RV140, which were isolated from two water samples collected in 1998–1999, strain R31^T, which was obtained from a water sample collected in 2005, and strains RS17 and RS28^T, which were isolated from two water samples collected in 2006, were found to represent three novel species.

This investigation revealed a wide diversity of yeasts in the waters of this mangrove forest. Investigations of yeasts in water from other mangrove forests in Thailand have identified *Candida globulata*, *C. membranifaciens*, *C. parapsilosis*, *C. phangngensis*, *C. picinguabensis*, *C. thaimueangensis*, *C. tropicalis*, *Geotrichum siamensis*, *Lodderomyces elongisporus*, *P. caribbica*, *P. guilliermondii* and *R. mucilaginosa* in water from Kho Lumpee-Haad Thaimueang National Park (Kaewwichian *et al.*, 2010; Limtong *et al.*, 2007, 2008) and *C. parapsilosis*, *C. phangngensis*, *Candida* cf. *glabrata* UWO (PS) 98-110.4, *P. caribbica*, *P. fabianii*, *P. guilliermondii* and *R. mucilaginosa* in water from Mu Ko Ra-Ko Prathong National Park (Limtong *et al.*, 2007, 2008). Comparison of the yeasts found in waters of the three mangrove forests indicated that most species found in this study were not present in the other mangrove forests. Only *C. parapsilosis*, *P. caribbica* and *R. mucilaginosa* were found from all three mangrove forests, while *C. thaimueangensis* and *P. fabianii* found in this study were present in one of the other mangrove forests. This may result from different environmental factors.

Novel species, delineation and ecology

The sequences of the D1/D2 domain of the LSU rRNA genes of strains RV5^T and RV140 differed only by one gap. They showed 1.1 % nucleotide substitutions (six nucleotide substitutions and four gaps out of 573 nt) in comparison with the sequence of the type strain of *C. berthetii*, the closest species. According to Kurtzman & Robnett (1998), yeast strains that show more than 1 % nucleotide substitution in the D1/D2 domain of the LSU rRNA gene usually represent different species. In the phylogenetic tree based on sequences of the D1/D2 domain of the LSU rRNA gene, the two strains were placed in the *Starmera* clade in a position distinct from other members of the clade (Fig. 1). They clustered with *C. berthetii*, supported by high bootstrap values. Analysis of ITS sequence similarity revealed that the two strains had identical sequences and differed from the closest species, *C. berthetii*, by 1.4 % nucleotide substitutions (seven nucleotide substitutions out of 512 nt). It was indicated by Sugita *et al.* (1999) that conspecific yeast strains usually have nucleotide differences of less than 1 % in

Fig. 1. Phylogenetic tree based on sequences of the D1/D2 domain of the LSU rRNA gene, showing the positions of *Candida laemsonensis* sp. nov. RV5^T and RV140, *Candida andamanensis* sp. nov. R31^T and *Candida ranongensis* sp. nov. RS17 and RS28^T with respect to closely related species. The tree was constructed from evolutionary distance data corrected by the two-parameter transformation of Kimura (1980), using the neighbour-joining method. Numbers indicate percentages of bootstrap resampling, derived from 1000 samples. Bar, K_{nuc} of 0.02.

the ITS region. From these results, we concluded that strains RV5^T and RV140 represent a single novel species.

Sequence similarity analysis of the D1/D2 domain of the LSU rRNA gene showed that strain R31^T was closely related to *C. conglobata*, with 0.9 % nucleotide substitutions (five nucleotide substitutions out of 530 nt), and to *Candida aaseri* and *C. butyri*, again with 0.9 % nucleotide substitutions (five nucleotide substitutions and one gap out of 531 nt). The phylogenetic tree based on sequences of the D1/D2 domain of the LSU rRNA gene demonstrated that strain R31^T was placed in a position distinct from *C. conglobata*, *C. aaseri* and *C. butyri* (Fig. 1). Therefore strain R31^T was considered to represent a distinct species. As the sequence differences in the D1/D2 domain of the LSU rRNA gene were relatively small, the ITS region was sequenced to confirm the novelty of this strain. In this region, the sequence of strain R31^T differed from those of

its closest relatives as follows: from *C. butyri* by 5.1 % nucleotide substitutions (31 substitutions and 11 gaps out of 607 nt), from *C. aaseri* by 5.5 % nucleotide substitutions (33 substitutions and 17 gaps out of 604 nt) and from *C. conglobata* by 5.8 % nucleotide substitutions (35 substitutions and 30 gaps out of 601 nt). These results supported the conclusion that strain R31^T represents a novel species.

Strains RS17 and RS28^T showed identical LSU rRNA gene D1/D2 domain sequences. The closest strains in terms of pairwise sequence similarity were *Candida* sp. BG02-7-17-001A-1-1 and *Candida* sp. BG02-7-21-004Q-1-2, two undescribed strains with identical sequences, which differed from strains RS17 and RS28^T by 5.1 % nucleotide substitutions (25 substitutions out of 492 nt), and *Candida scorzettiae*, which differed by 10.5 % nucleotide substitutions (53 substitutions and 48 gaps out of 506 nt). The phylogenetic tree based on sequences of the LSU rRNA

gene D1/D2 domain demonstrated that the two strains clustered with *Candida* sp. BG02-7-17-001A-1-1 and *Candida* sp. BG02-7-21-004Q-1-2 and were placed in a position distant from *C. scorzettiae* and other related species (Fig. 1). Therefore, the two novel strains were considered to represent another single novel, phylogenetically distinct species.

Cells of strains RV5^T and RV140 were subglobose, ellipsoidal to elongate, while cells of strains R31^T, RS17 and RS28^T were ovoidal to ellipsoidal (Fig. 2). The strains proliferated by multilateral budding. Pseudohyphae were formed, but true hyphae were not formed by the novel strains. No ascospores were produced from individual strains or strains paired on YM agar, 5 % malt extract agar, Fowell's acetate agar, cornmeal agar or Gorodkova agar after 6 weeks at 15 or 28 °C.

On the basis of morphological, biochemical, physiological and chemotaxonomic characteristics, sequence analysis of the LSU rRNA gene D1/D2 domain and the ITS region and phylogenetic analysis, we conclude that strains RV5^T and

RV140 represent a single novel species of *Candida*, for which the name *Candida laemsonensis* sp. nov. is proposed, and that strain R31^T represents a novel species that we name *Candida andamanensis* sp. nov. On the basis of morphological, biochemical, physiological and chemotaxonomic characteristics, sequence analysis of the LSU rRNA gene D1/D2 domain and phylogenetic analysis, strains RS17 and RS28^T were found to represent a third novel species, for which the name *Candida ranongensis* sp. nov. is proposed.

In practice, *C. laemsonensis* sp. nov. can be distinguished from *C. berthetii*, its closest phylogenetic relative, by only a few phenotypic characteristics, including its ability to assimilate sorbose and cellobiose, its ability to grow in 0.01 and 0.1 % cycloheximide and its ability to grow on 50 and 60 % glucose.

C. andamanensis sp. nov. can be distinguished from its closest relative *C. conglobata* by its inability to ferment D-glucose, D-galactose and trehalose, by its ability to assimilate sucrose, maltose, melezitose and methyl α -D-glucoside and

Fig. 2. Budding cells on YM agar after 4 days at 28 °C (a, c, g) and pseudohyphae formed on cornmeal agar after 7 days at 28 °C (b, d, e, f) by *Candida laemsonensis* sp. nov. RV5^T (a, b), *Candida andamanensis* sp. nov. R31^T (c–e) and *Candida ranongensis* sp. nov. RS28^T (f, g). Bars, 10 µm.

by its ability to grow in 0.01 % cycloheximide, 50 % glucose and at 37 °C. *C. andamanensis* sp. nov. can be distinguished from *C. aaseri* by its inability to assimilate lactose and citric acid, its ability to assimilate D-arabinose, its ability to grow in vitamin-free medium, in 0.01 % cycloheximide and at 40 and 42 °C (weak) and by its ability to grow on 50 and 60 % glucose. *C. andamanensis* sp. nov. can be separated from *C. butyri* by its inability to ferment D-glucose and to assimilate citric acid and by its ability to grow in vitamin-free medium and in 0.01 % cycloheximide and at 40 and 42 °C (weak).

C. ranongensis sp. nov. can be distinguished from *C. scorzettiae*, the closest described species, on the basis of the LSU rRNA gene D1/D2 domain sequence and some phenotypic characteristics, including its inability to assimilate cellobiose, lactose, salicin and citric acid and its ability to assimilate L-sorbose and to grow in 10 % NaCl and at 37, 40 and 42 °C (weak).

Strains RV5^T and RV140 of *C. laemsonensis* sp. nov. were isolated from different water samples collected in 1998–1999 which were characterized by pH of 7.7 and 7.4, temperatures of 25.3 and 25.2 °C and salinity of 0 p.p.t. Strains RS17 and RS28^T of *C. ranongensis* sp. nov. were derived from water samples collected in 2006 which were characterized by lower pH (6.9 and 7.0), higher temperatures (27.4 and 27.3 °C) and higher salinity (14 and 13 p.p.t.). Strain R31^T of *C. andamanensis* sp. nov. was obtained from a water sample collected in 2005 that was characterized by the highest pH (8.0), temperature (29.7 °C) and salinity (34 p.p.t.). This shows that the three novel species are not the dominant species in this mangrove forest and that each of them was present only in a specific natural environment that differed in pH, temperature and salinity. The three novel species were found in water only from a mangrove forest in Ranong Province, and not from the other mangrove forests in other areas of Thailand, indicating that they are not common species of these mangrove habitats.

Latin diagnosis of *Candida laemsonensis* Am-In, Limtong, Yongmanitchai et Jindamorakot sp. nov.

In medio liquido YM, *post dies* 3 *ad* 28 °C *cellulae subglobosae, ellipsoideae aut elongate* (3–6 × 3–7 µm), *singulae aut binae, per germinationem multipolarem reproducentes*. *In agar* YM, *post dies* 4 *ad* 28 °C, *cultura butyrosa, crenea, sublatum, glabra et margine labata minuta*. *Pseudohyphae formantur nec hyphae non formantur*. *Ascospores non formantur*. D-Glucosum (*infirmum*) fermentatur et non D-galactosum, sucrosum, maltosum, lactosum, trehalosum, raffinotum nec melibiosum. D-Glucosum, L-sorbosum (*lente*), cellobiosum, ethanolum, glycerolum, salicinum, D-glucono-δ-lactonum (*infirmum*), acidum DL-lacticum, acidum succinicum, acidum citricum, nitrosum, nitricum, ethylaminum, L-lysinum et cadaverinum assimilantur at non D-galactosum, sucrosum, maltosum, trehalosum, lactosum, melibiosum, raffinotum, melezitotum, inulinum, amyllum solubile, D-xylosum, L-arabiosum, D-arabiosum, D-ribosum, L-rhamnosum, N-acetyl-D-glucosa-

minum, methanolum, erythritolum, ribitolum, D-mannitolum, galactitolum, D-glucitolum, methyl-α-D-glucosinum, acidum D-gluconicum, acidum D-glucuronicum, acidum D-galacturonicum, acidum 2-keto-D-gluconicum, acidum 5-keto-D-gluconicum nec inositolum. *Vitamina externa ad crescentiam necessaria non sunt*. *Crescit in* 50 % glucosum, 60 % glucosum, 0.01 % cycloheximido et 0.1 % cycloheximido. *Non crescit in* 10 % NaCl/5 % glucosum, 15 % NaCl/5 % glucosum. *Crescere potest in temperatura* 20, 25, 37, 40 et 42 °C (*infirmum*) et *non crescit in temperatura* 45 °C. *Amyllum non formatur*. *Acidum formatur*. *Diazonium caeruleum B non respondens*. *Ureum non hydrolysatur*. *Ubiquinonum majus*: Q-7.

Holotypus: stirps RV5^T isolatus aqua, Ranong Provincia, Thailandia. *Cultura et conservatus in collectione culturarum in BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailandia ut BCC 35154^T; NITE Biological Resources Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japonia conservatus ut NBRC 105873^T et Centraalbureau voor Schimmelcultures (CBS), Utrecht, Hollandia ut CBS 11419^T.*

Description of *Candida laemsonensis* Am-In, Limtong, Yongmanitchai & Jindamorakot sp. nov.

Candida laemsonensis (laem.son.en'sis. N.L. fem. adj. *laemsonensis* referring to Laem Son National Park, Ranong Province, Thailand, where the first two strains were isolated).

After growth on YM agar for 3 days at 28 °C, cells are subglobose, ellipsoid to elongate (3–6 × 3–7 µm) and occur singly or in pairs (Fig. 2a). Budding is multilateral. The streak culture on YM agar after 4 days at 28 °C is butyrous, cream-coloured, raised, smooth-surfaced and has a finely lobed margin. In slide culture on cornmeal agar at 28 °C after 7 days, rudimentary pseudohyphae are formed but septate hyphae are not formed (Fig. 2b). No ascospores are produced from individual strains or strains paired on YM agar, 5 % malt extract agar, Fowell's acetate agar, cornmeal agar or Gorodkova agar after 6 weeks at 15 or 28 °C. Glucose is fermented weakly and D-galactose, sucrose, maltose, lactose, trehalose, raffinose and melibiose are not fermented. D-Glucose, L-sorbose (*latent*), cellobiose, ethanol, glycerol, salicin, D-glucono-1,5-lactone (*weak*), DL-lactic acid, succinic acid, citric acid, nitrate, nitrite, ethylamine, L-lysine and cadaverine are assimilated; D-galactose, sucrose, maltose, trehalose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, L- and D-arabinose, D-ribose, L-rhamnose, N-acetyl-D-glucosamine, methanol, erythritol, ribitol, D-mannitol, galactitol, D-glucitol, methyl α-D-glucoside, D-gluconic acid, D-glucuronic acid, D-galacturonic acid, 2-keto-D-gluconate, 5-keto-D-gluconate and inositol are not assimilated. Grows in vitamin-free medium and in the presence of 50 and 60 % glucose and 0.01 and 0.1 % cycloheximide. No growth in the presence of 10 % NaCl/5 % glucose or 15 % NaCl/5 % glucose. Growth is observed at 20, 25, 37, 40 and 42 °C (*weak*); no growth at 45 °C. Starch-like

compounds are not produced. Acid formation from glucose is positive. Diazonium blue B reaction is negative. Urea is not hydrolysed. Ubiquinone is Q-7.

Holotype: RV5^T is the holotype. The strain was isolated from estuarine water collected from a mangrove forest in Laem Son National Park, Ranong Province, Thailand. Living cultures from the type have been deposited at the BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailand, as BCC 35154^T, at the NITE Biological Resources Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japan, as NBRC 105873^T and at the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands, as CBS 11419^T.

Latin diagnosis of *Candida andamanensis* Am-In, Limtong, Yongmanitchai et Jindamorakot sp. nov.

In medio liquido YM, *post dies* 3 *ad* 28 °C *cellulae ovoideae aut ellipsoideae* (3–5 × 3–7 µm), *singulae aut binae, per germinationem multipolarem reproducentes*. *In agar* YM, *post dies* 3 *ad* 28 °C, *cultura butyrosa, crenea, sublatum, glabra et margine glabra vel undulato*. *Pseudohyphae formantur nec hyphae non formantur*. *Ascosporae non formantur*. *Fermentatio nulla*. D-Glucosum, D-galactosum, L-sorbose, sucrosum, maltosum, cellobiosum, trehalosum, melezitum, D-xylosum, L-arabinosum, D-arabiosum, D-ribosum, N-acetyl-D-glucosaminum, ethanolum, glycerolum, erythritolum, ribitolum, D-mannitolum, D-glucitolum, methyl-α-D-glucosidum, salicinum, acidum D-gluconicum, D-glucono-δ-lactonum, acidum succinicum assimilantur at non lactosum, melibiosum, raffinolum, inulinum, amyllum solubile, L-rhamnosum, methanolum, galactitolum, D-glucuronicum, acidum D-galacturonicum, acidum 2-keto-D-gluconicum, acidum 5-keto-D-gluconicum, acidum DL-lacticum, acidum citricum nec inositolum. Ethylaminum, L-lysinum et cadaverinum assimilantur at non nitrosum nec nitricum. Vitamina externa ad crescentiam necessaria non sunt. Crescit in 10 % NaCl/5 % glucosum, 15 % NaCl/5 % glucosum, 0.01 % cycloheximido, 50 % glucosum et 60 % glucosum. Non crescit in 0.1 % cycloheximido. Crescere potest in temperatura 20, 25, 37, 40 et 42 °C (infirmum) et non crescit in temperatura 45 °C. Diazonium caeruleum B non respondens. Ureum non hydrolysat. Acidum nec amyllum non formatur. Ubiquinonum majus: Q-9.

Holotypus: stirps R31^T isolatus aqua, Ranong Provincia, Thailandia. Cultura et conservatus in collectione culturarum in BCC, BIOTEC, Pathumthani, Thailandia ut BCC 25965^T; NBRC, Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japonia conservatus ut NBRC 103862^T et CBS, Utrecht, Hollandia ut CBS 10859^T.

Description of *Candida andamanensis* Am-In, Limtong, Yongmanitchai & Jindamorakot sp. nov.

Candida andamanensis (an.da.man.en'sis. N.L. fem. adj. *andamanensis* referring to the Andaman Sea, on the coast

of which the mangrove forest from which the type strain was isolated is located).

After 3 days of growth in YM broth at 28 °C, cells are ovoidal to ellipsoidal (3–5 × 3–7 µm) and occur singly or in pairs (Fig. 2c). Budding is multilateral. After 3 days of growth on YM agar at 28 °C, the streak culture is butyrous, cream-coloured and raised, with a smooth surface, and has an entire to undulating margin. Pseudohyphae are formed but true hyphae are not formed in slide culture on cornmeal agar after 7 days at 28 °C (Fig. 2d, e). Ascospores are not produced on YM agar, 5 % malt extract agar, Fowell's acetate agar, cornmeal agar or Gorodkova agar after 6 weeks at 15 or 28 °C. Fermentation is negative. D-Glucose, D-galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, melezitose, D-xylose, L- and D-arabinose, D-ribose, N-acetyl-D-glucosamine, ethanol, glycerol, erythritol, ribitol, D-mannitol, D-glucitol, methyl α-D-glucoside, salicin, D-gluconic acid, D-glucono-1,5-lactone and succinic acid are assimilated; lactose, melibiose, raffinose, inulin, soluble starch, L-rhamnose, methanol, galactitol, D-glucuronic acid, D-galacturonic acid, 2-keto-D-gluconate, 5-keto-D-gluconate DL-lactic acid, citric acid and inositol are not assimilated. Ethylamine, L-lysine and cadaverine are assimilated, but nitrate and nitrite are not. Grows in vitamin-free medium and in the presence of 10 % NaCl/5 % glucose, 15 % NaCl/5 % glucose, 0.01 % cycloheximide and 50 and 60 % glucose. No growth in the presence of 0.1 % cycloheximide. Growth is observed at 20, 25, 37, 40 and 42 °C (weak); no growth at 45 °C. Diazonium blue B reaction is negative. Urea is not hydrolysed. No acid formation from glucose. Starch-like compounds are not produced. Ubiquinone is Q-9.

Holotype: R31^T is the holotype. The strain was isolated from estuarine water collected from a mangrove forest in Laem Son National Park, Ranong Province, Thailand. Living cultures from the type have been deposited as BCC 25965^T, NBRC 103862^T and CBS 10859^T.

Latin diagnosis of *Candida ranongensis* Am-In, Limtong, Yongmanitchai et Jindamorakot sp. nov.

In medio liquido YM, *post dies* 3 *ad* 28 °C *cellulae ovoideae aut ellipsoideae* (3–4 × 4–7 µm), *singulae aut binae, per germinationem multipolarem reproducentes*. *In agar* YM, *post dies* 3 *ad* 28 °C, *cultura butyrosa, crenea, sublatum, glabra et margine glabra*. *Pseudohyphae formantur nec hyphae non formantur*. *Ascosporae non formantur*. *Fermentatio nulla*. D-Glucosum, D-galactosum, L-sorbose, maltosum, trehalosum, D-xylosum, N-acetyl-D-glucosaminum, ethanolum, glycerolum, ribitolum (lente), D-mannitolum, D-glucitolum, acidum D-gluconicum, D-glucono-δ-lactonum, acidum 2-keto-D-gluconicum (infirmum), acidum DL-lacticum, acidum succinicum assimilantur at non sucrosum, cellobiosum, lactosum, melibiosum, raffinolum, melezitum, inulinum, amyllum solubile, L-arabiosum, D-arabiosum, D-ribosum, L-rhamnosum, methanolum, erythritolum, galactitolum, methyl-α-D-glucosidum, salicinum,

D-glucuronicum, acidum D-galacturonicum, acidum 5-keto-D-gluconicum, acidum citricum nec inositolum. Ethylaminum, L-lysinum et cadaverinum assimilantur at non nitrosum nec nitricum. Vitamina externa ad crescentiam necessaria non sunt. Crescit in 10 % NaCl/5 % glucosum, 0.01 % cycloheximido, 50 % glucosum et 60 % glucosum. Non crescit in 15 % NaCl/5 % glucosum et 0.1 % cycloheximido. Crescere potest in temperatura 20, 25, 37, 40 et 42 °C (infirmum) et non crescit in temperatura 45 °C. Diazonium caeruleum B non respondens. Ureum non hydrolysatur. Acidum nec amyllum non formatur. Ubiquinonum majus: Q-9.

Holotypus: stirps RS28^T isolatus aqua, Ranong Provincia, Thailandia. Cultura et conservatus in collectione culturarum in BCC, BIOTEC, Pathumthani, Thailandia ut BCC 25964^T; NBRC, Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japonia conservatus ut NBRC 103861^T et CBS, Utrecht, Hollandia ut CBS 10861^T.

Description of *Candida ranongensis* Am-In, Limtong, Yongmanitchai & Jindamorakot sp. nov.

Candida ranongensis (ra.nong.en'sis. N.L. fem. adj. *ranongensis* referring to Ranong Province, where the mangrove forest that yielded the first strains is located).

After 3 days of growth in YM broth at 28 °C, cells are ovoidal to ellipsoidal (3–4 × 4–7 µm) and occur singly or in pairs (Fig. 2g). Budding is multilateral. After 3 days of growth on YM agar at 28 °C, the streak culture is butyrous, cream-coloured and raised, with a smooth surface, and has an entire margin. Pseudohyphae are formed but true hyphae are not formed in slide culture on cornmeal agar after 7 days at 28 °C (Fig. 2f). No ascospores are produced from individual strains or strains paired on YM agar, 5 % malt extract agar, Fowell's acetate agar, cornmeal agar or Gorodkova agar after 6 weeks at 15 or 28 °C. Fermentation is negative. D-Glucose, D-galactose, L-sorbose, maltose, trehalose, D-xylose, N-acetyl-D-glucosamine, ethanol, glycerol, ribitol (latent), D-mannitol, D-glucitol, D-gluconic acid, D-glucono-1,5-lactone, 2-keto-D-gluconate (weak), DL-lactic acid and succinic acid are assimilated; sucrose, cellobiose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, L- and D-arabinose, D-ribose, L-rhamnose, methanol, erythritol, galactitol, methyl α-D-glucoside, salicin, D-glucuronic acid, D-galacturonic acid, 5-keto-D-gluconate, citric acid and inositol are not assimilated. Ethylamine, L-lysine and cadaverine are assimilated, but nitrate and nitrite are not. Grows in vitamin-free medium and in the presence of 10 % NaCl/5 % glucose, 0.01 % cycloheximide and 50 and 60 % glucose. No growth in the presence of 15 % NaCl/5 % glucose or 0.1 % cycloheximide. Growth is observed at 20, 25, 37, 40 and 42 °C (weak); no growth at 45 °C. Diazonium blue B reaction is negative. Urea is not hydrolysed. No acid formation from glucose. Starch-like substances are not produced. Ubiquinone is Q-9.

Holotype: RS28^T is the holotype. The strain was isolated from estuarine water collected from a mangrove forest in

Laem Son National Park, Ranong Province, Thailand. Living cultures from the type have been deposited as BCC 25964^T, NBRC 103861^T and CBS 10861^T.

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