

# *Kodamaea transpacific* f.a., sp. nov., a yeast species isolated from ephemeral flowers and insects in the Galápagos Islands and Malaysia: further evidence for ancient human transpacific contacts

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Twenty-four yeast strains were isolated from ephemeral flowers of *Ipomoea* spp. and *Datura* sp. and their associated insects in the Galápagos Archipelago, Ecuador, and from *Ipomoea* spp. and associated insects in the Cameron Highlands, Malaysia. Sequences of the D1/D2 domains of the large subunit rRNA gene indicated that these strains belong to a novel yeast species of the *Kodamaea* clade, although the formation of ascospores was not observed. The closest relative is *Candida restingae*. The human-mediated dispersion of this species by transpacific contacts in ancient times is suggested. The name *Kodamaea transpacific* f.a., sp. nov. is proposed to accommodate these isolates. The type strain is CLQCA-24i-070<sup>T</sup> (=CBS 12823<sup>T</sup>=NCYC 3852<sup>T</sup>); MycoBank number MB 803609.

The *Kodamaea* clade consists, at the time of writing, of 20 recognized species, some of which are associated with ephemeral flowers and floricolous insects (Lachance *et al.*, 2001; Lachance & Kurtzman, 2011). Lachance and Kurtzman (2011) suggested that these yeasts enhance the nutritional value of the carbon and nitrogen compounds found in flowers by serving as a food source for floricolous insects. The type species of the genus, *Kodamaea ohmeri*, appears to be a generalist, but the other species are associated with specific flowers, mushrooms, beetles or drosophilids. *K. ohmeri* and *Candida* (iter. nom. *Kodamaea*) *mesenterica* are also found in clinical specimens (Lachance & Kurtzman, 2011; Lachance *et al.*, 2011).

During independent surveys of yeasts associated with ephemeral flowers and their associated insects in the Galápagos Archipelago (Ecuador) and in the Cameron Highlands (Malaysia) several isolates of a species of the *Kodamaea* clade were found. These isolates did not

produce ascospores alone or mixed in pairs on several culture media. Sequences of the D1/D2 domains of the large subunit rRNA gene suggested that these isolates represented a novel species closely related to *Candida* (iter. nom. *Kodamaea*) *restingae* and *Kodamaea nitidulidarum*. In this paper, we describe the novel anamorphic species as *Kodamaea transpacific* f.a., sp. nov. The species is described as an asexual form, and thus *forma asexualis* (f.a.) forms part of the title. This usage has been proposed by Lachance (2012) and was first applied to the description of *Yarrowia yakushimensis* by Groenewald & Smith (2013).

Samples were collected in October 2009 in Isabela, Floreana and San Cristóbal Islands in the Galápagos Archipelago. In these islands, 45 flowers of *Ipomoea* spp. (Convolvulaceae) and 10 of *Datura* sp. (Solanaceae) were aseptically sampled using sterile plastic bags. Yeasts were cultured on the same day, usually within a few hours of sampling. The nectar region of the flowers was scraped gently with a sterile loop and streak-inoculated on yeast extract-malt extract agar (YMA; glucose 1 %, peptone 0.5 %, malt extract 0.3 %, yeast extract 0.3 %, agar 2 % and chloramphenicol 10 mg%). Beetles of the family Nitidulidae and *Drosophila* flies visiting these flowers were captured using sterile plastic bags

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession number for the ITS region and the D1/D2 domains of the large subunit of the rRNA gene sequence of strain CLQCA-24i-070<sup>T</sup> is KF002564.

and placed in plates of the same medium and kept for 15–30 min before being removed. The Malaysian plants were roadside weeds known in Malay as tatampajan. Nitidulid beetles and *Drosophila* flies were collected from palmate *Ipomoea* sp. and *Ipomoea indica* flowers. The collection sites were located at an altitude of approximately 1500 m in the Cameron Highlands (4° 30' N 101° 24' E), where the climate is cool and humid year round with a temperature range of 14–23 °C (Lachance *et al.*, 2006). Insects were collected in sterile plastic bags and later allowed to walk on the surface of YMA plates. Representatives of the different colony morphotypes were purified by repeated streak inoculation on YMA and preserved at –80 °C or in liquid nitrogen for later identification. The yeasts were characterized using standard methods (Kurtzman *et al.*, 2011). Identities were determined by sequencing the D1/D2 domains and the internal transcribed spacer (ITS) region of the large subunit of the rRNA gene. The region spanning the ITSs, the 5.8S rRNA gene and the D1/D2 domains of the large subunit rRNA gene were amplified by PCR directly from whole yeast cells as described previously (Lachance *et al.*, 1999). The amplified DNA was concentrated and cleaned on QIAquick PCR columns (Qiagen) and sequenced using an ABI sequencer at the John P. Robarts Research Institute (London, Ontario, Canada). The sequences were assembled, edited and aligned with the program MEGA5 (Tamura *et al.*, 2011). Phylogenetic placement of the novel species was based on a neighbour-joining analysis of the sequences of the D1/D2 domains of the large subunit rRNA gene. The bootstrap consensus tree was produced from 1000 iterations using 407 aligned nucleotide positions.

### Species delineation and phylogenetic placement

A BLAST search (Altschul *et al.*, 1997) of the ITS–D1/D2 regions rRNA gene cluster identified *C. restingae* and *K. nitidulidarum* as close relatives on the basis of phenetic sequence distance, but maximum-parsimony analysis of the D1/D2 region (Fig. 1) suggests that the isolates represent a sister species to *C. restingae*. Neighbour-joining analysis gave similar results, but a maximum-likelihood tree had a less pronounced structure with respect to the clade that contains the novel species. Maximum-likelihood analysis of a combined ITS–D1/D2 dataset, where available, supported sisterhood to *C. restingae*. The novel species differs from *C. restingae* by seven substitutions in the D1/D2 domains and by five substitutions and as many gaps, depending on alignment, in the ITS region. We propose the name *Kodamaea transpacificae* f.a., sp. nov., to accommodate the isolates of this novel species.

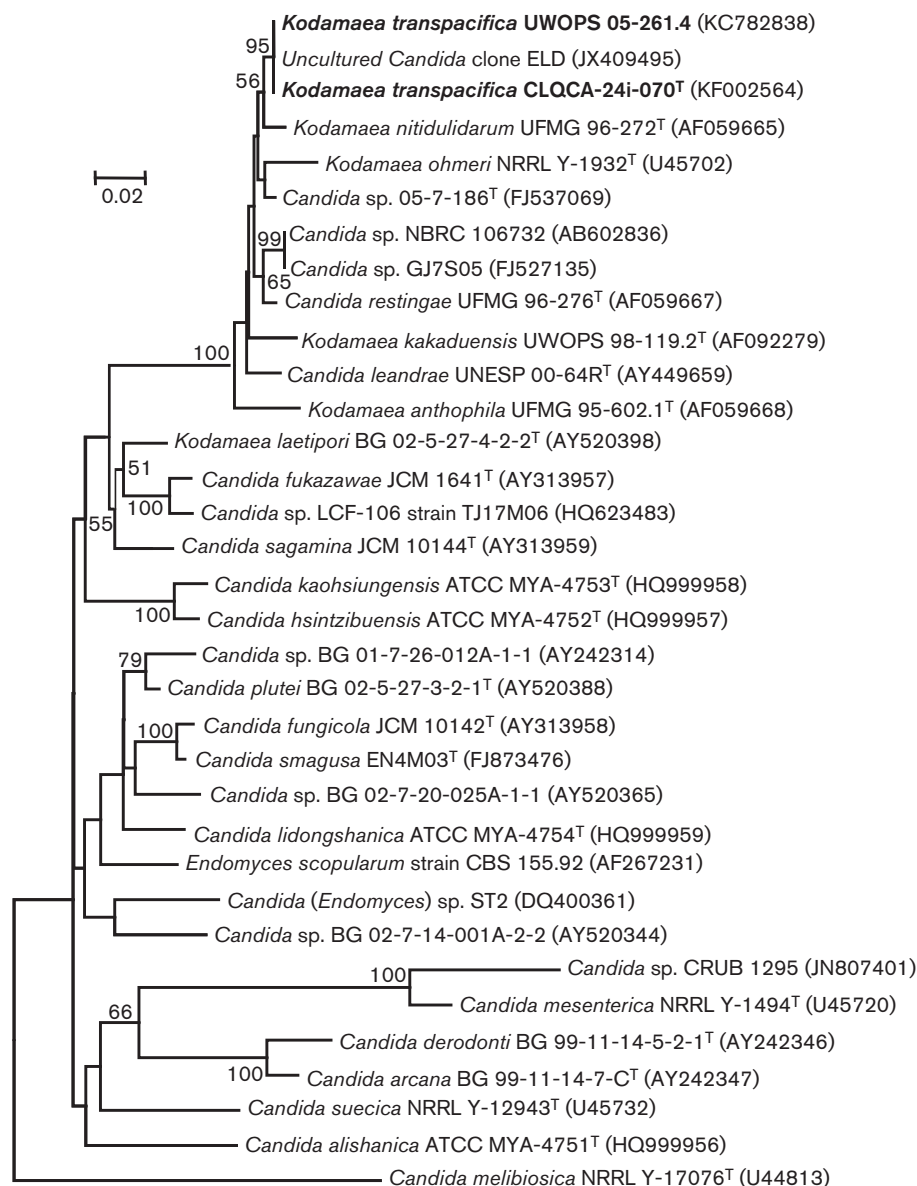
Isolates of *K. transpacificae* sp. nov. were examined individually or mixed in pairs on cornmeal, V8, dilute V8, 5% malt extract, yeast carbon base supplemented with 0.01% ammonium sulphate, acetate and Gorodkova agars, but no asci or signs of conjugation were observed. This result suggests that the novel species occurs as an asexual form in nature. *K. transpacificae* sp. nov. is difficult to distinguish from *C. restingae* on the basis of

growth tests and should be identified by sequence analysis.

*K. transpacificae* sp. nov. was isolated mostly from nitidulid beetles and *Drosophila* collected in *Ipomoea* spp. and *Datura* sp. flowers (Table 1). In *Datura* sp., the insects were observed on the inner corolla surface (Fig. 2) at the time of collection. Adaptation of some beetles to toxic tropane alkaloids such as atropine and scopolamine allows them to colonize *Datura* spp. flowers, which provide the insects with protection against possible competitors and predators (Kitamura *et al.*, 2004; Boros *et al.*, 2010). All parts of the *Datura* sp. contain toxic belladonna alkaloids, the concentration of which is highest in the petioles of the flowers (Krenzelok, 2010). As morning glory flowers are short-lived and not known to exhibit such toxicity, *K. transpacificae* sp. nov. should probably be regarded as a transient component of the *Datura* mycobiota, as the insects collected in these flowers may only be seeking protection.

### Yeast dispersal and history

The closest relative of *K. transpacificae* sp. nov., *C. restingae*, was isolated from various substrates, including cactus flowers and associated insects in Brazil and Costa Rica as well as nitidulid beetles from morning glory flowers in Malaysia (Lachance *et al.*, 2011). Most other close relatives in the *Kodamaea* clade were isolated from flowers and insects, mainly in tropical regions (Lachance & Kurtzman, 2011). *K. transpacificae* sp. nov. is apparently sympatric with *C. restingae* in Malaysia. Furthermore, a recent study of the intestinal yeast symbionts of *Drosophila* species recovered in a variety of localities identified DNA sequences that matched closely those of *K. transpacificae* sp. nov. (Fig. 1) in samples obtained from Taiwan, where they were most abundant, as well as the Seychelles Islands and Australia (Chandler *et al.*, 2012). The study employed DNA amplified from whole yeast communities of fly guts. The most widespread sequences in the gut community were those of *Hanseniaspora* species followed by *Saccharomyces* species, and *K. transpacificae* sp. nov. appeared unique in being restricted to the Eastern Hemisphere. Localities that did not yield evidence for the presence of the species included several sites in California, New York State, Hawaii and Mexico, which confirms observations made by one of us (M.-A.L.) on the basis of intense sampling in Central America and Hawaii. Our present report of isolates from the Galápagos Archipelago would be unexpected in this context. However, an intriguing parallel can be drawn with some recent observations made on *Saccharomycopsis fodiens*, a rare, floricolous beetle-associated species found in Australia, Costa Rica and the Galápagos Islands (Lachance *et al.*, 2012). It was suggested that the dispersal of such a rare and specialized organism could be attributed to human-mediated transport of American species of plants through the Pacific Ocean. For instance, *Datura*, a Mesoamerican-originated genus belonging to the family



**Fig. 1.** Neighbour-joining phylogram showing the phylogenetic placement of *Kodamaea transpacificae* sp. nov. based on the large subunit rRNA gene D1/D2 domains (407 positions). Bootstrap values above 50 % are shown. Bar, 0.02 changes per position.

Solanaceae, was dispersed somewhat in pre-Columbian times to India (Daunay *et al.*, 2010). This is supported by iconographic evidence as well as old Arabic and Indian texts, which are regarded as providing strong evidence for the pre-Columbian occurrence of this genus in the Old World. These lines of evidence suggest that in ancient times, transpacific human-mediated contacts between the Old and New Worlds may have occurred (Daunay *et al.*, 2010; Geeta & Gharaibeh, 2007). The present research supports the hypothesis that human-mediated dispersal could underlie the current biogeography of *K. transpacificae* sp. nov. We propose a relatively fast dispersal of the plant

species from the New World to the Old World by Polynesians as well as other peoples inhabiting the Pacific Ocean islands (i.e. Polynesia, Melanesia and Micronesia). Lachance *et al.* (2012) further stated that this hypothesis would receive support from the eventual discovery of a more abundant population of *S. fodiens* in Taiwan, which is recognized as an early stop of Austronesian peoples (the Lapita cultural complex) during their migration from South East Asia 4000 years ago and further dispersion towards the Pacific Ocean about 1200 AD (Kirch, 2010). These migrant populations expanded in the Pacific islands and eventually reached South American coasts in

**Table 1.** Origin of strains of *Kodamaea transpacific* sp. nov.

Strain	Source	Locality
UWOPS 05-261.4	Nitidulid in flower of palmate <i>Ipomoea</i> sp.	Road between Berincang and Teringkap, Cameron Highlands, Malaysia
UWOPS 05-263.1, UWOPS 05-264.2	<i>Drosophila</i> sp. in flower of palmate <i>Ipomoea</i> sp.	Road between Berincang and Teringkap, Cameron Highlands, Malaysia
UWOPS 05-265.3	Nitidulid in flower of palmate <i>Ipomoea</i> sp.	Road between Berincang and Teringkap, Cameron Highlands, Malaysia
UWOPS 05-271.1	Nitidulid in flower of <i>Ipomoea indica</i>	Kampom Baru, Cameron Highlands, Malaysia
CLQCA-24i-026, CLQCA-24i-051	<i>Drosophila</i> sp. in flower of <i>Datura</i> sp.	Trail of the volcano Sierra Negra, Isabela Island, Galápagos Islands, Ecuador
CLQCA-24i-070 <sup>T</sup>	Nitidulid in flower of <i>Ipomoea alba</i>	Trail of the volcano Sierra Negra, Isabela Island, Galápagos Islands, Ecuador
CLQCA-24i-147	Flower of <i>Datura</i> sp.	Trail of the volcano Sierra Negra, Isabela Island, Galápagos Islands, Ecuador
CLQCA-24i-158, CLQCA-24i-238	Nitidulid in flower of <i>Ipomoea alba</i>	Trail of the volcano Sierra Negra, Isabela Island, Galápagos Islands, Ecuador
CLQCA-24ST-003, CLQCA-24ST-004, CLQCA-24ST-018, CLQCA-24ST-024, CLQCA-24ST-088, CLQCA-24ST-094	Nitidulid in flower of <i>Ipomoea</i> sp.	Road in San Cristóbal Island, Galápagos Islands, Ecuador
CLQCA-24ST-060	<i>Drosophila</i> sp. in flower of <i>Datura</i> sp.	Road in San Cristóbal Island, Galápagos Islands, Ecuador
CLQCA-24ST-083	Nitidulid in flower of <i>Datura</i> sp.	Road in San Cristóbal Island, Galápagos Islands, Ecuador
CLQCA-24F-001, CLQCA-24F-007, CLQCA-24F-012, CLQCA-24F-015, CLQCA-24F-017	Nitidulid in flower of <i>Datura</i> sp.	Estación de protección de tortugas, Floreana Island, Galápagos Islands, Ecuador

pre-Columbian times, returning with the sweet potato, the bottle gourd (*Lagenaria siceraria*) (Kirch, 2010) and possibly *Datura* sp. In the course of these migrations some sailors may have ended up in the Galápagos Islands and neighbouring regions of Central and South America during the first millennium AD (Scaglione & Cordero, 2011). A sequence matching that of *S. fodiens* has now been deposited in GenBank (C. Y. Chen, JN581104) in support of a biodiversity study conducted in Taiwan. In the present

case, our results and those reported by Chandler *et al.* (2012) provide an even stronger case. Accordingly, we present information relevant to the dispersal of the host plants where *K. transpacific* sp. nov. has hitherto been recovered.

Ancient societies have documented their cultural features through iconographies, sometimes complemented by written information. These sources are useful in understanding the use of plants by ancient societies and can provide valuable biogeographical information about the plants (Daunay *et al.*, 2010). The genus *Datura* of the family Solanaceae originated in Mesoamerica, and so its occurrence in the Old World is an intriguing case of global anthropogenic dispersal. Archaeological evidence points to the intervention of Polynesian sailors and perhaps other peoples from the Pacific islands in the transport of other plant species such as sweet potato (*Ipomoea* sp.), an Andean species, from South America to the Pacific islands (Scaglione & Cordero, 2011). To conclude, it is stressed that global commerce in pre-Columbian times may have occurred not only between Asia and Europe but also bidirectionally across the Pacific Ocean with the Americas (Daunay *et al.*, 2010). The natural dispersal of species such as sweet potato across the Pacific Ocean is not supported by the strong genetic evidence for the Ecuadorian and Peruvian Andes origin of Polynesian sweet potato varieties (Roullier *et al.*, 2013).

The Andean-originated cultigen *Ipomoea batatas* has served as a key element in building a plausible case for



**Fig. 2.** Flower of *Datura* sp. with *Drosophila* flies and nitidulid beetles collected in San Cristoban Island, Galápagos Islands, Ecuador.



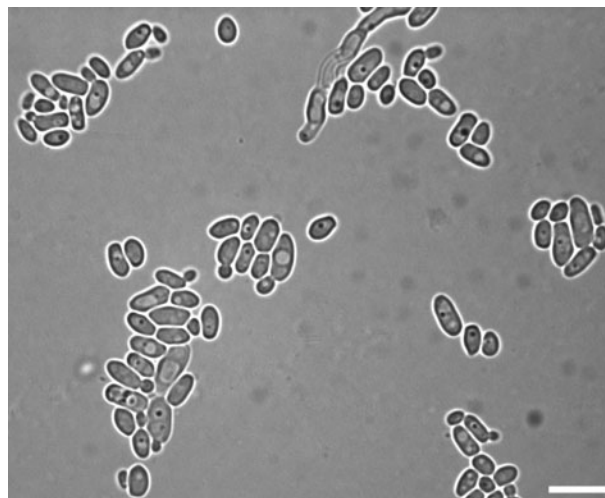
trans-Pacific contact. Excavations at Tangatatau, a large rock shelter in the Cook Islands (Polynesia), yielded several specimens of carbonized sweet potato tubers in prehistoric contexts from around 1000 AD. Anthropologists have found a remarkable similarity in the spelling and pronunciation of the words used in Andean Quichua and Polynesian languages to designate the sweet potato, namely *cumar* and *kumara*, respectively. Such cognates support the existence of an ancient contact between South American and Polynesian populations. Although an ancient contact is not part of written history, the linguistic evidence reinforces Paul Rivet's theory on the multiple origins of the American human populations (Rivet, 1957).

*K. transpacific* sp. nov. and *S. fodiens* join yet another recently discovered yeast species where dispersal may be linked to the activity of ancient human populations. *Candida theae* was described from two different isolates, one from an Indonesian tea bottle collected in 2009 and the second recovered during a microbial archaeology survey in Quito, Ecuador (Gomes *et al.*, 2009; Carvajal *et al.*, 2011). The yeast came from an ancient chicha fermentation vessel dated to 680 CE (Chang *et al.* 2012). *C. theae* is a member of the *Lodderomyces* clade, which contains a number of species that are frequently associated with humans, either as commensals or as opportunistic pathogens, leading to the hypothesis that dispersal occurred through historical human movements across a large array of islands that served as stepping stones between South America and the Old World.

### Description of *Kodamaaea transpacific* sp. nov. Freitas, Carvajal, Barahona, Lachance & Rosa

*Kodamaaea transpacific* (trans.pa.ci'fi.ca. L. prep. *trans* across; L. fem. adj. *pacific* peaceful, pertaining to the Pacific Ocean; NL. fem. adj. *transpacific* across the Pacific Ocean, referring to a plausible path of dispersal of the species).

After 3 days on YM agar at 25 °C, cells are ovoid to ellipsoid (2–3 × 3–5 µm) and occur singly or in pairs (Fig. 3). In older cultures, growth is pleomorphic and includes larger spherical cells (6–7 µm), pseudohyphae and true hyphae. A clumpy sediment is formed after 1 month, but no pellicle is observed. On YMA after 2 days at 25 °C, colonies are white, convex, smooth and opalescent. After 2 weeks, colonies are pleomorphic and tough, and form sectors that vary in sheen from semi-glossy to rugose or powdery. Asci or signs of conjugation are not seen on sporulation media. Glucose, sucrose and galactose fermentation are positive. Maltose and trehalose fermentation are negative. Glucose, inulin (variable), sucrose, raffinose (variable), galactose, trehalose, maltose, melezitose (variable), methyl  $\alpha$ -D-glucoside, cellobiose (variable), salicin, L-sorbose, D-xylose (sometimes weak), ethanol, glycerol, ribitol, xylitol, D-mannitol, D-glucitol, succinate, citrate, D-glucono- $\Delta$ -lactone, D-glucosamine (variable and slow), N-acetyl-D-glucosamine, ethylacetate (variable) and



**Fig. 3.** Budding cells of *Kodamaaea transpacific* sp. nov. grown on malt extract agar after 3 days at 25 °C. Bar, 5 µm.

hexadecane (variable) are assimilated. Melibiose, lactose, soluble starch, L-rhamnose, L-arabinose, D-arabinose, D-ribose, methanol, erythritol, galactitol, *myo*-inositol, DL-lactate, D-gluconate, acetone and 2-propanol are not assimilated. Lysine, ethylamine and cadaverine are utilized as sole nitrogen sources, but not nitrate or nitrite. Growth on vitamin-free medium is absent. Growth on amino acid-free medium is positive. No growth at 37 °C. Growth in the presence of 0.1 % cycloheximide is negative or weak. Growth in the presence of 10 % NaCl is absent. Growth in the presence of 50 % glucose is negative or weak. Growth in the presence of 1 % acetic acid is negative. Starch-like compounds are not produced. The diazonium blue B reaction is negative. Isolated from flowers of *Ipomoea* spp., *Datura* sp. and their associated insects in the Galápagos Archipelago (Ecuador) and Malaysia.

The type strain, CLQCA-24i-070<sup>T</sup> (=CBS 12823<sup>T</sup>=NCYC 3852<sup>T</sup>), was isolated from a nitidulid beetle collected from a flower of *Ipomoea alba* (Convolvulaceae) on a trail of the volcano Sierra Negra in the Isabela Island, Galápagos Islands, Ecuador. The name is registered in MycoBank under the number MB 803609.

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