Allobacillus salarius sp. nov., and Allobacillus saliphilus sp. nov., isolated from shrimp paste (ka-pi) in Thailand

Supalurk Yiamsombut1 · Pawina Kanchanasin2 · Wongsakorn Phongsopitanun2 · Nattakorn Kuncharoen2 · Ancharida Savarajara1 · Wenyu Shi3 · Linhuan Wu3 · Juncai Ma3 · Somboon Tanasupawat2

Received: 6 August 2021 / Revised: 5 November 2021 / Accepted: 8 November 2021 / Published online: 24 December 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract
Two strains of moderately halophilic, Gram-stain-positive and spore-forming rods, designated as SKP4-8T and SKP8-2T isolated from shrimp paste (Ka-pi), were taxonomic studied based on polyphasic approach. Strain SKP4-8T grew at pH 6.0–9.0 (optimum 7.0), at 25–45 °C (optimum 37 °C) and in 1–16% (w/v) NaCl (optimum 5–10%). Strain SKP8-2T grew at pH 6.0–9.0 (optimum 8.0), at 25–45 °C (optimum 37 °C) and in 0–20% (w/v) NaCl (optimum 3–10%). The strains contained meso-diaminopimelic acid in cell-wall peptidoglycan and the major menaquinone was MK-7. Strain SKP4-8T contained iso-C15:0, anteiso-C15:0 and iso-C17:0; and strain SKP8-2T contained anteiso-C15:0, iso-C15:0, iso-C16:0 and anteiso-C17:0 as major cellular fatty acids. Phosphatidylglycerol, diphosphatidylglycerol, unknown phospholipids and an unknown glycolipid were detected as major polar lipids. On the basis of 16S rRNA gene sequence analysis, strains SKP4-8T and SKP8-2T belonged to the genus Allobacillus and were closely related to Allobacillus halotolerans LMG 24826T with 98.8% and 99.3% similarity, respectively. The comparative genome analysis based on average nucleotide identity (ANI) and digital DNA–DNA hybridization revealed that both strains showed the values below 95 and 70%, from each other and from Allobacillus halotolerans LMG 24826T, respectively. Based on the data from this polyphasic study, strains SKP4-8T (= KCTC 33905T = LMG 30016T = TISTR 2499T) and Allobacillus saliphilus sp. nov. for SKP8-2T (= KCTC 33906T = LMG 29682T = TISTR 2558T).

Keywords Allobacillus salarius · Allobacillus saliphilus · Moderately halophile · Shrimp paste · Spore-forming bacteria

Introduction
Shrimp paste (Ka-pi), a dark brown, gray or pink brown in color with strong odor fermented shrimp is commonly used as a cooking ingredient. It is produced by fermentation of shrimp with salt for 1–3 months and has rich in various nutrients, particularly amino acids and peptides, and contains a high concentration of NaCl (Phithakpol et al. 1995; Tanasupawat and Komagata 2001). Moderately halophilic rod-shaped bacteria have been isolated from shrimp paste and was proposed as the new genus Allobacillus (Sheu et al. 2011). In Thailand, many halophilic species were isolated from fermented fish or shrimp paste such as Gracilibacillus thailandensis (Chamroensaksri et al. 2010), Lentibacillus kapialis (Pakdeeto et al. 2007a), Lentibacillus salicampi and Lentibacillus juripiscarius (Namwong et al. 2005), Oceanobacillus kapialis (Namwong et al. 2009), Piscibacillus salipiscarius (Tanasupawat et al. 2007), Salinicoccus siamensis (Pakdeeto et al. 2007b), Virgibacillus kapii (Daroonpunt et al. 2016) and Virgibacillus siamensis (Tanasupawat et al. 2010). This study, two strains, SKP4-8T and SKP8-2T were isolated from shrimp paste (ka-pi) in Thailand and found to represent the novel species of the genus Allobacillus and were subsequently evaluated by the means of a polyphasic taxonomy.

Communicated by Erko Stackebrandt.

* Somboon Tanasupawat
somboon.T@chula.ac.th

1 Biotechnology Program and Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
2 Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand
3 World Data Center for Microorganisms (WDCM), Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, People’s Republic of China
Materials and methods

Bacterial isolation

Strains SKP4-8T and SKP8-2T were isolated from shrimp paste collected from the market in Samut Sakhon province, Thailand, using spread-plate technique on modified JCM medium no.377 agar plates, contained (g/L): 5 g casamino acids, 5 g yeast extract, 1 g sodium glutamate, 3 g trisodium citrate, 20 g MgSO4·7H2O, 2 g KCl, 50 g NaCl, 0.036 g FeCl3·4H2O, 0.0036 g MnCl2·4H2O, 20 g agar (pH adjusted to 7.0–7.2) and incubated at 37 °C for 3–7 days.

Phenotypic characteristics

Cells grown on modified JCM medium no. 377 agar at 37 °C after 3 days were examined for their colony and cell morphology. Gram staining was performed as previously described (Barrow and Feltham 1993). Cell form and spore formation were observed under a light microscope (CH-2, Olympus) and electron microscope (JSM-5410LV, Japan). Flagella were examined as described by Forbes (1981). Anaerobic growth was tested by incubating the cultures on modified JCM medium no. 377 plates in an anaerobic jar with AnaeroPack-Anaero (Mitsubishi Gas Chemical, MGC). Catalase and oxidase activities and nitrate reduction were determined as described by Barrow and Feltham (Barrow and Feltham 1993). Hydrolysis of aesculin, gelatin, starch, skim milk and Tween 20, Tween 40, Tween 60 and Tween 80 were determined as described by Namwong et al. (2005). Arginine hydrolysis was performed using a reported medium (Thornley 1960). Acid production from carbohydrates was determined using basal medium composing (g/L) of 5 g carbohydrate, 1 g casamino acids, 1 g yeast extract, 1 g sodium glutamate, 3 g trisodium citrate, 20 g MgSO4·7H2O, 2 g KCl, 50 g NaCl, 0.036 g FeCl3·4H2O, 0.0036 g MnCl2·4H2O and was adjusted to pH 7.0 and incubated at 37 °C for 7 days. Phenol red was used as an indicator. Effects of growth at various NaCl concentrations were investigated in modified JCM medium no. 377 broth omitting MgSO4·7H2O with different concentrations of NaCl (0 and 1–20%, w/v with an interval of 1). Growth at different pH (4–10) was investigated in buffered medium (Sorokin 2005) and growth at different temperatures (10–50 °C) was investigated on modified JCM medium no. 377 agar plates. Antibiotic susceptibility was examined by disc diffusion assay (Acar and Goldstein 1991) on Mueller–Hinton agar (Difco) supplemented with 5% (w/v) NaCl. The enzyme activities were determined using API ZYM system (bioMérieux), according to the manufacturer’s instructions. All tests were carried out in media supplemented with 5% NaCl, except for the investigation of effects of growth at various NaCl concentrations.

Chemotaxonomy

The cell biomass for chemotaxonomic characterization was produced in modified JCM medium no. 377 at 30 °C for 3 days. Diaminopimelic acid in the cell wall and menaquinone component were determined as described by Komagata and Suzuki (1987). Polar lipids were extracted and analyzed using the procedure of as described by Minnikin et al. 1984 and identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Komagata and Suzuki 1987). For quantitative analysis of cellular fatty acid compositions, cells were cultivated on modified JCM medium no. 377 agar plates at 30 °C for 48 h. Fatty acid methyl esters (FAMEs) were prepared and identified according to the instructions of the Microbial Identification System (MIDI) (Sasser 1990).

Genotypic and phylogenetic analyses

Chromosomal DNA was isolated and purified from cells grown in modified JCM medium no. 377 according to the method of Tamaoka and Komagata (1984). The 16S rRNA gene was amplified, purified, and sequenced as described by Namwong et al. (2005). The sequences were determined by aligning with selected sequences obtained from the GenBank/EMBL/DDBJ database employing CLUSTAL_X version 1.83 (Thompson et al. 1997). The alignment was manually edited and positions with gaps bases were eliminated prior to construction of a phylogenetic tree. The phylogenetic trees based on the neighbor-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony methods (Kluge and Farris 1969) were constructed using MEGA version 6 (Tamura et al. 2013). The confidence values of branches of the phylogenetic tree were determined using bootstrap analyses (Felsenstein 1985) based on 1000 resamplings.

Whole genome sequence of strains SKP4-8T, SKP8-2T and Allobacillus halotolerans LMG 24826T were performed using an Illumina Miseq platform (Illumina, Inc., San Diego, US-CA) by the World Data Center for Microorganisms (WDCM) under the Global Catalogue of Microorganisms (GCM) 2.0 project. Assembling the reads to contigs was accomplished using SPAdes 3.12 (Bankevich et al. 2012). The assembled genome of strains SKP4-8T, SKP8-2T and Allobacillus halotolerans LMG 24826T were publicly available on the GenBank (accession numbers: VMHE00000000, JAGSIE00000000 and JAHLZF00000000, respectively). The genome was annotated using the DFAST server (Taniwaki et al. 2018) following the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). Average nucleotide identity (ANI) values were calculated with pairwise genome alignment of the draft genome sequences of Allobacillus
halotolerans LMG 24826T (JAHLF000000000) using the ANI-BLAST (ANIb) and ANI-MUMmer (ANIm) algorithms (Meier-Kolthoff and Göker 2019) implemented with the JSpeciesWS web service (Richter and Rosselló-Móra 2009). Calculation of the digital DNA–DNA hybridization (dDDH) values was achieved using the Genome-to-Genome Distance Calculator (GGDC 2.1) using the BLAST + method (Richter et al. 2016). Results were based on the recommended formula 2 (identities/HSP length), which is useful when dealing with incomplete draft genomes.

Results and discussion

Strains SKP4-8T and SKP8-2T were Gram-stain-positive, strictly aerobic and spore-forming rods. Endospores are produced at terminal position in swollen sporangia (Supplementary Fig. S1). Colonies were smooth, circular, low convex and cream in the color (1–2 mm in diameter) after grew on modified JCM medium no. 377 agar at 37 °C for 2 days. Motile by peritrichous flagella (Fig. S2). Strains SKP4-8T and SKP8-2T were differentiated from A. halotolerans LMG 24826T by growth in NaCl, nitrate reduction, aesculin hydrolysis, and acid production from D-fructose, D-galactose, D-glucose and D-mannose (Table 1), and the amount of fatty acids of iso-C15:0 and anteiso-C15:0 (Table S1).

The strains SKP4-8T and SKP8-2T contained meso-diaminopimelic acid in the cell wall peptidoglycan and the major menaquinone was MK-7. The major fatty acids found in strain SKP4-8T were iso-C15:0 (54.4%), anteiso-C15:0 (11.9%), and iso-C17:0 (11.6%). In addition, the other fatty acids, C16:0 (0.8%), iso-C14:0 (1.8%), iso-C16:0 (3.2%), anteiso-C17:0 (4.5%), C16:1ω11c (0.6%) C16:1ω7c alcohol (5.7%) and summed feature 4 (1.7%) were also detected and are different from strain SKP8-2T (Table S1). The major fatty acids found in strain SKP8-2T contained anteiso-C15:0 (36.3%), iso-C15:0 (19.2%), iso-C16:0 (16.5%) and anteiso-C17:0 (16.3%). In addition, the other fatty acids, C16:0 (0.7%), iso-C14:0 (4.4%), iso-C17:0 (3.2%), C16:1ω7c alcohol (1.4%) and summed feature 4 (0.6%) were also detected (Table S1). The polar lipids of strain SKP4-8T were phosphatidylglycerol (PG), diphasatidylglycerol (DPG), three unknown phospholipids (PL1, PL2 and PL3) and unknown glycolipid (GL) (Supplementary Fig. S3). Strain SKP8-2T showed major polar lipid similar to strain SKP4-8T which contained of PG, DPG, unknown glycolipid (GL), but showed two unknown phospholipids (PL1 and PL2) (Supplementary Fig. S4) while A. halotolerans LMG 24826T contained PG, DPG, unknown phospholipids and one aminolipid as minor lipids (Sheu et al. 2011).

On the basis of 16S rRNA gene sequence analysis, strains SKP4-8T (1471 nt) and SKP8-2T (1441 nt) were closely related to A. halotolerans LMG 24826T with 98.8 and 99.3%, respectively (Figs. 1, S5 and S6). Strain SKP4-8T exhibited 99.5% 16S rRNA gene sequence similarity with SKP8-2T. The draft genome sequence of strain SKP4-8T was 2.59 Mb in size with an average in silico DNA G + C content of 38.8 mol%. The draft genome sequence of strain SKP8-2T was 2.54 Mb in size with an average in silico DNA G + C content of 38.8 mol%. The draft genomic features of the strains SKP4-8T, SKP8-2T and that of A. halotolerans LMG 24826T are described in Table 2. The ANIb and ANIm values of the draft genomes between strains SKP4-8T and

### Table 1

| Characteristics                          | SKP4-8T       | SKP8-2T       | LMG 24826T     |
|------------------------------------------|---------------|---------------|---------------|
| Temperature range for growth (optimum) °C | 25–45 (37)    | 25–45 (37)    | 20–45 (37)    |
| NaCl range for growth (optimum) % (w/v) | 1–16 (5–10)   | 0–20 (3–10)   | 1–20 (5–10)   |
| Nitrate reduction                        | +             | –             | –             |
| Hydrolysis of Tween 80                   | +             | +             | –             |
| API ZYM tests                            | +             | –             | –             |
| C14 Lipase                               |               | –             |               |
| Trypsin                                  | +             | –             | –             |
| Acid phosphatase                         | +             | +             | –             |
| Acid production from                      |               |               |               |
| D-Fructose                               | –             | +             | –             |
| D-Galactose                              | –             | +             | –             |
| D-Mannose                                | –             | +             | –             |
| D-Ribose                                 | –             | +             | –             |

All data are from this study. + positive reaction; – negative reaction.
its closest related species, *A. halotolerans* LMG 24826\(^T\) (86.6 and 88.1%, respectively) are shown in Table 3. The ANIb and ANIm values of the draft genomes between strains SKP4-8\(^T\) and its closest related species, *A. halotolerans* LMG 24826\(^T\) (87.1 and 88.3%, respectively) are shown in Table 3. Strain SKP4-8\(^T\) exhibited 93.2 and 93.8% of ANIb and ANIm with SKP8-2\(^T\). Both ANI values are clearly lower than the 95–96% cut-off for species delineation (Meier-Kolthoff et al. 2013). The digital DNA–DNA hybridization (dDDH) value between the genomes of strains SKP4-8\(^T\) and *A. halotolerans* LMG 24826\(^T\) was 32.5% (C.I. model 30.1–35.1%). The digital DNA–DNA hybridization (dDDH) values between the genomes of the strains SKP8-2\(^T\) and *A. halotolerans* LMG 24826\(^T\) were 33.0% (C.I. model 30.6–35.5%) (Table 3), which are significantly lower than the threshold value of 70% commonly used to delineate separated species status (Wayne et al. 1987). It is concluded from the above results that strains SKP4-8\(^T\) and SKP8-2\(^T\) should be recognized as new species within the genus *Allobacillus* for which the name *Allobacillus salarius* sp. nov. and *Allobacillus saliphilus* sp. nov., respectively, are proposed.

### Description of *Allobacillus salarius* sp. nov.

*Allobacillus salarius* (sa.lu’ri.us. L. masc. adj. salarius, of or belonging to salt)

Cells are strictly aerobic Gram-stain-positive rods (0.25–0.30×0.8–3.5 μm). Motile by peritrichous flagella.
Spores with oval shape are located at terminal position. Colonies are circular, convex, entire and cream in color (1–2 mm in diameter). Growth occurs at 25–45 °C, pH 6.0–9.0 and 0–20% (w/v) NaCl (optimally at 37 °C, at pH 7.0 and in 5–10% NaCl). Positive for oxidase, catalase, urease, nitrate reduction and hydrolysis of aesculin, Tween 20, Tween 40, Tween 60, and Tween 80. Hydrolysis of arginine, gelatin, skim milk, starch and acid production from D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, L-rhamnose, D-ribose, D-salicin, D-trehalose and D-xylose. In the API ZYM system, positive reaction for alkaline phosphatase, C4 esterase, C8 esterase, C14 lipase, leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase. The polar lipid profile contains phosphotidylglycerol, diphosphatidylglycerol, two unknown phospholipids and an unknown glycolipid. Meso-diaminopimelic acid is presented in the cell wall peptidoglycan. The major cellular fatty acids are iso-C15:0, anteiso-C15:0, and iso-C17:0, and the major isoprenoid quinone is MK-7. The in silico DNA G + C content of the type strain is 39.0 mol%.

The type strain is SKP8-2 T (= KCTC 33906T = LMG 29682T = TISTR 2558T), isolated from shrimp paste (Kapi) collected from a market in Samut Sakhon province, Thailand.

The DDBJ accession numbers for the 16S rRNA gene sequence of strains SKP8-2 T is LC215449. The GenBank accession numbers of the draft genome of strain SKP8-2 T is JAGSIE000000000.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-021-02694-9.

Funding This study was supported by the Grant for International Research Integration: Research Pyramid, Ratchadapiseskompot Endowment Fund (GCURP_58_01_33_01), Chulalongkorn University and the International Partnership Program of Chinese Academy of Sciences (Grant No. 153211KYSB 20190021). Additional thanks to the Ratchadapiseskompot Endowment Fund, Chulalongkorn University for a post-doctoral fellowship to P. K.
Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

References

Acar JF, Goldstein FW (1991) Disk susceptibility testing. In: Lorian V (ed) Antibiotics in laboratory medicine, 3rd edn. Williams and Wilkins, Baltimore, pp 17–52
Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477
Barrow GI, Feltham RKA (1993) Cowan and Steel’s manual for the identification of medical bacteria. Cambridge University Press, Cambridge
Chamroensaksri N, Tanasupawat S, Akaracharanya A, Visessanguan W, Kudo T, Itoh T (2010) Gracilibacillus thailandensis sp. nov., from fermented fish (pla-ru). Int J Syst Evol Microbiol 60:944–948
Daroonpunt R, Tanasupawat S, Kudo T, Ohkuma M, Itoh T (2016) Virgibacillus kapii sp. nov., isolated from Thai shrimp paste (Kapi). Int J Syst Evol Microbiol 66:1832–1837
Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376
Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
Forbes L (1981) Rapid flagella stain. J Clin Microbiol 13:807–809
Kluge AG, Farris JS (1969) Quantitative Phyletics and the evolution of anurans. Syst Zool 18:1–32
Komagata K, Suzuki K (1987) Lipid and cell-wall analysis in bacterial systematics. Methods Microbiol 19:161–203
Meier-Kolthoff JP, Göker M (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182
Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60
Minnikin DE, O’Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241
Namwong S, Tanasupawat S, Smitinont T, Visessanguan W, Kudo T, Itoh T (2007) Lentibacillus kapialis sp. nov., from fermented fish paste in Thailand. Int J Syst Evol Microbiol 57:364–369
Pakdeeto A, Tanasupawat S, Thawai C, Moonmangmee S, Kudo T, Itoh T (2007a) Lentibacillus kapialis sp. nov., from fermented shrimp paste in Thailand. Int J Syst Evol Microbiol 59:2254–2259
Pakdeeto A, Tanasupawat S, Thawai C, Moonmangmee S, Kudo T, Itoh T (2007b) Salinicoccus siamensis sp. nov., isolated from fermented shrimp paste in Thailand. Int J Syst Evol Microbiol 57:2004–2008
Phithakpol B, Varanyanond W, Reungmaneepaatoon S, Wood H (1995) The Traditional fermented foods of Thailand. ASEAN Food Handling Bureau Level, Kuala Lumpur
Richter M, Rosselló-Móra R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA 106:19126–19131
Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J (2016) JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 32:929–931
Saitou N, Nei M (1987) The neighboring-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical note 101. MIDI Inc., Newark
Sheu SY, Arun AB, Jiang SR, Young CC, Chen WM (2011) Allobacillus halotolerans gen. nov., sp. nov. isolated from shrimp paste. Int J Syst Evol Microbiol 61:1023–1027
Sorokin DY (2005) Is there a limit for high-pH life? Int J Syst Microbiol 55:1405–1406
Tamaoka J, Komagata K (1984) Determination of DNA base composition by reversed-phase-high-performance liquid chromatography. FEMS Microbiol Lett 25:125–128
Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA 6: molecular evolutionary genetics analysis 6.0. Mol Biol Evol 30:2725–2729
Tanasupawat S, Komagata K (2001) Lactic acid bacteria in fermented foods in Southeast Asia. In: Nga BH, Tan HM, Suzuki K (eds) Microbial Diversity in Asia. Technology and Prospects, Singapore, World Scientific Publishing, Singapore, pp 43–59
Tanasupawat S, Namwong S, Kudo T, Itoh T (2007) Piscibacillus salipiscarius gen. nov., sp. nov., a moderately halophilic bacterium from fermented fish (pla-ru) in Thailand. Int J Syst Evol Microbiol 57:1413–1417
Tanasupawat S, Chamroensaksri N, Kudo T, Itoh T (2010) Identification of moderately halophilic bacteria from Thai fermented fish (plara) and proposal of Virgibacillus siamensis sp. nov. J Gen Appl Microbiol 56:369–379
Tanizawa Y, Fujisawa T, Nakamura Y (2018) DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. Bioinformatics 34:1037–1039
Thompson JD, Higgins DG, Gibson TJ (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882
Thorley MJ (1960) The differentiation of Pseudomonas from other Gram-negative bacteria on the basis of arginine metabolism. J Appl Bacteriol 23:37–52
Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Trüper HG (1987) Report of the ad hoc committee on their conciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.