International Journal of Systematic and Evolutionary Microbiology (2008), **58**, 1950–1960 **DOI** 10.1099/ijs.0.65548-0

Transfer of *Catellatospora koreensis* Lee *et al.* 2000 as *Catelliglobosispora koreensis* gen. nov., comb. nov. and *Catellatospora tsunoense* Asano *et al.* 1989 as *Hamadaea tsunoensis* gen. nov., comb. nov., and emended description of the genus *Catellatospora* Asano and Kawamoto 1986 emend. Lee and Hah 2002

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A polyphasic taxonomic analysis of all species of the genus *Catellatospora* with validly published names revealed morphological, phenotypic and chemotaxonomic heterogeneity. The type species, *Catellatospora citrea*, and *Catellatospora methionotrophica*, *Catellatospora chokoriensis*, *Catellatospora coxensis* and *Catellatospora bangladeshensis* had similar morphological and chemotaxonomical properties. Phylogenetic studies based on 16S rRNA gene sequences showed that *Catellatospora koreensis* and *Catellatospora tsunoense* were heterogeneous and were also heterogeneous with other species of the genus *Catellatospora* with respect to the menaquinone composition. DNA–DNA hybridization data showed that the type strains of *Catellatospora koreensis* and *Catellatospora tsunoense* were significantly different from each other and other recognized species in the genus *Catellatospora*. Therefore, on the basis of phenotypic, chemotaxonomic and genomic differences, two new genera with the names *Catelliglobosispora* gen. nov. and *Hamadaea* gen. nov. are proposed to accommodate two species classified originally as belonging to the genus *Catellatospora* and *Catelliglobosispora koreensis* gen. nov., comb. nov. and *Hamadaea tsunoensis* gen. nov., comb. nov. are described. The type species of the genus *Catelliglobosispora* is *Catelliglobosispora koreensis* (type strain LM 042^T=JCM 10976^T=DSM 44566^T=IMSNU 50729^T) and the type species of the genus *Hamadaea* is *Hamadaea tsunoensis* (type strain 6420-P^T=JCM 9105^T=DSM 44101^T=IMSNU 22005^T).

The genus *Catellatospora* was described by Asano & Kawamoto (1986) for actinomycete strains that produce short chains of non-motile spores borne directly on the substrate mycelium without the formation of aerial mycelium. This genus originally contained two species, *Catellatospora citrea* and *Catellatospora ferruginea*. Subsequently, *Catellatospora citrea* subsp. *methionotrophica* (Asano & Kawamoto, 1988), *Catellatospora matsumotoense*, *Catellatospora tsunoense* (Asano *et al.*, 1989) and *Catellatospora koreensis* (Lee *et al.*, 2000) were added. Later, *Catellatospora matsumotoense* was transferred to the genus *Micromonospora* as *Micromonospora matsumotoense*, based on 16S rRNA gene sequence analysis and phenotypic characteristics (Lee *et al.*, 1999), and *Catellatospora ferruginea* (Asano & Kawamoto, 1986) was transferred to the genus *Asanoa* as *Asanoa ferruginea* (Lee & Hah, 2002). Recently, *Catellatospora citrea* subsp. *methionotrophica* (Asano & Kawamoto, 1988) was transferred to *Catellatospora methionotrophica* based on DNA–DNA

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Abbreviations: DAP, diaminopimelic acid; DPG, diphosphatidylglycerol; OH-PE, hydroxy-phosphatidylethanolamine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Catellatospora koreensis* IMSNU 50729^T and *Catellatospora tsunoense* IMSNU 22005^T are AF171700 and AF152110, respectively.

Tables showing 16S rRNA gene sequence similarities and chemotaxonomic characteristics of *Catelliglobosispora koreensis* gen. nov., comb. nov. and *Hamadea tsunoensis* gen. nov., comb. nov. and recognized species of the genus *Catellatospora* are available as supplementary material with the online version of this paper.

hybridization data (Ara & Kudo, 2006) and *Catellatospora bangladeshensis*, *Catellatospora coxensis* and *Catellatospora chokoriensis* were described (Ara & Kudo, 2006). The chemical heterogeneity between members of the genus *Catellatospora* has also been reflected in comparative analysis of 16S rRNA gene sequences (Koch *et al.*, 1996; Lee *et al.*, 1999; Ara & Kudo, 2006). The chemical heterogeneity among members of this genus was also increased following the descriptions of *Catellatospora tsunoense* (Asano *et al.*, 1989) and *Catellatospora koreensis* (Lee *et al.*, 2000). The emergence of molecular systematics, numerical phenetic classification and chemotaxonomy has led to the assignment of seven species, *C. citrea*, *C. methionotrophica*, *C. bangladeshensis*, *C. coxensis*, *C. chokoriensis, C. koreensis* and *C. tsunoense* to the genus *Catellatospora* (Asano & Kawamoto, 1986; Asano *et al.*, 1989; Lee *et al.*, 1999; Ara & Kudo, 2006). The major aim of this study was to re-evaluate the taxonomic status of *Catellatospora koreensis* and *Catellatospora tsunoense* using a polyphasic approach and to emend the description of the genus *Catellatospora*. In addition, on the basis of the results of a polyphasic approach to bacterial classification, we propose the creation of two new genera, *Catelliglobosispora* gen. nov. and *Hamadaea* gen. nov.

The type strains of recognized species of the genus *Catellatospora* were obtained from the Japan Collection of Microorganisms (JCM) and maintained on yeast extract-malt extract agar (ISP medium 2) at 4 °C and as suspensions in 20 % (v/v) glycerol at −20 °C. Strains were grown on tap-water agar and sucrose-nitrate agar (Waksman no. 1) media at 28 °C for 21 days and then observed using light microscopy and scanning electron microscopy (model S-2400 Hitachi; Tokyo). The sample for scanning electron microscopy was prepared as described by Itoh *et al.* (1989) and Ara & Kudo (2007b, c). The phenotypic properties were examined using several standard methods. For cultural characterization, the isolates were grown for 21 days at 28 °C on various agar media as described by Waksman (1950, 1961), Shirling & Gottlieb (1966) and Asano & Kawamoto (1986). The *Color Harmony Manual* (Jacobson *et al.*, 1958) was used to determine the names and designations of colony colours. The temperature range and NaCl tolerance for growth were determined on yeast extract-starch agar. Utilization of carbohydrates as sole carbon sources was tested by using neutralized yeast nitrogen base without amino acids as a basal medium according to the method of Stevenson (1967). Production of melanoid pigments was examined using tyrosine agar (ISP medium 7).

For chemotaxonomic analyses, freeze-dried cells were obtained from cultures grown in yeast extract-starch broth on a rotary shaker at 28 °C. The isomers of diaminopimelic acid (DAP) present in the cell wall peptidoglycan were determined by using TLC as described by Staneck & Roberts (1974). Reducing sugars from whole-cell hydrolysates were analysed using the HPLC method of Mikami & Ishida (1983). The *N*-acyl group of muramic acid present in the peptidoglycan was determined using the method of Uchida & Aida (1984). Cell phospholipids were extracted and identified using the method of Minnikin *et al.* (1984). Methyl esters of cellular fatty acids were prepared and analysed according to the instructions of the Microbial Identification System (Sherlock Microbial Identification System; Hewlett Packard) (Sasser, 1990). Isoprenoid quinones were extracted using the method of Collins *et al.* (1977, 1984) and were analysed by HPLC with a Cosmosil $5C_{18}$ column (4.6 × 150 mm; Nacalai Tesque) (Tamaoka *et al.*, 1983) and mass spectrometry (GCMS QP5050; Shimadzu). The preparation and detection of methyl esters of mycolic acids were carried out as described by Tomiyasu (1982).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and sequencing of the PCR products were carried out as described by Nakajima *et al.* (1999) and Ara *et al.* (2007a, b). The sequences were multiply aligned with selected sequences (Fig. 1) obtained from GenBank/EMBL/DDBJ by using the CLUSTAL_X program package (Thompson *et al.*, 1997). The alignment was verified manually and adjusted prior to the construction of a phylogenetic tree. A phylogenetic tree constructed using the neighbour-joining method (Saitou & Nei, 1987) in the PAUP program package (version 4.0b10) (Swofford, 2001) and CLUSTAL_X program was based on the comparison of 1355 nucleotides present in all the strains as a result of elimination of gaps and ambiguous nucleotides from the sequences between positions 34 and 1491 (*Escherichia coli* position number). *Micromonospora chalcea* was used as an outgroup. The confidence values of branches of the phylogenetic tree were determined using bootstrap analyses based on 1000 resamplings (Felsenstein, 1985). The values for sequence similarity among *Catellatospora* strains were calculated manually after pairwise alignment using the CLUSTAL_X package.

DNA was isolated from biomass using the methods of Tamaoka (1994) and Saito & Miura (1963) with minor modifications as follows: achromopeptidase crude (Wako Pure Chemicals), *N*-acetylmuramidase SG (Seikagaku Kogyo) and lysozyme were used for lysing cells (Kudo *et al.*, 1998). In case cells were not lysed by these enzymes, the cells were freeze-dried and mechanically ground as described by Raeder & Broda (1985). The G + C content of the DNA was determined using the HPLC method of Tamaoka & Komagata (1984). An equimolar mixture of nucleotides for analysis of DNA base composition (Yamasa Shoyu, Japan) was digested with bacterial alkaline phosphatase and used as the quantitative standard. DNA–DNA relatedness was measured fluorometrically using the microplate hybridization method devised by Ezaki *et al.* (1989). Hybridization was carried out at 55 °C for 2 h.

Almost-complete 16S rRNA gene sequences of the type strains of recognised species were determined and compared with those of representatives of the genera of the family *Micromonosporaceae* (Ørskov, 1923; Couch, 1950;

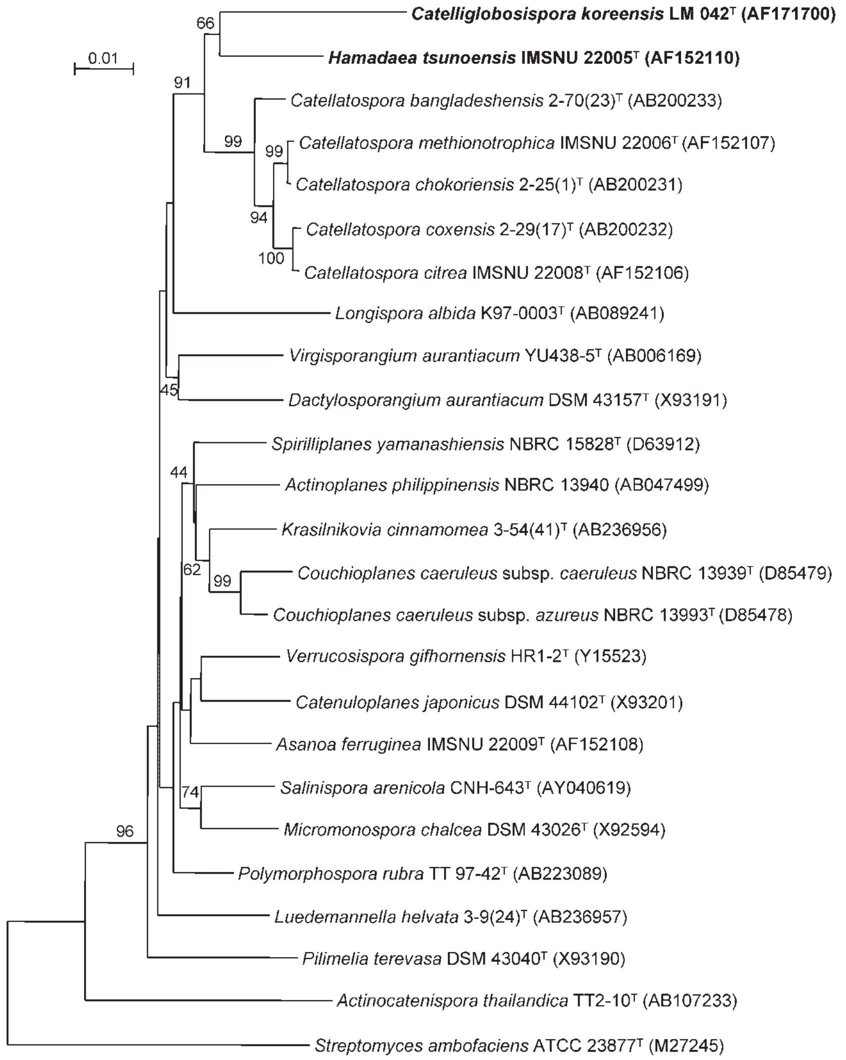

Fig. 1. Phylogenetic tree based on almost-complete 16S rRNA gene sequences showing the relationships between *Hamadaea tsunoensis* gen. nov., comb. nov. and *Catelliglobosispora koreensis* gen. nov., sp. nov. and type species of genera in the family *Micromonosporaceae* and type strains of the genus *Catellatospora*. *Streptomyces ambofaciens* ATCC 23877^T was used as an outgroup. The tree was reconstructed by using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap percentages based on 1000 replications are indicated at branch points (only values >40 % are given). Bar, 0.01 nucleotide substitutions per 100 nucleotides.

Kane, 1966; Thiemann *et al.*, 1967; Asano & Kawamoto, 1986; Yokota *et al.*, 1993; Tamura *et al.*, 1994, 1997, 2001, 2006; Rheims *et al.*, 1998; Lee & Hah, 2002; Matsumoto *et al.*, 2003; Maldonado *et al.*, 2005; Thawai *et al.*, 2006; Ara & Kudo, 2007a, d). The phylogenetic dendrogram based on the neighbour-joining algorithm (Fig. 1) showed that *Catellatospora koreensis* and *Catellatospora tsunoense* formed a distinct clade within the radiation encompassed by the family *Micromonosporaceae*. The clade was well separated from the *C. citrea* group of the genus *Catellatospora* and other genera of the family *Micromonosporaceae*. This relationship was supported by a bootstrap value of 91 % (Fig. 1) and parsimony analysis showed similar topologies for a tree obtained using a distance-based algorithm (data not shown). The two organisms *Catellatospora koreensis* and *Catellatospora tsunoense* were distantly associated with the *C. citrea* clade of the genus *Catellatospora*, with sequence similarities of 94.7–95.1 and 94.8–96.6 %, respectively (see Supplementary Table S1 available in IJSEM Online).

The chemical properties of *Catellatospora koreensis* and *Catellatospora tsunoense* support the conclusion that the two species have been misclassified as members of the genus *Catellatospora*. *Catellatospora koreensis* is characterized by the possession of MK-10(H_4) (Lee *et al.*, 2000 and this study) and *Catellatospora tsunoense* by the presence of MK-9(H_6) (this study) as major menaquinones (see Supplementary Table S2 in IJSEM Online). *Catellatospora tsunoense* contained hydroxy-phosphatidylethanolamine (OH-PE) as a diagnostic component of the polar lipids that also included phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylinositol (PI) and phosphatidylinositol mannosides (PIMs) (Supplementary Table S2 available in IJSEM Online). The phospholipid OH-PE was not detected in *Catellatospora koreensis* or in other members of the genus *Catellatospora*. On the other hand, members of the *C. citrea* clade in the genus *Catellatospora* have the major menaquinone MK-9(H_4) and are characterized by the presence of *meso*- and 3-OH-DAP and the polar lipids PE, DPG, PI and PIMs

(Supplementary Table S2). *Catellatospora koreensis* and *Catellatospora tsunoense* could be readily differentiated from other members of the genus *Catellatospora* with regard to the the fatty acid profiles (Table 1). In this study, the fatty acid profiles of members of the genus *Catellatospora* showed slight differences from the results of Lee *et al.* (2000). Our results showed that *Catellatospora koreensis* contained iso-$C_{16:0}$ (44.5 %), iso-$C_{14:0}$ (10.8 %) and iso-$C_{15:0}$ (8.5 %) and that *Catellatospora tsunoensis* contained iso-$C_{15:0}$ (29.5 %), anteiso-$C_{17:0}$ (15.6 %) and iso-$C_{16:0}$ (10.4 %) as major fatty acids. Similar fatty acid profiles were not detected in the *Catellatospora citrea* clade (Table 1). In addition, 10-methyl branched fatty acids were detected in *Catellatospora tsunoense* but not in the *Catellatospora koreensis* clade (Table 1). The other members of the genus *Catellatospora* contained large amounts of iso-$C_{15:0}$ and iso-$C_{16:0}$; this combination of fatty acids corresponds to fatty acid type 3b (Kroppenstedt, 1985). *Catellatospora koreensis* and *Catellatospora tsunoense* could be distinguished from members of other genera of the family *Micromonosporaceae* by using a combination of chemical characteristics (Table 2) and phylogenetic evidence, supporting the conclusion that these two organisms merit the recognition of two new genera.

Table 1. Cellular fatty acid compositions (%) of *Catelliglobosispora koreensis* gen. nov., comb. nov., *Hamadaea tsunoensis* gen. nov., comb. nov. and type strains of the genus *Catellatospora*

Species: 1, *Catelliglobosispora koreensis* JCM 10976^T; 2, *Hamadaea tsunoensis* JCM 9105^T; 3, *Catellatospora chokoriensis* 2-25$(1)^T$; 4, *Catellatospora coxensis* 2-29$(17)^T$; 5, *Catellatospora bangladeshensis* 2-70$(23)^T$; 6, *Catellatospora citrea* JCM 7542^T; 7, *Catellatospora methionotrophica* JCM 7543^T. Values are percentages of total fatty acids. Values <1.0 % are not shown.

Fatty acid	1	2	3	4	5	6	7
$C_{15:0}$		1.0	2.2	4.8	1.7	3.9	3.0
$C_{16:0}$	1.2	3.1		1.6		1.5	1.4
$C_{17:0}$	1.2	7.6	10.9	14.4	3.2	8.1	8.9
$C_{18:0}$	2.6	2.9	4.3	1.8		2.2	1.7
$C_{19:0}$				1.2			
2-OH-$C_{16:1}$	2.9					5.5	1.4
$C_{17:1}\omega6c$		2.7					
$C_{17:1}\omega8c$		1.7	3.5	8.0	8.8	7.8	9.3
$C_{18:1}\omega9c$				1.6	3.1	2.2	3.0
iso-$C_{14:0}$	10.8		3.7	4.8	5.2	2.4	1.5
iso-$C_{15:0}$	8.5	29.5	30.3	22.2	19.8	28.4	37.6
anteiso-$C_{15:0}$	3.7	8.8	6.3	7.4	2.5	5.3	7.5
iso-$C_{15:1}$						2.6	
iso-$C_{16:1}$	8.6		2.4		3.7	1.8	
iso-$C_{16:0}$	44.5	10.4	22.9	18.5	35.4	10.5	5.7
anteiso-$C_{17:1}\omega9c$	1.5						
iso-$C_{17:1}\omega9c$					1.8	1.4	3.2
iso-$C_{17:0}$	1.7	5.4	5.5	2.2	2.8	3.3	4.5
anteiso-$C_{17:0}$	6.6	15.6	7.9	4.5	2.1	4.3	5.0
iso-$C_{18:0}$	3.0				1.2		
10-Methyl $C_{17:0}$		3.6			2.3	1.0	
10-Methyl $C_{18:0}$		1.5					
Summed feature 3*		2.0			1.2		
Summed feature 6*						1.5	1.4

*Summed features represent groups of one or two fatty acids, which could not be separated by GLC with the MIDI system. Summed feature 3 contains 2-OH-i-$C_{15:0}$ and/or $C_{16:1}\omega7c$. Summed feature 6 contains $C_{19:1}\omega11c$ and/or $C_{19:1}\omega9c$.

Catellatospora koreensis and *Catellatospora tsunoense* produced a well-developed and branched substrate mycelium. Aerial mycelium was not formed on any agar media tested (Asano *et al.*, 1989; Lee *et al.*, 2000). Straight and relatively short chains of non-motile spores were borne directly on substrate mycelium. Each spore was cylindrical and its surface was smooth. Abundant globose bodies were observed on oatmeal agar for *Catellatospora koreensis* (Lee *et al.*, 2000), which were morphologically similar to the globose bodies of *Catellatospora tsunoense* (Asano *et al.*, 1989). It has been reported previously that globose bodies of *Catellatospora tsunoense*, which were morphologically similar to those of members of the genus *Dactylosporangium* described by Thiemann *et al.* (1967) and Sharples & Williams (1974) and were 0.4–0.6 μm in diameter. *Catellatospora tsunoense* sporulated only on tap-water agar and calcium-malate agar medium in 10 days to 1 month at 28 °C. It has been reported previously (Asano *et al.*, 1989; Lee & Hah, 2002) and in the present study that the level of sporulation decreased in cultures grown on poor media compared with cultures grown on rich complex organic media (Table 3). The genus *Catellatospora* clearly differs from other genera in the actinoplanate group by the morphology of the linear spore units formed directly from the vegetative mycelia. However, globose bodies were not observed in other species of the genus *Catellatospora* (Asano & Kawamoto, 1986; Asano *et al.*, 1989; Lee *et al.*, 2000; Ara & Kudo, 2006).

Physiologically, *Catellatospora koreensis* and *Catellatospora tsunoense* could be differentiated from each other by their utilization of D-arabinose, α-melibiose, L-rhamnose, trehalose, lactose and raffinose; growth requirement for thiamine; growth on 0.0001 % crystal violet; resistance to 50 μg gentamicin ml^{-1}; and growth at 37 °C and in 1 % NaCl (Asano *et al.*, 1989; Lee *et al.*, 2000) (Table 4). In the physiological tests described in this study, *Catellatospora koreensis* and *Catellatospora tsunoense* were different from other *Catellatospora* strains tested (Asano *et al.*, 1989; Lee *et al.*, 2000) (Table 4). *Catellatospora koreensis* and *Catellatospora tsunoense* were further compared by using DNA–DNA hybridization with other *Catellatospora* species (Table 5). *Catellatospora koreensis* and *Catellatospora*

Table 2. Morphological features and chemotaxonomic characteristics of *Hamadaea* gen. nov., *Catelliglobosispora* gen. nov. and other genera of the family *Micromonosporaceae*

Taxa: 1, *Hamadaea* gen. nov.; 2, *Catelliglobosispora* gen. nov.; 3, *Catellatospora*; 4, *Pseudosporangium*; 5, *Couchioplanes*; 6, *Krasilnikovia*; 7, *Actinoplanes*; 8, *Luedemannella*; 9, *Polymorphospora*; 10, *Salinispora*; 11, *Longispora*; 12, *Actinocatenispora*; 13, *Micromonospora*; 14, *Asanoa*; 15, *Catenuloplanes*; 16, *Dactylosporangium*; 17, *Pilimelia*; 18, *Spirilliplanes*; 19, *Verrucosispora*; 20, *Virgisporangium*. Data were from Vobis (1987), Stackebrandt & Kroppenstedt (1987), Horan & Brodsky (1986), Asano *et al.* (1989), Goodfellow *et al.* (1990), Yokota *et al.* (1993), Tamura & Hatano (2001), Tamura *et al.* (1994, 1997, 2001, 2006), Rheims *et al.* (1998), Kudo *et al.* (1999), Lee *et al.* (2000), Lee & Hah (2002), Matsumoto *et al.* (2003), Maldonado *et al.* (2005), Ara & Kudo (2006), Thawai *et al.* (2006), Ara & Kudo (2007a) and Ara & Kudo (2007d). +, Present; −, absent; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Sporangium	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	+	+	−	−	+
Spore motility	−	−	−	−	+	−	+	−	−	−	−	−	−	−	+	+	+	+	−	−
Diamino acid	*meso*- and 3-OH-DAP	*meso*-DAP	*meso*-DAP	*meso*- and 3OH-DAP	L-lysine	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	L-lysine	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP
Whole-cell sugars*	Xyl, Gal, Man, Rib, Ara, Rham, Glu	Rham, Man, Xyl, Gal, Glu	Xyl, Man, Gal, Ara, Rham, Rib	Gal, Glc, Rib, Man, Xyl, Ara	Ara, Gal, Xyl	Gal, Man, Xyl, Ara, Rib	Ara, Xyl	Xyl, Gal, Man, Rham, Rib, Ara	Xyl	Ara, Gal, Xyl	Ara, Gal, Xyl	Ara, Gal, Xyl	Ara, Xyl	Ara, Gal, Xyl	Xyl	Ara, Xyl	Ara, Xyl	Ara, Xyl	Man, Xyl	Ara, Gal, Xyl
Fatty-acid type†	3b	3b	3b	2d	2c	2d	2d	2d	2a	3a	2d	3b	3b	2d	2c	3b	2d	2d	2b	2d
Major menaquinone(s) (MK-)	$9(H_6)$	$10(H_4)$	$9(H_4)$	$9(H_6)$	$9(H_4)$	$9(H_{6,4,8})$	$9(H_4)$, $10(H_4)$	$9(H_{6,4,2,8})$	$10(H_{6,4})$ $9(H_{6,4})$	$9(H_4)$	$10(H_{4,6})$	$9(H_{4,6})$	$10(H_{4,6})$, $9(H_{4,6})$	$10(H_{6,8})$	$9(H_8)$, $10(H_8)$	$9(H_{4,6,8})$	$9(H_{4,2})$	$10(H_4)$	$9(H_4)$	$10(H_{4,6,8})$
Phospholipid type‡	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PIII	PII	PII	PII	PII	PII
DNA G+C content (mol%)	70	70	70–71	73	70–72	71	72–73	71	71	70–73	70	72	71–72	71–72	71–73	71–73	ND	69	70	71

* Ara, arabinose; Gal, galactose; Man, mannose; Rham, rhamnose; Rib, ribose; Xyl, xylose.

†According to the classification of Kroppenstedt (1985).

‡According to the classification of Lechevalier *et al.* (1981).

Table 3. Cultural characteristics of *Catelliglobosispora koreensis* gen. nov., comb. nov., *Hamadaea tsunoensis* gen. nov., comb. nov. and the type species of the genus *Catellatospora*

Cultures were incubated at 28 °C for 3 weeks. Aerial mycelium was not formed on any of the media used. Diffusible pigments were not formed on all of the agar media tested. Colour designations and codes in parentheses were taken from the *Color Harmony Manual* (Jacobson *et al.*, 1958). Growth and sporulation on substrate mycelium are scored as: ++, good; +, moderate; ±, poor; −, no growth and no spores formed.

Agar medium	***Catelliglobosispora koreensis* JCM 10976^T**			***H. tsunoensis* JCM 9105^T**			***Catellatospora citrea* JCM 7542^T**		
	Growth	**Reverse colour**	**Sporulation**	**Growth**	**Reverse colour**	**Sporulation**	**Growth**	**Reverse colour**	**Sporulation**
Glucose-asparagine	++	Colourless	−	±	Colourless	−	+	Cream (1½ca)	−
Glycerol-asparagine	±	Colourless	−	±	Colourless	+	+	Cream (1½ca)	−
Salts-starch	++	Honey gold (2ic)	−	±	Colourless	−	++	Light wheat (2ea)	−
Tyrosine	±	Cream (1½ca)	+	±	Light wheat (2ea)	−	+	Light tan (3gc)	++
Nutrient	±	Cream (1½ca)	−	±	Colourless	−	±	Light melon yellow (3ea)	−
Yeast-malt extract	+	Light amber (3ic)	−	++	Orange (4la)	−	++	Orange (4la)	−
Oatmeal	++	Light amber (3ic)	−	++	Bright maize (3la)	−	++	Light maize (2ea)	+
Bennett	++	Bamboo (2gc)	−	++	Bright marigold (3pa)	−	++	Bright marigold (3pa)	−
1/5 yeast extract-starch	+	Oatmeal sand (2ec)	−	++	Maize (3ga)	+	+	Light wheat (2ea)	+
Oatmeal-nitrate	+	Oatmeal sand (2ec)	−	+	Light wheat (2ea)	−	+	Cream (1½ca)	+
Glucose-yeast extract	++	Honey gold (2ic)	−	++	Bright marigold (3pa)	−	++	Russet orange (4pc)	−
Hickey-Tresner	++	Light tan (3gc)	−	+	Maize (2ga)	−	±	Melon yellow (3ga)	−
Tap-water	±	Colourless	++	±	Colourless	++	±	Colourless	++
Sucrose-nitrate	±	Colourless	++	±	Colourless	−	±	Colourless	++
Yeast extract-starch	++	Honey gold (2ic)	−	++	Bright marigold (3pa)	−	++	Bright marigold (3pa)	−

Table 4. Physiological characteristics of *Hamadaea tsunoensis* gen. nov., comb. nov., *Catelliglobosispora koreensis* gen. nov., comb. nov. and type strains of recognized *Catellatospora* species

Species: 1, *H. tsunoensis* JCM 9105^{T}; 2, *Catelliglobosispora koreensis* JCM 10976^{T}; 3, *Catellatospora chokoriensis* 2-25(1)^{T}; 4, *Catellatospora coxensis* 2-29(17)^{T}; 5, *Catellatospora bangladeshensis* 2-70(23)^{T}; 6, *Catellatospora citrea* JCM 7542^{T}; 7, *Catellatospora methionotrophica* JCM 7543^{T}. Cultures were incubated at 30 °C for 3 weeks. Growth: ++, abundant; +, positive; ±, weakly positive; −, no growth observed.

Characteristic	1	2	3	4	5	6	7
Utilization of:							
Glycerol	±	−	−	+	±	+	±
Erythritol	−	−	−	−	−	−	±
Adonitol	−	−	±	−	±	−	−
L-Arabinose	++	++	++	++	++	++	++
D-Ribose	−	−	−	+	−	+	−
D-Xylose	++	++	++	+	++	++	++
D-Fructose	++	++	−	±	−	+	−
L-Rhamnose	++	−	−	++	+	+	++
myo-Inositol	−	−	−	−	−	±	±
D-Mannitol	−	−	−	−	±	±	−
Methyl α-D-glucoside	−	−	−	±	−	+	−
Salicin	±	−	++	−	++	+	++
D-Lactose	++	++	++	++	++	++	+
α-(+)-D-Melibiose	++	−	++	++	++	+	−
Sucrose	++	++	+	++	++	++	++
(+)-D-Raffinose	++	++	−	−	−	−	±
Trehalose	++	−	++	++	++	++	++
Temperature range for growth (°C)	20–30	15–37	15–30	20–30	25–30	15–30	20–30
pH range for growth	6–9	6–9	6–9	6–9	6.8–7.2	6–9	6.8–7.2
Growth in 1 % NaCl	−	+	+	−	−	−	−

tsunoense exhibited very low levels of DNA–DNA relatedness to the other *Catellatospora* species tested (2–7 %), as well as to each other (2–5 %) (Table 5). Concerning the fatty acid profiles, these organisms showed significant differences in the amounts of iso-$C_{16:0}$, iso-C-$_{15:0}$ and anteiso-$C_{17:0}$. In addition, *C. koreensis* contained iso-$C_{14:0}$, iso-$C_{16:1}$, iso-$C_{18:0}$, 2-OH-$C_{16:1}$ and iso-$C_{17:1}\omega 9c$, whereas 10-methyl-$C_{17:0}$, 10-methyl-$C_{18:0}$, summed feature 3, $C_{17:1}\omega 6c$,

Table 5. DNA G+C content and DNA–DNA relatedness among *Catelliglobosispora koreensis* gen. nov., comb. nov., *Hamadaea tsunoensis* gen. nov., comb. nov. and type strains of the genus *Catellatospora*

Strain	G+C content (mol%)	Hybridization (%) with ^{3}H-labelled DNA from:						
		1	2	3	4	5	6	7
1. *Catellatospora chokoriensis* 2-25(1)^{T}	71	100	53	26	66	50	7	4
2. *Catellatospora coxensis* 2-29(17)^{T}	71	27	100	14	20	31	4	2
3. *Catellatospora bangladeshensis* 2-70(23)^{T}	71	26	35	100	30	31	6	5
4. *Catellatospora citrea* JCM 7542^{T}	71	50	47	28	100	44	6	5
5. *Catellatospora methionotrophica* JCM 7543^{T}	71	36	46	25	43	100	6	4
6. *H. tsunoensis* JCM 9105^{T}	70	9	13	7	8	7	100	5
7. *Catelliglobosispora koreensis* JCM 10976^{T}	70	8	11	5	6	6	2	100

$C_{18:1}\omega 8c$ and $C_{15:0}$ were present in the fatty acid profile of *Catellatospora tsunoense* (Table 1). The mean DNA G+C contents of *Catellatospora koreensis* and *Catellatospora tsunoense* were 70.4 and 70.3 mol%, respectively (Table 5).

The phylogenetic analysis, DNA–DNA hybridization studies, and chemical and morphological characteristics show that *Catellatospora koreensis* LM 042^T and *Catellatospora tsunoense* 6420-P^T can be differentiated from each other and from other members of the genus *Catellatospora* and other genera of the family *Micromonosporaceae*. Therefore, it is proposed that these organisms be classified as representing two new genera. On the basis of phenotypic and genotypic data, *Catelliglobosispora koreensis* gen. nov., comb. nov. and *Hamadaea tsunoensis* gen. nov., comb. nov. are proposed.

Description of *Catelliglobosispora* gen. nov.

Catelliglobosispora [Ca.tel.li.glo′bo.si.spo′ra. L. n. *catella* small chain; L. adj. *globosus* spherical; Gr. fem. n. *spora* a seed and in biology a spore; N.L. fem. n. *Catelliglobosispora* (organism forming) small chain and spherical spores].

The description is based on data taken from previous studies (Lee *et al.*, 2000; Ara & Kudo, 2006) and this study. Aerobic. Gram-positive. Catalase-positive. Urease-negative. Nitrate is reduced to nitrite. H_2S is not produced. Mesophilic. Vegetative hyphae are branched but not fragmented. Aerial mycelia are absent. Short chains of spores are borne directly on substrate mycelium. Spores are cylindrical and non-motile and spore surfaces are smooth. Substrate mycelia are cream to light yellow. Globose bodies are formed. Soluble pigments are not produced. Grow at pH 6.0–9.0. Temperature range for growth is 20–37 °C. Cell walls contain *meso*-DAP, rhamnose, ribose, galactose, xylose, mannose and glucose (type II cell wall and a variant of sugar pattern D). Acyl type of muramic acid is glycolyl. Predominant menaquinone is MK-10(H_4), with minor amounts of MK-9(H_4) and MK-10(H_6). Diagnostic phospholipids comprise PE, DPG and PI. Glucosamine-containing phospholipid and phosphatidylcholine are absent. Mycolic acids are absent. Predominant fatty acids (>10.0 % of total) are iso-$C_{16:0}$ and iso-$C_{14:0}$; significant amounts of saturated, unsaturated and anteiso-branched fatty acids are also present. The G+C content of the DNA of the type species is 70.4 % (determined by HPLC). The type species is *Catelliglobosispora koreensis.*

Description of *Catelliglobosispora koreensis* comb. nov.

Catelliglobosispora koreensis (ko.re.en′sis. N.L. fem. adj. *koreensis* of Korea, pertaining to soil from Korea from which the organism was isolated).

Basonym: *Catellatospora koreensis* Lee *et al.* 2000.

Morphological, chemotaxonomic and general characteristics are given above for the genus description. The description is based on data taken from previous studies (Lee *et al.*, 2000) and our own studies. Straight, relatively short chains of non-motile spores are borne directly on substrate mycelium. Each spore is cylindrical and has a smooth surface. Abundant globose bodies are observed on oatmeal agar and ISP medium 4. Good growth occurs on ISP medium 3, ISP medium 4, glucose-asparagine agar, glucose-yeast extract agar, Hickey-Tresner agar, yeast extract-starch agar and Bennett agar. Moderate growth occurs on yeast extract-malt extract agar, 1/5 yeast extract-starch agar, oatmeal-nitrate agar and poor growth occurs on glycerol-asparagine agar, nutrient agar, ISP medium 7, tap-water agar and sucrose-nitrate agar. Melanin pigment production on ISP medium 7 is negative. Abundant sporulation occurs on tap-water agar and sucrose-nitrate agar and moderate sporulation occurs on ISP medium 7. In general, vegetative mycelia are cream to honey-gold in colour and aerial mycelia are not present. Utilizes L-arabinose, cellobiose, D-glucose, D-galactose, D-mannose, maltose, D-fructose, D-xylose, D-lactose, sucrose, starch and raffinose as carbon sources. Does not utilize D-arabinose, dextran, gluconate, inulin, melezitose, melibiose, glycerol, erythritol, D-ribose, L-rhamnose, salicin, L-sorbose, *myo*-inositol, *meso*-inositol, 2-propanol, propanol, D-mannitol, sorbitol, D-xylitol, adonitol, dulcitol, butanol, *meso*-erythritol, ethanol, methyl α-D-glucoside or trehalose. Does not degrade adenine, hippurate, hypoxanthine, DL-tyrosine or xanthine. Hydrolyses casein, gelatin and starch, but not DNA. Grows on 0.001 % brilliant green and 0.0001 % crystal violet. No growth in 3 % NaCl and 0.01 % lysozyme. Susceptible to novobiocin (50 $\mu g\ ml^{-1}$), vancomycin (20 $\mu g\ ml^{-1}$), streptomycin (100 $\mu g\ ml^{-1}$) and tetracycline (10 $\mu g\ ml^{-1}$), and resistant to gentamicin (50 $\mu g\ ml^{-1}$) and neomycin (5 $\mu g\ ml^{-1}$). Predominant fatty acids are iso-$C_{16:0}$ (44.5 %), iso-$C_{14:0}$ (10.8 %), iso-$C_{16:1}$ (8.6 %), iso-$C_{15:0}$ (8.5 %), with minor amounts of anteiso-$C_{17:0}$ (6.6 %), anteiso-$C_{15:0}$ (3.7 %), iso-$C_{18:0}$ (3.0 %), 2-OH-$C_{16:1}$ (2.9 %), $C_{18:0}$ (2.6 %), iso-$C_{17:0}$ (1.7 %), anteiso-$C_{17:1}\omega 9c$ (1.5 %), $C_{16:0}$ (1.2 %) and $C_{17:0}$ (1.2 %).

The type strain, LM 042^T (=JCM 10976^T=DSM 44566^T=IMSNU 50729^T), was isolated from soil from a gold-mine cave.

Description of *Hamadaea* gen. nov.

Hamadaea (Ha.ma.da.e′a. N.L. fem. n. *Hamadaea* of Hamada, named after Masa Hamada, the Japanese microbiologist who made a tremendous contribution to actinomycete research).

The description is based on data from previous studies (Asano *et al.*, 1989; Lee *et al.*, 2000) and our own studies. Aerobic. Gram-positive. Catalase-positive. Urease-negative. Nitrate is reduced to nitrite. H_2S is not produced. Mesophilic. Vegetative hyphae are branched but not fragmented. Aerial mycelia are absent. Short chains of spores are borne directly on substrate mycelium. 3-O-Methyl-rhamnose is lacking in the cell walls. Require thiamine for growth. Cell walls contain *meso*- and 3-OH-DAP, xylose, galactose, mannose, ribose, arabinose, rhamnose and glucose (type II cell wall and a variant of sugar pattern D). Acyl type of muramic acid is glycolyl. Major menaquinone is MK-9(H_6), with minor amounts of MK-9(H_4) and MK-9(H_8). Diagnostic phospholipids include phosphatidylethanolamine, OH-PE, DPG, PI and PIMs. Glucosamine-containing phospholipid and phosphatidylcholine are absent. Mycolic acids are absent. The G + C content of the DNA of the type species is 70.3 % (as determined by HPLC). Member of the family *Micromonosporaceae*. The type species is *Hamadaea tsunoensis*.

Description of *Hamadaea tsunoensis* comb. nov.

Hamadaea tsunoensis (tsu.no.en′sis. N.L. fem. adj. *tsunoensis* of Tsuno-gun, Yamaguchi, Japan, the origin of the soil sample from which the type strain was isolated).

Basonym: *Catellatospora tsunoense* Asano *et al.* 1989.

Morphological, chemotaxonomic and general characteristics are given above for the genus description. The description is based on data taken from previous studies (Asano *et al.*, 1989) and our own studies. Good growth occurs on ISP medium 2, ISP medium 3, Bennett agar, 1/5 yeast-starch agar, glucose-yeast extract agar and yeast extract-starch agar. Moderate growth occurs on oatmeal-nitrate agar, Hickey-Tresner agar and poor growth on glucose-asparagine agar, glycerol-asparagine agar, ISP medium 4, ISP medium 7, nutrient agar, tap-water agar and sucrose-nitrate agar. Colony mass is pale yellow to bright marigold. Spores are cylindrical and non-motile. Spore surface is smooth. Abundant globose bodies are present on tap-water agar medium and are 0.4–0.6 μm in diameter. Abundant sporulation occurs on tap-water agar and moderate sporulation on glycerol-asparagine and 1/5 yeast extract-starch agar medium. Sporulates on tap-water agar medium in 10 days to 1 month at 28 °C. No soluble and melanin-like pigments are produced. No growth occurs in the presence of 3 % NaCl, lysozyme (0.005 %), crystal violet (0.001 %) or sodium lauryl sulfate (0.01 %). Susceptible to 6-demethyltetracycline (500 μg ml^{-1}), novobiocin (50 μg ml^{-1}), gentamicin (50 μg ml^{-1}), streptomycin (100 μg ml^{-1}) and vancomycin (50 μg ml^{-1}), and resistant to neomycin (5 μg ml^{-1}). Does not liquefy gelatin or peptonize milk. Positive for activities of leucine arylamidase, β-galactosidase, α-glucosidase and β-glucosidase, but low or negative for activities of cystine arylamidase, α-fucosidase and β-glucuronidase. Utilizes L-arabinose, D-xylose, D-fructose, L-rhamnose, α-melibiose, sucrose, raffinose, trehalose, cellobiose, D-galactose, D-glucose, maltose, lactose, mannose and starch. Poor growth occurs on glycerol, salicin and no growth on erythritol, adonitol, *myo*-inositol, dextran, methyl α-D-glucoside, D-ribose or D-mannitol. Predominant fatty acids are iso-$C_{15:0}$ (29.5 %), anteiso-$C_{17:0}$ (15.6 %), iso-$C_{16:0}$ (10.4 %), anteiso-$C_{15:0}$ (8.8 %) and $C_{17:0}$ (7.6 %), with small amounts of iso-$C_{17:0}$ (5.4 %), 10-methyl-$C_{17:0}$ (3.6 %), $C_{16:0}$ (3.1 %), $C_{18:0}$ (2.9 %), $C_{17:1}\omega 6c$ (2.7 %), summed feature 3 (2.0 %), $C_{17:1}\omega 8c$ (1.7 %), 10-methyl-$C_{18:0}$ (1.5 %) and $C_{15:0}$ (1.0 %).

The type strain, 6420-P^T (=JCM 9105^T=DSM 44101^T=IMSNU 22005^T), was isolated from woodland soil.

Emended description of *Catellatospora* Asano and Kawamoto 1986 emend. Lee and Hah 2002

Aerobic. Gram-positive. Catalase-positive. Urease-negative. Nitrate is reduced to nitrite. H_2S is not produced. Mesophilic. Vegetative hyphae are branched but not fragmented. Colony masses are bright yellow. Aerial mycelia are not formed. Chains of non-motile spores arise singly or in tufts from vegetative hyphae on the surface of agar media. Globose bodies are not produced. Contain *meso*- and 3-OH-DAP as diamino acids, a glycolylated peptidoglycan and whole-cell sugars xylose, mannose, galactose, arabinose, ribose and glucose. Rhamnose is variable according to species. Predominant menaquinone is MK-9(H_4). PE, DPG, PI and PIMs are present as diagnostic phospholipids. Fatty acid pattern is type 3b. Mycolic acids are absent. The G + C content of the DNA is 70.71–71.44 mol%. Members of this genus form a coherent group within the radiation of the family *Micromonosporaceae* on the basis of 16S rRNA sequence data. The type species is *Catellatospora citrea*.

Acknowledgements

I. A. was supported financially by the Japan Society for the Promotion of Science (JSPS) for her research fellowship. We are very grateful to Dr J. P. Euzéby for the nomenclature of the two genera.

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