Taibaiella smilacinae gen. nov., sp. nov., an endophytic member of the family *Chitinophagaceae* isolated from the stem of *Smilacina japonica*, and emended description of *Flavihumibacter petaseus*

Lei Zhang,[1,2] Yang Wang,[3] Linfang Wei,[1] Yao Wang,[1] Xihui Shen[1] and Shiqing Li[1,2]

Correspondence
Xihui Shen
xihuishen@nwsuaf.edu.cn

[1]State Key Laboratory of Crop Stress Biology for Arid Areas and College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, PR China

[2]State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Northwest A&F University, Yangling, Shaanxi 712100, PR China

[3]Hubei Institute for Food and Drug Control, Wuhan 430072, PR China

A light-yellow-coloured bacterium, designated strain PTJT-5^T, was isolated from the stem of *Smilacina japonica* A. Gray collected from Taibai Mountain in Shaanxi Province, north-west China, and was subjected to a taxonomic study by using a polyphasic approach. The novel isolate grew optimally at 25–28 °C and pH 6.0–7.0. Flexirubin-type pigments were produced. Cells were Gram-reaction-negative, strictly aerobic, rod-shaped and non-motile. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain PTJT-5^T was a member of the phylum *Bacteroidetes*, exhibiting the highest sequence similarity to *Lacibacter cauensis* NJ-8^T (87.7 %). The major cellular fatty acids were iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ and iso-$C_{17:0}$ 3-OH. The only polyamine was homospermidine and the major polar lipid was phosphatidylethanolamine. The only respiratory quinone was MK-7 and the DNA G+C content was 40.3 mol%. Based on the phenotypic, phylogenetic and genotypic data, strain PTJT-5^T is considered to represent a novel species of a new genus in the family *Chitinophagaceae*, for which the name *Taibaiella smilacinae* gen. nov., sp. nov. is proposed. The type strain of *Taibaiella smilacinae* is PTJT-5^T (=CCTCC AB 2013017^T=KCTC 32316^T). An emended description of *Flavihumibacter petaseus* is also proposed.

The family *Chitinophagaceae* within the phylum *Bacteroidetes* was proposed by Ludwig *et al.* (2008) and its description was formally established by Kämpfer *et al.* (2011). At the time of writing, the family *Chitinophagaceae* includes 14 genera: *Chitinophaga* (Kämpfer *et al.*, 2006), *Ferruginibacter* (Lim *et al.*, 2009), *Filimonas* (Shiratori *et al.*, 2009), *Flavihumibacter* (Zhang *et al.*, 2010), *Flavisolibacter* (Yoon & Im, 2007), *Flavitalea* (Wang *et al.*, 2011), *Hydrotalea* (Kämpfer *et al.*, 2011), *Lacibacter* (Qu *et al.*, 2009), *Niabella* (Kim *et al.*, 2007), *Niastella* (Weon *et al.*, 2006), *Parasegetibacter* (Zhang *et al.*, 2009), *Sediminibacterium* (Qu & Yuan, 2008), *Segetibacter* (An *et al.*, 2007) and *Terrimonas* (Xie & Yokota, 2006).

Endophytes residing within plant tissues are considered to be a rich source of genetic diversity, and remain relatively unexplored as potential sources of novel species and novel natural products (Strobel & Daisy, 2003). During the course of a study of the diversity of culturable bacterial communities associated with traditional Chinese medicinal plants, strain PTJT-5^T was isolated from the stem of *Smilacina japonica* A. Gray. Comparative analysis of 16S rRNA gene sequences showed that strain PTJT-5^T had <88 % sequence similarity to all recognized bacterial species, with *Lacibacter cauensis* NJ-8^T as its closest phylogenetic relative (87.7 % sequence similarity). Data from further polyphasic taxonomic study indicate that this strain represents a novel species of a new genus within the family *Chitinophagaceae*.

An apparently healthy plant sample of *Smilacina japonica* A. Gray was collected from Taibai Mountain (33° 57′ N 107° 45′ E) in Shaanxi Province, north-west China, stored in the dark at 4 °C and subjected to isolation of endophytic bacteria within 48 h. The plant was washed

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PTJT-5^T is KC571459.

A supplementary figure is available with the online version of this paper.

carefully in running water to remove external soil and the roots, stems and leaves were separated. After drying at room temperature, the tissue segments were subjected to surface sterilization by using the following steps: a 1 min wash in 70 % ethanol followed by a 3 min wash in sodium hypochlorite solution (2 % available Cl^-), a 0.5 min wash in 70 % ethanol and two rinses in sterilized distilled water. After surface disinfection, the leaf, stem or root tissue was cut into small fragments and macerated using a sterile pestle and mortar in sterile distilled water. The macerated samples were serially diluted in sterile distilled water, spread on 0.1 × trypticase soy agar (TSA; Difco) or R2A agar (Difco) supplemented with 50 μg Imazalil ml^{-1} and incubated at 25 °C for 2 weeks. Isolate PTJT-5^T was obtained from a stem sample inoculated on 0.1 × TSA, routinely cultivated on the same medium at 28 °C and maintained as a glycerol suspension (20 %, v/v, in distilled water) at −80 °C.

Extraction of genomic DNA was performed with a commercial genomic DNA extraction kit (ChaoShi-Bio). The 16S rRNA gene was amplified by PCR using the primer pair 27f and 1492r (Lane, 1991) and sequencing of the purified PCR product was carried out as described by Lin *et al.* (2004). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012b). Multiple sequence alignments were performed with CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic trees were reconstructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods in MEGA version 4.1 software (Tamura *et al.*, 2007) with bootstrap values based on 1000 replications (Felsenstein, 1985). The evolutionary distance matrix for the neighbour-joining method was generated according to Kimura's two-parameter model (Kimura, 1980) and the close-neighbour-interchange algorithm (search level=2, random additions=100) was used in the maximum-parsimony method. Relationships among taxa were also established with the maximum-likelihood algorithm in the PHYLIP package version 3.66 (Felsenstein, 2006).

The nearly complete 16S rRNA gene sequence (1397 bp) of strain PTJT-5^T was determined and subjected to phylogenetic analysis, which revealed that strain PTJT-5^T was most closely related to members of the family *Chitinophagaceae*. The closest relative of strain PTJT-5^T was *Lacibacter cauensis* NJ-8^T, but the sequence similarity between them was only 87.7 %. Lower sequence similarities (83.3–87.3 %) were found to the type strains of described species of the genera *Chitinophaga*, *Ferruginibacter*, *Niabella*, *Segetibacter*, *Terrimonas*, *Sediminibacterium*, *Flavitalea*, *Niastella*, *Filimonas*, *Flavisolibacter*, *Parasegetibacter*, *Flavihumibacter* and *Hydrotalea*. In the phylogenetic tree based on the neighbour-joining algorithm, the novel strain formed a distinct cluster with all members of family *Chitinophagaceae*, with a bootstrap value of 100 % (Fig. 1). The overall topologies of the maximum-likelihood and maximum-parsimony trees were essentially the same as that of the neighbour-joining tree (not shown). These results suggest that strain PTJT-5^T represents a novel genus within the family *Chitinophagaceae*.

Cell morphology was examined by transmission electron microscopy (Hitachi HT7700) with cells grown for 36 h at 28 °C on 0.1 × TSA. Cells were negatively stained with 1 % (w/v) phosphotungstic acid and grids were examined after being air-dried. Gram staining of cells was carried out according to the classical Gram procedure described by Doetsch (1981). Gliding motility was determined as described by Bowman (2000). The presence of flexirubin-type pigments was detected using the bathochromatic shift test with 20 % (w/v) KOH (McCammon & Bowman, 2000; Bernardet *et al.*, 2002). Growth at 4, 7, 10, 15, 25, 28, 30, 33, 37 and 42 °C was investigated on 0.1 × TSA for up to 1 week. Tolerance of NaCl was determined on 0.1 × TSA supplemented with 0–5.0 % (w/v) NaCl at 1.0 % intervals. The pH range and optimum for growth were tested in 0.1 × trypticase soy broth (TSB) adjusted to pH 4.0–11.0 at intervals of 1 pH unit (Xu *et al.*, 2005). Growth under anaerobic conditions was determined after 1 week of incubation on 0.1 × TSA in an anaerobic jar by using AnaeroGen anaerobic system envelopes (Oxoid). In addition to 0.1 × TSA and R2A, growth was also evaluated at 28 °C on TSA, nutrient agar (NA), marine agar 2216 (MA) and MacConkey agar (all from Difco). Oxidase activity was determined using 1 % (w/v) tetramethyl *p*-phenylenediamine and catalase activity was determined by assessing bubble production in 3 % (v/v) H_2O_2. Hydrolysis of starch (0.2 %, w/v; Bodi Chemical Co.), chitin from crab shells (1 %, w/v; Sigma), casein (5 % skimmed milk, w/v; Difco), CM-cellulose (0.5 %, w/v; Sinopharm Chemical Reagent Co.) and L-tyrosine (0.5 %, w/v; BBI) was tested by using 0.1 × TSA as the basal medium according to the methods of Kim *et al.* (2012a). Hydrolysis of gelatin and aesculin was determined according to the methods of Smibert & Krieg (1994). Activities of constitutive enzymes and other physiological properties were determined by using the API 20E, API 20NE and API ZYM strips (bioMérieux) according to the manufacturer's instructions, except that the incubation temperature was 28 °C.

Cells of strain PTJT-5^T were Gram-reaction-negative, strictly aerobic, non-motile and rod-shaped. Other phenotypic features of strain PTJT-5^T are summarized in the genus and species descriptions. Differential characteristics of the novel strain and type species of genera of the family *Chitinophagaceae* are shown in Table 1. Strain PTJT-5^T could be distinguished clearly from its closest phylogenetic relative *Lacibacter cauensis* NJ-8^T by a number of phenotypic characteristics, including production of flexirubin-type pigments, temperature range for growth, nitrate reduction, aesculin hydrolysis, assimilation of carbohydrates and enzyme activities.

For the determination of DNA G+C content, genomic DNA of strain PTJT-5^T was prepared according to the

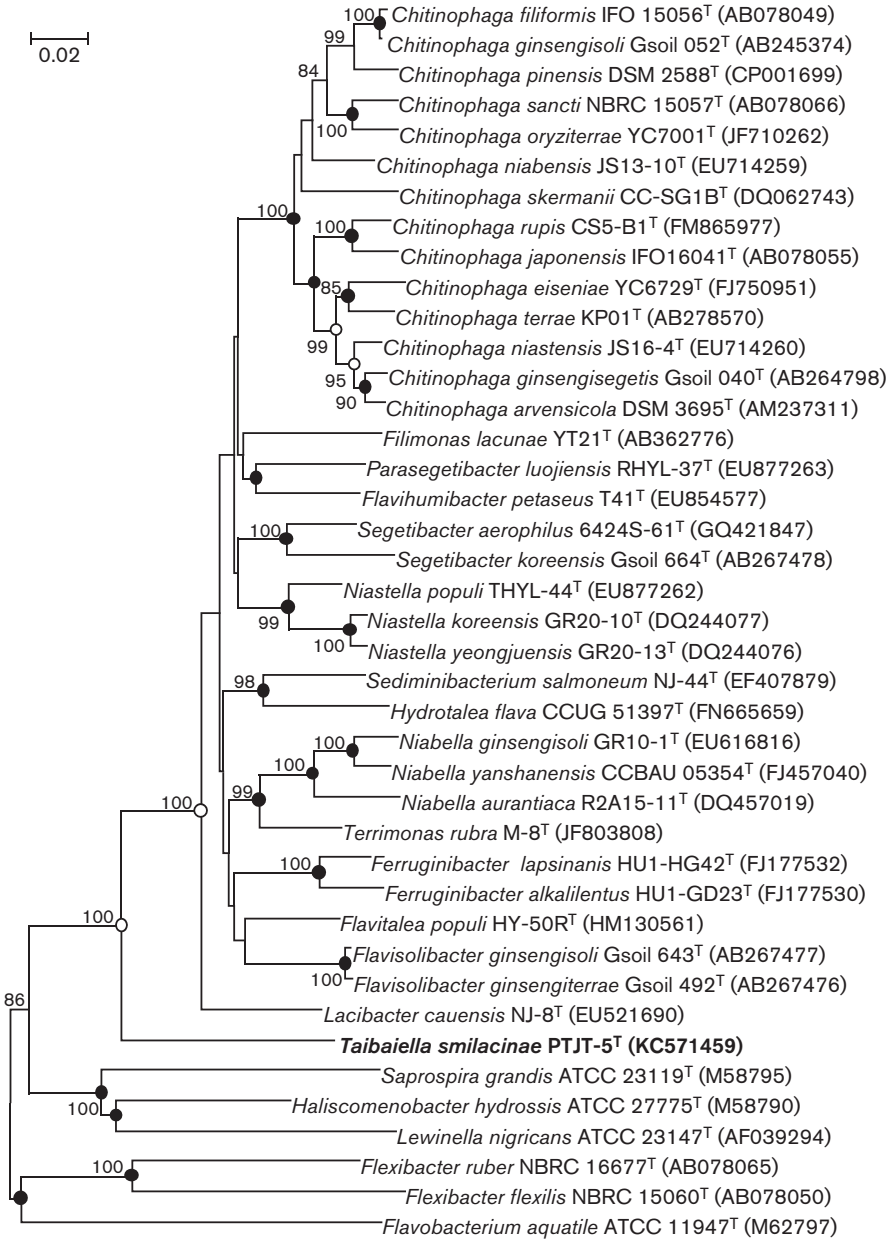

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strain PTJT-5^T and related taxa. Numbers at nodes indicate bootstrap percentages (based on 1000 resampled datasets); values below 70 % are not shown. Filled circles indicate that the corresponding nodes were also recovered in trees reconstructed with the maximum-parsimony and maximum-likelihood algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree reconstructed with the maximum-parsimony algorithm. Bar, 0.02 substitutions per nucleotide position.

procedure of Wilson (1987) with the modification of Cleenwerck *et al.* (2002). The DNA G+C content was determined by HPLC according to the method of Mesbah *et al.* (1989). Respiratory quinones of strain PTJT-5^T were extracted from 100 mg freeze-dried cells with chloroform/methanol (2 : 1, v/v), purified by TLC and identified by HPLC as described by Xie & Yokota (2003). Polyamines of strain PTJT-5^T were extracted and analysed according to Busse & Auling (1988) and Schenkel *et al.* (1995), respectively. Polar lipids of strain PTJT-5^T were extracted, resolved by two-dimensional TLC and identified as described by Tindall (1990). The total lipids and specific functional groups were detected using 5 % ethanolic molybdatophosphoric acid (total lipids), Zinzadze reagent (phosphate), ninhydrin (free amino groups), periodate Schiff (α-glycols), Dragendorff (quaternary nitrogen) and anisaldehyde-sulfuric acid (glycolipids). For cellular fatty acid analysis, strain PTJT-5^T and 13 reference strains were

Table 1. Differential characteristics of strain PTJT-5^T and representatives of related genera of the family *Chitinophagaceae*

Strains: 1, PTJT-5^T; 2, *Lacibacter cauensis* CGMCC 1.7271^T (unless indicated, data from Qu *et al.*, 2009; Wang *et al.*, 2011); 3, *Chitinophaga pinensis* DSM 2588^T (Kämpfer *et al.*, 2006; Weon *et al.*, 2009; Zhang *et al.*, 2009); 4, *Ferruginibacter alkalilentus* KCTC 22306^T (Lim *et al.*, 2009); 5, *Terrimonas ferruginea* DSM 30193^T (Weon *et al.*, 2006; Xie & Yokota, 2006); 6, *Flavitalea populi* CCTCC AB 208255^T (Wang *et al.*, 2011); 7, *Filimonas lacunae* DSM 21054^T (Shiratori *et al.*, 2009); 8, *Flavisolibacter ginsengiterrae* DSM 18136^T (Yoon & Im, 2007); 9, *Parasegetibacter luojiensis* CCTCC AB 208240^T (Zhang *et al.*, 2009); 10, *Niabella aurantiaca* DSM 17617^T (Kim *et al.*, 2007); 11, *Segetibacter koreensis* KCTC 12655^T (An *et al.*, 2007; Kämpfer *et al.*, 2011; Wang *et al.*, 2011); 12, *Niastella koreensis* DSM 17620^T (Weon *et al.*, 2006); 13, *Sediminibacterium salmoneum* CGMCC 1.6845^T (Qu & Yuan, 2008; Wang *et al.*, 2011; Kim *et al.*, 2013); 14, *Flavihumibacter petaseus* CCTCC AB 2010373^T (Zhang *et al.*, 2010); 15, *Hydrotalea flava* CCUG 51397^T (Kämpfer *et al.*, 2011; Albuquerque *et al.*, 2012). +, Positive; −, negative; w, weakly positive, ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Gliding motility	−	+	+	−	−	−	+	−	+	−	−	+	+	−	−
Production of flexirubin-type pigments	+	−	+	−	−	−	−	−	−	+	−	−	−	+*	−
Nitrate reduction	+	−	−*	−	−*	−	−	−	−	−	−	−	−	−	−
Growth at 37 °C	−	+	+	−	+	+	−	−	+	−	−	+	+	+	+
Oxidase	+	+	−	+	+	−	+	+	+	−	+	−	+	w	+
Catalase	+	+	+	+	w	+	+	−	+	+	+	−	+	+	+
Hydrolysis of:															
Gelatin	+	+	+	+	+	−	+	+	+	−	−	+	+	+	ND
Starch	+	+	−	−	+	−	−	−	+	−	−	−	−	−	ND
Casein	+	+	+	−	−	−	−	−	−	+	−	+	−	+	ND
Aesculin	−	+*	+	+	+*	+	+	+	+	+	+	+	+	+*	ND
Chitin	−	−	+	−	−	−	−	−	−	−	−	+	−	−*	ND
Assimilation of (API 20NE):†															
D-Glucose	+	−	+	−	+	−	+	−	+	+	−	−	−	+	ND
L-Arabinose	+	−	+	−	+	−	+	−	+	+	−	−	−	+	ND
D-Mannose	+	−	+	−	+	−	+	−	+	+	−	−	−	+	ND
D-Mannitol	+	−	−	−	−	−	−	−	−	−	−	−	−	−	ND
N-Acetylglucosamine	−	+	+	−	−	−	−	−	+	+	−	−	−	+	ND
Maltose	−	+	+	−	+	−	+	−	+	+	−	−	−	+	ND
Potassium gluconate	+	−	−	−	−	−	−	−	−	−	−	−	−	−	ND
Malic acid	+	−	−	−	−	−	−	−	−	−	−	−	−	−	ND
Trisodium citrate	+	−	−	−	−	−	−	−	−	−	−	−	−	−	ND
Enzyme activities (API ZYM)†															
Esterase (C4)	+	+	w	+	+	+	+	+	w	+	+	+	w	+	+
Esterase lipase (C8)	+	−	w	w	+	+	+	+	w	+	+	+	w	w	+
Lipase (C14)	−	−	−	−	−	−	−	−	w	−	−	−	−	−	−
Valine arylamidase	w	+	+	+	+	+	w	+	+	w	+	+	+	+	+
Cystine arylamidase	w	+	w	−	+	+	w	+	+	w	w	+	+	+	+
Trypsin	−	+	+	−	−	−	−	−	+	−	−	−	+	−	+
α-Chymotrypsin	+	−	−	−	−	−	−	+	+	−	−	−	−	−	+
α-Galactosidase	−	+	w	−	−	+	−	+	−	+	+	+	w	+	+
β-Galactosidase	w	+	+	+	+	+	+	+	+	+	−	+	+	+	+
β-Glucuronidase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
α-Glucosidase	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+
β-Glucosidase	w	w	+	+	−	+	+	w	+	+	−	+	+	+	+
N-Acetyl-β-glucosaminidase	−	+	+	−	+	+	w	+	+	+	+	+	+	+	+
α-Mannosidase	−	w	w	−	−	+	−	−	+	+	−	+	+	+	+
α-Fucosidase	−	−	+	−	−	−	−	−	−	+	w	+	−	+	+
DNA G+C content (mol%)	40.3	46.6	45.2	39.4	48.9	46.8	45.2	43.0	39.7	45.0	40.4	45.8	43.4	48.1	37.0

*Data from this study.
†Data in columns 1–14 are from this study.

grown on R2A agar plates at 28 °C and the biomass was standardized for physiological age at the point of harvest according to the protocol given by MIDI (Sasser, 1990). Fatty acids were saponified, methylated and extracted using the standard protocol of the MIDI Sherlock Microbial Identification System (version 6.0), analysed by GC

(Hewlett Packard 6890N) and identified using the TSBA6 database of the Microbial Identification System (Sasser, 1990).

The DNA G+C content of strain PTJT-5^T was 40.3 mol%, a value within the range reported for members of the family *Chitinophagaceae* (Table 1). The only respiratory quinone was MK-7, again in line with other members of the family *Chitinophagaceae* (Kämpfer *et al.*, 2011). Homospermidine was the only polyamine detected in strain PTJT-5^T. The polar lipid profile included phosphatidylethanolamine, an unidentified aminophosphoglycolipid, an unidentified aminophospholipid, an unidentified phospholipid and three unidentified polar lipids (L1–L3) (Fig. S1, available in IJSEM Online). The major fatty acids of strain PTJT-5^T were iso-$C_{15:0}$ (31.1 %), iso-$C_{15:1}$ G (26.5 %), iso-$C_{17:0}$ (12.7 %) and iso-$C_{17:0}$ 3-OH (12.3 %). The presence of iso-$C_{17:1}\omega10c$ and significant differences in the proportions of iso-$C_{17:0}$, anteiso-$C_{17:0}$, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and summed feature 4 (anteiso-$C_{17:1}$ B and/or iso-$C_{17:1}$ I) distinguished strain PTJT-5^T clearly from the type species of phylogenetically related genera (Table 2).

On the basis of the physiological, biochemical and chemotaxonomic data and the distinctness of its 16S rRNA gene sequence and phylogenetic position, strain PTJT-5^T was distinct from all other recognized genera of the family *Chitinophagaceae*. Therefore, we suggest that strain PTJT-5^T represents a novel species of a new genus within the family *Chitinophagaceae*, for which the name *Taibaiella smilacinae* gen. nov., sp. nov. is proposed. On the basis of new data obtained in this study, an emended description of *Flavihumibacter petaseus* is also proposed.

Description of *Taibaiella* gen. nov.

Taibaiella (Tai.bai.el′la. N.L. dim. fem. n. *Taibaiella* named after Taibai Mountain in Shaanxi Province, China, where the type strain of the type species was isolated).

Cells are Gram-reaction-negative, strictly aerobic, rod-shaped and non-motile. Catalase- and oxidase-positive. Flexirubin-type pigments are produced. Nitrate is reduced to nitrite. The only respiratory quinone is MK-7. The only polyamine is homospermidine and the major polar lipid is phosphatidylethanolamine. The major fatty acids are iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ and iso-$C_{17:0}$ 3-OH. The DNA G+C content of the type strain of the type species is 40.3 mol%. Phylogenetically, the genus is a member of the family *Chitinophagaceae* in the phylum *Bacteroidetes*. The type species is *Taibaiella smilacinae*.

Description of *Taibaiella smilacinae* sp. nov.

Taibaiella smilacinae (smi.la.ci′na.e. N.L. n. *Smilacina* a botanical genus name; N.L. gen. *smilacinae* of the plant genus *Smilacina*).

Displays the following properties in addition to those described for the genus. Cells are 0.4–0.6 × 0.8–1.5 μm. Colonies grown on 0.1 × TSA for 2 days are light yellow, smooth, circular, convex and 1–2 mm in diameter with regular margins. Good growth on R2A agar and 0.1 × TSA; moderate growth on NA; no growth on MacConkey agar, MA or TSA. Growth occurs at 7–33 °C (optimum, 25–28 °C), at pH 5.0–9.0 (optimum, pH 6.0–7.0) and in the presence of 0–1.0 % (w/v) NaCl (optimum growth in the absence of NaCl). Hydrolyses starch, casein, gelatin and tyrosine, hydrolyses CM-cellulose weakly and does not hydrolyse aesculin or chitin. Degradation of tyrosine is accompanied by production of a dark-red pigment. Positive for acetoin production (Voges–Proskauer reaction). Negative for H_2S and indole production, glucose acidification and arginine dihydrolase and urease activities. Assimilates D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, malic acid and trisodium citrate (API 20NE). Does not assimilate *N*-acetylglucosamine, maltose, capric acid, adipic acid or phenylacetic acid (API 20NE). In the API ZYM gallery, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase, weakly positive for valine arylamidase, cystine arylamidase, β-galactosidase and β-glucosidase and negative for lipase (C14), trypsin, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. In addition to phosphatidylethanolamine, an unidentified aminophosphoglycolipid, an unidentified aminophospholipid, an unidentified phospholipid and three unidentified polar lipids are also detected. In addition to the major fatty acids cited in the genus description, significant amounts of anteiso-$C_{15:0}$, $C_{16:0}$, iso-$C_{15:0}$ 3-OH, iso-$C_{17:1}\omega10c$, anteiso-$C_{17:0}$, summed feature 4 (anteiso-$C_{17:1}$ B and/or iso-$C_{17:1}$ I) and summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) are also present. The detailed fatty acid composition of the type strain is given in Table 2.

The type strain, PTJT-5^T (=CCTCC AB 2013017^T=KCTC 32316^T), was isolated from a surface-sterilized stem of *Smilacina japonica* A. Gray collected from Taibai Mountain in Shaanxi Province, north-west China.

Emended description of *Flavihumibacter petaseus* Zhang *et al.* 2010

The description is as given by Zhang *et al.* (2010) with the following amendments. Flexirubin-type pigments are produced. Aesculin is hydrolysed; chitin is not hydrolysed. In the API 20NE strip, the following tests are positive: hydrolysis of aesculin, gelatin and *p*-nitrophenyl α-D-glucopyranoside and assimilation of D-glucose, L-arabinose, D-mannose, *N*-acetylglucosamine and maltose. The following API 20NE tests are negative: nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and assimilation of D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. In the API ZYM strip, the following activities are present: alkaline phosphatase,

Table 2. Cellular fatty acid profiles of strain PTJT-5^T and the type strains of the type species of related genera

Taxa: 1, strain PTJT-5^T; 2, *Lacibacter cauensis* CGMCC 1.7271^T; 3, *Chitinophaga pinensis* DSM 2588^T; 4, *Ferruginibacter alkalilentus* KCTC 22306^T; 5, *Terrimonas ferruginea* DSM 30193^T; 6, *Flavitalea populi* CCTCC AB 208255^T; 7, *Filimonas lacunae* DSM 21054^T; 8, *Flavisolibacter ginsengiterrae* DSM 18136^T; 9, *Parasegetibacter luojiensis* CCTCC AB 208240^T; 10, *Niabella aurantiaca* DSM 17617^T; 11, *Segetibacter koreensis* KCTC 12655^T; 12, *Niastella koreensis* DSM 17620^T; 13, *Sediminibacterium salmoneum* CGMCC 1.6845^T; 14, *Flavihumibacter petaseus* CCTCC AB 2010373^T; 15, *Hydrotalea flava* CCUG 51397^T (data from Kämpfer *et al.*, 2011). Data are from this study unless indicated (strains were cultured on R2A agar at 28 °C and cells at late-exponential growth phase were used). Values are percentages of the total fatty acids; fatty acids amounting to <1 % of the total fatty acids in all strains studied are not listed; TR, Trace (<1 %), −, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
iso-$C_{13:0}$	−	TR	−	TR	TR	4.6	TR	TR	TR	TR	TR	TR	TR	−	4.2
Unknown 13.565	−	TR	−	TR	−	TR	2.2	TR	−	−	−	TR	TR	−	8.4
iso-$C_{14:0}$	−	TR	−	TR	TR	−	TR	1.2	TR	−	5.6	TR	1.1	TR	−
$C_{14:0}$	−	TR	1.8	−	TR	−	2.1	TR	1.2	1.5	−	−	1.5	TR	−
iso-$C_{13:0}$ 3-OH	TR	TR	1.0	2.4	TR	5.9	TR	TR	4.9	−	2.2	TR	2.3	−	−
iso-$C_{15:1}$ G	26.5	11.3	−	15.9	22.2	9.8	19.1	6.4	19.5	23.4	−	14.4	19.8	17.3	8.2
iso-$C_{15:1}$ F	−	−	TR	−	−	−	−	−	−	−	1.8	−	−	−	−
anteiso-$C_{15:1}$ A	TR	TR	TR	1.1	TR	2.5	TR	4.3	3.9	TR	TR	TR	6.5	1.1	−
iso-$C_{15:0}$	31.1	30.5	25.7	26.6	32.1	16.7	22.5	30.4	22.6	28.6	13.6	24.7	18.3	29.5	34.8
anteiso-$C_{15:0}$	1.2	TR	TR	3.2	1.9	3.3	TR	7.9	3.1	2.6	19.7	4.2	12.5	1.9	1.7
$C_{15:0}$	−	4.0	TR	3.6	5.8	TR	TR	1.4	2.6	6.3	1.3	2.2	TR	7.3	TR
iso-$C_{16:1}$ H	−	−	−	−	−	−	−	1.3	−	−	3.7	−	−	−	−
iso-$C_{16:0}$	TR	1.2	TR	2.6	TR	1.1	2.3	2.9	4.3	TR	9.9	1.0	1.0	2.1	TR
$C_{16:1}\omega 11c$	−	−	4.1	−	−	−	−	−	−	−	−	−	−	−	−
$C_{16:1}\omega 5c$	−	2.5	25.3	−	−	3.4	1.1	−	−	−	2.3	−	TR	7.4	−
$C_{16:0}$	2.5	4.8	3.3	4.3	3.9	1.9	5.4	5.6	7.1	4.5	3.6	3.2	3.4	2.3	TR
iso-$C_{15:0}$ 3-OH	2.7	3.6	5.2	6.7	2.4	−	5.2	1.2	−	2.8	1.2	1.4	5.5	4.1	4.0
$C_{15:0}$ 2-OH	−	TR	−	1.1	TR	−	−	TR	TR	1.5	TR	TR	1.4	TR	TR
iso-$C_{17:1}\omega 10c$	1.0	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Unknown 16.582	1.3	1.2	TR	TR	1.2	1.4	TR	TR	1.4	1.6	TR	1.3	TR	TR	1.7
iso-$C_{17:0}$	12.7	1.5	TR	TR	TR	5.7	TR	TR	TR	−	−	1.0	−	−	−
anteiso-$C_{17:0}$	1.9	−	TR	TR	TR	TR	−	TR	TR	TR	−	TR	−	TR	−
$C_{17:1}\omega 6c$	−	TR	−	TR	−	1.1	−	3.7	1.0	−	TR	TR	−	1.4	−
iso-$C_{16:0}$ 3-OH	−	TR	TR	6.3	TR	TR	1.3	TR	TR	TR	10.9	TR	7.2	TR	1.2
$C_{16:0}$ 3-OH	−	TR	1.3	2.9	2.3	−	3.2	TR	1.3	1.8	TR	1.5	5.9	2.5	TR
$C_{18:1}\omega 9c$	−	−	1.5	2.0	TR	1.3	−	TR	1.5	−	1.7	−	−	−	−
$C_{18:1}\omega 5c$	−	−	−	TR	−	TR	−	−	TR	−	1.1	−	−	−	−
$C_{18:0}$	−	TR	TR	TR	TR	TR	TR	TR	1.8	TR	3.7	TR	TR	TR	−
iso-$C_{17:0}$ 3-OH	12.3	19.7	11.7	12.8	14.3	19.8	17.2	8.7	15.8	15.2	3.0	27.3	9.1	13.2	16.9
$C_{17:0}$ 2-OH	TR	TR	TR	1.6	TR	TR	TR	TR	TR	TR	2.0	2.7	2.1	TR	TR
$C_{17:0}$ 3-OH	−	TR	TR	TR	TR	−	−	TR	−	TR	−	1.3	−	1.0	−
Summed features*															
1	−	−	TR	−	−	5.2	−	3.6	−	−	1.2	−	−	−	−
3	TR	13.2	11.0	2.9	9.5	6.7	14.8	9.9	4.1	8.8	3.9	7.6	TR	8.6	4.7
4	3.8	−	−	−	−	1.5	−	1.4	−	−	−	−	−	−	1.0
5	−	−	−	−	TR	2.1	−	TR	TR	−	−	TR	−	−	−
8	1.3	−	1.0	1.2	−	TR	−	−	−	−	1.3	−	−	−	−
9	−	−	−	−	−	−	−	TR	1.3	−	2.1	−	−	−	5.9

*Summed features are groups of two or three fatty acids that cannot be separated by the MIDI system. Summed feature 1 comprised $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$ H; summed feature 3 comprised $C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$; summed feature 4 comprised anteiso-$C_{17:1}$ B and/or iso-$C_{17:1}$ I; summed feature 5 comprised anteiso-$C_{18:0}$ and/or $C_{18:2}\omega 6,9c$; summed feature 8 comprised $C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$; summed feature 9 comprised iso-$C_{17:1}\omega 9c$ and/or 10-methyl $C_{16:0}$.

esterase (C4), esterase lipase (C8) (weakly), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. The following activities are absent in tests with

the API ZYM strip: lipase (C14), trypsin, α-chymotrypsin and β-glucuronidase.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (grant no. 31100001) and the Natural Science Foundation of Shaanxi Province, China (grant no. 2012JQ3006).

References

Albuquerque, L., Rainey, F. A., Nobre, M. F. & da Costa, M. S. (2012). *Hydrotalea sandarakina* sp. nov., isolated from a hot spring runoff, and emended descriptions of the genus *Hydrotalea* and the species *Hydrotalea flava*. *Int J Syst Evol Microbiol* **62**, 1603–1608.

An, D. S., Lee, H. G., Im, W. T., Liu, Q. M. & Lee, S. T. (2007). *Segetibacter koreensis* gen. nov., sp. nov., a novel member of the phylum *Bacteroidetes*, isolated from the soil of a ginseng field in South Korea. *Int J Syst Evol Microbiol* **57**, 1828–1833.

Bernardet, J.-F., Nakagawa, Y., Holmes, B. & Subcommittee on the taxonomy of Flavobacterium and Cytophaga-like bacteria of the International Committee on Systematics of Prokaryotes (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* **52**, 1049–1070.

Bowman, J. P. (2000). Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int J Syst Evol Microbiol* **50**, 1861–1868.

Busse, H.-J. & Auling, G. (1988). Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* **11**, 1–8.

Cleenwerck, I., Vandemeulebroecke, K., Janssens, D. & Swings, J. (2002). Re-examination of the genus *Acetobacter*, with descriptions of *Acetobacter cerevisiae* sp. nov. and *Acetobacter malorum* sp. nov. *Int J Syst Evol Microbiol* **52**, 1551–1558.

Doetsch, R. N. (1981). Determinative methods of light microscopy. In *Manual of Methods for General Bacteriology*, pp. 21–33. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Felsenstein, J. (2006). PHYLIP (phylogeny inference package), version 3.66. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

Fitch, W. M. (1971). Towards defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Kämpfer, P., Young, C. C., Sridhar, K. R., Arun, A. B., Lai, W. A., Shen, F. T. & Rekha, P. D. (2006). Transfer of [*Flexibacter*] *sancti*, [*Flexibacter*] *filiformis*, [*Flexibacter*] *japonensis* and [*Cytophaga*] *arvensicola* to the genus *Chitinophaga* and description of *Chitinophaga skermanii* sp. nov. *Int J Syst Evol Microbiol* **56**, 2223–2228.

Kämpfer, P., Lodders, N. & Falsen, E. (2011). *Hydrotalea flava* gen. nov., sp. nov., a new member of the phylum *Bacteroidetes* and allocation of the genera *Chitinophaga*, *Sediminibacterium*, *Lacibacter*, *Flavihumibacter*, *Flavisolibacter*, *Niabella*, *Niastella*, *Segetibacter*, *Parasegetibacter*, *Terrimonas*, *Ferruginibacter*, *Filimonas* and *Hydrotalea* to the family *Chitinophagaceae* fam. nov. *Int J Syst Evol Microbiol* **61**, 518–523.

Kim, B. Y., Weon, H. Y., Yoo, S. H., Hong, S. B., Kwon, S. W., Stackebrandt, E. & Go, S. J. (2007). *Niabella aurantiaca* gen. nov., sp. nov., isolated from a greenhouse soil in Korea. *Int J Syst Evol Microbiol* **57**, 538–541.

Kim, B.-C., Poo, H., Lee, K. H., Kim, M. N., Kwon, O.-Y. & Shin, K.-S. (2012a). *Mucilaginibacter angelicae* sp. nov., isolated from the rhizosphere of *Angelica polymorpha* Maxim. *Int J Syst Evol Microbiol* **62**, 55–60.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012b). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Kim, Y. J., Nguyen, N. L., Weon, H. Y. & Yang, D. C. (2013). *Sediminibacterium ginsengisoli* sp. nov., isolated from soil of a ginseng field, and emended descriptions of the genus *Sediminibacterium* and of *Sediminibacterium salmoneum*. *Int J Syst Evol Microbiol* **63**, 905–912.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Lane, D. J. (1991). 16S–23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 125–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Lim, J. H., Baek, S.-H. & Lee, S.-T. (2009). *Ferruginibacter alkalilentus* gen. nov., sp. nov. and *Ferruginibacter lapsinanis* sp. nov., novel members of the family '*Chitinophagaceae*' in the phylum *Bacteroidetes*, isolated from freshwater sediment. *Int J Syst Evol Microbiol* **59**, 2394–2399.

Lin, Y.-C., Uemori, K., de Briel, D. A., Arunpairojana, V. & Yokota, A. (2004). *Zimmermannella helvola* gen. nov., sp. nov., *Zimmermannella alba* sp. nov., *Zimmermannella bifida* sp. nov., *Zimmermannella faecalis* sp. nov. and *Leucobacter albus* sp. nov., novel members of the family *Microbacteriaceae*. *Int J Syst Evol Microbiol* **54**, 1669–1676.

Ludwig, W., Euzéby, J. & Whitman, W. B. (2008). Draft taxonomic outline of the *Bacteroidetes*, *Planctomycetes*, *Chlamydiae*, *Spirochaetes*, *Fibrobacteres*, *Fusobacteria*, *Acidobacteria*, *Verrucomicrobia*, *Dictyoglomi*, and *Gemmatimonadetes*. http://www.bergeys.org/outlines/Bergeys_Vol_4_Outline.pdf

McCammon, S. A. & Bowman, J. P. (2000). Taxonomy of Antarctic *Flavobacterium* species: description of *Flavobacterium gillisiae* sp. nov., *Flavobacterium tegetincola* sp. nov., and *Flavobacterium xanthum* sp. nov., nom. rev. and reclassification of [*Flavobacterium*] *salegens* as *Salegentibacter salegens* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **50**, 1055–1063.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Qu, J. H. & Yuan, H. L. (2008). *Sediminibacterium salmoneum* gen. nov., sp. nov., a member of the phylum *Bacteroidetes* isolated from sediment of a eutrophic reservoir. *Int J Syst Evol Microbiol* **58**, 2191–2194.

Qu, J. H., Yuan, H. L., Yang, J. S., Li, H. F. & Chen, N. (2009). *Lacibacter cauensis* gen. nov., sp. nov., a novel member of the phylum *Bacteroidetes* isolated from sediment of a eutrophic lake. *Int J Syst Evol Microbiol* **59**, 1153–1157.

Saitou, N. & Nei, M. (1987). The neighbor-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. Newark, DE: MIDI Inc. http://www.microbialid.com/PDF/TechNote_101.pdf

Schenkel, E., Berlaimont, V., Dubois, J., Helson-Cambier, M. & Hanocq, M. (1995). Improved high-performance liquid chromatographic method for the determination of polyamines as their benzoylated derivatives: application to P388 cancer cells. *J Chromatogr B Biomed Appl* **668**, 189–197.

Shiratori, H., Tagami, Y., Morishita, T., Kamihara, Y., Beppu, T. & Ueda, K. (2009). *Filimonas lacunae* gen. nov., sp. nov., a member of the phylum *Bacteroidetes* isolated from fresh water. *Int J Syst Evol Microbiol* **59**, 1137–1142.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Strobel, G. A. & Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiol Mol Biol Rev* **67**, 491–502.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Tindall, B. J. (1990). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.

Wang, Y., Cai, F., Tang, Y., Dai, J., Qi, H., Rahman, E., Peng, F. & Fang, C. (2011). *Flavitalea populi* gen. nov., sp. nov., isolated from soil of a Euphrates poplar (*Populus euphratica*) forest. *Int J Syst Evol Microbiol* **61**, 1554–1560.

Weon, H. Y., Kim, B. Y., Yoo, S. H., Lee, S. Y., Kwon, S. W., Go, S. J. & Stackebrandt, E. (2006). *Niastella koreensis* gen. nov., sp. nov. and *Niastella yeongjuensis* sp. nov., novel members of the phylum *Bacteroidetes*, isolated from soil cultivated with Korean ginseng. *Int J Syst Evol Microbiol* **56**, 1777–1782.

Weon, H. Y., Yoo, S. H., Kim, Y. J., Son, J. A., Kim, B. Y., Kwon, S. W. & Koo, B. S. (2009). *Chitinophaga niabensis* sp. nov. and *Chitinophaga niastensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **59**, 1267–1271.

Wilson, K. (1987). Preparation of genomic DNA from bacteria. In *Current Protocols in Molecular Biology*, pp. 241–245. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Greene Publishing and Wiley Interscience.

Xie, C. H. & Yokota, A. (2003). Phylogenetic analyses of *Lampropedia hyalina* based on the 16S rRNA gene sequence. *J Gen Appl Microbiol* **49**, 345–349.

Xie, C. H. & Yokota, A. (2006). Reclassification of [*Flavobacterium*] *ferrugineum* as *Terrimonas ferruginea* gen. nov., comb. nov., and description of *Terrimonas lutea* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **56**, 1117–1121.

Xu, P., Li, W.-J., Tang, S.-K., Zhang, Y.-Q., Chen, G.-Z., Chen, H.-H., Xu, L.-H. & Jiang, C.-L. (2005). *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family '*Oxalobacteraceae*' isolated from China. *Int J Syst Evol Microbiol* **55**, 1149–1153.

Yoon, M. H. & Im, W. T. (2007). *Flavisolibacter ginsengiterrae* gen. nov., sp. nov. and *Flavisolibacter ginsengisoli* sp. nov., isolated from ginseng cultivating soil. *Int J Syst Evol Microbiol* **57**, 1834–1839.

Zhang, K. D., Tang, Y. L., Zhang, L., Dai, J., Wang, Y., Luo, X. S., Liu, M., Luo, G. C. & Fang, C. X. (2009). *Parasegetibacter luojiensis* gen. nov., sp. nov., a member of the phylum *Bacteroidetes* isolated from a forest soil. *Int J Syst Evol Microbiol* **59**, 3058–3062.

Zhang, N. N., Qu, J.-H., Yuan, H.-L., Sun, Y. M. & Yang, J.-S. (2010). *Flavihumibacter petaseus* gen. nov., sp. nov., isolated from soil of a subtropical rainforest. *Int J Syst Evol Microbiol* **60**, 1609–1612.