Bartonella apis sp. nov., a honey bee gut symbiont of the class Alphaproteobacteria

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Here, we report the culture and characterization of an alphaproteobacterium of the order Rhizobiales, isolated from the gut of the honey bee Apis mellifera. Strain PEB0122T shares >95 % 16S rRNA gene sequence similarity with species of the genus Bartonella, a group of mammalian pathogens transmitted by bloodsucking arthropods. Phylogenetic analyses showed that PEB0122T and related strains from the honey bee gut form a sister clade of the genus Bartonella. Optimal growth of strain PEB0122T was obtained on solid media supplemented with defibrinated sheep blood under microaerophilic conditions at 35–37 °C, which is consistent with the cultural characteristics of other species of the genus Bartonella. Reduced growth of strain PEB0122T also occurred under aerobic conditions. The rod-shaped cells of strain PEB0122T had a mean length of 1.2–1.8 μm and revealed hairy surface structures. Strain PEB0122T was positive for catalase, cytochrome c oxidase, urease and nitrate reductase. The fatty acid composition was comparable to those of other species of the genus Bartonella, with palmitic acid (C16 : 0) and isomers of 18- and 19-carbon chains being the most abundant. The genomic DNA G + C content of PEB0122T was determined to be about 45.5 mol%. The high 16S rRNA gene sequence similarity with species of Bartonella and its close phylogenetic position suggest that strain PEB0122T represents a novel species within the genus Bartonella, for which we propose the name Bartonella apis sp. nov. The type strain is PEB0122T (=NCIMB 14961T=DSM 29779T).

Non-culture-based analyses of 16S rRNA gene sequences have shown that the hindgut of adult honey bees (genus Apis) is inhabited by a relatively small number of bacterial species including two members of the Firmicutes, two gammaproteobacteria, two alphaproteobacteria, one bifidobacterium and one betaproteobacterium (Cox-Foster et al., 2007; Martinson et al., 2011; Moran et al., 2012; Sabree et al., 2012). These bacteria are consistently present in honey bees worldwide (Ahn et al., 2012; Babendreier et al., 2007; Jeyaprakash et al., 2003), and a subset has also been found in the gut of various bumble bee species (genus Bombus) (Cariveau et al., 2014; Martinson et al., 2011). In other environments, these bacteria have so far not been detected, which suggests specific adaptation to the gut of social bees. Genome sequencing projects have provided the first insights into the functional capabilities of the bee gut bacteria (Ellegaard et al., 2015; Engel et al., 2012; Kwong et al., 2014a, b). Furthermore, cultures of most community members have been established and species names proposed, such as Snodgrassella alvi (Beta-proteobacteria), Gilliamella apicola and Frischella perrara (Gammaproteobacteria), Bifidobacterium asteroides (Actinobacteria) and Lactobacillus apis and Lactobacillus mellis (Firmicutes) (Engel et al., 2013; Killer et al., 2014; Kwong & Moran, 2013; Olofsson et al., 2014). However, species descriptions of the two alphaproteobacteria are still lacking. Based on 16S rRNA gene sequence analyses, they seem to belong to distinct lineages of the Alphaproteobacteria and were therefore referred to as ‘Alpha-1’ and ‘Alpha-2’ (Cox-Foster et al., 2007). ‘Alpha-2’ was further subdivided into ‘Alpha-2.1’ and ‘Alpha-2.2’, representing two distinct clades within the family Acetobacteraceae (Corby-Harris et al., 2014; Martinson et al., 2011). Phylogeny-type ‘Alpha-1’ belongs to the order Rhizobiales and appears to be closely related to the genus Bartonella (Martinson et al., 2011). The latter constitutes a group of facultatively intracellular pathogens that persist in the bloodstream of a wide range of mammals. Some species of Bartonella can colonize human hosts and cause severe diseases such as Carrion’s disease or bacillary angiomatosis (Harms &

Abbreviation: TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession numbers of the 16S rRNA gene sequences and protein-coding gene sequences of strains PEB0122T, PEB0149, PEB0150, BBC0104 and BBC0108 from Apis mellifera and the uncultured Rhizobiales bacterium from Herpagnathos saltator are KP987849–KP987886 and KT315729–KT315734.

Two supplementary tables and one supplementary figure are available with the online Supplementary Material.
Dehio, 2012). Transmission of these mammalian pathogens is facilitated by bloodsucking arthropods. To our knowledge, all currently described species of Bartonella have been isolated either from the bloodstream of mammals or from bloodsucking arthropods. In contrast, ‘Alpha-1’ has so far only been detected in the gut of honey bees.

Another group of the Rhizobiales that seems to be closely related to ‘Alpha-1’ are bacteria detected in the gut of herbivorous tropical ants from diverse species. The function of these ant-associated members of the Rhizobiales has so far remained elusive, but several lines of evidence suggest that they might play a role in the nitrogen uptake of their host (Russell et al., 2009).

Here, we report the cultivation and characterization of ‘Alpha-1’ strain PEB0122T from the Western honey bee, Apis mellifera, and propose the name Bartonella apis sp. nov.

Strain PEB0122T was isolated together with strains PEB0149 and PEB0150 from homogenized guts of A. mellifera. Adult worker bees were captured in West Haven, CT, USA, and immobilized by chilling at 4 °C. Thereafter, the guts were dissected with sterile forceps and homogenized in PBS by bead-beating. Diluted homogenates were plated on tryptic soy agar supplemented with 5 % defibrinated sheep blood (blood agar; Hardy Diagnostics) and incubated at 37 °C in an atmosphere enriched with 5 % CO2. Bacterial colonies were visible after 2–3 days and were identified by amplification and sequencing of the partial 16S rRNA gene. To this end, PCR was performed on bacterial colonies with the universal 16S rRNA gene primers 27F (5′-AGAGTTTGATCCTG-GCTCAG-3′) and 1492R (5′-GTTACCTTGTAGACT-3′). DNA from colonies was denatured in a thermocycler by boiling at 95 °C for 10 min, followed by 35 cycles of amplification (95 °C for 20 s, 54 °C for 30 s and 72 °C for 40 s) and 5 min of final elongation at 72 °C. Sequencing of amplicons was performed using the dideoxy chain-termination method and capillary gel electrophoresis on an Applied Biosystems 3730xl DNA Genetic Analyzer. The resulting sequences were trimmed based on the ABI sequencer traces.

Clustal W alignments (Thompson et al., 1994) revealed that the 16S rRNA gene sequences of strains PEB0122T, PEB0149 and PEB0150 are identical. We next used the sequences to search with MegabLAST against the non-redundant nucleotide collection database of NCBI. All three strains shared >97% similarity with 16S rRNA gene sequences deposited as ‘uncultured Rhizobiales bacteria’ from A. mellifera and related bee species. The closest described taxa in the MegabLAST analysis were species of the genus Bartonella, including several human pathogens.

To conduct a more detailed comparative analysis, we retrieved the full-length sequences of the 16S rRNA, gltA and rpoB genes of strains PEB0122T, PEB0149 and PEB0150 from ongoing genome sequencing projects (data not shown). Clustal W alignments confirmed that strains PEB0122T, PEB0149 and PEB0150 (1528 sites) have identical 16S rRNA gene sequences and share high sequence similarity with members of the genus Bartonella, ranging from 95.9% (Bartonella chomelii A828T) to 97.6% (Bartonella tamiae Th239) (Table S1, available in the online Supplementary Material). In contrast, sequence similarities for conserved fragments of the gltA gene (327 bp fragment) and the rpoB gene (825 bp fragment) (La Scola et al., 2003) were much lower (Table S1). Strain PEB0122T exhibited sequence similarities with other species of Bartonella ranging from 77.4 to 82.0% for the gltA fragment and from 75.6 to 79.1% for the rpoB fragment. This is clearly below the sequence similarity cut-offs of 96.0% (gltA fragment) and 95.4% (rpoB fragment) that were proposed by La Scola et al. (2003) to discriminate species of Bartonella from each other. In contrast to the comparison with previously described species of Bartonella, sequence similarities between strain PEB0122T and the other two strains isolated from the bee gut, PEB0149 and PEB0150, were much higher, ranging from 98.2 to 98.8% (gltA fragment) and 98.7 to 99.5% (rpoB fragment), suggesting that they belong to the same species (Table S1).

A phylogenetic tree inferred from aligned 16S rRNA gene sequences corroborated the findings from the sequence similarity analyses. Strains PEB0122T, PEB0149 and PEB0150 clustered together with 16S rRNA gene sequences previously detected in species of Apis and with two strains, BBC0104 and BBC0108, that we had isolated from the gut of honey bees in Switzerland (Fig. 1). The honey bee-specific strains form a clade that is basal to species of the genus Bartonella. The phylogenetic clustering of strains from honey bees and the basal position of this cluster within the genus Bartonella are both supported by bootstrap values ≥80%. The most closely related member of Bartonella is ‘B. tamiae’, forming a sister clade to the sequences from honey bees (Fig. 1). However, this relationship is not supported by the bootstrap analysis (i.e. values <80%), probably because the 16S rRNA gene sequence similarities between the honey bee strains and different species of Bartonella are all in the same range (Table S1). The 16S rRNA gene phylogeny also showed that sequences of members of the Rhizobiales from various ant species are more distant from the honey bee-specific strains than are species of Bartonella. They form a separate monophyletic group basal to the honey bee-specific clade and the genus Bartonella (Fig. 1). Accordingly, pairwise 16S rRNA gene sequence similarities were lower for strain PEB0122T with the ant strains (93.3–93.9%) than with members of the genus Bartonella (95.9–97.6%) (Table S1).

Because of the high similarity of 16S rRNA gene sequences and the resulting low bootstrap values for most branches in the tree, we inferred another phylogeny based on concatenated nucleotide sequences of eight phylogenetic marker genes (alaS, COG0013; uvrC, COG0322; recN, COG 0497; pyrG, COG0504; fflh/sp, COG0541; uvrB, COG0556; radA, COG1066; typA, COG1217). These genes occur in a single copy in the majority of bacteria and have proven...
Fig. 1. Neighbour-joining tree based on a CLUSTAL W alignment of cropped 16S rRNA gene sequences (1364 aligned sites). The tree was inferred with MEGA 6.0 (Tamura et al., 2013) using the Jukes–Cantor model; 1000 bootstrap trees were inferred and values ≥80 % are shown above branches. Numbers below branches indicate bootstrap values ≥95 %. Accession numbers of sequences are listed in Table S2. Bar, 0.005 substitutions per site.

**Major mammalian or insect host**

- Bartonella elizabethae FN15-2T: Brown rat (Rattus norvegicus)
- Bartonella pachyvomys F9251T: Fat-tail gerbil (Pachyuromys duprasi)
- Bartonella florenciae R4T: White-toothed shrew (Crocidura russula)
- Bartonella tribocorum IBS 506T: Black rat (Rattus rattus)
- Bartonella acromydis KS2-1T: Golden spiny mouse (Acomys russatus)
- Bartonella queenslandensis AUST/NH4T: Australian rat (Uromys, Melomys and Rattus spp)
- Bartonella grahamii V2T: Bank vole (Clethrionomys glareolus)
- Bartonella birtlesii IBS 325T: Wood mouse (Apodemus spp.)
- Bartonella doshiare R18T: Field vole (Microtus agrestis)
- Bartonella taylori M6T: Wood mouse (Apodemus spp.)
- Bartonella vinsonii subsp. vinsonii BakerT: Meadow vole (Microtus pennsylvanicus)
- Bartonella vinsonii subsp. arupensis OK94-513T: White-footed mouse (Peromyscus leucopus)
- Bartonella alsatica IBS382T: European rabbit (Oryctolagus cuniculus)
- Bartonella vinsonii subsp. berkholfii 93-C01T: Dog (Canis familiaris)
- Bartonella henselae Houston-10T: Cat (Felis domesticus)
- Bartonella koehlerae C-29T: Soft tick (Ornithodoros sonrai)
- Bartonella capreoli IBS 193T: Roe deer (Capreolus capreolus)
- Bartonella bacilliformis KC589T: Human (Homo sapiens)
- Bartonella bovis 91-4T: Cattle (Bos taurus)
- Bartonella chomelii A828T: Roe deer (Capreolus capreolus)
- Bartonella schoenbuchensis R1T: Human (Homo sapiens)
- Bartonella cooperpslainsensis AUST/NH20T: Australian rat (Uromys, Melomys and Rattus spp)
- Bartonella japonica Fuji-18T: Small Japanese field mouse (Apodemus argentatus)
- Bartonella silvatica Fuji-23-1T: Large Japanese field mouse (Apodemus speciosus)
- Bartonella callosciuri BR11-1T: Plantain squirrel (Callosciurus notatus)
- Bartonella jaculu FullerT: Greater Egyptian jerboa (Jaculus orientalis)
- Bartonella quintana FullerT: Louse (Pediculus humanus), Human (Homo sapiens)
- Bartonella australis Aust/NH1: Kangaroo (Macropus giganteus)
- Bartonella claridgeiae Houston-2T: Cat (Felis domesticus)
- Bartonella rochalimae ATCC BAA-1498T: Human (Homo sapiens)
- Bartonella tamiiae Th239: Human (Homo sapiens)
- Uncultured Rhizobiales bacterium (1): Honey bee from Asia (Apis dorsata)
- Uncultured Rhizobiales bacterium (2): Honey bee from Africa (Apis mellifera capensis)
- Uncultured Bartonella sp.: Honey bee from Asia (Apis mellifera)
- Uncultured Bartonella sp.: Ant (Procryptocerus batesi)
- Uncultured Bartonella sp.: Ant (Procryptocerus batesi)
- Uncultured Bartonella sp.: Ant (Terataner sp.)
- Uncultured Bartonella sp.: Ant (Herpognathos saltator)
- Uncultured Bartonella sp.: (5)
- Uncultured Bartonella sp.: (6)
- Uncultured Bartonella sp.: (7)
- Uncultured Bartonella sp.: (8)

**Host species of Bartonella**

- Honey bee (Apis mellifera)
- Ant (Procryptocerus batesi)
- Ant (Procryptocerus batesi)
- Ant (Terataner sp.)
- Ant (Herpognathos saltator)
to be suitable for taxonomic classification of bacteria in previous publications (Ciccarelli et al., 2006; Engel et al., 2012, 2013, 2014; Mende et al., 2013; Sorek et al., 2007). Sequences of PEB0122T, PEB0149 and PEB0150 and an uncultured member of the Rhizobiales from the ant Herpagnathos saltator were obtained from ongoing genome sequencing projects. The phylogenetic marker gene tree (Fig. 2) confirmed the overall topology of the 16S rRNA gene tree: (i) the honey bee strains formed a clade basal to species of the genus Bartonella, (ii) ‘B. tamiae’ was the next most closely related species, and (iii) the member of the Rhizobiales from the ant H. saltator formed a more distant sister lineage basal to the honey bee-specific strains and the genus Bartonella. Because of the limited number of sequences available, fewer taxa were included in this analysis than in the 16S rRNA gene analysis. In summary, our phylogenies and comparative sequence analyses suggest that strains of ‘Alpha-1’ belong to the genus Bartonella (i.e. 16S rRNA gene sequence identity >95 %), but form a distinct clade that is basal to other members of Bartonella. Sequence similarities of the gltA and rpoB gene fragments are clearly below the defined species cut-off (La Scola et al., 2003), indicating that the strains isolated from the honey bee gut represent a novel species of Bartonella. Accession numbers of sequences used for these analyses are listed in Table S2. At 45.5 mol%, strains PEB0122T, PEB0149 and PEB0150 exhibit a markedly elevated DNA G+C content compared with other members of Bartonella, which typically exhibit DNA G+C contents in the range 37.8–41.8 mol%. DNA G+C content information for strains PEB0122T, PEB0149 and PEB0150 was obtained from the unpublished genome sequencing projects.

To describe the honey bee-specific strains further, we studied growth characteristics and conducted a number of phenotypic and morphological analyses. Because of the identical 16S rRNA gene sequences of the three isolated strains, we restricted our analyses to strain PEB0122T. Growth of strain PEB0122T was tested under different conditions and compared with ‘B. tamiae’ Th239 (Kosoy et al., 2008) and Bartonella henselae Houston-1T (Söder et al., 1995). For the inoculation of agar plates, equal volumes of bacteria were resuspended in PBS and adjusted to the same OD$_{600}$. The following growth media were tested: Colombia agar base (bioMérieux), tryptic soy agar (TSA; BD), brain heart infusion agar (BHIA; BD), heart infusion

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**Fig. 2.** Maximum-likelihood tree based on eight concatenated phylogenetic marker genes: aalS, uvrC, recN, pyrG, ffh/srp, uvrB, radA and tyrA. Nucleotide sequences of the eight genes were aligned at the protein level with CLUSTAL W, back-translated, cropped and concatenated in Geneious 6.1.8 (Kearse et al., 2012). The tree was inferred using the GTR model (+I=0.319, +G=0.884); 100 bootstrap trees were calculated and values ≥80 are shown above branches. Accession numbers of sequences are listed in Table S2. Bar, 0.05 substitutions per site.
agar (HIA; BD) and mannitol medium (per litre: 1.25 g yeast extract, 6.25 g mannitol, 3.75 g agar, 0.75 g Bacto peptone) with or without 5% defibrinated sheep blood. For all strains, optimal growth was observed on Colombia agar base with 5% defibrinated sheep blood (CBA) at 37 °C in a 5% CO2 incubator (i.e. microaerophilic conditions). On media without blood, growth of strain PEB0122T and the other two strains of Bartonella could not be observed. The dependence on blood to grow on agar plates is a typical characteristic of members of the genus Bartonella. For strain PEB0122T, formation of opaque white colonies with a diameter of 0.2–0.4 mm was observed after 2–3 days of incubation. In contrast, colonies of ‘B. henselae’ Th239 and B. henselae Houston-1T were only observed after 4 and 5 days, respectively, and were generally smaller compared with those of strain PEB0122T (Fig. S1). Notably, strain PEB0122T also exhibited slow growth under aerobic conditions, i.e. in air, while neither ‘B. tamiiae’ Th239 nor B. henselae Houston-1T showed reasonable growth under these conditions. None of the three strains grew under anaerobic conditions. Temperature sensitivity was tested on CBA in CO2Gen Compact sachets (Oxoid), creating a microaerophilic environment with about 15% O2 and 6% CO2. In the tested temperature range of 25–40 °C, all strains grew optimally at 35–37 °C. Haemolytic activity was not observed under any of the tested growth conditions during incubation on CBA. All subsequent assays and analyses were performed with bacteria grown under optimal conditions, i.e. on CBA in a 5% CO2 incubator at 37 °C.

Cell morphology of strain PEB0122T was analysed with differential interference contrast microscopy and transmission electron microscopy (TEM). For the former, cells were harvested from plates after they had grown for 2 days, resuspended in PBS (OD600 ~1), placed on a microscope slide covered with a thin layer of agar and incubated for 1 h with DAPI (5 mg ml−1) in order to stain the DNA. Rod-shaped cells were the predominant morphology of strain PEB0122T, ‘B. tamiiae’ Th239 and B. henselae Houston-1T (Fig. S1). However, with cell lengths of 1.2–1.8 μm, the rods of PEB0122T were shorter than those of ‘B. tamiiae’ Th239 (1.2–2 μm) but longer than those of B. henselae Houston-1T (0.7–1.4 μm). We did not observe any filamentous or coccoid morphologies for strain PEB0122T. For the TEM studies, cells were grown for 2 days and then fixed in 2.5% glutaraldehyde and 4% formaldehyde in 0.1 M phosphate buffer, pH 7.4, post-fixed in 1% aqueous osmium oxide and stained en bloc with 1% uranyl acetate. The samples were then dehydrated in a graded series of ethanol and embedded in Epon. Ultrathin sections were post-stained with uranyl acetate and lead citrate and observed in a TEM at 80 keV (CM10; FEI). Pictures were taken using a Morada camera (Olympus). Cells of PEB0122T appeared rod-shaped in TEM and revealed a two-layered cell envelope typical of Gram-negative bacteria (Fig. 3). Cells were 1.2–1.8 μm long, confirming results from differential interference contrast microscopy. The width was about 0.5 μm. Hairy structures could be detected on the surface of some cells covering parts of the cell envelope. The presence of filamentous structures in the extracellular matrix of bacterial cells could suggest the presence of flagella. Several members of Bartonella are known to harbour flagella (Dehio et al., 2001; Engel & Dehio, 2009), making their presence also likely in strain PEB0122T. Electron-dense spots were observed in the cytoplasm of cells of strain PEB0122T. These could originate from metal deposits.

For biochemical and metabolic characterization, we performed several standard assays on strains PEB0122T and PEB0149, ‘B. tamiiae’ Th239 and B. henselae Houston-1T (Table 1). Catalase activity was tested by the direct addition of bacteria onto a drop of 3% H2O2. The observation of foaming as a result of O2 gas production was used as an indication for a catalase-positive strain. The presence of cytochrome-c oxidase was determined by spreading bacteria on Whatman paper with a drop of 1% N,N,N′,N′-tetramethyl p-phenylenediamine (TMPD) and observing the development of a characteristic blue–violet colour that indicates oxidation of TMPD by cytochrome-c oxidase. PEB0122T, PEB0149 and ‘B. tamiiae’ Th239 were
positive for catalase activity, while *B. henselae* Houston-1\textsuperscript{T} was negative. All four strains were positive for cytochrome-c oxidase. Using the Microgen GnA + B-ID System kit (Microgen Bioproducts), we further tested fermentation of different substrates (glucose, mannitol, xylose, inositol, sorbitol, rhamnose, sucrose, lactose, arabinose, adonitol, raffinose and salicin), reduction of nitrate, H\textsubscript{2}S production, the activity of urease, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, tryptophan deaminase and proteolytic enzymes, indole production from tryptophan, acetoin production from glucose, utilization of citrate (as the only carbon source) and \(\beta\)-galactosidase hydrolysis of ONPG. The two ‘Alpha-1’ strains PEB0122\textsuperscript{T} and PEB0149 revealed the same metabolic characteristics in these tests. In contrast to ‘*B. tamiae*’ Th239 and *B. henselae* Houston-1\textsuperscript{T}, they were positive for xylose and arabinose fermentation, nitrate reduction and urease activity. Tests that gave differential results between the four tested strains are summarized in Table 1.

We also tested antibiotic susceptibility of strains PEB0122\textsuperscript{T} and PEB0149, ‘*B. tamiae*’ Th239 and *B. henselae* Houston-1\textsuperscript{T}. Filter discs (BD Sensi-Discs) were spotted with defined amounts of antibiotics and put directly onto CBA plates inoculated with approximately the same number of bacteria. The following antibiotics were tested: kanamycin, tetracycline, oxytetracycline, chloramphenicol and rifampicin, at concentrations of 10, 20 and 30 \(\mu\)g per disc, and ampicillin, erythromycin, gentamicin and streptomycin, at 10 and 20 \(\mu\)g per disc. Plates were cultivated for 6 days before they were inspected for zones without bacterial growth around the discs. The antibiotic-susceptible strain *Escherichia coli* K-12 MG1655 was cultivated on TSA under aerobic conditions at 37 °C for 1 day and used as a positive control for the activity of the antibiotics. Strains were considered to be resistant when no clearance zone appeared around the filter disc. Strain PEB0122\textsuperscript{T} was found to be resistant to oxytetracycline (20 \(\mu\)g) and, as indicated by slower growth, weakly resistant to tetracycline (20 \(\mu\)g), ampicillin (20 \(\mu\)g) and chloramphenicol (20 \(\mu\)g) (Table 1). Strain PEB0122\textsuperscript{T} was susceptible to all other tested antibiotics at all tested concentrations. A similar pattern was found for strain PEB0149, while ‘*B. tamiae*’ Th239 and *B. henselae* Houston-1\textsuperscript{T} showed marked differences in resistance (Table 1). Fatty acid analyses of strains PEB0122\textsuperscript{T} and ‘*B. tamiae*’ Th239 were carried out by the Identification Service of the DSMZ, Braunschweig, Germany, and compared with previously published profiles of other members of the genus *Bartonella*, including *B. henselae* Houston-1\textsuperscript{T}, *B. quintana* WA-1 and *B. bacilliformis* ATCC 35685\textsuperscript{T} (Clarridge *et al.*, 1995). The most abundant cellular fatty acids of strain PEB0122\textsuperscript{T} were palmitic acid (C\textsubscript{16 : 0}), C\textsubscript{18 : 1} \(\text{cis}7\)c and C\textsubscript{19 : 1} cyclo \(\omega8\)c. This was also the case for ‘*B. tamiae*’ Th239, except that the proportions were shifted towards markedly larger amounts of C\textsubscript{19 : 1} cyclo \(\omega8\)c and smaller amounts of palmitic acid. Previously published fatty acid compositions of members of the genus *Bartonella* (Clarridge *et al.*, 1995) showed similar results, with palmitic acid (C\textsubscript{16 : 0}) and isomers of 18- or 19-carbon fatty acids being the most abundant (Table 2). Overall, strain PEB0122\textsuperscript{T}

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**Table 1.** Differential characteristics of strains PEB0122\textsuperscript{T} and PEB0149, ‘*B. tamiae*’ Th239 and *B. henselae* Houston-1\textsuperscript{T}

<table>
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<td>MA, (A)</td>
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<td>+</td>
<td>+</td>
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<td><strong>Urease</strong></td>
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<td>+</td>
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<td><strong>Reduction of nitrate</strong></td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>Positive fermentation substrates</strong>\†</td>
<td>Glc, Xyl, Ara</td>
<td>Glc, Xyl, Ara</td>
<td>Glc, Suc</td>
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<tr>
<td><strong>Antibiotic susceptibility</strong>\‡</td>
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<td>s</td>
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<td>30 (w)</td>
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<td>20</td>
<td>30 (w)</td>
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\*A, Aerobe; MA, microaerophile.
\†Ara, Arabinose; Glc, glucose; Suc, sucrose, Xyl, xylose.
\‡S, Susceptible. Numbers correspond to the concentrations of antibiotic (\(\mu\)g per disc) to which the tested strains were resistant or weakly resistant (w); amounts lower than 10 \(\mu\)g and more than 30 \(\mu\)g were not tested.
probably comprises isomers of 18- and 19-carbon chains.

In conclusion, sequence similarity and phylogenetic position suggest that the closely related strains PEB0122T, PEB0149 and PEB0150 belong to a novel species of the genus Bartonella. Distinct growth characteristics and a number of biochemical properties discriminate strains PEB0122T and PEB0149 from other species of the genus Bartonella. Thus, we conclude that these strains can be classified within a novel species of the genus Bartonella, for which we propose the name Bartonella apis sp. nov.

### Description of Bartonella apis sp. nov.

Bartonella apis (a’pis, L. gen. fem. n. apis from a honey bee, the genus name of the honey bee Apis mellifera, referring to the insect host of this species).

Cells of this Gram-staining-negative bacterium are rod-shaped, 1.2–1.8 μm long and 0.5 μm wide. Optimal growth is achieved on CBA under microaerophilic atmosphere enriched with 5% CO₂ at 35–37 °C. Suboptimal growth is also observed under aerobic conditions.

No growth is observed under anaerobic conditions. After 2–3 days of cultivation, smooth, round, opaque-white colonies with a diameter of 0.2–0.4 mm are formed. Positive for catalase and cytochrome-c oxidase. Positive for urease, nitrate reductase and fermentation of glucose, xylose and arabinose, but negative for H₂S production, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, tryptophan deaminase, proteolysis, indole production from tryptophan, acetoin production from glucose, utilization of citrate, β-galactosidase hydrolysis of ONPG and fermentation of mannitol, inositol, sorbitol, rhamnose, sucrose, lactose, adonitol, raffinose and salicin. The most abundant cellular fatty acids are palmitic acid (C₁₆:0) and isomers of 18- and 19-carbon chains.

The type strain is PEB0122T (=NCIMB 14961T=DSM 29779T), isolated from the gut of a Western honey bee, Apis mellifera, from West Haven, CT, USA. The genomic DNA G+C content of the type strain is 45.5 mol%.

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