Campylobacter iguaniorum sp. nov., isolated from reptiles

Maarten J. Gilbert,[1] Marja Kik,[2] William G. Miller,[3] Birgitta Duim[1,4] and Jaap A. Wagenaar[1,4,5]

Correspondence
Maarten J. Gilbert
m.j.gilbert@uu.nl

[1]Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3508 TD Utrecht, The Netherlands

[2]Department Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3508 TD Utrecht, The Netherlands

[3]Produce Safety and Microbiology Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Albany, USA

[4]WHO Collaborating Centre for Campylobacter/OIE Reference Laboratory for Campylobacteriosis, Utrecht, The Netherlands

[5]Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands

During sampling of reptiles for members of the class *Epsilonproteobacteria*, strains representing a member of the genus *Campylobacter* not belonging to any of the established taxa were isolated from lizards and chelonians. Initial amplified fragment length polymorphism, PCR and 16S rRNA sequence analysis showed that these strains were most closely related to *Campylobacter fetus* and *Campylobacter hyointestinalis*. A polyphasic study was undertaken to determine the taxonomic position of five strains. The strains were characterized by 16S rRNA and *atpA* sequence analysis, matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry and conventional phenotypic testing. Whole-genome sequences were determined for strains 1485E^T and 2463D, and the average nucleotide and amino acid identities were determined for these strains. The strains formed a robust phylogenetic clade, divergent from all other species of the genus *Campylobacter*. In contrast to most currently known members of the genus *Campylobacter*, the strains showed growth at ambient temperatures, which might be an adaptation to their reptilian hosts. The results of this study clearly show that these strains isolated from reptiles represent a novel species within the genus *Campylobacter*, for which the name *Campylobacter iguaniorum* sp. nov. is proposed. The type strain is 1485E^T (=LMG 28143^T=CCUG 66346^T).

Strains of species of the genus *Campylobacter* have been isolated from various vertebrate hosts, including mammals, birds and reptiles. In general, whereas mammals and birds are endothermic and have more constant body temperatures (homeothermic), reptiles are ectothermic and largely dependent on external heat sources for their body temperatures, which can show considerable fluctuations (poikilothermic). Consequently, species of the genus *Campylobacter* occurring in poikilothermic reptiles have to be adapted to larger temperature ranges and on average lower temperatures than species of the genus *Campylobacter* occurring in homeothermic animals. All currently known species of the genus *Campylobacter* are predominantly associated with mammals and birds. The only known species of the genus *Campylobacter* isolated from reptiles is a distinct genetic variant of *Campylobacter fetus* (Harvey & Greenwood, 1985; Wang *et al.*, 2013; Fitzgerald *et al.*, 2014), which has been shown to cause systemic infections in humans (Tu *et al.*, 2004; Patrick *et al.*, 2013). Here we describe a novel species of the genus *Campylobacter* exclusively isolated from reptiles, distinct from all currently known species of the genus *Campylobacter*.

In 2003, a diagnostic screening for members of the genus *Campylobacter* in a large variety of avian, mammalian and reptilian species was performed (unpublished results). Cloacal and rectal swabs were obtained from the animals and were plated directly onto blood agar, CCDA and

Abbreviations: AAI average amino acid identity; ANI, average nucleotide identity; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 1485E^T, 2463D, 12S02285-1, 12S02842-24 and 13S00387-3 are KF425533, KF425532, KF787891, KF787892 and KF787893, respectively. The complete annotated genome sequence of strain 1485E has been deposited in GenBank with the accession numbers CP009043 (chromosome) and CP009044 (megaplasmid).

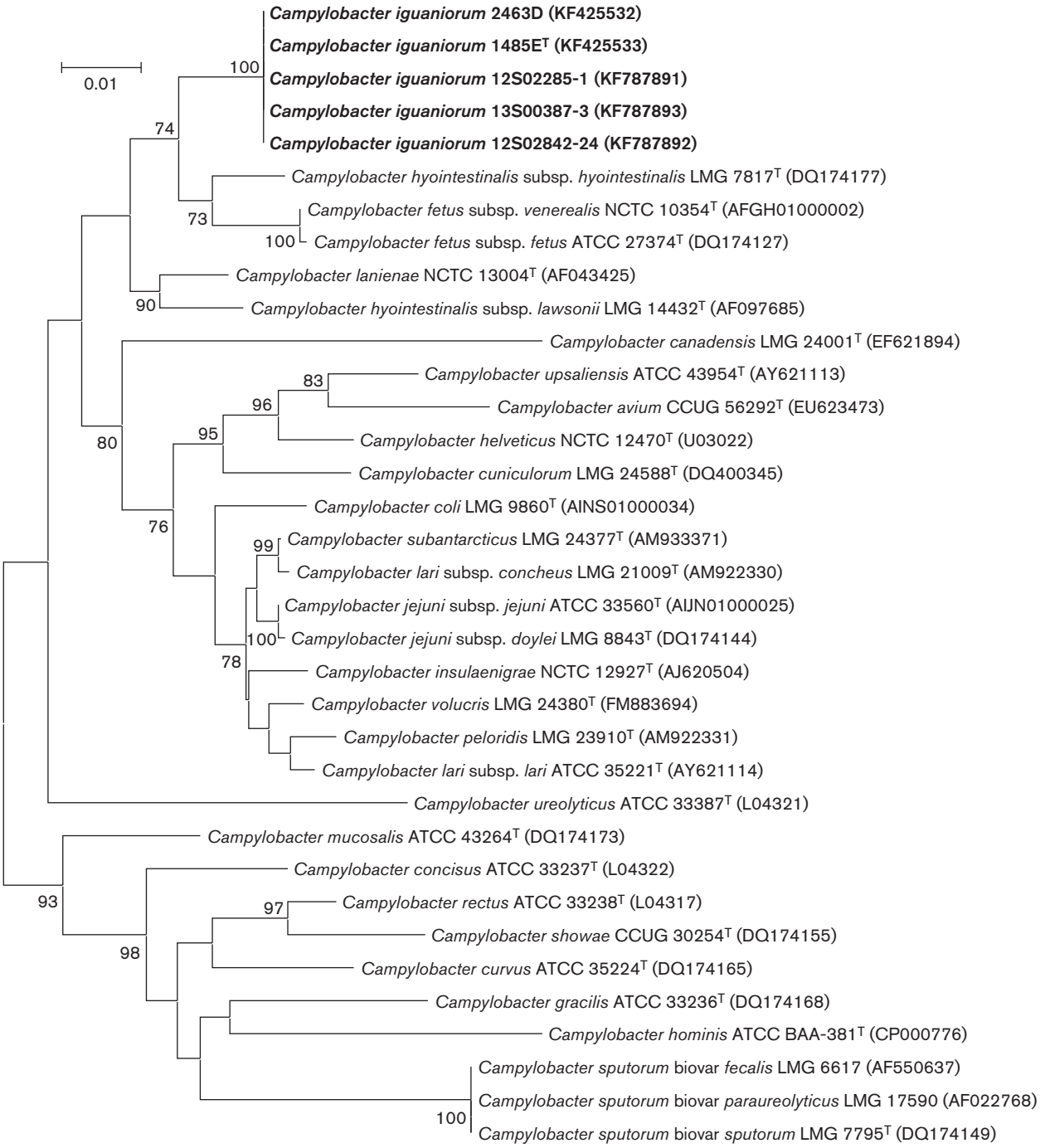

Fig. 1. Neighbour-joining phylogenetic dendrogram based on 16S rRNA sequences. Bootstrap values ($\geqslant$70 %) based on 500 replications are indicated at the nodes. The scale bar represents the number of base substitutions per site.

Skirrow agar plates (Biotrading) and incubated in a microaerobic atmosphere (83.3 % N_2, 7.1 % CO_2, 3.6 % H_2 and 6 % O_2) at 37 °C for 48 h. Eight isolates resembling members of the genus *Campylobacter* were obtained from two lizards kept in captivity: a bearded dragon (*Pogona vitticeps*) and a green iguana (*Iguana iguana*). Initial characterization using PCR with primers targeting members of the genera *Campylobacter*, *Arcobacter* and *Helicobacter* (Marshall *et al.*, 1999), amplified fragment length polymorphism analysis (Duim *et al.*, 1999) and 16S rRNA gene sequencing (Maiwald, 2004) indicated that these isolates were different from all other species of the genus *Campylobacter*, but related to *C. fetus* and *Campylobacter hyointestinalis*. Two strains, 1485E^T and 2463D, originating

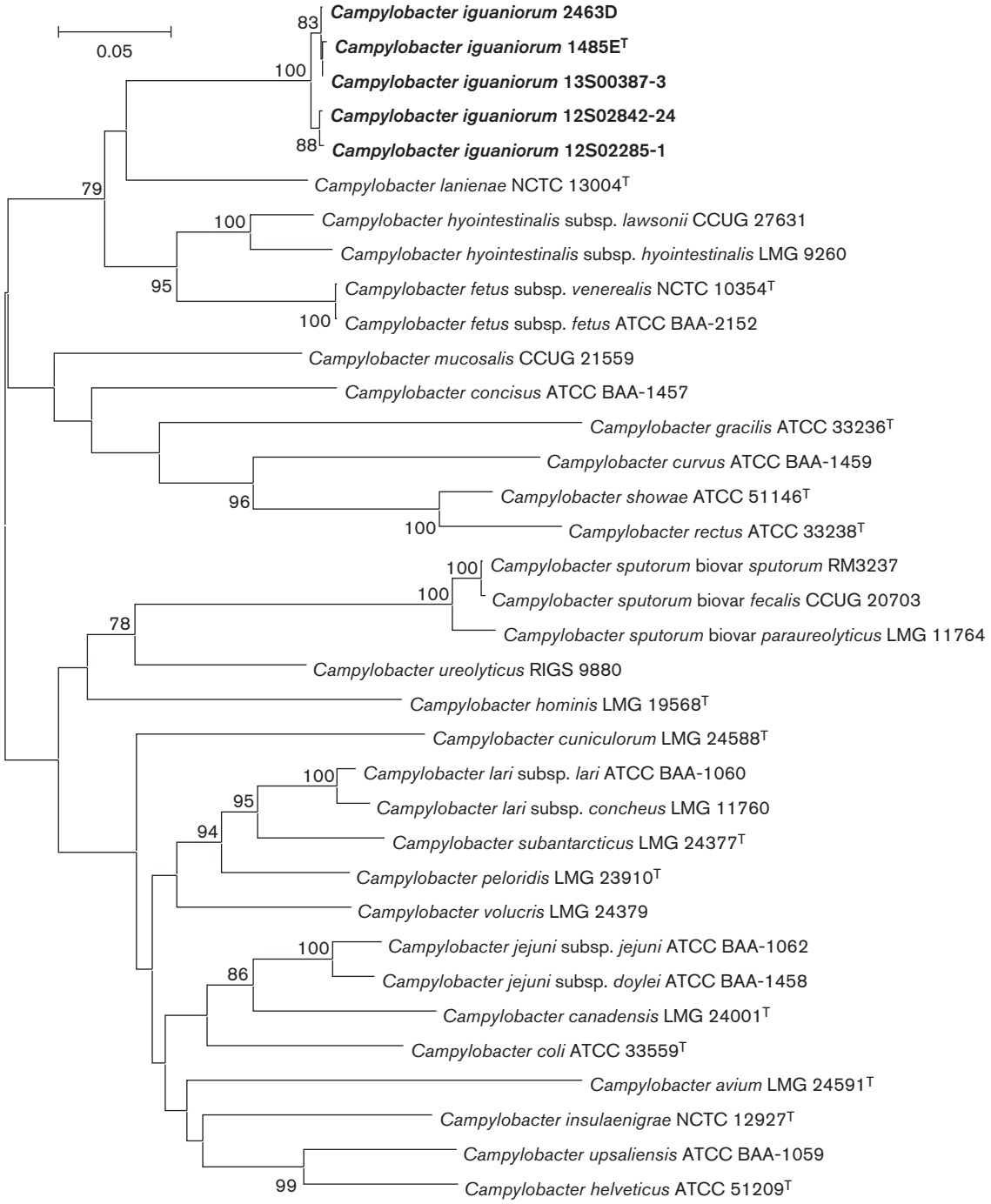

Fig. 2. Neighbour-joining phylogenetic dendrogram based on partial *atpA* sequences (489 bp). Bootstrap values ($\geqslant$70 %) based on 500 replications are indicated at the nodes. The scale bar represents the number of base substitutions per site.

from the bearded dragon and green iguana, respectively, were used for further characterization. A strain with highly similar 16S rRNA sequence has been isolated from a leopard tortoise (*Stigmochelys pardalis*) in an independent study (Benejat *et al.*, 2014). During a subsequent screening (2011–2013) for members of the class *Epsilonproteobacteria* in reptiles (Gilbert *et al.*, 2014a), another 95 isolates representing members of the genus *Campylobacter* with 16S rRNA gene sequences identical to 1485E^T and 2463D were obtained from lizards and chelonians. Three strains, 12S02285-1, 12S002842-24 and 13S00387-3, obtained from a chuckwalla (*Sauromalus ater*), an Aldabra giant tortoise (*Aldabrachelys gigantea*) and a red-footed tortoise (*Chelonoidis carbonaria*), respectively, were selected for

further analysis in support of strains 1485E^T and 2463D. A polyphasic study was undertaken to determine the taxonomic position of these selected strains.

Strains 1485E^T and 2463D were examined by whole-genome sequencing. Based on the whole-genome sequences, the average nucleotide and amino acid identities were determined for these strains. For all strains, comparisons based on 16S rRNA and *atpA* gene sequences and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS were made to determine genotypic and phenotypic characters, respectively. In addition to this, phenotypic characteristics were determined by conventional biochemical testing for all strains.

Strains were grown on Colombia agar with 5 % sheep blood (Oxoid) in a microaerobic atmosphere at 37 °C for 48 h. Bacterial genomic DNA was isolated following the Gram-negative bacteria protocol of the High Pure PCR template preparation kit (Roche Diagnostics).

Whole-genome sequences of strains 1485E^T and 2463D were obtained with a Roche 454 FLX Genome sequencer (Gilbert *et al.*, 2014b). A minimum of 292 000 mate-paired and shotgun reads were assembled for each strain to provide draft genome sequences with an average coverage of 66×. All 454 base calls were validated using Illumina MiSeq reads, adding an additional average coverage of 220×. Using Perl scripts, draft contigs were assembled into single predicted contiguous sequences. Sequences across the contig junctions were confirmed by PCR and Sanger sequencing. Polymeric tracts and putative split genes were validated using Sanger sequencing and high-depth MiSeq reads. The complete annotated genome sequence of strain 1485E^T has been deposited in GenBank with the accession numbers CP009043 (chromosome) and CP009044 (megaplasmid).

The taxonomic position of all strains was determined by 16S rRNA gene sequence comparison. The 16S rRNA gene sequences (⩾1333 bp) were obtained from the whole-genome sequences of strains 1485E^T and 2463D; sequenced as described by Gilbert *et al.* (2014a) for strains 12S02285-1, 12S002842-24 and 1300387-3; and obtained from GenBank for the other species of the genus *Campylobacter*. Sequence alignment and tree reconstruction were performed using Muscle and MEGA v.5.2 (Tamura *et al.*, 2011). A neighbour-joining tree containing all species of the genus *Campylobacter* with validly published names was reconstructed. Bootstrap values were determined using 500 repetitions. The 16S rRNA sequence similarity between the strains was 100 %, while the sequence similarity between the strains and the closest relatives *C. fetus* (ATCC 27374^T) and *C. hyointestinalis* (LMG 7817^T) was 97 %. The strains formed a distinct clade with *C. fetus*, *C. hyointestinalis* and *Campylobacter lanienae* (Fig. 1).

For improved species resolution (Miller *et al.*, 2014), partial *atpA* sequences (489 bp) extracted from the whole-genome sequences of strains 1485E^T and 2463D, sequenced as described by Gilbert *et al.* (2014a) for strains 12S02285-1, 12S002842-24 and 1300387-3, and obtained from the MLST database for the genus *Campylobacter* (http://pubmlst.org/campylobacter) for other species of the genus *Campylobacter*, were aligned with Muscle. A neighbour-joining phylogenetic tree was reconstructed with MEGA v.5.2 and bootstrap values were determined using 500 repetitions. In line with the 16S rRNA comparison, the strains formed a clade distinct from other species of the genus *Campylobacter* (Fig. 2).

The average nucleotide identity (ANI) can be used as an alternative to DNA–DNA hybridization (DDH) (Konstantinidis & Tiedje, 2005a; Konstantinidis *et al.*, 2006). A DDH species delineation of 70 % corresponds to 95 % ANI (Goris *et al.*, 2007). Using JSpecies v.1.2.1 (Richter & Rosselló-Móra, 2009) and BLAST v.2.2.26, ANI values based on whole-genome sequences were calculated for strains 1485E^T and 2463D and the most closely related species *C. fetus* (strain 82-40), *C. hyointestinalis* subsp. *hyointestinalis* (strain LMG 9260), *C. hyointestinalis* subsp. *lawsonii* (strain CCUG 27631) and *C. lanienae* (strain NCTC 13004^T). The BLAST parameters were: X=150, q=−1, F=F, e=1e−15 and a=2. Strains 1485E^T and 2463D were highly homologous (ANI ⩾99 %) and above the 95 % species cut-off (Table 1). The ANI between strains 1485E^T and 2463D and *C. fetus*, *C. hyointestinalis* and *C. lanienae* had a maximum value of 76 %, which is well below the 95 % species cut-off and similar to ANI values observed between the other species.

In support of the ANI values, the predicted proteomes (based on the whole-genome sequences) of strains 1485E^T and 2463D were determined and compared with those of other related species of the genus *Campylobacter* using pairwise BLASTP analysis. The core proteomes (i.e. proteins conserved across all tested taxa) for these members of the genus *Campylobacter* were identified and the average

Table 1. Average nucleotide identity (ANI) values (in percentages) based on BLAST for *C. iguaniorum* sp. nov. and the most closely related members of the genus *Campylobacter*

Strains: 1, *C. iguaniorum* sp. nov. 1485E^T; 2, *C. iguaniorum* sp. nov. 2463D; 3, *C. fetus* subsp. *fetus* 82-40; 4, *C. fetus* subsp. *venerealis* 97/608; 5, *C. hyointestinalis* subsp. *hyointestinalis* LMG 9260; 6, *C. hyointestinalis* subsp. *lawsonii* CCUG 27631; 7, *C. lanienae* NCTC 13004^T.

Strain	1	2	3	4	5	6	7
1	100	99	75	75	75	76	72
2	99	100	75	75	76	76	72
3	75	75	100	100	78	77	72
4	75	75	100	100	78	77	72
5	75	76	78	78	100	94	72
6	75	75	77	77	94	100	74
7	72	72	72	72	72	74	100

Table 2. Average amino acid identity (AAI) values (in percentages) based on BLAST for *C. iguaniorum* sp. nov. and the most closely related members of the genus *Campylobacter*

Strains: 1, *C. iguaniorum* sp. nov. 1485E^T; 2, *C. iguaniorum* sp. nov. 2463D; 3, *C. fetus* subsp. *fetus* 82-40; 4, *C. fetus* subsp. *venerealis* 97/608; 5, *C. hyointestinalis* subsp. *hyointestinalis* LMG 9260; 6, *C. hyointestinalis* subsp. *lawsonii* CCUG 27631; 7, *C. lanienae* NCTC 13004^T.

Strain	1	2	3	4	5	6	7
1	100	100	78	78	78	78	70
2	100	100	78	78	78	78	70
3	78	78	100	100	79	78	71
4	78	78	100	100	79	78	71
5	79	79	79	79	100	95	70
6	78	78	79	79	95	100	71
7	71	70	72	71	71	72	100

amino acid identity (AAI) of these core proteins between any two taxa was used as a determinant of genetic divergence (Konstantinidis & Tiedje, 2005b), following Lan & Reeves (2000) who proposed that the core genome is the principle genomic unit defining bacterial species. An AAI of 78 % was observed between the proteomes common to both strains and the most closely related known species *C. fetus* and *C. hyointestinalis*. Analyses indicated a high degree of both synteny and similarity in the core proteomes of strains 1485E^T and 2463D, which were >99 % similar on average (Table 2). The AAI for both strains and the most closely related species *C. fetus* and *C. hyointestinalis* was <80 %, while the two latter species share 78–80 % AAI. These results indicate that strains 1485E^T and 2463D form a distinct cluster, which is clearly separated from the most closely related species of the genus *Campylobacter*.

The chromosomal DNA G+C content was determined based on the whole-genome sequences using Artemis v.13.2 (Wellcome Trust Sanger Institute) (Rutherford *et al.*, 2000). Strains 1485E^T and 2463D had a DNA G+C content of 35.9 % and 35.7 %, respectively, which is within the range reported for members of the genus *Campylobacter* (29–47 %) (Debruyne *et al.*, 2008).

In accordance with suggestions made by Stackebrandt *et al.* (2002), phenotypic characterization of all unidentified strains and type strains of selected species of the genus *Campylobacter* was performed using MALDI-TOF MS. Spectra were acquired with a MicroFlex LT mass spectrometer (Bruker Daltonics) and recorded in a mass range from 2000 to 20 000 kDa. For each strain of a member of the genus *Campylobacter*, three technical replicates were spotted on the target plate. Two spectra were acquired per spot, resulting in six spectra per strain. Each spectrum was exported and examined visually with BioNumerics v.7.1 (Applied Maths). After processing (baseline subtraction and smoothing), six spectra were summarized in a newly generated spectrum. Peak-based clustering (Pearson correlation; UPGMA) with all species of the genus *Campylobacter* present in the Bruker database confirmed phenotypic identification of these strains as representatives of a unique taxon related to *C. fetus* and *C. hyointestinalis* (Fig. 3).

Additional phenotypic testing was performed as described previously (Elharrif & Mégraud, 1986a, 1986b; On & Holmes, 1991a, b, 1992; Ursing *et al.*, 1994). Oxidase activity, catalase activity, nitrate reduction, indoxyl acetate hydrolysis, urea hydrolysis, hippurate hydrolysis and H_2S production on TSI agar were determined. In addition to this, growth with 1 % glycine, γ-glutamyltransferase activity, α-haemolysis, H_2 requirement and susceptibility to nalidixic acid (30 μg) and cefalotin (30 μg) were evaluated. Strains were grown at various temperatures and under various atmospheres. The strains displayed phenotypic characters distinct from all other members of the genus *Campylobacter*. In contrast to *C. fetus* and *C. hyointestinalis*, all strains showed γ-glutamyltransferase activity. All strains showed growth at 25 °C and 37 °C, but no growth at 42 °C. The only other members of the genus *Campylobacter* showing growth at 25 °C after 72 h are *C. fetus* and *C. hyointestinalis* subsp. *hyointestinalis* (On *et al.*, 1996). Most *C. fetus* subsp. *fetus*, but not *C. fetus* subsp. *venerealis* and all *C. hyointestinalis* strains are able to grow at 42 °C. Additional testing showed

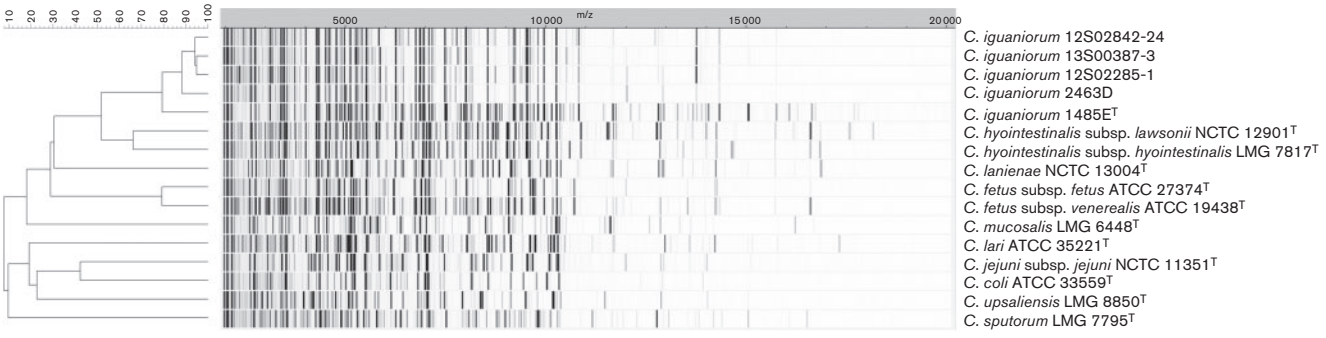

Fig. 3. Dendrogram based on MALDI-TOF MS spectra of *C. iguaniorum* sp. nov. and selected members of the genus *Campylobacter*.

Table 3. Characteristics differentiating the novel strains from other species of the genus *Campylobacter* Strain designations and the number of strains are only indicated for *C. iguaniorum*, which is in line with previous Campylobacter species descriptions. For the other species, strain designations and the number of strains can be found in the original species descriptions and the publications referred to.

Taxa: 1, *C. iguaniorum* sp. nov. (n=5); 2, *C. avium*; 3, *C. canadensis*; 4, *C. coli*; 5, *C. concisus*; 6, *C. cuniculorum*; 7, *C. curvus*; 8, *C. fetus* subsp. *fetus*; 9, *C. fetus* subsp. *venerealis*; 10, *C. gracilis*; 11, *C. helveticus*; 12, *C. hominis*; 13, *C. hyointestinalis* subsp. *hyointestinalis*; 14, *C. hyointestinalis* subsp. *lawsonii*; 15, *C. insulaenigrae*; 16, *C. jejuni* subsp. *doylei*; 17, *C. jejuni* subsp. *jejuni*; 18, *C. lanienae*; 19, *C. lari* subsp. *concheus*; 20, *C. lari* subsp. *lari*; 21, *C. mucosalis*; 22, *C. peloridis*; 23, *C. rectus*; 24, *C. showae*; 25, *C. sputorum*; 26, *C. subantarcticus*; 27, *C. upsaliensis*; 28, *C. volucris*. Characteristics of reference taxa were adapted from On *et al.* (1996), Debruyne *et al.* (2009) and Debruyne *et al.* (2010). +, 90–100 % trains positive; (+), 75–89 % strains positive; V, 26–74 % strains positive; (−), 11–25 % strains positive; −, 0–10 % strains positive; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Oxidase	+	+	+	+	V	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	V	+	+	+	+
Catalase	+	+	V	+	−	−	−	+	(+)	(−)	−	−	+	+	+	V	+	+	+	+	−	+	(−)	+	V**	+	−	+
Urease	−	−	V	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	ND	−	−	V†	ND	−	ND
Nitrate reduction	+	+	V	+	(−)	+	+	+	(+)	(+)	+	V	+	+	−	−	+	+	+	+	(−)	ND	+	+	(+)	+	+	+
Hippurate hydrolysis	−	+	−	−	−	−	(−)	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−
Indoxyl acetate hydrolysis	−	+	−	+	−	+	V	−	−	(+)	+	−	−	−	−	+	+	−	ND	−	−	ND	+	V	−	+	−	−
γ-Glutamyltransferase	+	−	+	−	−	−	ND	−	−	ND	−	ND	−	−	ND	−	−	ND	ND	−	ND	ND	ND	ND	−	ND	−	ND
H_2S production (TSI)	+	−	V	−	−	−	(−)	−	−	−	−	−	+	+	−	−	−	−	ND	−	+	ND	−	V	+	−	−	ND
α-Haemolysis	+	−	−	(−)	(−)	+	(−)	−	V	−	+	ND	V	V	ND	+	+	+	ND	+	−	ND	+	+	+	+	+	ND
Growth at/in/on:																												
18–22 °C (microaerobic)	+	ND	ND	−	−	ND	−	(+)	(−)	−	−	ND	(−)	−	ND	−	−	ND	ND	−	−	ND	−	−	−	ND	−	−
25 °C (microaerobic)	+	−	−	−	−	−	−	+	+	−	−	−	(−)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
37 °C (microaerobic)	+	+	+	+	+	+	V	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	V	+	+	+	+
42 °C (microaerobic)	−	+	+	+	(+)	(+)	V	(+)	−	V	+	(−)	+	+	−	−	+	+	+	+	+	+	(−)	V	+	+	+	+
37 °C (anaerobic)	+*	−	+	−	+	−	+	(−)	V	+	−	+	−	+	−	−	−	+	ND	−	+	ND	+	+	+	ND	−	+*
37 °C (aerobic)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	ND	−	−
CCDA	+	−	+	+	(−)	(+)	(+)	+	+	V	+	ND	+	+	ND	+	+	ND	+	+	+	+	−	+	(+)	ND	+	ND
Glycine (1 %)	+	−	V	(+)	(−)	−	+	+	(−)	+	V	+	+	V	−	(−)	+	−	(+)	+	(−)	+	+	V	+	(+)	+	−
Resistance to:																												
Nalidixic acid (30 μg)	+	−	V	−	(+)	V	+	+	V	V	−	V	+	+	+	−	−	+	−	(+)	(+)	(+)	(+)	−	(+)	+	−	+
Cefalotin (30 μg)	−	+	−	+	−	(+)	−	−	−	−	−	−	(−)	−	+	−	+	+	+	+	−	(−)	−	−	−	−	(−)	+
H_2 requirement	−	V	−	−	+	−	+	−	−	+	−	+	V	V	ND	−	−	−	ND	−	+	ND	+	+	−	ND	−	ND
S-layer present	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
DNA G+C content (mol%)	36	35	ND	31	37–41	32	45–46	33–35	33–34	44–46	34	32–33	35–36	31–33	ND	31	30–31	36	30	29–30	36–38	29	45–46	44–46	29–33	30	32–36	29

*Weak growth.

†Test results differ between *C. sputorum* biovars *sputorum* (catalase and urease-negative), *paraureolyticus* (catalase-negative, urease-positive) and *fecalis* (catalase-positive, urease-negative).

that all unidentified strains, but not *C. hyointestinalis* subsp. *hyointestinalis* (strains LMG 7817^T, LMG 8540, LMG 9276, LMG 15912 and CCUG 14170), showed growth at room temperature (18–22 °C) after 72 h. In addition to this, the strains could be distinguished from *C. fetus* by H_2S production on TSI agar and by the absence of an S-layer, a proteinaceous external layer characteristic of *C. fetus*, which can be assessed by either Western blotting or PCR (Blaser *et al.*, 2008). Differentiating characteristics for the strains tested and other members of the genus *Campylobacter* are summarized in Table 3.

In conclusion, the results from this polyphasic taxonomic study clearly indicate that the isolates recovered from the faecal contents from reptiles represent a novel species distinct from other currently known species of the genus *Campylobacter*, on the basis of 16S rRNA, *atpA* and whole-genome sequence comparison and MALDI-TOF MS, growth temperature and biochemical properties. To this date, reptiles have been shown to be the primary and only reservoir, representing a unique niche for members of the genus *Campylobacter*. The name *Campylobacter iguaniorum* sp. nov. is proposed.

Description of *Campylobacter iguaniorum* sp. nov.

Campylobacter iguaniorum (i.gua.ni.o′rum. N.L. pl. gen. n. *iguaniorum* of Iguania, the lizard suborder comprising agamid, chameleon and iguana families).

Gram-stain-negative slightly curved to spiral-shaped rods. After incubation on Colombia agar (5 % sheep blood) in a microaerobic atmosphere at 37 °C for 48 h, colonies are beige to taupe, glossy, slightly convex, round with smooth margins, 1–1.5 mm in diameter and show α-haemolysis. Grows under microaerobic and anaerobic conditions (although growth is scanty anaerobically), but not under aerobic conditions. H_2 is not required. After 72 h, growth can be observed on Colombia agar (5 % sheep blood) at room temperature, 25 °C and 37 °C, but not at 42 °C. Oxidase, catalase and γ-glutamyltransferase activity are observed in all tested strains. All strains show nitrate reduction and H_2S production on TSI agar, but no indoxyl acetate hydrolysis, urea hydrolysis or hippurate hydrolysis. Displays normal growth in the presence of 1 % glycine, on IST agar and slightly inhibited growth on CCD, Mueller–Hinton, Preston and Skirrow agar at 37 °C. All strains are resistant to nalidixic acid (30 μg) but susceptible to cefalotin (30 μg).

Pathogenicity is unknown; strains have been recovered both from reptiles with and without clinical signs of disease (Benejat *et al.*, 2014; Gilbert *et al.*, 2014a). Strains have been recovered from lizard and chelonian intestinal contents. The type strain is 1485E^T (=LMG 28143^T= CCUG 66346^T), which was isolated from a bearded dragon (*Pogona vitticeps*) in 2003.

Acknowledgements

We thank Jean Euzéby (École Nationale Vétérinaire, France) for support in naming the novel species, Jan Sinnige (University Medical Center Utrecht, The Netherlands) for support with the MALDI-TOF MS analysis and Linda van der Graaf-van Bloois (Utrecht University, The Netherlands) for support in phenotypic analysis.

References

Benejat, L., Gravet, A., Sifré, E., Ben Amor, S., Quintard, B., Mégraud, F. & Lehours, P. (2014). Characterization of a *Campylobacter fetus*-like strain isolated from the faeces of a sick leopard tortoise (*Stigmochelys pardalis*) using matrix-assisted laser desorption/ionization time of flight as an alternative to bacterial 16S rDNA phylogeny. *Lett Appl Microbiol* **58**, 338–343.

Blaser, M. J., Newell, D. G., Thompson, S. A. & Zechner, E. L. (2008). Pathogenesis of *Campylobacter fetus*. In *Campylobacter*, pp. 401–428. Edited by I. Nachamkin, C. M. Szymanski & M. J. Blaser. Washington, DC: American Society for Microbiology.

Debruyne, L., Gevers, D. & Vandamme, P. (2008). Taxonomy of the family *Campylobacteraceae*. In *Campylobacter*, pp. 3–25. Edited by I. Nachamkin, C. M. Szymanski & M. J. Blaser. Washington, DC: American Society for Microbiology.

Debruyne, L., On, S. L., De Brandt, E. & Vandamme, P. (2009). Novel *Campylobacter lari*-like bacteria from humans and molluscs: description of *Campylobacter peloridis* sp. nov., *Campylobacter lari* subsp. *concheus* subsp. nov. and *Campylobacter lari* subsp. *lari* subsp. nov. *Int J Syst Evol Microbiol* **59**, 1126–1132.

Debruyne, L., Broman, T., Bergström, S., Olsen, B., On, S. L. & Vandamme, P. (2010). *Campylobacter volucris* sp. nov., isolated from black-headed gulls (*Larus ridibundus*). *Int J Syst Evol Microbiol* **60**, 1870–1875.

Duim, B., Wassenaar, T. M., Rigter, A. & Wagenaar, J. (1999). High-resolution genotyping of *Campylobacter* strains isolated from poultry and humans with amplified fragment length polymorphism fingerprinting. *Appl Environ Microbiol* **65**, 2369–2375.

Elharrif, Z. & Mégraud, F. (1986a). Characterization of thermophilic *Campylobacter*. I. Carbon-substrate utilization tests. *Curr Microbiol* **13**, 117–122.

Elharrif, Z. & Mégraud, F. (1986b). Characterization of thermophilic *Campylobacter*. II. Enzymatic profiles. *Curr Microbiol* **13**, 317–322.

Fitzgerald, C., Tu, Z. C., Patrick, M., Stiles, T., Lawson, A. J., Santovenia, M., Gilbert, M. J., van Bergen, M., Joyce, K. & other authors (2014). *Campylobacter fetus* subsp. *testudinum* subsp. nov., isolated from humans and reptiles. *Int J Syst Evol Microbiol* **64**, 2944–2948.

Gilbert, M. J., Kik, M., Timmerman, A. J., Severs, T. T., Kusters, J. G., Duim, B. & Wagenaar, J. A. (2014a). Occurrence, diversity, and host association of intestinal *Campylobacter*, *Arcobacter*, and *Helicobacter* in reptiles. *PLoS ONE* **9**, e101599.

Gilbert, M. J., Miller, W. G., Yee, E., Kik, M., Wagenaar, J. A. & Duim, B. (2014b). Complete genome sequence of *Campylobacter iguaniorum* strain 1485E^T, isolated from a bearded dragon (*Pogona vitticeps*). *Genome Announc* **2**, e00844-14.

Goris, J., Konstantinidis, K. T., Klappenbach, J. A., Coenye, T., Vandamme, P. & Tiedje, J. M. (2007). DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* **57**, 81–91.

Harvey, S. & Greenwood, J. R. (1985). Isolation of *Campylobacter fetus* from a pet turtle. *J Clin Microbiol* **21**, 260–261.

Konstantinidis, K. T. & Tiedje, J. M. (2005a). Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci U S A* **102**, 2567–2572.

Konstantinidis, K. T. & Tiedje, J. M. (2005b). Towards a genome-based taxonomy for prokaryotes. *J Bacteriol* **187**, 6258–6264.

Konstantinidis, K. T., Ramette, A. & Tiedje, J. M. (2006). The bacterial species definition in the genomic era. *Philos Trans R Soc Lond B Biol Sci* **361**, 1929–1940.

Lan, R. & Reeves, P. R. (2000). Intraspecies variation in bacterial genomes: the need for a species genome concept. *Trends Microbiol* **8**, 396–401.

Maiwald, M. (2004). Broad-range PCR for detection and identification of bacteria. In *Molecular Microbiology: Diagnostics Principles and Practice*, pp. 379–390. Edited by D. H. Persing, F. C. Tenover, J. Versalovic, Y.-W. Tang, D. Relman & T. J. White. Washington, DC: American Society for Microbiology.

Marshall, S. M., Melito, P. L., Woodward, D. L., Johnson, W. M., Rodgers, F. G. & Mulvey, M. R. (1999). Rapid identification of *Campylobacter*, *Arcobacter*, and *Helicobacter* isolates by PCR-restriction fragment length polymorphism analysis of the 16S rRNA gene. *J Clin Microbiol* **37**, 4158–4160.

Miller, W. G., Yee, E., Jolley, K. A. & Chapman, M. H. (2014). Use of an improved *atpA* amplification and sequencing method to identify members of the *Campylobacteraceae* and *Helicobacteraceae*. *Lett Appl Microbiol* **58**, 582–590.

On, S. L. & Holmes, B. (1991a). Effect of inoculum size on the phenotypic characterization of *Campylobacter* species. *J Clin Microbiol* **29**, 923–926.

On, S. L. & Holmes, B. (1991b). Reproducibility of tolerance tests that are useful in the identification of campylobacteria. *J Clin Microbiol* **29**, 1785–1788.

On, S. L. & Holmes, B. (1992). Assessment of enzyme detection tests useful in identification of campylobacteria. *J Clin Microbiol* **30**, 746–749.

On, S. L., Holmes, B. & Sackin, M. J. (1996). A probability matrix for the identification of campylobacters, helicobacters and allied taxa. *J Appl Bacteriol* **81**, 425–432.

Patrick, M. E., Gilbert, M. J., Blaser, M. J., Tauxe, R. V., Wagenaar, J. A. & Fitzgerald, C. (2013). Human infections with new subspecies of *Campylobacter fetus*. *Emerg Infect Dis* **19**, 1678–1680.

Richter, M. & Rosselló-Móra, R. (2009). Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* **106**, 19126–19131.

Rutherford, K., Parkhill, J., Crook, J., Horsnell, T., Rice, P., Rajandream, M. A. & Barrell, B. (2000). Artemis: sequence visualization and annotation. *Bioinformatics* **16**, 944–945.

Stackebrandt, E., Frederiksen, W., Garrity, G. M., Grimont, P. A., Kämpfer, P., Maiden, M. C., Nesme, X., Rosselló-Mora, R., Swings, J. & other authors (2002). Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Tu, Z. C., Zeitlin, G., Gagner, J. P., Keo, T., Hanna, B. A. & Blaser, M. J. (2004). *Campylobacter fetus* of reptile origin as a human pathogen. *J Clin Microbiol* **42**, 4405–4407.

Ursing, J. B., Lior, H. & Owen, R. J. (1994). Proposal of minimal standards for describing new species of the family *Campylobacteraceae*. *Int J Syst Bacteriol* **44**, 842–845.

Wang, C. M., Shia, W. Y., Jhou, Y. J. & Shyu, C. L. (2013). Occurrence and molecular characterization of reptilian *Campylobacter fetus* strains isolated in Taiwan. *Vet Microbiol* **164**, 67–76.