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TAXONOMIC DESCRIPTION
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Proposal of new combination, *Cutibacterium acnes* subsp. *elongatum* comb. nov., and emended descriptions of the genus *Cutibacterium*, *Cutibacterium acnes* subsp. *acnes* and *Cutibacterium acnes* subsp. *defendens*

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Abstract

In 2016, division of the genus *Propionibacterium* into four distinct genera was proposed. As a consequence, the species *Propionibacterium acnes* was transferred to *Cutibacterium* gen. nov. as *Cutibacterium acnes* comb. nov. The three recently proposed subspecies of *P. acnes* were not, however, accommodated in this proposal. Following a very recent validation of a new combination for *C. acnes* subsp. *defendens* and an automatically created *C. acnes* subsp. *acnes*, we now propose the new combination, *C. acnes* subsp. *elongatum* comb. nov. The type strain of *Cutibacterium acnes* subsp. *elongatum* is JCM 18919^T (=NCTC 13655^T). On the basis of further genomic and phenotypic (haemolysis and MALDI-TOF mass spectrometry) analyses of these subspecies, we also provide emended descriptions of the genus *Cutibacterium* Scholz and Kilian 2016, *C. acnes* subsp. *acnes* (Gilchrist 1900) Nouioui *et al.* 2018, and *C. acnes* subsp. *defendens* (McDowell *et al.* 2016) Nouioui *et al.* 2018.

The bacterial species *Cutibacterium acnes* (basonym: *Propionibacterium acnes*) is one of the most abundant residents of the human skin microbiota. There has been a rich history of investigation into the medical relevance of this organism since its successful isolation from acne pus [1] and its proposal as a novel species in the late nineteenth century [2]. Three distinct groups of *C. acnes*, designated types I, II, and III, have been well described based on a combination of genetic, phenotypic, and morphological differences, as well as pathogenic profile [3–9] (Fig. 1). These differences have ultimately led to the proposal of *P. acnes* subsp. *acnes* for type I [10, 11], *P. acnes* subsp. *defendens* for type II [11], and *P. acnes* subsp. *elongatum* for type III [10].

In parallel to the proposal of these distinct phylogroups as subspecies, it has recently been proposed to divide the genus *Propionibacterium* into four genera based on whole genome sequence analysis and source of isolation [12]. As part of this proposal, *P. acnes* was placed within the new genus *Cutibacterium*, although this taxonomic re-appraisal has not met with universal acceptance [13]. Due to the simultaneous timelines of these independent proposals, the three subspecies were not described under the proposal of *Cutibacterium acnes* comb. nov. However, very recently, *C. acnes* subsp. *defendens* was proposed [14] and validated with automatically created *C. acnes* subsp. *acnes* as part of the latest Validation List [15]. As a consequence, this leaves *P. acnes* subsp. *elongatum*, which we now propose as *C. acnes* subsp. *elongatum*.

The 16S rRNA gene dendrogram generated from the DDBJ/EMBL/GenBank database entries of type strains of all three *C. acnes* subspecies and all other *Cutibacterium* species and '*Propionibacterium humerusii*', which has not been validated yet, outlined the clade formation of the three *C. acnes* subspecies within genus *Cutibacterium* (Fig. 1). Upon phylogenetic analysis of concatenated protein-encoding gene sequences from all known *C. acnes* sequence types (STs) in the MLST$_8$ database (http://pubmlst.org/pacnes/), *C. acnes* subsp. *elongatum* clearly forms a highly distinct clade from *C. acnes* subsp. *acnes* and *C. acnes* subsp. *defendens* (Fig. 2), consistent with its proposal as a distinct subspecies [10].

Further analysis based on *in silico* DNA–DNA hybridization (using the Genome-to-Genome Distance Calculator 2.1) of

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Keywords: *Cutibacterium*; *Cutibacterium acnes* subspecies *elongatum*; genome; haemolysis; subspecies; MALDI-TOF mass spectrometry.
Abbreviations: MALDI-TOF MS, matrix-assisted laser desorption and ionization time-of-flight mass spectrometry; MLST, multilocus sequence typing.
The DDBJ/EMBL/GenBank accession numbers for the 16S rRNA gene and the draft genome sequence of the type strain of *C. acnes* subsp. *elongatum comb.* nov. (JCM 18919^T) are AB777861 and BFFM01000001–BFFM01000226, respectively.
One supplementary figure is available with the online version of this article.

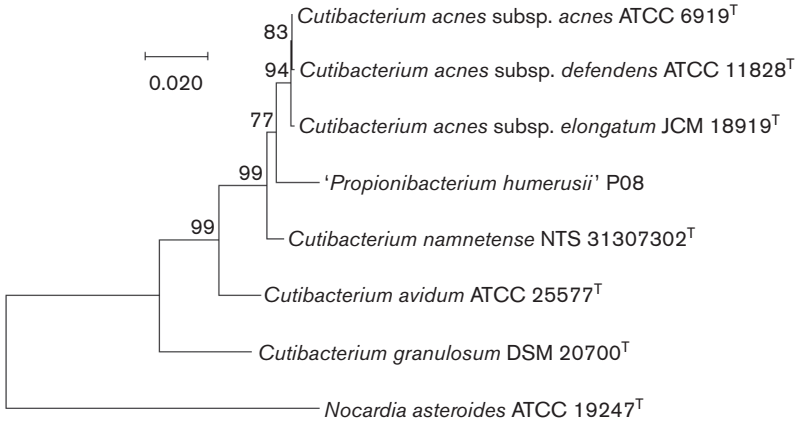

Fig. 1. Minimum evolution phylogenetic tree generated from 16S rRNA gene sequences of all three *Cutibacterium acnes* subspecies and all other *Cutibacterium* species.

the DDBJ/EMBL/GenBank database entries revealed that type strains representing *C. acnes* subsp. *elongatum* had DNA–DNA hybridization values that ranged from 72–74 % when analysed against type strains from types I and II, consistent with its proposal as a distinct subspecies based on a 79–80 % similarity boundary [16] (Table 1). Strains within each subspecies also shared high DNA–DNA hybridization scores (89–99 %) in keeping with their shared type or phylogroup status. While comparison of all 11 *C. acnes* genomes revealed DNA–DNA hybridization values above the 70 % cut-off value currently used for bacterial species demarcation, values of 36 % or lower were determined when compared to the type strains of bacterial species *Cutibacterium avidum*, *Cutibacterium granulosum*, *Cutibacterium namnetense* and '*Propionibacterium humerusii*'. In line with their classification as *C. acnes*, all subspecies demonstrated high levels of synteny upon whole genome alignments, although some differences with respect to inversions and translocations were noted (Fig. S1, available in the online version of this article).

The genome analysis of the complete genome sequences of *C. acnes* subsp. *acnes* and *C. acnes* subsp. *defendens* and the draft whole genome of *C. acnes* subsp. *elongatum* revealed all three subspecies shared a similar G+C content of 60.0 mol%, but the genome length is slightly longer for

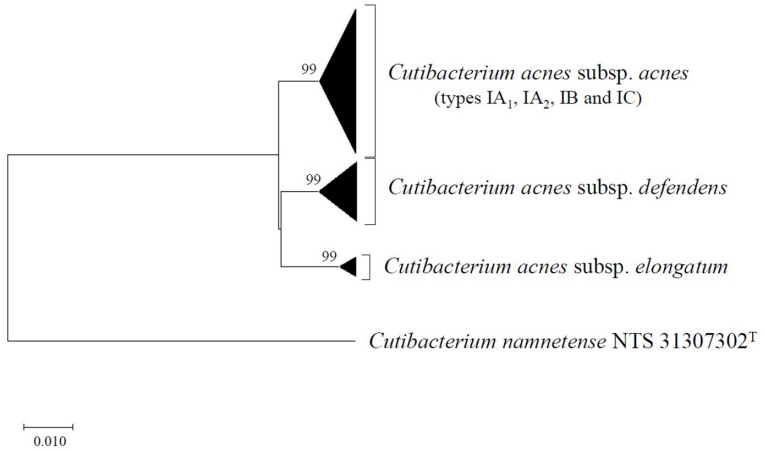

Fig. 2. Minimum-evolution phylogenetic tree of concatenated gene sequences (4253 bp) from all sequence types (STs) currently represented in the $MLST_8$ database (http://pubmlst.org/pacnes/). Genes analysed are *aroE*, *atpD*, *gmk*, *guaA*, *lepA*, *recA*, *sodA*, *tly* and *camp2*. The closely related sister taxon, *Cutibacterium namnetense* (NTS 31307302^T), was also analysed as an outgroup. Sequence input order was randomized, and bootstrapping resampling statistics were performed using 500 data sets. Bootstrap values (≥70 %) are shown on the arms of the tree. Horizontal bar represents genetic distance. The different subspecies of *Cutibacterium acnes* are indicated. All *C. acnes* strains analysed were as listed previously [6]. The $MLST_8$ scheme provides high resolution typing of *C. acnes* and strain clustering which is consistent with that obtained by whole genome analysis [6].

Table 1. *In silico* DNA–DNA hybridization values

Whole genome *in silico* DNA–DNA hybridization (using DSMZ site Genome-to-Genome Distance Calculator 2.1) was performed using genome data in DDBJ/EMBL/GenBank database. DNA–DNA hybridization value >70 % indicates same species, DDH value >79 % indicates same subspecies.

		C. acnes **Type IA_1** **ATCC 6919^T**	***C. acnes*** **Type II** **ATCC 11828^T**	***C. acnes*** **Type III** **JCM 18919^T**
C. acnes				
Type IA_1	ATCC 6919^T	100	75.6	73.2
	JCM 18916	98.5	74.1	72.0
Type IA_2	P.acn33	96.7	76.7	73.4
Type IB	KPA171202	93.1	78.4	73.8
	JCM 18918	91.2	76.8	72.4
Type IC	PRP-38	89.4	74.1	72.4
Type II	ATCC 11828^T	75.5	100	74.1
	JCM 18920	75.3	96.3	73.8
Type III	JCM 18919^T	73.1	74.1	100
	Asn12	73.1	74.0	98.7
	JCM 18909	72.0	72.9	97.6
C. avidum	ATCC 25577^T	19.6	23.5	23.3
C. granulosum	NCTC 11865^T	22.5	22.2	21.6
C. namnetense	NTS 31307302^T	36.0	35.3	35.1
'*P. humerusii*'	P08	31.6	32.3	31.4

C. acnes subsp. *elongatum* (Table 2). The results of *C. acnes* subsp. *acnes* and *C. acnes* subsp. *defendens* are consistent with the emended description of *C. acnes* by Nouioui *et al.* [14].

Previously, we described that some strains of *C. acnes* are haemolytic [10]. The description of the genus *Cutibacterium*, however, states that the species is non-haemolytic [12]. To clarify this, we examined the haemolytic features of 45 strains representing all three subspecies (Table 3). We observed that 23 out of 25 *C. acnes* subsp. *acnes* isolates were β-haemolytic under anaerobic conditions (92 %), while all 16 strains of *C. acnes* subsp. *defendens* and *C. acnes* subsp. *elongatum* were non-haemolytic. This finding is consistent with previous descriptions of *P. acnes* subspecies [10, 11], but not with the description of genus *Cutibacterium* [12].

We also examined differences between the proposed *C. acnes* subsp. *elongatum* and *C. acnes* subsp. *acnes* and *C. acnes* subsp. *defendens* based on MALDI-TOF mass spectrometry profiles previously reported [10, 17]. Analysis of three strains of type IA_1 within *C. acnes* subsp. *acnes* (strains K107=JCM 18916, M5, M18), using an MALDI Biotyper (Bruker), showed predominant peaks of 3450–3650 Da window at 3516 and 3589 Da, and those of 6950–7300 Da window at 7034 and 7180 Da (Table 4). The predominant peaks of three strains of types IA_2 and IB within *C. acnes* subsp. *acnes* (strains K115=JCM 18918, M1, W27) were also similar to type IA_1, but with differences, at 3503, 3516, and 3589 Da (3450–3650 Da window), and at 7005 and 7180 Da (6950–7300 Da window). This is also consistent with previous observations that type IA_2 and IB share other characteristics including *recA* and *tly* alleles, which suggests conjugal transfer of large genomic regions [18].

The spectra of five strains of *C. acnes* subsp. *defendens* (strains K80=JCM 18911, K127=JCM 18920, M13, M17, M33) were largely different than *C. acnes* subsp. *acnes*, with predominant peaks at 3493 and 3632 Da (3450–3650 Da window), and 6985 and 7265 Da (6950–7300 Da window). This was also the case for five strains of *C. acnes* subsp. *elongatum* (strains K124=JCM 18919^T, K57=JCM 18909, K55), with predominant peaks at 3619 Da (3450–3650 Da window), and 7237 Da (6950–7300 Da window). The results added the description of the mass ion peaks of *C. acnes* subsp. *defendens* but not that of *C. acnes* subsp. *elongatum* (the two peaks are 2–3 Da different than previous description [10] but we concluded it is not worth amending this as the differences are relatively small.)

Table 2. Genome comparison of the type strains of three subspecies of *C. acnes*

Subspecies name	Strain	Size (Mb)	GC %	Gene number
C. acnes subsp. *acnes* (type I)	ATCC 6919^T	2.495	60.0	2509
C. acnes subsp. *defendens* (type II)	ATCC 11828^T	2.489	60.0	2477
C. acnes subsp. *elongatum* (type III)	JCM 18919^T	2.618*	60.0*	2660*

*Estimation based on the draft whole genome.

Table 3. *β*-Haemolysis features of *C. acnes* strains

UK, United Kingdom; JP, Japan; NG, no growth.

Isolate	Source	Type	β-Haemolysis	
			Anaerobic	Aerobic
C. acnes subsp. *acnes* (type I)				
NCTC 737^T	Facial acne, UK	IA_1	+	−
JCM 6495	Unknown	IA_1	+	−
K107=JCM 18916	Facial skin, JP	IA_1	+	−
K161=JCM 18922	Facial skin, JP	IA_1	+	−
K282=JCM 18924	Facial skin, JP	IA_1	+	−
K51=JCM 18907	Facial skin, JP	IA_2	+	−
K56=JCM 18908	Facial skin, JP	IA_2	−	−
K72=JCM 18910	Facial skin, JP	IA_2	+	−
K94=JCM 18912	Facial skin, JP	IA_2	−	−
K12	Facial skin, JP	IB	+	−
K13	Facial skin, JP	IB	+	−
K24	Facial skin, JP	IB	+	−
K81	Facial skin, JP	IB	+	+
K86	Facial skin, JP	IB	+	+
K114=JCM 18917	Facial skin, JP	IB	+	−
K115=JCM 18918	Facial skin, JP	IB	+	−
K280=JCM 18923	Facial skin, JP	IB	+	−
S16=JCM 18927	Axilla skin, JP	IB	+	−
W27	Facial acne, JP	I	+	+
W31	Facial acne, JP	I	+	−
W32	Facial acne, JP	I	+	−
W33	Facial acne, JP	I	+	−
W34	Facial acne, JP	I	+	−
W35	Facial acne, JP	I	+	−
W36	Facial acne, JP	I	+	−
C. acnes subsp. *defendens* (type II)				
JCM 6473^T	Abscess	II	−	−
K80=JCM 18911	Facial skin, JP	II	−	−
K96=JCM 18913	Eczema skin, JP	II	−	−
K104=JCM 18914	Facial skin, JP	II	−	−
K106=JCM 18915	Facial skin, JP	II	−	−
K127=JCM 18920	Eczema skin, JP	II	−	−
K145=JCM 18921	Facial skin, JP	II	−	−
S13=JCM 18926	Axilla skin, JP	II	−	−
K15	Facial skin, JP	II	−	−
K79	Facial skin, JP	II	−	−
K128	Eczema skin, JP	II	−	−
S14	Axilla skin, JP	II	−	−
W37	Facial acne, JP	II	−	−
C. acnes subsp. *elongatum* (type III)				
K124^T = JCM 18919^T	Facial skin, JP	III	−	NG
K57=JCM 18909	Facial skin, JP	III	−	NG
K290=JCM 18925	Forearm skin, JP	III	−	NG

Table 4. Characteristic MALDI-TOF peaks of *C. acnes* subspecies

Subspecies	Prominent peaks (Da)	
	3450–3650 Da	6950–7300 Da
C. acnes subsp. *acnes* (type I)		
Type IA_1	3516, 3589	7034, 7180
Types IA_2 and IB	3503, 3516, 3589	7005, 7180
C. acnes subsp. *defendens* (type II)	3493, 3632	6985, 7265
C. acnes subsp. *elongatum* (type III)	3619	7237

DESCRIPTION OF *CUTIBACTERIUM ACNES* SUBSP. *ELONGATUM* COMB. NOV.

Cutibacterium acnes subsp. *elongatum* (e.lon.ga′tum. L. neut. part. adj. *elongatum* elongated, named after the characteristic cell shape).

Basonym: *Propionibacterium acnes* subsp. *elongatum* Dekio *et al.* 2015.

Description is as given for *Propionibacterium acnes* subsp. *elongatum* [10] with the following additions. Strains can be isolated from the human skin surface, but are infrequently recovered from the face and are more common on the back, especially the lower back, which appears the preferred habitat [19]. Strains are not associated with acne vulgaris, but have been shown to be associated with progressive macular hypomelanosis [19, 20]. The G+C content of the type-strain genome is approximately 60.0 mol% and its approximate size is 2.62 Mbp, based on the draft whole genome.

The type strain is $K124^T$ (JCM 18919^T=NCTC 13655^T), isolated from normal-looking facial skin of an atopic dermatitis patient in Wako, Japan, 2003.

EMENDED DESCRIPTION OF *CUTIBACTERIUM* SCHOLZ AND KILIAN 2016

The description is as given before [12] with the following modification. Some strains of *C. acnes* are β-haemolytic under anaerobic condition, and in some cases, also under aerobic condition.

EMENDED DESCRIPTION OF *CUTIBACTERIUM ACNES* SUBSP. *ACNES* (GILCHRIST 1900) NOUIOUI *ET AL.* 2018

The description is as given for *Propionibacterium acnes* subsp. *acnes* [11] with the following modifications. The G+C content of the type-strain genome is 60.0 mol%, its approximate size 2.50 Mbp, Prominent mass ions obtained by MALDI-TOF MS at 3516, 3589, 7034, and 7179 Da are characteristic for type IA_1, and those at 3503, 3516, 3589, 7005, and 7179 Da are characteristic for type IA_2 and type IB.

EMENDED DESCRIPTION OF *CUTIBACTERIUM ACNES* SUBSP. *DEFENDENS* (MCDOWELL 2016) NOUIOUI *ET AL.* 2018

The description is as given before [14] with the following modifications. The G+C content of the type-strain genome is 60.0 %, its approximate size 2.49 Mbp. Prominent mass ions obtained by MALDI-TOF MS at 3493, 3632, 6985 and 7265 Da are characteristic.

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

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