Description of *Wautersiella falsenii* gen. nov., sp. nov., to accommodate clinical isolates phenotypically resembling members of the genera *Chryseobacterium* and *Empedobacter*

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A total of 26 isolates of non-fermenting, Gram-negative rods, obtained between 1980 and 2004 by various clinical laboratories in Belgium, with phenotypic characteristics resembling those of members of the genera *Chryseobacterium* and *Empedobacter* (indole-positive) and a biochemical profile resembling that of CDC group II-h, but urease-positive, were collected at the Université Catholique de Louvain Microbiology Laboratory, Belgium. The 16S rRNA gene sequences were determined for most of the isolates and showed 94–95% similarity with the type strain of *Empedobacter brevis* as the closest relative, indicating that these isolates might belong to a separate genus. Furthermore, the 16S rRNA gene sequences of the isolates were similar, but two clusters (genomovars) could be distinguished. The sequence similarities were 99.5–100% for the 14 isolates of genomovar 1 and 99.4–100% for the 12 isolates of genomovar 2. The similarity between the two clusters was 98.3–99.5%. The presence of two clearly different groups was corroborated by using tRNA intergenic length polymorphism analysis, which also enabled differentiation of the novel species from all other species studied thus far using this technique. DNA–DNA hybridization results excluded a close relatedness to *Empedobacter brevis*. The DNA G + C contents of the reference strains of genomovars 1 and 2 were 33.8 ± 0.4 and 34.4 ± 0.2 mol%, respectively. The name *Wautersiella falsenii* gen. nov., sp. nov., is proposed for this group, comprising two closely related genomovars. The type strain of the species and reference strain for genomovar 1 is NF 993^T (= CCUG 51536^T = CIP 108861^T), which was isolated from a surgical wound. The reference strain for genomovar 2 is NF 770 (= CCUG 51537 = CIP 108860), which was isolated from blood.

The family *Flavobacteriaceae*, emended by Bernardet *et al.* (2002), comprises several genera of which *Bergeyella*, *Chryseobacterium* and *Riemerella* form a separate branch

Abbreviation: tDNA-PCR, tRNA intergenic length polymorphism analysis.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Wautersiella falsenii* genomovar 1 strains NF 993^T, NF 1182, NF 880, NF 777, NF 869, NF 289, NF 1240, NF 1241, NF 835, NF 1242, NF 1243 and NF 1244 are AM084341 and AM238680–AM238690, respectively, those of *Wautersiella falsenii* genomovar 2 strains NF 770 (reference strain), NF 622, NF 1159, NF 1249, NF 1140, NF 1084, NF 1080, NF 1136, NF 203, NF 316 and NF 58 are AM084342 and AM238670–AM238679, respectively, and those of *Empedobacter brevis* strain LMG 4011^T and group CDC II-h strain NF 802 are AM177497 and AM261868, respectively.

on the basis of rRNA cistron similarity (Vandamme *et al.*, 1994; Bernardet *et al.*, 2002) and whereby the genus *Empedobacter* (Vandamme *et al.*, 1994) represents a separate line of descent comprising only one species, *Empedobacter brevis*. We propose a new genus within the *Flavobacteriaceae*, designated *Wautersiella* gen. nov., comprising the species *Wautersiella falsenii* sp. nov., with genomovars 1 and 2 to accommodate two groups of closely related isolates from clinical origins and with phenotypic resemblance to isolates of the genera *Chryseobacterium*, *Weeksellia* and *Empedobacter* and of CDC groups II-e and II-h (Schreckenberger *et al.*, 2003).

The 26 isolates, which were obtained between 1980 and 2004 by at least ten clinical laboratories in Belgium, originated from various human clinical samples, including blood,

wounds, pus, respiratory tract, ear discharge, vaginal swab and pleural fluid (Table 1) and were cultivated on tryptic soy agar containing 5% sheep blood. Strains of *Empedobacter brevis*, *Weeksella virosa* and CDC group II-h used in this study were isolated from different patients in various Belgian hospitals.

16S rRNA gene sequences were determined for most of the isolates as described previously (Wauters *et al.*, 2001) and tree construction was done as described by Nemec *et al.* (2001).

The results of sequence determination and cluster analysis of the 16S rRNA genes are presented in Fig. 1. The *Wautersiella falsenii* isolates clearly affiliated separately and the 16S rRNA gene sequences showed 94–95% similarity with the type strain of *Empedobacter brevis* as the closest relative, indicating that the isolates might belong to a separate genus. Furthermore, the 16S rRNA gene sequences of the isolates were similar, but two clusters (genomovars) could be distinguished. The sequence similarities were 99.5–100% for the 14 isolates of genomovar 1 and 99.4–100% for the 12 isolates of genomovar 2. The similarity between the two clusters was 98.3–99.5%.

It should be noted that the 16S rRNA gene sequence of an isolate designated *Flavobacterium* sp. (GenBank accession no. AY363052), submitted to GenBank in August 2003 by the Institut für Klinische Mikrobiologie of the University of Erlangen, Germany, showed 100% similarity with some of the 16S rRNA gene sequences determined for the *Wautersiella falsenii* genomovar 2 isolates.

tRNA intergenic length polymorphism analysis (tDNA-PCR) was carried out as described previously (Baele *et al.*, 2000). Fig. 2 presents the tDNA-PCR fingerprints as obtained for representative strains of *Wautersiella falsenii* genomovars 1 and 2, *Empedobacter brevis*, *Weeksella virosa* and CDC group II-h. The tDNA-PCR fingerprints of isolates from the two genomovars had the following fragments in

common: 57.6 bp (SD for 11 isolates, 0.3 bp), 90.7 (0.1), 101.3 (0.1) and 221.4 bp (0.2 bp), but all genomovar 2 isolates had an additional tRNA spacer of 132.9 bp (0.1 bp for 5 isolates).

The three *Empedobacter brevis* strains (LMG 4011^T, NF 285 and NF 1052) had tRNA spacers with lengths of 102.4 (0.1), 103.5 (0.1), 112.0 (0.1), 113.1 (0.1) and 114.1 bp (0.1 bp) in common. All except strain NF 285 also had spacers with lengths of 99.2 (0.2) and 242.7 bp (0.1 bp) in common and all except strain NF 1052 had spacers of 111.0 (0.1) and 241.0 bp (0.7 bp) in common.

The three *Weeksella virosa* strains tested (NF 135, NF 309 and NF 323) had tRNA spacers with lengths of 57.6 (0.5), 88.0 (0.1), 98.4 (0.5), 99.3 (0.5) and 104.0 bp (0.1 bp) in common, with strains NF 135 and NF 309 having a spacer of 91.3 bp (0.1 bp) in common and NF 135 and NF 323 having spacers of 230.4 (0.1) and 291.1 bp (0.0 bp) in common.

All eight CDC II-h strains tested (NF 675, NF 690, NF 696, NF 802, NF 974, NF 1141, NF 1146 and NF 1335) had tRNA spacers with lengths of 57.5 (0.2), 81.1 (0.1), 90.2 (0.1), 92.3 (0.1), 110.6 (0.1) and 218.5 bp (0.1 bp) in common. As such, tDNA-PCR enabled differentiation between *Wautersiella falsenii* and the most closely related species.

Biochemical and morphological tests were performed as described previously (Laffineur *et al.*, 2002) and by Schreckenberger *et al.* (2003). Assimilation–alkalinization of organic compounds was detected on Simmons' citrate agar base by replacing citrate with 0.2% (w/v) of various organic substrates, according to Martin *et al.* (1981). For casein hydrolysis, 1% (v/v) of skimmed milk was added to nutrient agar plates. Enzymic reactions were carried out using diagnostic tablets from Rosco. Utilization of carbohydrates was performed using API 50 CH strips (bioMérieux), which were read after 1, 2 and 3 days. The KOH test was used to detect flexirubin pigment (Bernardet *et al.*, 2002).

Table 1. Clinical origins of the *Wautersiella falsenii* isolates

NF, Designation for non-fermenting strains from the collection of Georges Wauters, Université Catholique de Louvain, Brussels, Belgium.

Clinical origin	Genomovar 1	Genomovar 2
Blood	NF 880	NF 770, NF 1136, NF 1159, NF 1249
Ear discharge	NF 835	
Oral cavity	NF 976	
Pleural fluid	NF 777	
Pus	NF 1242	NF 622
Respiratory tract	NF 869, NF 1240	
Surgical wound	NF 993 ^T	NF 1080, NF 1084, NF 1138, NF 1140
Vaginal swab	NF 1243	
Wound		
Unknown	NF 289, NF 987, NF 1182, NF 1241, NF 1244	NF 58, NF 203, NF 316

Fig. 1. Rooted tree based on 16S rRNA gene sequences of strains of *Wautersiella falsenii* gen. nov., sp. nov. and related genera. Cluster analysis was performed using Genebase (Applied Maths) and was based on the neighbour-joining method, with the 16S rRNA gene sequence of *Flavobacterium aquatile* as an outgroup. Percentage bootstrap values ($n=100$) are shown at branch points. Bar, 2% dissimilarity.

All isolates were Gram-negative, non-motile, non-fermenting rods and were oxidase- and catalase-positive. Some strains displayed yellow-pigmented colonies after prolonged incubation, but the pigment was not of the flexirubin type.

Acid was produced from glucose and maltose. Lysine and ornithine decarboxylases and arginine dihydrolase were negative. Indole was produced and urease was positive using Christensen's urea broth. Nitrate reduction was negative.

Fig. 2. tDNA-PCR fingerprints of representative strains of the two genomovars of *Wautersiella falsenii* gen. nov., sp. nov., *Empedobacter brevis*, *Weeksella virosa* and CDC group II-h. (a) *Wautersiella falsenii* genomovar 1, NF 998^T; (b) *Wautersiella falsenii* genomovar 2, NF 1080; (c) *Empedobacter brevis* LMG 4011^T; (d) *Weeksella virosa* NF 309; (e) CDC group II-h, NF 802.

and nitrite reduction was mostly negative. Gelatinase production varied from weak to strong and was in general faster and stronger for genomovar 1 isolates. Casein hydrolysis was negative or very weak. Growth did not occur at 42 °C. Assimilation and alkalization of acetate, aspartate, L-alanine and L-serine were positive on Simmons' agar base, whereas citrate and most of the other organic salts were negative. Isolates were variable for galacturonate. Aesculin hydrolysis was variable. Alkaline phosphatase, trypsin, pyrrolidonyl aminopeptidase, α -glucosidase and *N*-acetyl-glucosaminidase were positive, whereas α -galactosidase, α -mannosidase, α -fucosidase and β -xylosidase were negative. β -galactosidase (ONPG) was variable. On API 50 CH strips, only maltose, glycerol and starch were assimilated within 3 days. Glucose was assimilated by some of the isolates. Table 2 summarizes some of the biochemical characteristics that differ between the two genomovars;

aesculin, ONPG and galacturonate yielded characteristic reactions that separated the two genomovars, but exceptions occurred and isolate NF 1138, belonging to genomovar 2, even had the biochemical profile of genomovar 1. *Empedobacter brevis* differed from all *Wautersiella falsenii* isolates in being urease-negative and having strong casein hydrolysis activity (Table 2). All CDC II-h isolates were urease-negative, in contrast to the *Wautersiella falsenii* isolates.

Fatty acid analysis was carried out as described previously (Kämpfer & Kroppenstedt, 1996; Kämpfer *et al.*, 2003). The most abundant fatty acid was 15:0 iso, followed by summed feature 4 (15:0 iso 2-OH and/or 16:1 ω 7 ct), 17:0 iso 3-OH and 15:0 iso 3-OH. The fatty acid profiles of strains of the two genomovars were different from those of *Empedobacter brevis* and *Chryseobacterium* species (Table 3).

Table 2. Differential biochemical characteristics for *Wautersiella falsenii* genomovars 1 and 2, *Empedobacter brevis* and *Weeksella virosa*

Results are given as the percentage of positive isolates. Strains used were: *Wautersiella falsenii* (as listed in Table 1); *Empedobacter brevis* LMG 4011^T, NF 285 and NF 1052; and *Weeksella virosa* LMG 12995^T, LMG 8349, NF 309, NF 637, NF 805, NF 947 and NF 1063.

Characteristic	<i>Wautersiella falsenii</i>		<i>Empedobacter brevis</i> (n=3)	<i>Weeksella virosa</i> (n=7)
	Genomovar 1 (n=14)	Genomovar 2 (n=12)		
Aesculin hydrolysis (4 h)	100	8	0	0
Galacturonate alkalization	93	8	0	0
Gelatin hydrolysis (24 h)	100	8 (weak: 92)	100	100
β -Galactosidase (ONPG)	0	75	0	0
Casein hydrolysis (within 8 h)	0	0	100	0
Urease	100	100	0	0
Colistin resistance	100	100	100	0
Acid from glucose and maltose	100	100	100	0

Table 3. Long-chain fatty acid composition of *Wautersiella falsenii* gen. nov., sp. nov. and related bacteria

Taxa: 1, *Wautersiella falsenii* genomovar 1 ($n=5$: strains NF 993^T, NF 835, NF 869, NF 976 and NF 987; data from this study); 2, *Wautersiella falsenii* genomovar 2 ($n=5$: strains NF 770, NF 1080, NF 1136, NF 1138 and NF 1159; this study); 3, *Empedobacter brevis* ($n=6$); 4, *Chryseobacterium taichungense* ($n=1$); 5, *Chryseobacterium formosense* ($n=1$); 6, *Chryseobacterium defluvii* ($n=1$); 7, *Chryseobacterium joostei* ($n=11$); 8, *Chryseobacterium gleum* ($n=5$); 9, *Chryseobacterium indologenes* ($n=45$); 10, *Chryseobacterium balustinum* ($n=1$); 11, *Chryseobacterium indoltheticum* ($n=1$); 12, *Chryseobacterium scophthalmum* ($n=2$); 13, *Elizabethkingia meningoseptica* ($n=1$); 14, *Elizabethkingia miricola* ($n=1$); 15, *Bergeyella zoohelcum* ($n=1$); 16, *Weeksellia virosa* ($n=18$). Data from Hollis *et al.* (1995), Hugo *et al.* (2003), Kämpfer *et al.* (2003), Kim *et al.* (2005) and Young *et al.* (2005). Values are percentages of total fatty acids. Where applicable means $\pm$ SD are given. tr, Trace (less than 1.0%); ND, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Unknown 11:543*	1.25 $\pm$ 0.15	0.88 $\pm$ 0.45														
13:0 iso	tr		tr	tr	3.6	2.8	tr	tr	tr	tr	ND	tr	1.3 $\pm$ 0.3	2.0 $\pm$ 0.5	1.5	1
13:1 AT 12-13†	1.16 $\pm$ 0.31	1.4 $\pm$ 0.43														
14:1 ω 5c	tr	tr														
14:0	1.38 $\pm$ 0.21	1.1 $\pm$ 0.1														tr
14:0 2-OH	tr	tr														
Unknown 13:566*	4.24 $\pm$ 1.4	6.55 $\pm$ 2.21	1.9 $\pm$ 1.4	6.7	tr	tr	1.1 $\pm$ 0.2	1.2 $\pm$ 0.4	2.1 $\pm$ 0.7	1.6	1.7	2.9 $\pm$ 0.2	1.9 $\pm$ 0.3	1.5 $\pm$ 0.2	1.8	2
15:0 iso	31.62 $\pm$ 0.86	25.83 $\pm$ 1.8	23.6 $\pm$ 2.2	35.4	52.2	58.5	34.6 $\pm$ 2.0	35.4 $\pm$ 2.9	34.3 $\pm$ 4.9	32.3	29.4	35.0 $\pm$ 0.7	43.9 $\pm$ 2.0	46.4 $\pm$ 2.2	47.8	46
15:0 iso 2-OH																10
15:0 iso 3-OH	8.2 $\pm$ 2.0	5.58 $\pm$ 0.58	4.8 $\pm$ 0.7	4.3	1.8	2.6	2.9 $\pm$ 0.3	2.5 $\pm$ 0.1	2.6 $\pm$ 0.2	2.7	2.3	2.7 $\pm$ 0.1	2.8 $\pm$ 0.3	3.0 $\pm$ 0.6	4.0	5
15:0 anteiso			tr	0.7	2.1	3.2	tr	tr	tr	tr	5.9	tr	1.1 $\pm$ 0.8	1.0 $\pm$ 0.6	ND	ND
15:0	tr	tr														ND
15:1 ω 6c	tr	tr														
16:0	3.2 $\pm$ 1.36	4.98 $\pm$ 0.73	3.8 $\pm$ 0.4	1.3	1.5	1.3	tr	1.3 $\pm$ 0.4	tr	1.6	1.0	1.2 $\pm$ 0.2	tr	1.2 $\pm$ 0.1	tr	4
16:0 iso																1
16:0 3-OH	4.58 $\pm$ 0.95	3.05 $\pm$ 0.50	3.6 $\pm$ 0.7	2.6	tr	tr	1.2 $\pm$ 0.2	1.1 $\pm$ 0.1	1.0 $\pm$ 0.2	1.4	tr	1.0 $\pm$ 0.1	2.6 $\pm$ 0.4	3.0 $\pm$ 0.6	ND	1
16:0 iso 3-OH	0.96 $\pm$ 0.16	1.23 $\pm$ 0.38	tr	1.4	1.1	tr	ND	ND	ND	tr	1.3	ND	tr	tr	ND	ND
16:1 ω 5c	4.74 $\pm$ 1.03	5.55 $\pm$ 0.77														1
Unknown 16:580*	1.24 $\pm$ 0.24	1.5 $\pm$ 0.07	1.4 $\pm$ 0.1	1.7	1.0	ND	1.6 $\pm$ 0.1	1.7 $\pm$ 0.1	1.7 $\pm$ 0.2	1.3	1.3	1.5 $\pm$ 0.1	1.6 $\pm$ 0.1	1.3 $\pm$ 0.6	1.4	
17:0 2-OH	tr	tr	tr	ND	ND	ND	ND	ND	ND	tr	3.0	tr	ND	ND	ND	
17:0 iso	1.1 $\pm$ 0.45	1.23 $\pm$ 0.48	tr	0.8	2.3	2.0	tr	1.6 $\pm$ 0.6	tr	1.0	tr	tr	tr	tr	ND	3
17:0 iso 3-OH	13.32 $\pm$ 1.67	13.85 $\pm$ 1.86	17.1 $\pm$ 2.1	22.4	10.9	14.1	20.1 $\pm$ 1.2	21.8 $\pm$ 0.3	19.2 $\pm$ 1.8	16.8	14.0	16.3 $\pm$ 0.1	14.6 $\pm$ 1.0	15.3 $\pm$ 0.2	13.5	7
17:1 iso ω 8c																5
17:1 iso ω 9c	1.22 $\pm$ 0.31	2.3 $\pm$ 0.47	3.9 $\pm$ 0.5	8.9	4.3	4.8	22.9 $\pm$ 1.9	20.2 $\pm$ 3.9	24.2 $\pm$ 3.1	27.1	25.6	24.8 $\pm$ 0.4	7.8 $\pm$ 1.3	6.6 $\pm$ 0.2	17.5	
17:1 iso ω 12t																8
18:0																1

Table 3. cont.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
18:1 ω 5c	ND	ND	ND	ND	ND	ND	tr	tr	tr	tr	tr	tr	tr	tr	1.5	1
18:1 ω 9c																2
18:2																
Summed features:‡																
1	1.78±0.19	0.83±0.28														
2	tr	tr														
4	13.96±2.65	15.35±1.99	19.4±1.6	13.8	6.5	8.4	12.1±1.3	11.8±0.8	11.1±1.3	9.2	11.2	11.5±0.3	19.6±1.0	17.0±1.3	9.0	
5	3.44±0.44	6.1±0.39	6.7±0.8	ND	ND	ND	tr	tr	tr	tr	ND	tr	tr	ND	1.5	

*Unknown fatty acid; numbers indicate equivalent chain-length.
†Not further specified by the Microbial Identification System (MIDI; Microbial ID) software.
‡Fatty acids that could not be separated by GC using the MIDI system; summed feature 1, 15:1 iso H and/or 13:0 3-OH; summed feature 2, 16:1 iso I and/or 14:0 3-OH; summed feature 4, 15:0 iso 2-OH and/or 16:1 ω 7c; summed feature 5, 17:1 iso I and/or 17:1 anteiso B.

The DNA G+C contents, determined according to the method of Peña *et al.* (2005), of the type strain of genomovar 1 and the reference strain of genomovar 2 were 33.8±0.4 and 34.4±0.2 %, respectively, in agreement with values obtained for the closely related genera *Chryseobacterium* and *Empedobacter* (Bernardet *et al.*, 2002).

For DNA–DNA hybridization, a microplate method was used, modified after Lind & Ursing (1986), and as described by Ziemke *et al.* (1998). Reciprocal DNA–DNA hybridizations, i.e. with one of the two strains labelled first and the other labelled subsequently, were carried out between the two reference strains of *Wautersiella falsenii* and *Empedobacter brevis* LMG 4011^T. Values obtained were 29.4 % (respectively 25.5 % reciprocal) for *Wautersiella falsenii* genomovar 1 strain NF 993^T versus *Empedobacter brevis* LMG 4011^T and 33.0 % (respectively 35.8 % reciprocal) for *Wautersiella falsenii* genomovar 2 strain NF 770 versus *Empedobacter brevis* LMG 4011^T, indicating that the reference strains of the two genomovars do not belong to *Empedobacter brevis*.

DNA–DNA hybridization values of 104.6 % (respectively reciprocal 103.0 %) were obtained for genomovar 1 strains NF 993^T and NF 835, 86.6 % (respectively reciprocal 93.9 %) for strains NF 993^T and NF 869 and 88.2 % and 93.5 % for genomovar 2 reference strain NF 770 with, respectively, isolates NF 1084 and NF 1138. A value of 63.6 % was obtained for strain NF 993^T (labelled) versus strain NF 770, indicating that the two strains represent separate genomovars and borderline the same species.

Although two separate groups are clearly present within the genus *Wautersiella*, at present it is not possible to describe them as separate species, because of the lack of a phenotypic characteristic that can unambiguously differentiate between the two groups. Therefore it was decided to describe the two groups as genomovars.

Description of *Wautersiella* gen. nov.

Wautersiella (Wau.ter'si.ella. N.L. fem. dim. n. *Wautersiella* named after Georges Wauters, a contemporary Belgian microbiologist, who first recognized this group of organisms as a separate entity and to honour him for his lifelong contribution to bacterial taxonomy).

Non-motile, Gram-negative rods, 2–3 µm in length, growing aerobically at 20, 30 and 37 °C on standard media such as tryptic soy agar or blood agar. Colonies are approximately 2 mm in diameter after growth on blood agar for 48 h at 30 °C. Main cellular fatty acids are 15:0 iso, 17:0 iso 3-OH, summed feature 4 and 15:0 iso 3-OH. The G+C content of the DNA is 33.8–34.4 mol%. The type and only species is *Wautersiella falsenii*.

Description of *Wautersiella falsenii* sp. nov.

Wautersiella falsenii (fal.sen'i.i. N.L. gen. n. *falsenii* of Falsen, to honour the contemporary Norwegian microbiologist,

Enevold Falsen, for his lifelong interest in bacterial taxonomy and for his systematic characterization of bacteria at the CCUG, Göteborg, Sweden).

Oxidative acidification of glucose and maltose. Positive for urease and for indole production. Nitrates are not reduced, but nitrite reduction is variable. Lysine and ornithine decarboxylases and arginine dihydrolase are not present. Citrate is negative. Alkaline phosphatase, trypsin (benzyl-arginine arylamidase) and pyrrolidonyl aminopeptidase are positive. Gelatin hydrolysis is positive or weakly and delayed positive. Casein hydrolysis is negative or weakly and delayed positive. Comprises two genomovars that differ in 16S rRNA gene sequence and in tDNA-PCR pattern. All genomovar 1 isolates ($n=14$) rapidly hydrolyse gelatin and aesculin, are β -galactosidase (ONPG)-negative and all but one alkalize galacturonate. All genomovar 2 isolates ($n=12$) but one are aesculin- and galacturonate-negative. Most isolates are ONPG-positive and slightly gelatin-positive. Fatty acids are as described for the genus, with only minor differences between the two genomovars.

The type strain of the species and reference strain for genomovar 1 is NF 993^T (= CCUG 51536^T = CIP 108861^T), which was isolated from a surgical wound. The reference strain for genomovar 2, NF 770 (= CCUG 51537 = CIP 108860), was isolated from blood.

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