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Riemerella anatipestifer gen. nov., comb. nov., the Causative Agent of Septicemia Anserum Exsudativa, and Its Phylogenetic Affiliation within the *Flavobacterium-Cytophaga* rRNA Homology Group

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The phylogenetic position of the causative agent of septicemia anserum exsudativa, now most often referred to as [*Moraxella*] *anatipestifer* (brackets indicate a generically misnamed taxon) or "[*Pasteurella*] anatipestifer," was established by performing rRNA cistron similarity studies. [*Moraxella*] *anatipestifer* belongs to rRNA superfamily V, together with the genera *Flavobacterium*, *Cytophaga*, *Flexibacter*, *Weeksella*, *Capnocytophaga*, and *Sphingobacterium*. The detailed structure of rRNA superfamily V, which now contains five major rRNA homology groups, is described. An analysis of various phenotypic parameters, including new data (cellular proteins and fatty acids) and previously published data (respiratory quinones, enzyme activities, and classical phenotypic features), revealed that [*Moraxella*] *anatipestifer* differs in many aspects from its closest relatives, *Flavobacterium indologenes*, *Flavobacterium gleum*, *Flavobacterium indoltheticum*, *Flavobacterium balustinum*, *Flavobacterium meningosepticum*, and *Weeksella zoohelcum*. The combined genotypic and phenotypic data indicate that this organism should be placed in a separate genus; the name *Riemerella anatipestifer* gen. nov., comb. nov. is proposed for this bacterium. The specific epithet *anatipestifer* is kept in order to avoid nomenclatural confusion. However, it should be emphasized that the illness caused by this organism is a septicemic disease which is not restricted to ducks.

The epizootic infectious disease of domestic birds known as septicemia anserum exsudativa was first described by Riemer in 1904 (30). The taxonomic status of few other bacteria has been changed as often as the taxonomic status of the organism causing this disease. The phylogenetic position of this organism remained unsettled (it was classified in various genera with several specific epithets) from 1904 until 1986, when Piechulla and coworkers described for the first time its correct affiliation with the *Flavobacterium-Cytophaga* group (29). An overview of this nomenclatural odyssey was given by Floren et al. (14). At present, the organism is most often referred to as [*Moraxella*] *anatipestifer* (brackets indicate a generically misnamed taxon) or "[*Pasteurella*] *anatipestifer*," and it is considered a species incertae sedis in *Bergey's Manual of Systematic Bacteriology* (5, 24). The numerous similarities between this organism and members of the *Flavobacterium-Cytophaga* group (lack of flagellation, low DNA base ratio, presence of menaquinones as the sole respiratory quinones, presence of branched-chain fatty acids in high percentages, absence of carbohydrate fermentation, and similar hydrolytic enzyme patterns) were significant parameters used to determine genomic relatedness, as shown by Rossau and coworkers (31). Rossau et al. also showed by performing a DNA-rRNA hybridization analysis that [*Moraxella*] *anatipestifer* indeed is not a close relative of the *Moraxella* or *Pasteurella* rRNA homology clusters but belongs to the *Flavobacterium-Cytophaga* rRNA homology cluster (31).

The phylogenetic relationships of the genera *Flavobacterium*, *Cytophaga*, and *Flexibacter* have been the subject of an on-going DNA-rRNA hybridization study performed during the past decade by members of the Ghent research group. Preliminary data were published by Bauwens and De Ley in 1981 (4), while a second set of data was presented recently during the Second International Symposium on *Flavobacterium-Cytophaga* and Related Bacteria in 1992 (32). However, only a phylogenetic tree was presented, with no hybridization values or strain numbers (32). Therefore, we describe in this paper the previous hybridization results and an extensive number of new data obtained from DNA-rRNA hybridization analyses and determinations of DNA base ratios. These results, together with those obtained from the protein and fatty acid analyses, are used below to show that the causative agent of the disease known as septicemia anserum exsudativa constitutes a separate taxon within the *Flavobacterium-Cytophaga* rRNA homology cluster. We propose the name *Riemerella anatipestifer* for this taxon and use this name below.

MATERIALS AND METHODS

Bacterial strains and growth conditions. All of the strains studied in the protein and fatty acid analyses are listed along with their sources in Table 1. The strains included in the DNA-rRNA hybridization analysis are listed in Table 2. The *R. anatipestifer* strains and all of the reference strains included in the protein and fatty acid analyses except *Flavobacterium balustinum* LMG 8329^T (T = type strain) were grown on Trypticase soy agar (catalog no. 11768; BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.) and incubated at 36 to 37°C in a microaerobic atmosphere containing 5% O_2, 10% CO_2, and 85% N_2; *Flavobacterium balustinum* LMG 8329^T was incubated at 28°C. For

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TABLE 1. Strains studied in the protein and fatty acid analyses

Strain[a]	Other designation(s)[a]	Source, year, and place of isolation
R. anatipestifer LMG 10957	Hommez 3-8	Duck, Belgium
R. anatipestifer LMG 10988	Hommez 5-25	Chicken, Belgium
R. anatipestifer LMG 11054^T	CCUG 14215^T, ATCC 11845^T, MCCM 00568^T	Duck blood, United States
R. anatipestifer LMG 11056	CCUG 25000, HPRS 1795	Duck, United Kingdom, 1966–1969
R. anatipestifer LMG 11057	CCUG 25001, HPRS 2591	Duck, United Kingdom, 1966–1969
R. anatipestifer LMG 11059	CCUG 25005, HPRS 2336	Duck, United Kingdom, 1966–1969
R. anatipestifer LMG 11060	CCUG 25054, HPRS 2560	
R. anatipestifer LMG 11524	CCUG 25006, HPRS 1785	Duck, United Kingdom, 1966–1969
R. anatipestifer LMG 11525	CCUG 25008, HPRS 2174	Duck, United Kingdom, 1966–1969
R. anatipestifer LMG 11526	CCUG 25010, HPRS 2528	
R. anatipestifer LMG 11600	MCCM 00761	Turkey heart, Italy, 1987
R. anatipestifer LMG 11601	MCCM 00762, K.-H. Hinz 46	Duck, Germany, 1985
R. anatipestifer LMG 11602	MCCM 00771, K.-H. Hinz 47	Goose, Germany, 1985
R. anatipestifer LMG 11603	MCCM 00772	United States
R. anatipestifer LMG 11604	MCCM 00773	United Kingdom, 1976
R. anatipestifer LMG 11605	MCCM 00793, K.-H. Hinz 1	Duck, Germany, 1981
Flavobacterium gleum LMG 8334^T	CCUG 14555^T, NCTC 11432^T	Vaginal specimen
Flavobacterium indologenes LMG 8337^T	CCUG 14556^T	Trachea
Flavobacterium indoltheticum LMG 4025^T	ATCC 27950^T	Marine mud
Flavobacterium balustinum LMG 8329^T	NCTC 11212^T, LMG 4010^T	Fish heart
Flavobacterium meningosepticum LMG 12279^T	CCUG 214^T, ATCC 13253^T	Human cerebrospinal fluid
W. zoohelcum LMG 8351^T	CCUG 12568^T	Sputum, United States

[a] ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection of the University of Göteborg, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; Hommez, J. Hommez, Provinciaal Verbond voor Dierenziektenbestrijding, Torhout, Belgium; HPRS, Houghton Poultry Research Station, Houghton, United Kingdom; LMG, Culture Collection of the Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium; MCCM, Medical Culture Collection of Microorganisms, Marburg, Germany; NCTC, National Collection of Type Cultures, London, United Kingdom.

DNA and rRNA preparation, *R. anatipestifer* and *Capnocytophaga* strains were incubated as described above; all other strains were incubated aerobically at 30°C.

Bacteriological purity was checked by plating and examining living and Gram-stained cells. For mass cultures, cells were grown in Roux flasks.

Preparation of high-molecular-weight DNA. High-molecular-weight native DNA was prepared as described previously (39).

DNA base compositions. All of the guanine-plus-cytosine (G+C) values were determined by thermal denaturation and were calculated by using the equation of Marmur and Doty (25), as modified by De Ley (10).

Preparation of rRNA. To prepare radioactively labelled rRNA, ^{3}H-labelled adenine or ^{14}C-labelled uracil was added to early-log-phase cultures. In vivo radioactively labelled rRNAs from *R. anatipestifer* LMG 11054^T, *Flavobacterium indologenes* LMG 8337^T, and *Weeksella zoohelcum* LMG 8351^T were prepared by using a slight modification of the procedure of Aiba et al. (1), as described by Vandamme et al. (38). In vivo radioactively labelled rRNAs from *Flavobacterium breve* LMG 4011^T, *Flavobacterium aquatile* LMG 4008^T, *Capnocytophaga ochracea* LMG 11546^T, *Cytophaga heparina* LMG 10339^T, and *Sphingobacterium spiritivorum* LMG 8347^T were prepared as described by De Ley and De Smedt (11). Radioactively labelled rRNA from *Cytophaga johnsonae* LMG 1341^T was prepared in a previous study (4). *Flavobacterium aquatile* LMG 4008^T cells were grown on agar slopes in Roux flasks, whereas the other strains were grown in broth cultures as described by De Ley and De Smedt (11).

DNA-rRNA hybridization experiments. The methods used for fixation of single-stranded DNA on membrane filters, chemical determination of the amount of DNA on a filter, saturation hybridization, RNase treatment, and thermostability measurement of the hybrids were the methods described by De Ley and De Smedt (11).

Polyacrylamide gel electrophoresis of whole-cell proteins. Cells were grown for 48 h on one or two petri dishes. Whole-cell protein extracts were prepared, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as described by Vauterin et al. (40).

Numerical analysis of the protein gel electropherograms. A densitometric analysis, normalization and interpolation of the protein profiles, and a numerical analysis were performed by using the GelCompar software package (Applied Maths, Kortrijk, Belgium). The profiles were recorded and stored on a PC-AT computer. The levels of similarity between pairs of traces were expressed by the Pearson product moment correlation coefficient converted for convenience to a percentage. The stacking gel-separation gel interface and the final part of the protein profile (the region containing proteins having molecular weights less than 20,100 [marker, trypsin inhibitor]) (see Fig. 2) were not used in the numerical analysis.

Fatty acid methyl ester analysis. Cells were grown for 48 h on one or two petri dishes. A loopful of well-grown cells was harvested, and fatty acid methyl ester extracts were prepared and analyzed by using the Microbial Identification System (Microbial ID, Inc., Newark, Del.) as described previously (39).

RESULTS

DNA base compositions. The average DNA base ratios determined are shown in Table 2.

DNA-rRNA hybridizations. The DNA-rRNA hybridization results are given in Table 2 and are shown as a dendrogram based on the melting temperatures of elution [$T_{m(e)}$] of the hybrids in Fig. 1; each DNA-rRNA hybrid was characterized by the $T_{m(e)}$ value (the temperature at which 50% of the DNA-rRNA hybrid was denatured). A homologous duplex was formed between the DNA and rRNA of the same strain; a heterologous hybrid was formed between DNA and rRNA

TABLE 2. $T_{m(e)}$ values of DNA-rRNA hybrids

Organism used for DNA isolation	G + C content (mol%)	$T_{m(e)}$ (°C) with rRNA from:								
		R. anatipestifer LMG 11054^T	*Flavobacterium indologenes* LMG 8337^T	*W. zoohelcum* LMG 8351^T	*Capnocytophaga ochracea* LMG 11546^T	*Cytophaga johnsonae* LMG 1341^T	*Flavobacterium aquatile* LMG 4008^T	*Flavobacterium breve* LMG 4011^T	*Cytophaga heparina* LMG 10339Ta	*Sphingo-bacterium spiritivorum* LMG 8347^T
Bacteroides fragilis NCTC 9343^T	54				60.0		58.8			
Bacteroides oralis ATCC 33269^T	43				56.4		58.2			
Capnocytophaga ochracea LMG 11546^T	39	61.8	62.0		75.7		63.7			
Capnocytophaga sputigena LMG 11518^T	38				74.7		68.4			
Capnocytophaga gingivalis LMG 11514^T	40				70.3	65.7	66.3	61.2		
Cytophaga hutchinsonii LMG 10844	40						62.2		60.4	
Cytophaga heparina LMG 10339^T	43						63.1	59.8	80.0	67.1
Cytophaga johnsonae LMG 1341^T	34				66.3	76.9	72.8	62.9	58.5	
C. johnsonae LMG 1342	35					75.1	73.2			
Cytophaga marinoflava LMG 1345^T	37					71.6	71.0			
Cytophaga lytica LMG 1344^T	33					70.3	70.0			
Cytophaga salmonicolor LMG 1346^T	42					68.0				
Cytophaga uliginosa LMG 3809^T	37					67.8	68.8	63.4		
Flavobacterium acidificum LMG 8364	54						54.1			
Flavobacterium acidurans LMG 8388	66						54.6	55.6		
Flavobacterium aquatile LMG 4008^T	33		63.5		65.9	73.1	77.0	64.7		55.7
Flavobacterium balustinum LMG 8329^T	35	72.1	73.7	70.6	62.4		66.3	64.9		
Flavobacterium breve LMG 4011^T	35	64.0			63.2	66.3	65.2	76.4		58.3
F. breve LMG 4012	31					66.2	66.7	75.1		
F. breve LMG 4013	32					66.5	67.5			
F. breve LMG 4014	32					65.8	66.0			
"Flavobacterium dehydrogenans" LMG 4015^T	73						59.0			
"Flavobacterium denitrificans" LMG 4016	64						55.2			
Flavobacterium ferrugineum LMG 10403^T	46						58.0			55.7
"Flavobacterium fuscum" LMG 8391	65						58.7			
Flavobacterium gleum LMG 8333	38	70.8	74.4	71.7				66.1		
F. gleum LMG 8334^T	37				63.5		64.9	65.5		
Flavobacterium indologenes-like strain LMG 8336	36	72.9	74.0	71.8			65.3	66.3		
Flavobacterium indologenes LMG 8337^T	38	71.7	78.1	72.5	62.8		65.1	66.4		
Flavobacterium indoltheticum LMG 4025^T	30		75.3	73.1						
Flavobacterium marinotypicum LMG 8374	59						52.9			
"Flavobacterium marinovirosum" LMG 8375	47							59.6		
Flavobacterium meningosepticum LMG 12279^T	35	72.7	72.2	71.9	64.5		65.2	67.2		
F. meningosepticum LMG 12873		72.3	72.3	72.4			66.7	66.8		
F. meningosepticum NCTC 10585	37					67.0	68.0			
F. meningosepticum NCTC 10016^T					64.5	65.2	66.4	67.2		
F. meningosepticum LMG 12967	37							66.9		
Flavobacterium odoratum LMG 4028	34					72.0	73.5			

Continued on following page

TABLE 2—*Continued*

Organism used for DNA isolation	G + C content (mol%)	$T_{m(e)}$ (°C) with rRNA from:								
		R. anatipestifer LMG 11054^T	*Flavobacterium indologenes* LMG 8337^T	*W. zoohelcum* LMG 8351^T	*Capnocytophaga ochracea* LMG 11546^T	*Cytophaga johnsonae* LMG 1341^T	*Flavobacterium aquatile* LMG 4008^T	*Flavobacterium breve* LMG 4011^T	*Cytophaga heparina* LMG 10339Ta	*Sphingo-bacterium spiritivorum* LMG 8347^T
F. odoratum LMG 4029	32					71.3	73.3			
F. odoratum LMG 1233^T	37					71.3	71.4			
Flavobacterium okeanokoites LMG 4030^T	46						55.5	53.4		
"*Flavobacterium pectinovorum*" LMG 4031	35						73.3			
"*Flavobacterium proteus*" ATCC 12841	49						57.2			
Flavobacterium resinovorum LMG 8367^T	66						57.3			
Flavobacterium thalpophilum LMG 11520^T	44									69.0
F. thalpophilum LMG 11521	44									68.2
Flavobacterium thermophilum NCTC 11526^T							55.7	54.9		
Flavobacterium yabuuchiae LMG 11523	38									76.6
Flexibacter canadensis LMG 8368^T	38									67.9
Flexibacter flexilis LMG 10845^T	38		58.8							65.2
"*Flexibacter elegans*" LMG 10750^T	38									66.0
"*Flexibacter aurantiacus* subsp. *excathedrus*" LMG 3986^T	34					67.5	69.5			
Flexibacter roseolus LMG 3993^T	39					62.2	60.7			
Flexibacter ruber LMG 3994^T	37					62.3	58.2			
Heparinase-producing strain LMG 9527[a]	38								77.0	67.4
Heparinase-producing strain LMG 10351[a]	41								74.2	
Heparinase-producing strain LMG 10337[a]	37								70.9	67.6
Riemerella anatipestifer LMG 11054^T	35	77.5	72.4	72.7	64.5	62.7	65.0	64.5		
R. anatipestifer LMG 10957	35	77.7								
R. anatipestifer LMG 10988	35	77.6								
R. anatipestifer LMG 11600		77.5								
R. anatipestifer LMG 11601	35[b]	77.5								
R. anatipestifer LMG 11602	29[b]	77.6	72.6							
R. anatipestifer LMG 11603	35[b]	77.5								
R. anatipestifer LMG 11604	35	77.6								
R. anatipestifer LMG 11605	33	77.7								
R. anatipestifer IPDH 475/75					64.9		66.7	65.8		
R. anatipestifer IPDH 220/79					65.1		65.7	65.3		
Saprospira grandis LMG 10407^T	46						61.2			57.1
Sphingobacterium spiritivorum LMG 8347^T	40		59.3				60.7	61.6	69.5	77.2
S. spiritivorum LMG 8348										76.7
Sphingobacterium multivorum LMG 8342^T	41						62.4	60.7	67.4	72.6
S. multivorum LMG 8354										73.0
Sphingobacterium mizutae LMG 8340^T	39								67.3	71.2
S. mizutae LMG 8341										72.3
Weeksella virosa LMG 8349	37	62.4		64.8			63.9	69.1		
W. virosa LMG 8350	37			64.4				68.6		
Weeksella zoohelcum LMG 8351^T	37	70.9	73.1	75.0	63.9		66.1	65.4		
W. zoohelcum LMG 8352	37	67.1	73.8	76.8			65.5	65.4		

[a] A number of hybridization values for these organisms were published in reference 35.
[b] Data from reference 27.

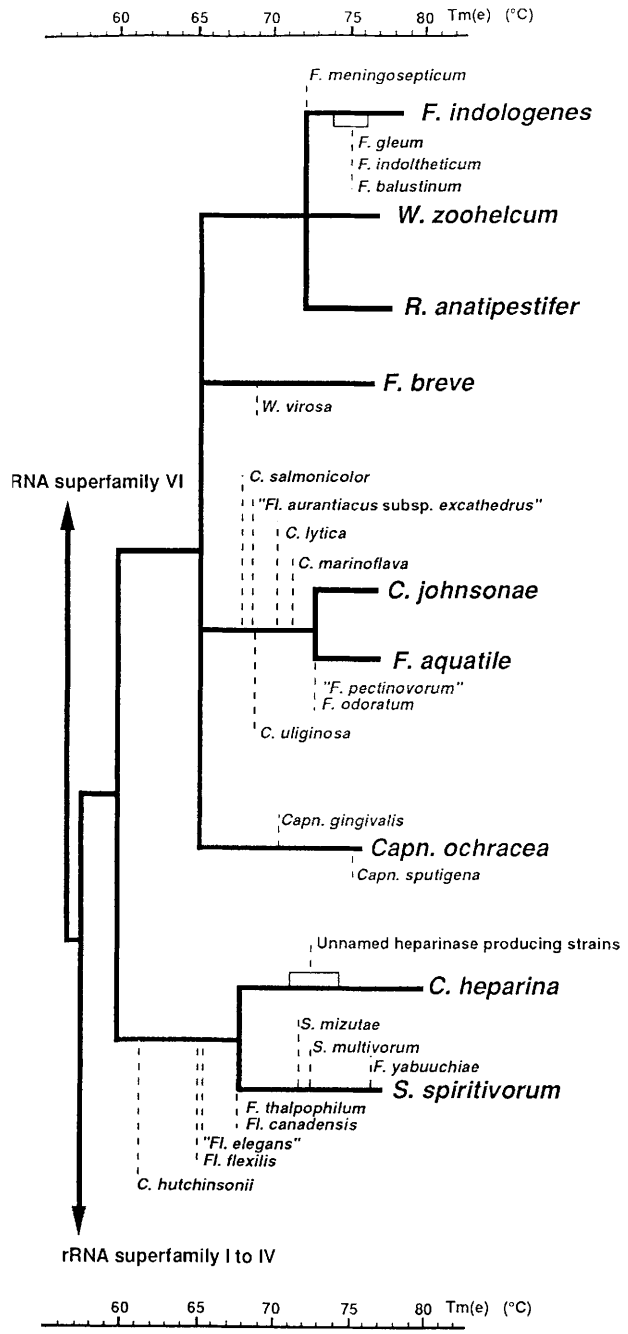

FIG. 1. Simplified rRNA cistron similarity dendrogram of rRNA superfamily V. Abbreviations: *C.*, *Cytophaga*; *Capn.*, *Capnocytophaga*; *F.*, *Flavobacterium*, *Fl.*, *Flexibacter*; *R.*, *Riemerella*; *S.*, *Sphingobacterium*; *W.*, *Weeksella*.

of different strains. The $T_{m(e)}$ values from the reciprocal hybridization experiments obtained by using all strains of each rRNA branch were used to calculate the average linkage level between each pair of rRNA branches.

Polyacrylamide gel electrophoresis of whole-cell proteins. Duplicate protein extracts were prepared to check the reproducibility of the growth conditions and the preparation of the extracts. The correlation level between duplicate protein patterns was ≥0.95 (Pearson product moment correlation coefficient).

The protein profiles of several *R. anatipestifer* strains were determined along with the profiles of reference strains belonging to the nearest phylogenetic neighbors of *R. anatipestifer* (Fig. 1). Figure 2 shows the protein profiles and the resulting numerical analysis data for all of the strains analyzed. All *R. anatipestifer* strains cluster together above a similarity level of 85%. At this similarity level, all reference strains occupy separate positions.

Cellular fatty acid compositions. The fatty acid profiles of *R. anatipestifer* strains and reference strains belonging to the nearest phylogenetic neighbors of *R. anatipestifer* were determined. The average fatty acid composition of *R. anatipestifer* strains and the fatty acid compositions of the reference strains are shown in Table 3. Generally, branched fatty acids account for the majority of the fatty acid contents of all of the strains studied. Fatty acids 13:0 iso, 15:0 iso, 15:0 iso 3OH, and 17:0 iso 3OH are the major fatty acids in *R. anatipestifer*. The *Flavobacterium* and *Weeksella* species studied lack high percentages of fatty acids 13:0 iso and 15:0 iso 3OH. Furthermore, the latter organisms are characterized by the presence of two additional major fatty acids, iso 17:1ω9c and "summed feature 4." As explained in Table 3, summed feature 4 comprises fatty acid 15:0 iso 2OH or 16:1ω7t or both. We cannot eliminate one of these fatty acids as both have been reported to be present in *Flavobacterium* species (26, 42).

DISCUSSION

The results of the DNA-rRNA hybridization analysis indicate that most members of the genera *Flavobacterium*, *Cytophaga*, *Flexibacter*, *Sphingobacterium*, *Capnocytophaga*, and *Weeksella* and some other organisms constitute a separate eubacterial lineage within the group of gram-negative bacteria. This lineage has been named rRNA superfamily V (12, 31). Comparisons of 16S rRNA sequences of some of these taxa led to similar conclusions (15, 41). So far, we have found five major rRNA homology groups within this rRNA superfamily. The first rRNA homology group contains four different rRNA branches (Fig. 1). *R. anatipestifer* and *W. zoohelcum* each constitutes a separate rRNA branch; *Flavobacterium indologenes*, *Flavobacterium gleum*, *Flavobacterium indoltheticum*, and *Flavobacterium balustinum* constitute a third rRNA branch, while *Flavobacterium meningosepticum* occupies a separate position at the linkage level of the three other rRNA branches and as such constitutes a separate rRNA branch.

At present, the second rRNA homology group contains a single rRNA branch comprising *Flavobacterium breve* and *Weeksella virosa* (Fig. 1). The difference in $T_{m(e)}$ of 7.5°C in comparisons with rRNA of *Flavobacterium breve* reflects a considerable genomic divergence between the two taxa.

The third rRNA homology group contains two rRNA branches and includes species belonging to the genera *Flavobacterium*, *Cytophaga*, and *Flexibacter*. Again, the large differences in $T_{m(e)}$ values found in comparisons with the two types of rRNAs revealed that there is wide genomic heterogeneity within this rRNA cluster. Only two of the *Flavobacterium* species belonging to this rRNA homology group, *Flavobacterium aquatile* and *Flavobacterium odoratum*, are described as valid species in *Bergey's Manual of Systematic Bacteriology* (18). Furthermore, the taxonomic status of

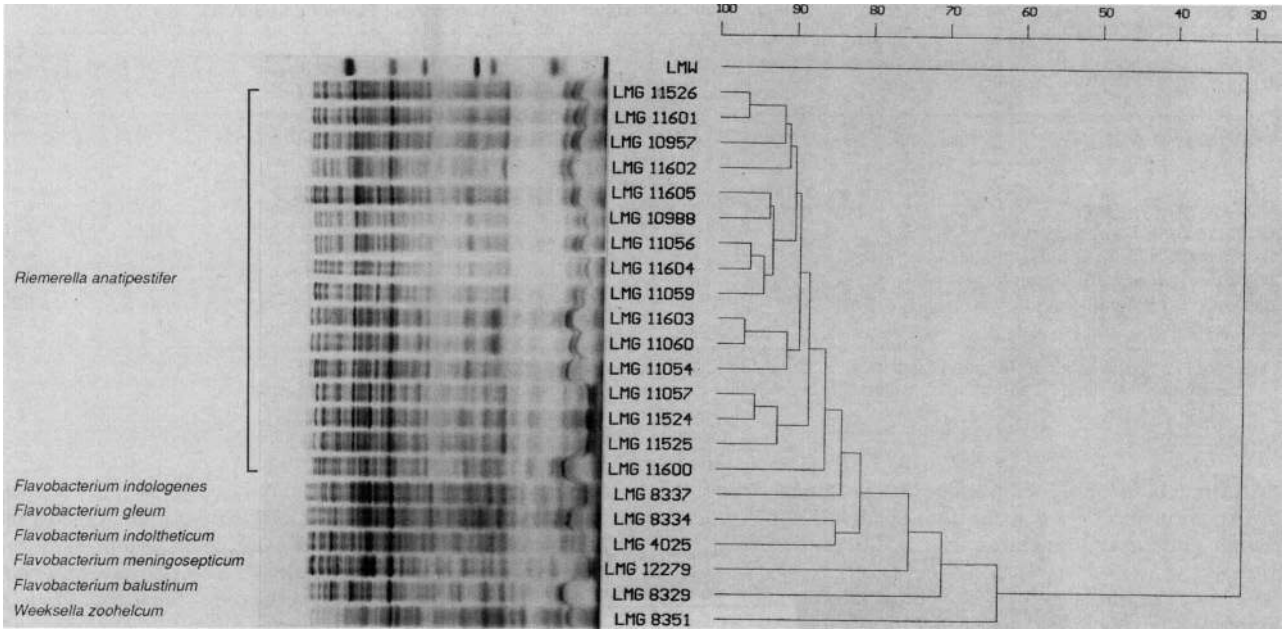

FIG. 2. Protein profiles of strains listed in Table 1 and corresponding dendrogram derived from unweighed pair group average linkage of Pearson product moment correlation coefficients (expressed for convenience as percentages). The positions of the molecular weight markers (track LMW) were as follows (from right to left): lysozyme, 14,500; trypsin inhibitor, 20,100; trypsinogen, 24,000; carbonic anhydrase, 29,000; glyceraldehyde-3-phosphate dehydrogenase, 36,000; egg albumin, 45,000; and bovine albumin, 66,000.

Flavobacterium aquatile as the type species of the genus *Flavobacterium* has been the subject of much controversy; most often this organism is not considered a true *Flavobacterium* species (16, 17). The type species of the genera *Cytophaga* and *Flexibacter*, *Cytophaga hutchinsonii* and *Flexibacter flexilis*, respectively, do not belong to this rRNA homology group, which implies that all *Cytophaga* and *Flexibacter* species belonging to this rRNA homology group are generically misnamed. Obviously, the remaining *Cytophaga* and *Flexibacter* species should be included in an analysis, and more labelled rRNAs have to be prepared in order to determine the detailed genotypic structure of this complex rRNA homology group. Only then can a significant taxonomic revision be proposed.

The *Capnocytophaga* species studied so far form a separate fourth rRNA homology group represented by a single rRNA branch (Fig. 1). *Capnocytophaga sputigena* and *Capnocytophaga ochracea* cluster together at the top of this

TABLE 3. Fatty acid compositions of the organisms studied[a]

Fatty acid	% in:						
	R. anatipestifer (16 strains)	*Flavobacterium gleum* LMG 8334^T	*Flavobacterium indologenes* LMG 8337^T	*Flavobacterium balustinum* LMG 8329^T	*Flavobacterium indoltheticum* LMG 4025^{Tb}	*Flavobacterium meningosepticum* LMG 12279^T	*W. zoohelcum* LMG 8351^{Tc}
13:0 iso	14.9 ± 3.6 (16)	Tr	Tr	Tr	Tr	1.9	3.6
ECL 13.566[d]	1.5 ± 0.6 (16)	2.1	2.2	2.6	Tr	1.4	1.5
15:0 iso	52.8 ± 4.9 (16)	35.5	34.8	31.1	34.1	42.1	61.9
15:0 anteiso	5.8 ± 1.8 (16)	ND	Tr	1.1	5.3	1.9	ND
SF 4[e]	ND	13.3	11.6	8.9	10.2	17.3	6.4
16:0	Tr (8)	1.0	1.2	2.7	1.3	Tr	ND
15:0 iso 3OH	8.0 ± 2.8 (16)	3.0	2.8	2.6	2.0	3.7	4.1
iso 17:1ω9c	ND	19.1	19.8	26.3	21.7	4.6	9.1
ECL 16.580	Tr (12)	1.9	1.6	1.4	1.4	1.7	ND
17:0 iso	ND	1.3	1.1	1.7	1.0	Tr	Tr
16:0 iso 3OH	0.9 ± 0.6 (15)	Tr	Tr	Tr	Tr	1.1	ND
16:0 3OH	ND	1.1	1.2	1.5	1.1	2.3	ND
17:0 iso 3OH	13.1 ± 3.5 (16)	20.6	20.5	15.1	15.4	17.6	9.9

[a] Those fatty acids which account for less than 1% of the total fatty acids in all strains studied are not shown. Therefore, the percentages do not total 100%. For *R. anatipestifer*, the means, standard deviations, and numbers of strains containing the fatty acids (values in parentheses) are shown. Tr, trace (less than 1.0%); ND, not detected.

[b] *Flavobacterium indoltheticum* LMG 4025^T also contains 2.7% 17:0 2OH.

[c] *W. zoohelcum* LMG 8351^T also contains 1.5% summed feature 5 (i.e., 17:1 iso I or 17:1 anteiso B or both).

[d] ECL, equivalent chain length. The identity of the fatty acid is unknown.

[e] SF 4, summed feature 4 (15:0 iso 2OH or 16:1ω7t or both).

TABLE 4. Differentiating phenotypic characteristics for *R. anatipestifer* and allied bacteria[a]

Species	Production of indole	Pigment production	Nitrate reduction	β-Galactosidase activity	Urease activity	Optimal growth conditions	Growth at 42°C	Growth on MacConkey agar
R. anatipestifer	−[b]	−	−	−	d	Microaerobic with CO_2 enrichment	(+)	−
Flavobacterium gleum	+	+	d	d	d	Aerobic	d	+
Flavobacterium indologenes	+	+	d	d	−	Aerobic	d	d
Flavobacterium balustinum	+	+	+	−	−	Aerobic	−	+
Flavobacterium indoltheticum	+	+	−	−	−	Aerobic	−	+
Flavobacterium meningosepticum	d	+	(−)	(+)	d	Aerobic	d	+
W. zoohelcum	+	−	−	−	+	Aerobic	(−)	−

[a] Data for *Flavobacterium and Weeksella* species were obtained from references 6, 19, 20, and 42.
[b] +, present in all strains; −, absent in all strains; (+), present in most strains; (−), absent in most strains; d, strain dependent.

rRNA branch, which corroborates the previously reported close relationship between the two species (34). *Capnocytophaga gingivalis* branches off at a difference in $T_{m(e)}$ of about 5°C.

The average linkage level for these four major rRNA homology groups is a $T_{m(e)}$ of 65.1 ± 1.5°C. This value is the average from 66 hybridizations between representatives of the four groups. It is obvious that organisms belonging to the different rRNA homology groups [with differences in $T_{m(e)}$ of about 11 to 14°C] cannot belong to a single genus. Even within most of the rRNA homology groups, the range of $T_{m(e)}$ values is so wide that, theoretically, several genera can be delineated, provided that the division is based on solid chemotaxonomic or other phenotypic grounds.

A fifth rRNA homology group is linked to the other groups at $T_{m(e)}$ of 59.9 ± 2.0°C (average of 11 values). Until now, this cluster contained two rRNA branches. The first rRNA branch consists of *Cytophaga heparina* and various other, as-yet-unnamed, heparin-degrading isolates (35, 36). The second rRNA branch contains the three *Sphingobacterium* species and *Flavobacterium yabuuchiae*, which exhibits high DNA homology values with *Sphingobacterium spiritivorum* (21). *Flavobacterium thalpophilum* and *Flexibacter canadensis* are situated at the base level between the two rRNA branches. Clearly, *Flavobacterium thalpophilum*, which is also a sphingophospholipid-producing organism, can be included in the genus *Sphingobacterium* on genotypic grounds as well. Finally, *Flexibacter flexilis*, "*Flexibacter elegans*," and *Cytophaga hutchinsonii* occupy separate positions on the $T_{m(e)}$ dendrogram (Fig. 1).

For a considerable number of taxa included in this study, the 16S rRNA sequence of a representative strain has been determined (15). Sequence comparison of 16S rRNAs is a more powerful tool than DNA-rRNA hybridization analysis for resolving taxonomic relationships at the deeper phylogenetic level (41). However, the detailed structure of the various subclusters is strongly influenced by the choice of outgroup, and the clustering sequence can be altered by another outgroup taxon (28). Despite the differences in branching order, the major groups obtained in the two studies are similar and, logically, lead to the same conclusions: the genera *Flavobacterium*, *Cytophaga*, *Flexibacter*, and *Weeksella* are polyphyletic genera which should be redefined, whereas the genera *Capnocytophaga*, *Sphingobacterium*, and *Riemerella* are genotypically well defined. A revision of the taxonomy of the former genera is overdue but would be premature until all present members of these genera are studied.

The following taxa exhibited only very low $T_{m(e)}$ values in comparisons with all of the reference strains studied (Table 2): *Flavobacterium acidificum*, *Flavobacterium acidurans*, "*Flavobacterium dehydrogenans*," "*Flavobacterium denitrificans*," *Flavobacterium ferrugineum*, "*Flavobacterium fuscum*," *Flavobacterium marinotypicum*, "*Flavobacterium marinovirosum*," *Flavobacterium okeanokoites*, "*Flavobacterium proteus*," *Flavobacterium resinovorum*, *Flavobacterium thermophilum*, *Flexibacter roseolus*, *Flexibacter ruber*, *Saprospira grandis*, *Bacteroides fragilis*, and *Bacteroides oralis*. The exact phylogenetic position of these taxa is not the subject of this paper and will be dealt with separately.

Differentiation of *R. anatipestifer* from its closest relatives. *Flavobacterium gleum*, *Flavobacterium indologenes*, *Flavobacterium indoltheticum*, *Flavobacterium balustinum*, *Flavobacterium meningosepticum*, and *W. zoohelcum* belong to the same rRNA homology group as *R. anatipestifer*. These taxa are situated on the dendrogram at a difference in $T_{m(e)}$ of about 5°C. This difference in $T_{m(e)}$ reflects a genomic divergence between *R. anatipestifer* and its allies which is large enough to warrant separate generic status for *R. anatipestifer*, provided that a sufficient number of differentiating chemotaxonomic or other features are available. *R. anatipestifer* can be distinguished from its neighbors by its capnophilic metabolism, by the absence of pigments, and by its fatty acid (Table 3) and protein (Fig. 2) contents. The overall fatty acid pattern is in agreement with previously published data (22, 37). Furthermore, the type strain of *R. anatipestifer* contains menaquinone 7 as its sole respiratory quinone (13), whereas the flavobacteria belonging to this rRNA homology group contain menaquinone 6 (9). No data on the quinone content of *W. zoohelcum* are available. Additional phenotypic features useful for differentiating *R. anatipestifer* from its closest neighbors are shown in Table 4. These phenotypic data and the features listed in the description given below were obtained from references 3, 6 through 8, 13, 27, 29, and 33.

Clearly, *R. anatipestifer* is very different from its relatives in many aspects. We therefore concluded that a separate genus for this taxon is justified on the basis of genotypic and phenotypic criteria. DNA-DNA hybridization studies with *R. anatipestifer* revealed high levels of intraspecies DNA homology (2, 27, 29).

Description of the genus *Riemerella* gen. nov. *Riemerella* (Rie.me.rel'la. proper name Riemer; L. dim. suf. *-ella*; N. L. fem. n. *Riemerella*, named in honor of Riemer, who first described *R. anatipestifer* infections in geese in 1904 and

referred to the disease as septicemia anserum exsudativa [30]). Cells are gram-negative, nonsporulating rods. Most strains grow aerobically and microaerobically after primary isolation in a CO_2-enriched atmosphere. A number of carbohydrates are weakly acidified or fermented. At present it is not possible to give a general biochemical profile for the genus as only a single species is known.

The type species is *R. anatipestifer*. The DNA base composition ranges from 29 to 35 mol% G+C.

Description of *Riemerella anatipestifer* comb. nov. *Riemerella anatipestifer* (a.na.ti.pes'ti.fer. L. n. *anas*, duck; L. n. *pestifer*, trouble bearer; N. L. n. *anatipestifer*, cause of a disease in ducks). It must be emphasized that the specific epithet *anatipestifer* is kept in order to avoid nomenclatural confusion; the illness caused by this organism is a septicemic disease which is not restricted to ducks. The description of *R. anatipestifer* is the same as the description of the genus. *R. anatipestifer* cells are gram-negative, nonmotile, nonsporulating rods that are 0.3 to 0.5 μm wide and 1 to 2.5 μm long and occur singly, in pairs, or in short chains. Gliding motility is not observed. Smooth, nonpigmented colonies are developed within 2 days during microaerobic incubation on rich peptone, peptone-blood, or chocolate agar at 36°C. Optimal growth occurs at 37°C; most strains grow at 45°C but not at 4°C. Requires thiamine, but low concentrations of pyrithiamine and amprolium are inhibitory. Nitrates are not reduced. Urease and chymotrypsin activities, hemolysis, and the litmus milk reaction are strain dependent. Indole is not produced. Most strains liquefy gelatin, Löffler's blood serum, and coagulated egg medium. Growth occurs in Huddleson's thionine medium, in Huddleson's basic fuchsin medium, and on agar containing 10% bile in serum. No growth occurs on agar containing 40% bile in serum, citrate agar, or MacConkey agar or in KCN broth or glycerol phosphate medium. Hydrogen sulfide is not produced. The following enzyme activities are present: oxidase, catalase, alkaline and acid phosphatase, ester lipase C8, leucine arylamidase, valine arylamidase, cystine arylamidase, phosphoamidase, α-glucosidase, and esterase C4. The following enzyme activities are absent: α- and β-galactosidases, β-glucuronidase, β-glucosidase, α-mannosidase, β-glucosaminidase, lipase C14, fucosidase, trypsin, ornithine and lysine decarboxylases, and phenylalanine deaminase. Highly susceptible to penicillin and highly resistant to polymixin B and kanamycin.

Menaquinone 7 is the sole respiratory quinone detected in the type strain. The fatty acids include branched fatty acids 13:0 iso, 15:0 iso, 15:0 anteiso, 15:0 iso 3OH, and 17:0 iso 3OH and an unidentified fatty acid with an equivalent chain length of 13.566.

Isolated from ducks, geese, turkeys, and waterfowl with septicemic disease. *R. anatipestifer* is important in veterinary medicine as it is distributed worldwide and causes serious problems in commercial duck, goose, and turkey flocks (23, 27). Furthermore, strains have also been isolated from muscovy ducks, chickens, pheasants, quails, and wild free-living waterfowl.

The type strain is LMG 11054 (= ATCC 11845 = MCCM 00568 = CCUG 14215), which was isolated from a duck in the United States. Its G+C content is 35 mol%.

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