Relatedness of Three Species of “False Neisseriae,” *Neisseria caviae*, *Neisseria cuniculi*, and *Neisseria ovis*, by DNA-DNA Hybridizations and Fatty Acid Analysis

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DNA-DNA hybridization was used to determine the levels of genomic relatedness of the three species of “false neisseriae,” *Neisseria caviae*, *Neisseria cuniculi*, and *Neisseria ovis*. The reference strains of these species exhibited high levels of intraspecies relatedness (93 to 100% for *N. caviae*, 79 to 100% for *N. cuniculi*, and 68 to 100% for *N. ovis*) but low levels of interspecific relatedness (less than 34%) to each other and to various species belonging to the β subclass of the *Proteobacteria* (*Kingella kingae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Oligella urethralis*) or to the γ subclass (*Branhamella catarrhalis*, *Kingella indologenes*, *Moraxella atlantae*, *Moraxella bovis*, *Moraxella lacunata* subsp. *lacunata*, *Moraxella lacunata* subsp. *liquefaciens*, *Moraxella nonliquefaciens*, *Moraxella osloensis*, and *Moraxella phenylpyruvica*). However, the levels of DNA-DNA hybridization for the three species of “false neisseriae” were significantly higher with the species belonging to the γ subclass (average, 13.7%) than with the species belonging to the β subclass (average, 4.5%). These data suggest that *N. caviae*, *N. cuniculi*, and *N. ovis* are three separate genomic species in the γ subclass. An ascendant hierarchical classification based only on fatty acid profiles distinguished four main classes containing (i) most of the “classical moraxellae,” the “false neisseriae,” and *B. catarrhalis*, (ii) only *Acinetobacter* spp., (iii) *M. nonliquefaciens* and “misnamed moraxellae” (*M. atlantae*, *M. osloensis*, and *M. phenylpyruvica*), and (iv) the “true neisseriae,” the three *Kingella* species, and *O. urethralis*. Fatty acids that distinguish these four classes were identified. The fatty acid profiles of the two strains of *Psychrobacter immobilis* which we studied are not very similar to the profiles of the other taxa. Our results support the hypothesis that the three species of “false neisseriae,” *B. catarrhalis*, the “classical moraxellae,” and *Acinetobacter* spp. should be included in the same family.

The genus *Branhamella*, containing *Branhamella catarrhalis* as the only species, was proposed by Catlin in 1970 to accommodate *Neisseria catarrhalis*, which was more closely related to *Moraxella* spp. than to *Neisseria* spp. as determined by genetic transformations (12). In 1979, Bøvre proposed that *B. catarrhalis* should be included in the genus *Moraxella* and that this genus should be divided into two subgenera, subgenus *Moraxella* for rod-shaped bacteria and subgenus *Branhamella* for coccidial organisms, including *Moraxella* (*Branhamella*) *catarrhalis* (4). In 1984 Bøvre recognized the following four genera in the family *Neisseriaceae*: *Neisseria*, *Moraxella* (with two subgenera), *Acinetobacter*, and *Kingella* (5).

The genus *Neisseria*, as described by Vedros (47), contains 11 species that are regarded in this paper as the “true neisseriae” and the following 4 species regarded as species incertae sedis: *Neisseria caviae*, *Neisseria ovis*, and *Neisseria cuniculi* (the “false neisseriae”), as well as *Neisseria animalis*. In the genus *Moraxella*, Bøvre placed six species in subgenus *Moraxella* and four species in subgenus *Branhamella* (*Moraxella* [*Branhamella*] *catarrhalis*, *Moraxella* [*Branhamella*] *caviae*, *Moraxella* [*Branhamella* med] *ovis*, and *Moraxella* [*Branhamella*] *cuniculi*); “*Moraxella urethralis*” was regarded as a species incertae sedis (6).

More recently, *Neisseria* species and related species have been studied intensively to establish a phylogenetic classification. Numerous gram-negative taxa that are referred to as

the “purple bacteria and their relatives” are now placed in the new class *Proteobacteria* (43), in which several RNA phylogenetic subclasses (α , β , γ , and δ) have been distinguished on the basis of the results of comparisons of rRNA sequences (50). Using rRNA-DNA hybridization data, DeLey and coworkers (15, 36, 39) divided the majority of the gram-negative bacteria into four rRNA superfamilies, superfamilies I to IV; the α , β , and γ phylogenetic subclasses appear to correspond to superfamilies IV, III, and I plus II, respectively (43).

The phylogenetic heterogeneity of the family *Neisseriaceae* was emphasized as early as 1986 by Rossau et al. (39), who demonstrated that this family contains at least five unrelated groups. On the basis of the results of Rossau et al. and some more recent studies (17, 37, 38), the relevant species (which were included in this study) could be distributed into two phylogenetic groups, the β subclass and the γ subclass.

The β subclass (superfamily III) contains the “true neisseriae,” *Kingella kingae*, *Kingella denitrificans*, and “*M. urethralis*.” For the last species, the new genus *Oligella* has been proposed, with *Oligella urethralis* as the type species (36), because this organism is not related to the genus *Moraxella* (39), is rather distantly related to the genus *Neisseria*, and is most closely related to *Taylorella equigenitalis* and to the family *Alcaligenaceae* (36). Thus, the definition of the family *Neisseriaceae* should be restricted to the genera *Neisseria* (“true neisseriae”) and *Kingella* (39).

The γ subclass (superfamily II) contains the genera *Moraxella* and *Acinetobacter*, the “false neisseriae,” *B. ca-*

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tarrhalis, and *Kingella indologenes*. For the last species, which is related to neither *K. kingae* nor *K. denitrificans* (37, 39), the new genus *Suttonella* has been created; this genus is assigned to the new family *Cardiobacteriaceae* (18). In the γ subclass, Rossau et al. defined a large group, the "Moraxellaceae rRNA cluster," whose members exhibit high levels of DNA-rRNA relatedness (melting temperatures of elution: 69.9 to 80.0°C) (38). This cluster contains the genus *Acinetobacter* and the *Moraxella*-*Psychrobacter* group. The *Moraxella*-*Psychrobacter* group is divided into the following four subgroups: the *Moraxella lacunata* subgroup (the "classical moraxellae" [6], including *Moraxella lacunata* subsp. *lacunata*, *Moraxella lacunata* subsp. *liquefaciens*, *Moraxella bovis*, and *Moraxella nonliquefaciens*), the *Moraxella osloensis* subgroup, the *Moraxella atlantae* subgroup, and the *Psychrobacter*-*Moraxella phenylpyruvica* subgroup. The "false neisseriae" and *B. catarrhalis* belong to the *M. lacunata* subgroup (38).

Recently, to accommodate the genus *Moraxella* and related genera, two proposals for bacterial family names were published simultaneously. In one proposal, based on the work of Rossau et al., both the genus *Branhamella* and the "false neisseriae" are assigned to the genus *Moraxella* in the new family *Moraxellaceae* (38); and in the other proposal, based on the work of Catlin, the genera *Moraxella* and *Branhamella* are considered separate genera within the new family *Branhamaceae* and the "false neisseriae" are assigned to the genus *Branhamella* (13).

Throughout all of these studies and proposals, the nomenclatural status of the three species of "false neisseriae" has been debated. In *Bergey's Manual of Systematic Bacteriology* (5), these species were either regarded as species incertae sedis in the genus *Neisseria* (47) or assigned to the genus *Moraxella* in the subgenus *Branhamella* (6). Another disagreement has occurred in the proposals of Rossau et al. (38) and Catlin (13) for a family name. In this study, we took a polyphasic approach (29) to the relationships between the "false neisseriae" and some related species by performing DNA-DNA hybridization experiments and comparing fatty acid profiles. Below we discuss the taxonomic status of these organisms. The chromatographic procedures and some of the fatty acid results have been described previously (26).

MATERIALS AND METHODS

Bacterial strains. The 61 bacterial strains used in this study are listed in Table 1. These organisms were received as members of 23 named species (including the three species of "false neisseriae") and two genomic groups belonging to the genus *Acinetobacter* (3). Except for *K. denitrificans*, each named species was represented by its type strain. For four of the species (the three species of "false neisseriae" and *B. catarrhalis*), the type strains were studied in duplicate; one sample came from the Collection de l'Institut Pasteur, and the other sample was obtained directly from the National Collection of Type Cultures.

Cultural conditions. For DNA extractions, the following two agar media were used: Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) and GGA (32). For the fatty acid analysis, all of the strains were cultivated on GGA, which was required for reproducibility. GGA (32) consisted of "milieu de base pour isolement des gonocoques et des méningocoques" (Sanofi-Diagnostics Pasteur, Marnes-la-Coquette, France) supplemented with 27% (vol/vol) supplement G. Supplement G (Sanofi-Diagnostics Pasteur) contains 330 ml of horse plasma, 200 ml of yeast extract, 470 ml

of "extrait globulaire," and 1.3 g of glucose. The "extrait globulaire" was prepared as described previously (45).

The strains were cultivated under different conditions, according to their requirements. *N. caviae*, *N. cuniculi*, *N. ovis*, and *B. catarrhalis* were cultivated on Mueller-Hinton agar (for DNA extraction) or on GGA (for fatty acids analysis), and *N. gonorrhoeae*, *N. meningitidis*, *M. phenylpyruvica*, and *Kingella* spp. were cultivated on GGA. *M. bovis*, *M. nonliquefaciens*, and *O. urethralis* were cultivated on Mueller-Hinton agar (for DNA extraction) or on GGA (for fatty acids analysis). *M. lacunata*, *M. lacunata* subsp. *liquefaciens*, *M. osloensis*, *M. atlantae*, and *Acinetobacter* spp. were cultivated on GGA. The first eight species were incubated at 37°C in humid air containing 10% CO₂. The other species were incubated at 30°C under aerobic conditions without CO₂. *Psychrobacter immobilis* was incubated at room temperature under aerobic conditions without CO₂ on GGA.

The strains were preserved frozen at -80°C in buffered medium containing 5% glycerol (1). The methods used for microscopic examination and biochemical tests have been described previously (32, 45, 46).

DNA-DNA hybridizations. The methods described by Brenner et al. were used for extraction, purification, and shearing of unlabeled DNA (10). The procedures used for in vitro labeling of DNA with tritium-labeled nucleotides and for the hybridization experiments (S1 nuclease-trichloroacetic procedure) have been described previously (19). The temperature (T_m) at which 50% of a reassociated DNA became hydrolyzable by S1 nuclease was determined as described by Crosa et al. (14). The difference (ΔT_m) between the T_m of the homoduplex and the T_m of the heteroduplex is an estimate of the divergence between two DNAs (9).

FAME extracts. Whole-cell fatty acids were analyzed as fatty acid methyl esters (FAMES). FAME extracts were prepared by using the technique described by Miller and Berger (27), modified only in the extraction solvent used (hexane instead of a mixture of hexane and ethyl ether). Briefly, the technique consisted of the following five steps: (i) approximately 50 mg (wet weight) of cells was harvested after 24 h of incubation; (ii) to release the fatty acids, the whole bacterial cells were saponified with 1 ml of a hydromethanolic solution of NaOH for 30 min in a boiling water bath; (iii) the free fatty acids were methylated with 2 ml of a hydromethanolic solution of HCl for 10 min in an 80°C water bath; (iv) the FAMES were then extracted with 1.25 ml of hexane; and (v) before injection, the hexane extract was washed with 3 ml of an aqueous 0.3 M NaOH solution.

Gas chromatographic procedure. FAMES were separated by capillary gas-liquid chromatography, using a model DI 200 chromatograph (Delsi-Nermag, Argenteuil, France) equipped with a 30-m SPB1 fused-silica capillary column (Supelco, St.-Germain-en-Laye, France) and a flame ionization detector. The column temperature was increased from 150 to 250°C at a rate of 4°C/min. Data acquisition, peak integration, and data analysis were performed with a model ENICA 21 integrator (Delsi-Nermag) connected to a Macintosh computer (Apple Computer, Les Villis, France).

Identification of FAMES. In every sample, FAME retention times were normalized by using two internal standards. The peaks were then identified by comparing their normalized retention times with the normalized retention times of known FAME contained in a calibrating standard (bacterial acid methylesters; catalog no. 4-7080; Supelco).

Data processing. Levels of similarity between FAME

TABLE 1. Strains used in this study

Species or subspecies	Strain ^a	Rank ^b	Other designation(s) ^c	Source (year)	Refer- ence	Received from ^c :
<i>Acinetobacter baumannii</i> ^d	bauC-92C ^T	36	ATCC 19606 ^T , CIP 7034 ^T			Bouvet
	bauD-92D	37	SEIP 8659			Bouvet
<i>Acinetobacter calcoaceticus</i> ^d	calA-92A ^T	31	ATCC 23055 ^T , CIP 8108 ^T			Bouvet
	calB-92B	39	ATCC 14987, CIP 6633			Bouvet
	calS-92S	32	ATCC 33308			Bouvet
<i>Acinetobacter haemolyticus</i> ^d	haeG-92G ^T	40	ATCC 17906 ^T , CIP 643 ^T			Bouvet
	haeH-92H	41	ATCC 17977, CIP 53116			Bouvet
<i>Acinetobacter johnsonii</i> ^d	johL-92L ^T	27	ATCC 17909 ^T , CIP 646 ^T			Bouvet
	johM-92M	28	ATCC 17946, CIP 7023			Bouvet
<i>Acinetobacter junii</i> ^d	junJ-92J ^T	33	ATCC 17908 ^T , CIP 645 ^T			Bouvet
	junK-92K	38	SEIP 884			Bouvet
<i>Acinetobacter lwoffii</i> ^d	lwoN-92N ^T	29	NCTC 5866 ^T , CIP 6410 ^T			Bouvet
	lwoP-92P	30	ATCC 17985, CIP 7017			Bouvet
<i>Acinetobacter species 12</i> ^d	s12Q-92Q	42	SEIP 1281			Bouvet
	s12R-92R	43	Adam Ac 176 Pm K49			Bouvet
<i>Acinetobacter species 3</i> ^d	sp3E-92E	35	ATCC 19004, CIP 7029			Bouvet
	sp3F-92F	34	Adam 202 OKI 55732			Bouvet
<i>B. catarrhalis</i>	cat1-86BK ^T	4	CIP 7321 ^T , ATCC 25238 ^T			Riou
	cat2-86BL	2	LNP 3108	Nose		Riou
	cat3-86CA	5	LNP 3034	Sputum		Riou
	cat4-86CB	3	LNP 4124	Sputum		Riou
	cat5-87N ^T	6	NCTC 11020 ^T , ATCC 25238 ^T			NCTC
<i>K. denitrificans</i> ^d	K.dX-92X	57	CIP 103473			CIP
<i>K. indologenes</i>	K.i1-86CW ^d	58	LNP 4102			Riou
	K.i2-86CX ^T	59	CIP 8020 ^T , ATCC 25869 ^T			Riou
<i>K. kingae</i>	K.k1-86CT	53	CIP 7301	Blood (1973)		Riou
	K.k2-86CV ^T	54	CIP 8016 ^T , ATCC 23330 ^T			Riou
<i>M. atlantae</i>	at1W-92W ^T	44	CIP 8225 ^T , ATCC 29525 ^T			CIP
<i>M. bovis</i>	bov1-86CF	15	CIP 649, ATCC 17947			Riou
	bov2-86CG ^T	16	CIP 7040 ^T , ATCC 10900 ^T			Riou
<i>M. lacunata</i> subsp. <i>lacunata</i>	lac1-86CC	45	LNP 4104			Riou
	lac2-86CD	46	CIP 5493, ATCC 17970			Riou
	lac3-87H ^T	20	NCTC 11011 ^T , ATCC 17967 ^T			NCTC
<i>M. lacunata</i> subsp. <i>liquefaciens</i>	Mliq-86CE	7	CIP 5413, ATCC 17972			Riou
<i>M. nonliquefaciens</i>	nli1-86CH	51	CIP A166	Piéchaud (1953)		Riou
	nli2-86CJ ^T	52	CIP 6836 ^T , ATCC 19975 ^T			Riou
<i>M. osloensis</i>	osl1-86CK	49	CIP 6814	Henriksen 5718	7	Riou
	osl2-86CL ^T	48	ATCC 19976 ^T , CIP 6835 ^T			Riou
<i>M. phenylpyruvica</i>	phe1-86CM	50	CIP 6816	Henriksen 752/52	8	Riou
	phe2-86CN ^T	47	ATCC 23333 ^T , CIP 8227 ^T			Riou
<i>N. caviae</i>	cav1-86BM ^T	17	CIP 7319 ^T , ATCC 14659 ^T	Pelczar GP11	30	Riou
	cav2-86BN	13	LNP 451	Reyn CPH 20, throat		Riou
	cav3-86DB ^c	12	LNP 452, Reyn CPH 21	Berger K29	2	Riou
	cav4-87K ^T	10	NCTC 10293 ^T , ATCC 14659 ^T			NCTC
	cav5-87L	11	NCTC 10891	Berger 1, guinea pig		NCTC
<i>N. cuniculi</i>	cun1-86BW ^T	8	CIP 7317 ^T , ATCC 14688 ^T			Riou
	cun2-87M ^T	9	NCTC 10297 ^T , ATCC 14688 ^T			NCTC

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profiles were calculated by using Euclidian distances. A cluster analysis was performed by using the unweighted average pair group method (41). The computer programs used in this work were written in our laboratory.

RESULTS

DNA relatedness. Labeled DNAs from strains cav4-87K, cun1-86BW, and ovi7-86BX (the type strains of *N. caviae*, *N. cuniculi*, and *N. ovis*, respectively) were used for the homology studies. The T_m values of homoduplexes (in 0.42

M NaCl) ranged from 93.3 to 93.8°C (*N. caviae*) and from 92.8 to 93.0°C (*N. ovis*). These three labeled DNAs were used with unlabeled DNAs 95 times for hybridizations and 9 times for ΔT_m determinations (Table 2).

An estimate of hybridization quality was possible because several pairs of unlabeled DNA samples were prepared from strains that had the same reference number but came from different collections (Table 1); these samples were hybridized under optimal conditions with the same sample of labeled DNA (Table 2). These controls were judged to be

TABLE 1—Continued

Species or subspecies	Strain ^a	Rank ^b	Other designation(s) ^c	Source (year)	Refer- ence	Received from ^c :
<i>N. gonorrhoeae</i>	gono-86BY ^T	60	CIP 7918 ^T , ATCC 19424 ^T			Riou
<i>N. meningitidis</i>	meni-86BZ ^T	61	CIP 7310 ^T , ATCC 13077 ^T			Riou
<i>N. ovis</i>	ovi1-86BP	18	CIP 7320, ATCC 19575	Lindqvist	31	Riou
	ovi2-86BQ	24	LNP 3010	Riou, lamb eye		Riou
	ovi3-86BR	22	LNP 3261	Berger		Riou
	ovi4-86BS	19	LNP 3263	Berger 37 (1973)		Riou
	ovi5-86BT	14	LNP 5111	Riou, cow eye (1985)		Riou
	ovi6-86BV	21	LNP 5178	Riou, cow eye (1985)		Riou
	ovi7-86BX ^T	25	CIP 8109 ^T , ATCC 33078 ^T			Riou
	ovi8-87J ^T	23	NCTC 11227 ^T , ATCC 33078 ^T			NCTC
<i>O. urethralis</i>	Oli1-86CQ	55	CIP 7513	Yourassowsky 4375		Riou
	Oli2-86CS ^T	56	CIP 8133 ^T , ATCC 17964 ^T			Riou
<i>P. immobilis</i> ^d	PimT-92T ^T	1	CIP 102557 ^T , ATCC 43116 ^T			CIP
	PimV-92V	26	CIP 1025354			CIP

^a Working designations of the strains.^b Strain positions on the dendrogram (Fig. 1).^c Adam, M. M. Adam, Budapest, Hungary; ATCC, American Type Culture Collection, Rockville, Md.; Bouvet, P. J. M. Bouvet, Institut Pasteur, Paris, France; CIP, Collection de l'Institut Pasteur, Paris, France; LNP, Laboratoire de Référence sur les Neisseria, Institut Pasteur, Paris, France; NCTC, National Collection of Type Cultures, London, United Kingdom; Reyn, Alice Reyn, Copenhagen, Denmark; Riou, J. Y. Riou, Institut Pasteur, Paris, France; SEIP, Service des Entérobactéries, Institut Pasteur, Paris, France.^d Taxa studied only in the fatty acid analysis.^e Strain cav3-86DB was received as a *Neisseria* strain and was assigned to *N. caviae* in this study on the basis of DNA-DNA hybridization results (Table 2).

satisfactory when we used labeled DNAs from *N. caviae* and *N. ovis*, for which the differences between the hybridization percentages were less than or equal to 9%, as follows: 6% for labeled *N. caviae* DNA and unlabeled *N. caviae* DNAs (ΔT_m , 0.8°C), 5% for labeled *N. ovis* DNA and unlabeled *N. ovis* DNAs (ΔT_m , 1.1°C), and 9% for labeled *N. caviae* DNA and unlabeled *B. catarrhalis* DNAs. However, the results obtained for the labeled *N. cuniculi* DNA control were less satisfactory (difference between labeled *N. cuniculi* DNA and unlabeled *N. cuniculi* DNAs, 21%).

The genomic relationships among the 15 strains originally assigned to the "false neisseriae" were evaluated by performing 31 hybridization tests (Table 2). All of the levels of intraspecific reassociation were high, varying from 68 to 105%, and the divergence values were low (ΔT_m , 0.1 to 2.6°C), whereas the levels of interspecific reassociation were very low, ranging from 10 to 19%. Strain cav3-86DB, which was received as a *Neisseria* sp. strain and had DNA that was strongly reactive with the DNA of *N. caviae* cav4-87K^T (T = type strain) (level of relatedness, 93%; ΔT_m , 0.1°C), was assigned in this study to the species *N. caviae*.

For the 25 remaining strains belonging to species other than the species of "false neisseriae," the levels of relatedness with the three species of "false neisseriae" (64 tests) were always less than or equal to 34% (Table 2). Therefore, the three species classified as "false neisseriae" were clearly distinct from *Branhamella*, *Kingella*, *Moraxella*, *Neisseria*, and *Oligella* species.

Phenotypic characteristics. The properties described below were common to the 15 strains of "false neisseriae" studied. The cells were gram negative cocci, although they often had a tendency to resist Gram decolorization. Swimming motility was absent. All strains grew aerobically and gave positive reactions in oxidase and catalase tests and for tributyrin hydrolysis. The following properties were uniformly absent: glucose, maltose, and sucrose acidification and gamma-glutamyltransferase production. Some characteristics that distinguish the three species are shown in Table 3. *N. caviae* cav3-86DB did not produce DNase.

General results of fatty acid profile studies. With the method which we used, the standard retention times of the peaks were fairly reproducible (variation coefficient, 0.06 to 0.75%). In a given profile, the quantity of each fatty acid was estimated as a percentage of the total peak surface area. A compound with a surface area value less than 0.1% was considered absent. Among the 61 strains studied, 36 fatty acids (32 identified and 4 unidentified) were encountered; each fatty acid occurred in from 1 to 61 strains (Table 4). All of the strains contained 16:1, 16:0, 18:2, and 18:0 fatty acids, and more than 90% of the strains contained 12:0, 3-OH 12:0, and 18:1 *cis*9 fatty acids. The average surface area values for individual fatty acids ranged from 0.1 to 37.6%. Only three fatty acids had average surface area values greater than 10% (16:1, 16:0, and 18:1 *cis*9 fatty acids). Unidentified compound x-1 occurred in 80% of the strains, and unidentified compounds x-2 to x-4 occurred in less than 20% of the strains; in every profile, the total surface area value for these unidentified compounds was less than 2%.

Strain classification on the basis of fatty acid profiles. The strains studied were classified by comparing the surface area values for the fatty acids in each profile. The level of dissimilarity between each pair of strains was estimated by using the Euclidian distances between the relative surface area values for the 36 fatty acids. From this distance matrix for the 61 profiles, four hierarchical ascendant classification schemes were developed by performing cluster analyses, using the single-linkage, complete-linkage, weighted-average-linkage, and unweighted-average-linkage methods (41); for these four analyses, the cophenetic correlation coefficients (35) were 0.88, 0.81, 0.85, and 0.89, respectively. Consequently, in this study the unweighted-average-linkage analysis appeared to be the best method for preserving the Euclidian distances initially calculated between two profiles. A dendrogram corresponding to this classification scheme is shown in Fig. 1. On this dendrogram the level of dissimilarity between two strains is expressed as a hierarchical level standardized between 0 and 100%; a value of 100% corresponds to the maximum distance calculated for

TABLE 2. Levels of DNA relatedness among "false neisseriae" and related species^a

Unlabeled DNA from:	³ H-labeled DNA from <i>N. caviae</i> cav4-87K ^T		³ H-labeled DNA from <i>N. cuniculi</i> cun1-86BW ^T : relative binding ratio (%)		³ H-labeled DNA from <i>N. ovis</i> ovi7-86BX ^T	
	Relative binding ratio (%)	ΔT_m (°C)	Relative binding ratio (%)	ΔT_m (°C)	Relative binding ratio (%)	ΔT_m (°C)
"False neisseriae"						
<i>N. caviae</i>						
cav1-86BM ^T	94	0.8	12		18	ND ^b
cav2-86BN	96	0.1	12		17	ND
cav3-86DB ^c	93	0.1	13		19	ND
cav4-87K ^T	100	0.0	ND		ND	ND
cav5-87L	96	0.8	ND		ND	ND
<i>N. cuniculi</i>						
cun1-86BW ^T	ND	ND	100		19	ND
cun2-87M ^T	18	ND	79		ND	ND
<i>N. ovis</i>						
ovi1-86BP	ND	ND	10		92	ND
ovi2-86BQ	ND	ND	13		92	ND
ovi3-86BR	ND	ND	15		87	ND
ovi4-86BS	ND	ND	11		89	ND
ovi5-86BT	ND	ND	10		68	2.6
ovi6-86BV	ND	ND	10		68	1.8
ovi7-86BX ^T	ND	ND	11		100	0.0
ovi8-87J ^T	16	ND	ND		105	1.1
Other species and subspecies						
<i>B. catarrhalis</i>						
cat1-86BK ^T	4	ND	13		7	ND
cat2-86BL	10	ND	11		14	ND
cat3-86CA	9	ND	19		14	ND
cat4-86CB	16	ND	17		20	ND
cat5-87N ^T	13	ND	ND		ND	ND
<i>K. indologenes</i> K.i2-86CX ^T	4	ND	7		7	ND
<i>K. kingae</i>						
K.k1-86CT	ND	ND	4		3	ND
K.k2-86CV ^T	0	ND	6		3	ND
<i>M. atlantae</i> atlW-92W ^T	18	ND	ND		34	ND
<i>M. bovis</i>						
bov1-86CF	ND	ND	9		18	ND
bov2-86CG ^T	13	ND	12		20	ND
<i>M. lacunata</i> subsp. <i>lacunata</i>						
lac1-86CC	ND	ND	18		19	ND
lac2-86CD	ND	ND	13		15	ND
lac3-87H ^T	14	ND	ND		14	ND
<i>M. lacunata</i> subsp. <i>liquefaciens</i> Mliq-86CE	12	ND	30		22	ND
<i>M. nonliquefaciens</i>						
nli1-86CH	ND	ND	19		15	ND
nli2-86CJ ^T	19	ND	10		22	ND
<i>M. osloensis</i>						
osl2-86CL ^T	8	ND	10		22	ND
osl1-86CK	ND	ND	9		16	ND
<i>M. phenylpyruvica</i>						
phe1-86CM	ND	ND	5		12	ND
phe2-86CN ^T	2	ND	3		4	ND
<i>N. gonorrhoeae</i> gono-86BY ^T	0	ND	3		5	ND
<i>N. meningitidis</i> meni-86BZ ^T	13	ND	3		11	ND
<i>O. urethralis</i>						
Oli1-86CQ	3	ND	4		6	ND
Oli2-86CS ^T	2	ND	5		6	ND

^a The strains are designated by their working designations (Table 1).^b ND, not determined.^c On the basis of these DNA-DNA hybridization results, strain cav3-86DB, which was received as a *Neisseria* strain, was placed in the species *N. caviae*.

the two clusters that are farthest apart (maximum distance, 60.01).

Direct observation of the dendrogram permitted us to define taxa or classes by choosing the clusters that were separated by the greatest distances. Effectively, the majority of the strains were clustered into four main classes, classes

A to D (Fig. 1); only the two *Psychrobacter* strains were loosely related to these classes. The compositions of the four classes are shown in Table 5. Class A contains 24 strains (ranks 2 to 25 on the dendrogram) belonging to the genus *Moraxella* (4 strains), to the species of "false neisseriae" (15 strains), and to *B. catarrhalis* (5 strains). Class B (ranks 27 to

TABLE 3. Biochemical characteristics that are useful for differentiating the three species of "false neisseriae"

Species	Hemolysis (horse blood)	Nitrate reduction	Nitrite reduction	DNase
<i>N. caviae</i>	0/5 ^a	5/5	3/5	4(w)/5 ^b
<i>N. cuniculi</i>	0/2	0/2	0/2	1/2
<i>N. ovis</i>	8/8	8/8	0/8	0/8

^a Number of strains positive/total number of strains tested.^b (w), weak activity.

43) contains the 17 strains belonging to the genus *Acinetobacter*. Class C (ranks 44 to 52) contains the nine remaining strains of the genus *Moraxella*, including two atypical strains of *M. lacunata* (which are distantly related to *M. atlantae*). Finally, class D (nine strains; ranks 53 to 61) contains five *Kingella* strains, two strains of the "true neisseriae," and two *Oligella* strains.

Mean fatty acid profiles and discriminating characteristics of the classes. The occurrence and the mean surface area values for the peaks detected in the classes are shown in

TABLE 4. General occurrence rates for the 36 fatty acids found in the 61 strains studied

Fatty acid ^a	Occurrence		Surface area (% of profile)	
	No. of strains	% of strains	Avg $\pm$ SD	Range
10:0	43	70.5	2.2 $\pm$ 1.7	0.03–8.69
i-11:0	6	9.8	1.0 $\pm$ 0.9	0.09–2.68
11:0	6	9.8	0.3 $\pm$ 0.3	0.04–0.73
12:0	60	98.4	4.2 $\pm$ 2.3	0.62–9.32
x-1	49	80.3	0.6 $\pm$ 0.3	0.18–1.13
i-13:0	4	6.6	0.2 $\pm$ 0.0	0.13–0.19
13:0	6	9.8	0.3 $\pm$ 0.4	0.04–1.23
2-OH 12:0	27	44.3	1.1 $\pm$ 1.0	0.06–3.65
3-OH 12:0	59	96.7	4.1 $\pm$ 1.7	1.53–11.62
x-2	5	8.2	0.2 $\pm$ 0.1	0.11–0.24
x-3	12	19.7	0.6 $\pm$ 0.6	0.04–1.92
14:1	7	11.5	0.1 $\pm$ 0.1	0.04–0.32
14:0	54	88.5	3.4 $\pm$ 8.6	0.27–50.55
x-4	5	8.2	0.2 $\pm$ 0.1	0.07–0.35
i-15:0	3	4.9	0.2 $\pm$ 0.1	0.04–0.29
a-15:0	3	4.9	0.3 $\pm$ 0.1	0.14–0.35
15:0	33	54.1	0.6 $\pm$ 0.7	0.05–3.68
2-OH 14:0	5	8.2	0.2 $\pm$ 0.2	0.05–0.51
3-OH 14:0	39	63.9	2.5 $\pm$ 1.1	0.17–4.78
i-16:0	14	23.0	1.0 $\pm$ 0.5	0.12–1.86
16:1	61	100.0	13.8 $\pm$ 9.0	0.58–42.49
16:0	61	100.0	14.7 $\pm$ 8.9	0.73–43.20
i-17:0	17	27.9	0.6 $\pm$ 0.5	0.06–1.70
a-17:0	50	82.0	3.0 $\pm$ 3.0	0.36–16.39
17:0 cycl	3	4.9	0.1 $\pm$ 0.0	0.09–0.19
17:0	45	73.8	1.0 $\pm$ 1.2	0.04–4.78
3-OH 16:0	11	18.0	1.1 $\pm$ 1.4	0.08–3.78
i-18:0	1	1.6	0.1 $\pm$ 0.0	0.13–0.13
18:2	61	100.0	7.8 $\pm$ 9.9	0.12–40.84
18:1 cis9	59	96.7	37.6 $\pm$ 18.2	0.47–67.50
18:1 cis11	48	78.7	4.7 $\pm$ 10.0	0.13–49.27
18:0	61	100.0	3.8 $\pm$ 3.7	0.16–25.69
3-OH i-17:0	6	9.9	0.5 $\pm$ 0.1	0.31–0.56
19:0 cycl	2	3.3	0.1 $\pm$ 0.0	0.06–0.14
19:0	14	23.0	0.3 $\pm$ 0.2	0.04–0.80
20:0	10	16.4	0.3 $\pm$ 0.3	0.05–1.18

^a The fatty acids are designated by their shorthand chemical symbols; their systematic and trivial names have been published previously (24, 44). x-1, x-2, x-3, and x-4 are unidentified compounds.

Table 6. For each class, only the peaks that occurred in more than 20% of the strains are shown in Table 6. Some other peaks were observed, but they always occurred in less than 20% of the strains of a given class; all of these occasional peaks had surface area values of less than 1.2%, and in 90% of the cases the surface area values were less than 0.3%. In classes C and D, some major compounds had surface area values with broad ranges around the mean, and these broad ranges were related to dissimilarity levels that were higher for these classes than for classes A and B (Fig. 1).

The diagnostic ability coefficients (DAC) of each fatty acid were calculated by considering within each class the frequency of surface area values greater than a given surface area value threshold (16). For each fatty acid, the surface area value threshold giving the maximum DAC was defined by computation. As determined by this method, the best fatty acids (the fatty acids that had the maximum DAC values) for discriminating classes A to D are shown in Table 7. Within the scope of this study, the four most discriminating fatty acids (the surface area thresholds are indicated in parentheses) were as follows: 14:0 (4%), 18:2 (15%), 18:1 cis9 (8%), and 18:1 cis9 (44%). Other fatty acids (Table 7) strengthened the differentiation of classes A, B, and C (2-OH 12:0 [1%] and 16:0 [14%]) or the differentiation of class D from the other classes (a-17:0, which is absent in class D).

The DAC of each fatty acid were also calculated for the 24 strains belonging to class A. Only the following fatty acid data could be used for differential diagnosis: *B. catarrhalis* does not produce 3-OH 14:0 and produces less than 7% 16:1 and more than 60% 18:1 cis9, in contrast to the great majority of the strains of "false neisseriae" and "classical moraxellae." No fatty acid differentiates the "false neisseriae" from the "classical moraxellae."

DISCUSSION

Species delineation of the "false neisseriae." Only a few DNA-DNA hybridization values have been published previously for the "false neisseriae" (Table 8). In the majority of the studies described previously, the interspecific hybridization values did not exceed 35% (25, 34, 38). Only Hoke and Vedros (20) and Vedros et al. (48) reported higher values (up to 58%). Nevertheless, it was clear that the "false neisseriae" could not be included in any other species since the hybridization values were less than 70%, the accepted threshold for delineating a species (49). However, until now the hybridization data available for the "false neisseriae" themselves were too limited to determine possible levels of relatedness among these organisms. According to our results (Table 2), the 15 strains of "false neisseriae" which we studied are distributed in three separate genomic groups, which exhibit high intragroup relative binding ratios (equal to or greater than 68% under optimal conditions) and low intergroup relative binding ratios (range, 10 to 19%). Thus, each genomic group is consistent with the genomic definition of a bacterial species (49).

None of the three species of "false neisseriae" exhibits a significant level of DNA relatedness to any other species studied; in particular, these organisms are loosely but equally related to *Moraxella* spp. and to *B. catarrhalis*. However, it is possible to demonstrate that the "false neisseriae" are more closely related to the phylogenetic γ subclass than to the β subclass. To do this, the average values for DNA-DNA interspecific hybridization between

FIG. 1. Dendrogram showing the hierarchical ascendant classification of the 61 strains studied, as determined by the unweighted-average-pair-group method. The main clusters define classes A to D.

the labeled DNAs of "false neisseriae" and the unlabeled DNAs of the other species (Table 2) were calculated, and these values were divided into two groups on the basis of the species affiliation with the phylogenetic β and γ subclasses of the *Proteobacteria*. The value for the strains belonging to the β subclass (17 values) was $4.5 \pm 3.4\%$ (mean $\pm$ standard deviation), and the value for the strains belonging to the γ subclass was $13.7 \pm 6.7\%$; these two means are significantly different (Student's bilateral test; $P = 0.0001$; 62 df).

Study of fatty acid profiles. The clusters resulting from the

numerical classification based on fatty acid analysis data (Table 5) can also be interpreted according to the present phylogenetic classification of *Neisseria* and related species. All of the class A strains are members of the *M. lacunata* subgroup ("authentic moraxellae") (38) in the phylogenetic γ subclass (43), including the "false neisseriae" and *B. catarrhalis*. Class B corresponds exactly to the genus *Acinetobacter* in the γ subclass. Class C contains the nine remaining strains of *Moraxella* (γ subclass) (that is, the two strains of *M. nonliquefaciens* which we studied and seven

TABLE 5. Strain compositions of the four classes defined by numerical classification on the basis of fatty acid profiles^a

Class	No. of strains	Species or subspecies	Strain(s)
A	24	<i>B. catarrhalis</i> <i>M. bovis</i> <i>M. lacunata</i> subsp. <i>lacunata</i> <i>M. lacunata</i> subsp. <i>liquefaciens</i> <i>N. caviae</i> <i>N. cuniculi</i> <i>N. ovis</i>	cat1-86BK ^T , cat2-86BL ^T , cat3-86CA, cat4-86CB, cat5-87N ^T bov1-86CF, bov2-86CG ^T lac3-87H ^T Mliq-86CE cav1-86BM ^T , cav2-86BN, cav3-86DB, cav4-87K ^T , cav5-87L cun1-86BW ^T , cun2-87M ^T ovi1-86BP, ovi2-86BQ, ovi3-86BR, ovi4-86BS, ovi5-86BT, ovi6-86BV, ovi7-86BX ^T , ovi8-87J ^T
B	17	<i>Acinetobacter baumannii</i> <i>Acinetobacter calcoaceticus</i> <i>Acinetobacter haemolyticus</i> <i>Acinetobacter johnsonii</i> <i>Acinetobacter junii</i> <i>Acinetobacter lwoffii</i> <i>Acinetobacter</i> species 12 <i>Acinetobacter</i> species 3	bauC-92C ^T , bauD-92D calA-92A ^T , calB-92B, calS-92S haeG-92G ^T , haeH-92H johL-92L ^T , johM-92M junJ-92J ^T , junK-92K lwoN-92N ^T , lwoP-92P s12Q-92Q, s12R-92R sp3E-92E, sp3F-92F atlW-92W ^T
C	9	<i>M. atlantae</i> <i>M. lacunata</i> subsp. <i>lacunata</i> <i>M. nonliquefaciens</i> <i>M. phenylpyruvica</i> <i>M. osloensis</i>	lac1-86CC, lac2-86CD ^b nli1-86CH, nli2-86CJ ^T phe1-86CM, phe2-86CN ^T osl1-86CK, osl2-86CL ^T
D	9	<i>K. denitrificans</i> <i>K. indologenes</i> <i>K. kingae</i> <i>N. gonorrhoeae</i> <i>N. meningitidis</i> <i>O. urethralis</i>	K.dX-92X K.i1-86CW, K.i2-86CX ^T K.k1-86CT, K.k2-86CV ^T gono-86BY ^T meni-86BZ ^T Oli1-86CQ, Oli2-86CS ^T
Unclassified	2	<i>P. immobilis</i>	PimT-92T ^T , PimV-92V

^a The classes were defined by direct observation of the dendrogram (Fig. 1).^b These *M. lacunata* strains are atypical in some of their phenetic properties and their fatty acid profiles.

strains belonging to species designated "misnamed moraxellae" by Rossau et al. [38]), *M. atlantae*, *M. phenylpyruvica*, and *M. osloensis*. Finally, class D is composed of nine strains, including seven strains assigned to the β subclass (the two species of "true neisseriae," *K. kingae*, *K. denitrificans*, and *O. urethralis*) and the two strains of *K. indologenes* which we studied.

Furthermore, our analysis of the fatty acid profiles resulted in the following two observations (Fig. 1): (i) the genus *Acinetobacter* is more closely related to the *M. lacunata* subgroup than to the "misnamed moraxellae" subgroup, and (ii) the two strains of *Psychrobacter* which we studied are more closely related to classes A and B than to the other classes. These results are in agreement with the results of other reports. The heterogeneity of the *Psychrobacter* group and the phenotypic properties of these organisms, which are intermediate between the properties of the genus *Acinetobacter* and the properties of the genus *Moraxella*, have been emphasized previously by Rossau et al. (38). Russell (40) and Bryn et al. (11) found wax esters, represented by their fatty alcohols, in the chromatographic profiles of "psychrophilic strains" (*Psychrobacter*), *Acinetobacter* spp., "false neisseriae," and *Moraxella* spp. All of these observations support the proposal that the genera *Moraxella*, *Acinetobacter*, and *Psychrobacter* should be included in the same family, in the γ subclass, as proposed by Rossau et al. (38). The production of small amounts of 2-OH 12:0 by all or most *Acinetobacter* strains and the absence of this fatty acid in *Moraxella* spp. (21, 28, 36) do not appear to be appropriate arguments for excluding the genus *Acinetobacter* from the family containing the genus

Moraxella as proposed by Catlin (13), because we found that this fatty acid was also produced by some strains of *Moraxella* spp. *P. immobilis* and *M. phenylpyruvica*, which exhibit some DNA-rRNA relationships (38), seem loosely related by their fatty acid profiles (Table 6) and do not cluster together (Fig. 1).

Bryn et al. showed that, in contrast to *Psychrobacter* strains, waxes were not present in *M. phenylpyruvica* strains (11).

Finally, the results of the fatty acid analysis are in full agreement with the phylogenetic classification for 57 of the 61 strains studied. Discrepancies were observed in the two situations described below.

(i) *K. indologenes* was created as a new species in the genus *Kingella* by Snell and Lapage (42). Rossau et al. (39) separated *K. indologenes* from the other *Kingella* species on the basis of differences in levels of DNA-rRNA relatedness when rRNA from *Acinetobacter calcoaceticus* (superfamily I, γ subclass) was used and from *Alcaligenes denitrificans* and *Pseudomonas solanacearum* (superfamily III, β subclass). However, this proposal is not in accord with the results of our fatty acid study. Indeed, *K. indologenes* has fatty acid profiles that are very similar to the profiles of the other species in this genus, *K. kingae* and *K. denitrificans*, and also to the profiles of the "true neisseriae." All of these species are aggregated in a cluster (class D), and seven of the nine strains in this cluster can be clearly assigned to the phylogenetic β subclass (Fig. 1). The resemblance of the fatty acid profiles of the three *Kingella* species has been mentioned previously (11, 21). Bryn et al. found that *K. indologenes*, as well as *K. kingae* and the "true neisseriae,"

TABLE 6. Fatty acid profiles for the four classes obtained by numerical classification

Fatty acid	% of surface area (mean $\pm$ SD) in:				% of surface area in <i>P. immobilis</i> (2 strains)
	Class A (24 strains)	Class B (17 strains)	Class C (9 strains)	Class D (9 strains)	
10:0	2.3 $\pm$ 1.3 (24) ^a	0.8 $\pm$ 0.6 (7)	2.1 $\pm$ 0.8 (9)		5.7–8.7
i-11:0			1.5 $\pm$ 0.7 (4)		
11:0					0.5–0.7
12:0	3.2 $\pm$ 1.9 (24)	5.4 $\pm$ 1.4 (17)	2.6 $\pm$ 0.9 (9)	6.0 $\pm$ 3.2 (9)	0.0–5.4
x-1	0.4 $\pm$ 0.1 (22)	0.9 $\pm$ 0.1 (17)	0.6 $\pm$ 0.3 (8)		0.0–0.4
13:0		0.1 $\pm$ 0.1 (5)			
2-OH 12:0	0.2 $\pm$ 0.1 (7)	1.6 $\pm$ 0.9 (17)	0.2 $\pm$ 0.0 (2)		
3-OH 12:0	4.1 $\pm$ 1.5 (24)	3.9 $\pm$ 1.4 (17)	4.8 $\pm$ 2.6 (9)	3.3 $\pm$ 1.2 (7)	3.9–5.5
x-2	0.2 $\pm$ 0.1 (5)				
x-3	0.3 $\pm$ 0.1 (7)			1.0 $\pm$ 0.8 (5)	
14:1			0.2 $\pm$ 0.1 (2)		
14:0	0.6 $\pm$ 0.2 (23)	0.6 $\pm$ 0.3 (17)	1.3 $\pm$ 0.7 (5)	17.5 $\pm$ 14.5 (9)	
15:0	0.2 $\pm$ 0.1 (9)	0.7 $\pm$ 0.4 (13)	0.9 $\pm$ 0.3 (4)	0.9 $\pm$ 1.2 (7)	
3-OH 14:0	3.0 $\pm$ 0.8 (17)	1.6 $\pm$ 0.9 (11)	2.0 $\pm$ 0.7 (2)	2.8 $\pm$ 1.1 (9)	
i-16:0	1.2 $\pm$ 0.4 (8)		1.0 $\pm$ 0.5 (4)		
16:1	9.5 $\pm$ 4.8 (24)	19.1 $\pm$ 5.7 (17)	11.0 $\pm$ 10.3 (9)	16.8 $\pm$ 12.9 (9)	9.9–30.5
16:0	9.2 $\pm$ 2.1 (24)	19.2 $\pm$ 4.0 (17)	12.2 $\pm$ 9.5 (9)	26.5 $\pm$ 9.7 (9)	0.7–1.3
i-17:0	0.8 $\pm$ 0.5 (9)	0.3 $\pm$ 0.1 (6)			0.0–0.4
a-17:0	2.4 $\pm$ 1.3 (24)	3.2 $\pm$ 2.5 (16)	2.0 $\pm$ 1.9 (8)		11.3–16.4
17:0 cycl				0.1 $\pm$ 0.0 (3)	
17:0	0.4 $\pm$ 0.4 (16)	2.2 $\pm$ 1.4 (16)	0.3 $\pm$ 0.2 (8)	0.4 $\pm$ 0.2 (4)	0.0–0.2
3-OH 16:0	0.2 $\pm$ 0.1 (6)	1.5 $\pm$ 1.3 (3)		3.5 $\pm$ 0.3 (2)	
18:2	5.1 $\pm$ 3.6 (24)	2.9 $\pm$ 1.3 (17)	29.0 $\pm$ 8.1 (9)	3.9 $\pm$ 4.6 (9)	2.9–3.9
18:1 <i>cis</i> 9	53.6 $\pm$ 7.0 (24)	35.2 $\pm$ 6.1 (17)	25.6 $\pm$ 10.0 (9)	1.9 $\pm$ 1.1 (7)	34.9–55.1
18:1 <i>cis</i> 11	1.3 $\pm$ 0.4 (17)	2.2 $\pm$ 1.9 (17)	1.4 $\pm$ 0.4 (3)	17.9 $\pm$ 17.7 (9)	0.1–0.3
18:0	5.1 $\pm$ 1.8 (24)	1.7 $\pm$ 0.8 (17)	6.5 $\pm$ 7.5 (9)	2.2 $\pm$ 1.2 (9)	0.5–0.9
3-OH i-17:0			0.5 $\pm$ 0.1 (6)		
19:0	0.3 $\pm$ 0.2 (12)		0.6 $\pm$ 0.2 (2)		
20:0	0.1 $\pm$ 0.1 (9)				

^a The numbers in parentheses are the numbers of strains in which the fatty acids occurred.

do not produce waxes, in contrast to *B. catarrhalis*, the “false neisseriae,” and the “classical moraxellae” (11).

(ii) The two strains of *M. nonliquefaciens* studied have fatty acid profiles which resemble the profiles of “misnamed moraxellae” (in class C) more than they resemble the profiles of the “classical moraxellae” belonging to class A. The clustering of these strains results from large differences

in the surface area values for some fatty acid peaks (in particular, 18:2, 18:1 *cis*9, and 18:0, the average surface area values of which are 22.8, 21.4, and 0.9%, respectively, for the two strains of *M. nonliquefaciens* and 5.1, 53.6, and 5.1%, respectively, for the 24 class A strains). Of course, this unexpected situation could be due to the atypical characteristics of the two strains studied; however, Jantzen et al. showed that, in numerical classification schemes based on fatty acid profiles, *M. nonliquefaciens* and *M. phenylpyruvica* were the species that were least closely related to the cluster containing *M. lacunata*, *M. bovis*, *B. catarrhalis*, and the “false neisseriae” (22, 23).

Nomenclature of the “false neisseriae.” In this polyphasic taxonomic study we found that each of the three species of “false neisseriae” is a separate genomic species and that the fatty acid profiles of these organisms are very similar to those of both “classical moraxellae” and *B. catarrhalis*. However, because the classifications based on fatty acid profiles and on DNA-rRNA hybridization data are not completely correlated, we have no definitive arguments for including the species of “false neisseriae” in either candidate genus, *Branhamella* or *Moraxella*.

At the present time, we expect more complete data for nucleic acid sequences and more precise information concerning the phylogenetic relationships among the species and genera which have to be included in a single family, designated *Moraxellaceae* (38) or *Branhamaceae* (13). Then it seems hasty to resolve the confused nomenclatural status of both the genus *Branhamella* and the species of “false

TABLE 7. Fatty acids that distinguish the classes^a

Fatty acid	Threshold (%) ^b	DAC ^c	Frequency of occurrence (%) in ^d :				
			Class A	Class B	Class C	Class D	Classes A, B, and C
14:0	4	0.81	0	0	0	100	
18:2	15	0.81	0	0	100	0	
18:1 <i>cis</i> 9	8	0.81	100	100	100	0	
18:1 <i>cis</i> 9	44	0.73	95.8	0	0	0	
2-OH 12:0	1	0.56	0	76.5	0		
16:0	14	0.57	0	94.1	33.3		
a-17:0	0	0.88				0	96.0

^a There were 24 strains in class A, 17 strains in class B, 9 strains in class C, and 9 strains in class D. The strain compositions of the classes are shown in Table 5.

^b Limit value for dividing the surface area value range (from 0 to 100%) for each fatty acid into two qualitative modalities. The fatty acid is considered to be present if the observed surface area value is greater than the threshold value; otherwise, it is considered to be absent.

^c The DAC was calculated for the threshold indicated in each case (16).

^d Percentage of strains with surface area values greater than the threshold value.

TABLE 8. Previously published levels of DNA-DNA interspecific relatedness among "false neisseriae" and related taxa

Source of unlabeled DNA	% Hybridization with:				
	Unlabeled <i>N. caviae</i> DNA ^a	Unlabeled <i>N. cuniculi</i> DNA	Unlabeled <i>N. ovis</i> DNA	Labeled or unlabeled <i>B. catarrhalis</i> DNA	Labeled DNAs from "true neisseriae" ^b
<i>N. caviae</i>				33 ^c	10–15 ^d
<i>N. caviae</i>					1.5–3 ^e
<i>N. cuniculi</i>	30		58 ^f	32.5 ^g	1–1.5 ^e
<i>N. ovis</i>	14	22 ^a		39 ^g	0–1 ^e
<i>B. catarrhalis</i>	30	26 ^a	23 ^a		1–2 ^e
<i>B. catarrhalis</i>					15–27 ^d
"True neisseriae"		23.5 ^f	16–42 ^f	16–35 ^g	
"True neisseriae"		41 ^h	42 ^h	10–28 ^c	
"Classical moraxellae" ⁱ	16	21 ^a	14–23 ^a	18–25 ^j	

^a Data from reference 38.^b One to five species of "true neisseriae" were included in the studies cited. The "true neisseriae" include species 1 to 11 in *Bergey's Manual* (47), *Neisseria polysacchara* (33), and *Neisseria macacae* (48).^c Data from reference 25. The DNA from *B. catarrhalis* was labeled.^d Data from reference 25.^e Data from reference 34.^f Data from reference 20.^g Data from reference 20. The DNA from *B. catarrhalis* was not labeled.^h Data from reference 48.ⁱ *M. lacunata* subsp. *lacunata*, *M. lacunata* subsp. *liquefaciens*, and/or *M. nonliquefaciens*.^j Data from reference 38. The DNA from *B. catarrhalis* was not labeled.

neisseriae" by placing these organisms in the "classical moraxellae" or other related genera. Since the generic name of the "false neisseriae" must be changed, because these organisms do not belong to the genus *Neisseria* and have a coccoid shape, we propose that they should be called *Branhamella caviae*, *Branhamella cuniculi*, and *Branhamella ovis*, temporarily following Catlin's proposals (13).

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