Taxonomy of the Feline Mycoplasmas *Mycoplasma felifaucium*, *Mycoplasma feliminutum*, *Mycoplasma felis*, *Mycoplasma gateae*, *Mycoplasma leocaptivus*, *Mycoplasma leopharyngis*, and *Mycoplasma simbae* by 16S rRNA Gene Sequence Comparisons†

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The nucleotide sequence of the 16S rRNA gene of eight mycoplasmas isolated from felids was determined and used for taxonomic comparisons. A signature nucleotide sequence motif and overall sequence similarity to other mollicutes positioned *Mycoplasma felifaucium*, *Mycoplasma felis*, *Mycoplasma leocaptivus*, *Mycoplasma leopharyngis*, and *Mycoplasma simbae* in the *Mycoplasma fermentans* phylogenetic group of mollicutes. *Mycoplasma arginini* and *Mycoplasma gateae* were positioned in the *Mycoplasma hominis* phylogenetic group of mollicutes, and *Mycoplasma feliminutum* was positioned in the phylogenetically distant *Acholeplasma* group of mollicutes, showing that host family preference does not necessarily derive from bacterial phylogenetic closeness.

Mollicutes demonstrate marked host preference in some instances. Informal grouping of mollicutes based upon the host from which they were first or are frequently isolated can have clinical utility. At least eight well-characterized mycoplasma species have been isolated from felids. *Mycoplasma felifaucium*, *Mycoplasma feliminutum*, *Mycoplasma felis*, *Mycoplasma gateae*, *Mycoplasma leocaptivus*, *Mycoplasma leopharyngis*, and *Mycoplasma simbae* were isolated from the respiratory tract of a group of hosts that includes the domestic cat (*Felis catus*), lynx (*Felis lynx*), puma (*Felis concolor*), leopard (*Panthera pardus*), lion (*Panthera leo*), tiger (*Panthera tigris*), and cheetah (*Acinonyx jubatus*) (4, 9–12). In addition, a strain of *Mycoplasma arginini*, a commensal of several species of domestic animals, was briefly mistaken to be a separate feline mycoplasma when it was isolated from the lung of a lion (9, 23). It is not certain that mycoplasmas play other than a secondary role in respiratory disease of cats, but mycoplasmas are not normally present in the lower respiratory tract of healthy cats (17, 22). Two feline ureaplasma species, *Ureaplasma felinum* and *Ureaplasma cati*, also have been isolated from the oral cavity of cats (8). The conjunctivae and urogenital tract are other potential sites of mollicute infection of cats.

Though they share related hosts, the feline mollicutes are phenotypically diverse in morphology, nutritional requirements, and metabolism (9, 20). Feline mollicutes also have been isolated from a variety of other hosts including dogs, horses, and ruminants (6, 14, 20). These observations suggest that even if the preference of this group of mollicutes for a feline host derives from shared phenotypic characteristics, implying genetic similarity, it may not reflect their phylogeny. Taxonomies of mollicute species have been constructed recently on the basis of 16S rRNA gene nucleotide sequences,

which do reflect their phylogenetic relationships (7, 15, 25). We determined the 16S rRNA gene nucleotide sequences of *M. arginini*, *M. felifaucium*, *M. feliminutum*, *M. felis*, *M. gateae*, *M. leocaptivus*, *M. leopharyngis*, and *M. simbae* for comparison with previously published 16S rRNA sequences to position these feline mycoplasmas in the taxonomy of mollicutes.

MATERIALS AND METHODS

M. arginini (ATCC 25528), *M. felifaucium* (ATCC 43428), *M. feliminutum* (ATCC 25749), *M. felis* (ATCC 23391), *M. gateae* (ATCC 23392), *M. leocaptivus* (ATCC 49890), *M. leopharyngis* (ATCC 49889), and *M. simbae* (ATCC 49888) were obtained from the American Type Culture Collection. Each was a feline isolate. A crude proteinase K digest of cells resuspended in the respective appropriate liquid culture medium was the template for PCR amplification of the 16S rRNA gene. Amplification primers were complementary to terminal sequences (sense strand nucleotides 11 to 30, 5'-AGAGTTTGATCCTGGCT CAGGA-3'; antisense strand nucleotides 1512 to 1493, 5'-GGTAGGGATAC CTTGTTACGACT-3') highly conserved in mollicutes (19). The optimum magnesium chloride concentration was 2.0 mM with 1 µM each primer, 200 µM deoxynucleoside triphosphates, and 2.5 U of *Taq* DNA polymerase in a 50-µl volume. There were 50 cycles of template denaturation for 45 s at 94°C, primer annealing for 1 min at 59°C, and polymerization for 2 min at 72°C. Sensitivity, by visualization of 20 µl of the unpurified 1.5-kb PCR product electrophoresed through ethidium bromide-stained 0.8% agarose gels, was 5 pg of template DNA or about 10³ cells. The PCR products were purified by gel filtration spin chromatography and quantitated by spectrophotometry before automated fluorescent dideoxy nucleotide sequence determination (Applied Biosystems, Inc., model 373A, version 1.2.1) without molecular cloning. Both strands of every product were sequenced completely by primer walking.

Previously published mollicute 16S rRNA gene nucleotide sequences were obtained from GenBank. Nucleotide sequences were compared by using VAX computer version 1.4 of the basic local alignment search tool of the National Center for Biotechnology Information, National Institutes of Health (1, 5). The conventional nucleotide numbering system based on the 16S rRNA gene of *Escherichia coli* was used for reference, though sequence length polymorphisms were observed among species. Pairwise nucleotide sequence similarity scores were computed with default gap and gap length weights. A score of 0 indicated that the two sequences had only random sequence similarity, and a score of 1.0 indicated that the two sequences were identical.

Nucleotide sequence accession numbers. The nucleotide sequences described in this article were released to GenBank (National Center for Biotechnology Information) (2), to EMBL, and to the DNA Data Bank of Japan with accession numbers U15794 (*M. arginini*), U15795 (*M. felifaucium*), U16758 (*M. feliminu-*

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um), U09787 (*M. felis*), U15796 (*M. gateae*), U16759 (*M. leocaptivus*), U16760 (*M. leopharyngis*), and U16323 (*M. simbae*).

RESULTS AND DISCUSSION

Nucleotide sequences. Figure 1 is a compilation of the feline mollicute 16S rRNA gene variable region nucleotide sequences determined in this study. The approximate bracketing nucleotides of intervening conserved regions, numbered according to convention on the basis of the gene of *E. coli*, were identified by rRNA secondary structure modeling (16). The V4 region is absent in prokaryotic 16S rRNA. In the V1 region, the rRNA of *M. feliminutum* had a potential 18-bp stem-loop structure instead of the potential 9-bp stem-loop structure present in the other feline mollicutes examined in this study. This feature is not shared by its putative closest known relatives (see below). The V6 region of the rRNA of *M. felifaucium* was unusually short compared with the other feline mollicutes examined in this study, resulting in deletion of two short potential stem-loop structures otherwise conserved at this position in the 16S rRNA gene of gram-positive eubacteria (25). This feature is shared by at least two putative close relatives (see below). A genus-specific sequence immediately 3' to this site (24) was conserved. Length polymorphisms were not remarkable in other variable regions, though nucleotide substitutions were abundant.

The 16S rRNA gene nucleotide sequence of *M. arginini* ATCC 25528, isolated from a lion lung and originally proposed as the type strain of a separate species *Mycoplasma leonis* (9), was identical to the sequence, determined by Weisburg et al. (25), of the type strain *M. arginini* ATCC 23838 isolated from a mouse brain. This result provided molecular genetic proof of the synonymy of *M. leonis* and *M. arginini* (23).

Taxonomic affiliations. A characteristic nucleotide sequence motif at nucleotides 907 to 915, 5'-AAACTTAAA-3', including thymine at nucleotide 912, placed every feline mollicute species examined in this study except *M. feliminutum* in the *Mycoplasma hominis* phylogenetic group described by Weisburg et al. (25) on the basis of 16S rRNA gene sequence comparisons. That sequence motif is otherwise characteristic of archaeobacteria and eukaryotes and is rare in eubacteria. The *M. hominis* phylogenetic group includes the majority of mollicute species characterized to date by this method, except the *Spiroplasma* group whose hosts are primarily plants and insects. The taxonomy of Weisburg et al. (25) also included *Mycoplasma pneumoniae* and *Anaeroplasma* and *Asteroleplasma* mollicute groups.

The *M. hominis* phylogenetic group of Weisburg et al. (25) has been further subdivided by refinement of the 16S rRNA gene comparison methods by Maniloff (15) into the *Mycoplasma fermentans*, *M. hominis*, and *Mycoplasma hyorhinis* phylogenetic groups. The refinements included additional 16S rRNA sequence data and use of the neighbor-joining method of Saitou and Nei (21) to reconstruct the mollicute phylogenetic tree. Overall nucleotide sequence similarity scores placed five feline mollicutes examined in this study in the *M. fermentans* phylogenetic group and two in the *M. hominis* phylogenetic group. In the *M. fermentans* group, the 16S rRNA gene nucleotide sequence of *M. felifaucium* was similar to those of the ruminant mycoplasma *Mycoplasma agalactiae*, with a similarity score of 0.93, and the avian mycoplasma *Mycoplasma gallinarum*, with a score of 0.92. The sequence of *M. felis* was similar to those of the avian mycoplasma *Mycoplasma gallinaceum* (score, 0.92) and *M. felifaucium* (score, 0.86). The sequence of *M. leocaptivus* was similar to those of the avian

mycoplasmas *Mycoplasma coragypsum* (score, 0.92) and *M. gallinaceum* (score, 0.90). The sequence of *M. leopharyngis* was similar to those of the ruminant mycoplasmas *Mycoplasma bovis* Donetta (score, 0.95) and *M. agalactiae* (score, 0.95). The sequence of *M. simbae* was similar to those of the ruminant mycoplasma *Mycoplasma bovigenitalium* (score, 0.95) and the avian mycoplasma *Mycoplasma meleagridis* (score, 0.92). The overall nucleotide sequence similarity scores of these five feline mycoplasmas with *M. fermentans* ranged from 0.85 to 0.93. Other members of the *M. fermentans* phylogenetic group are *Mycoplasma californicum*, *Mycoplasma lipophilum*, and *Mycoplasma pulmonis*. For perspective, the similarity of the 16S rRNA gene nucleotide sequence of *M. fermentans* to that of *Lactobacillus casei* was 0.72, and the similarity of the sequence of *M. fermentans* to that of *E. coli* was 0.45.

In the *M. hominis* phylogenetic group, the nucleotide sequence of *M. arginini* was similar to those of *M. gateae* (score, 0.98) and *Mycoplasma arthritidis* (score, 0.96). The sequence of *M. gateae* was similar to those of *M. arginini* (score, 0.98) and *M. arthritidis* (score, 0.96), showing that these three species form a very tight cluster within this phylogenetic group. The overall nucleotide sequence similarity score for each of these two feline mycoplasmas with *M. hominis* was 0.94. Other members of the *M. hominis* phylogenetic group are *Mycoplasma orale* and *Mycoplasma salivarium*. For perspective, the similarity of the 16S rRNA gene nucleotide sequence of *M. fermentans* to that of *M. hominis* was 0.85, the similarity of the sequence of *M. hominis* to that of *L. casei* was 0.75, and the similarity of the sequence of *M. hominis* to that of *E. coli* was 0.47.

Overall 16S rRNA gene nucleotide sequence similarity placed *M. feliminutum* in the distant *Acholeplasma* phylogenetic group of Maniloff (15), a subdivision of the *Anaeroplasma* phylogenetic group of Weisburg et al. (25). Its sequence was somewhat similar to those of *Acholeplasma modicum* (score, 0.83) and *Acholeplasma laidlawii* (score, 0.80). This positioning might be unexpected on the basis of phenotypic considerations, but mollicute phenotypes are notoriously poor indicators of phylogenetic relationships (18). In particular, it was recently established that growth requirements for sterol or cholesterol are variable in the class Mollicutes (13).

Species identification. Definitive species identification of mollicutes previously has been achieved by using immunological methods including metabolic inhibition, growth inhibition, immunofluorescence, and immunobinding (3). These methods depend on labile antisera or monoclonal antibodies, not available commercially, which must be standardized against a very large number of mollicute species and strains within species. Interpretation of assay results is sometimes subjective. In contrast, species identification by PCR-based 16S rRNA gene comparison is specific, objective, accomplished entirely with commercially available reagents, and can be applied even to unculturable mollicutes. From initial broth cultures or clinical samples such as lavage fluid or exudate, we can obtain partial nucleotide sequences spanning two or three variable regions, sufficient for definitive species identification, in less than 1 week.

Cross-reaction of *M. felis* antigen with other feline mycoplasma antisera has been demonstrated (10). Also, many mollicute species exhibit significant intraspecies antigenic variation (26). These factors could further complicate species identification by using immunological methods. For example, we typed five samples from throat swabs of domestic cats presented to the University of Florida Veterinary Medical Teaching Hospital Clinic by both immunobinding (3) and 16S rRNA gene analysis. All five were identified as *M. felis* by immuno-

Species	V1 region (E. coli bracketing nt 64 to 104)
<i>M. arginini</i>	AGCGAGGTTCTTTGAACCTAGC
<i>M. felifaucium</i>	AGCGATGCTAGCAATAGCATAGC
<i>M. feliminutum</i>	AACGGAAATTTTAAATTTACTTCGGTAAGATAAAATTTTAGT
<i>M. felis</i>	AGCGGAGTTCTTTTAGGACTTAGC
<i>M. gateae</i>	AGCGAGGTTCTTTGAACCTAGC
<i>M. leocaptivus</i>	AGCGGAGTTCTTTTAGAAGCTTAGC
<i>M. leopharyngis</i>	AGCGAAGGTAGCAATACCTTAGC
<i>M. simbae</i>	AGCGAAGTACTGTACTTAGC
Species	V2 region (E. coli bracketing nt 178 to 221)
<i>M. arginini</i>	ATACGCATGGAATCGCATGATTCGGTTGTGAAAGGAGCCTTTAAAGCTCCG
<i>M. felifaucium</i>	ATACTTATTAAGATCGCATGATCATATAATAAAGAGCGTTTGCCTTCG
<i>M. feliminutum</i>	ATAATCTGTGAAAAGGCATCTTTTTCATTGTAAGGTCGGTTGGATCG
<i>M. felis</i>	ATACCTTTTATTTTCACATGAGATTAAATAAAAGGAACCTCAAAGTTTCA
<i>M. gateae</i>	ATACGCATGGAACCGCATGGTTCCGTTGTGAAAGGAGCCTCAAAGCTCCG
<i>M. leocaptivus</i>	ATACTTATATTTTTCGCATGAAAGATATATAAAGAGCGTTTCGCTTCA
<i>M. leopharyngis</i>	ATACTTATAGTTTTCGCATGAAATTAATATAAAGGAGCCTTTAAAGCTCCG
<i>M. simbae</i>	ATACTTGTATTAGATCGCATGATCTTTACATAAAGAGCGCTCAAAGCGTCG
Species	V3 region (E. coli bracketing nt 403 to 498)
<i>M. arginini</i>	GTGCACGATGAAGTCTTCGGATTGTAAAGTGCTGTATAGGAAAGAACACCTGGTTGAGGAAATGCTTC
<i>M. felifaucium</i>	CAGGCTGACGGTACCCTGTCAGA
<i>M. feliminutum</i>	GTGCAGGATGAAGGTCCTATGGATTGTAACTGCTGTGGTAAGGGAAGAAAAATAGTGTAGGAAATGACAT
<i>M. felis</i>	TATCTTGACGGTACCTTATTAGA
<i>M. gateae</i>	GTGAATGATGAAGTATTTTCGGTATGTAAATTTCTTTTATTAGGGAAGAACGATATTTGTAGGAAATGACAAA
<i>M. leocaptivus</i>	TAAGTGACGTTTACCTTAATGAAT
<i>M. leopharyngis</i>	GTGCAGGATGAAGGCTTTCGGGTTGTAAACTGCTGTATAAGGGAAGNNNAATATTATAAGAAAAGATAAT
<i>M. simbae</i>	ATCTTGACGGTACCTTATCAGA
<i>M. gateae</i>	GTGCACGATGAAGGTCCTTCGGATTGTAAAGTGCTGTATAGGGAAGAACACTTGGTTGAGGAAATGCTTCC
<i>M. leocaptivus</i>	AAGCTGACGGTACCTGTCAGA
<i>M. leopharyngis</i>	GTGCAGGATGAAGGCTTTCGGGTTGTAAACTGCTGTATAAGGGAAGAAAAACATTTATAGGAAATGATAGT
<i>M. simbae</i>	GCCTTGACGGTACCTTGTTCAGA
<i>M. gateae</i>	GTGCAGGATGAAGGTCCTATGATTTGTAACTGCTGTGGTAAGGGAAGAAAAAGTAACCTTAGGAAATGAAGT
<i>M. leocaptivus</i>	TACCTTGACGGTACCTTATTAGA
<i>M. leopharyngis</i>	GTGCAGGATGAAGGCTTTCGGGTTGTAAACTGCTGTGGTAAGGGAATAAAAATAGTATAGGAAATGATAC
<i>M. simbae</i>	TATATTGAATGTACCTTATTAGA
Species	V5 region (E. coli bracketing nt 821 to 866)
<i>M. arginini</i>	ATCATTAGTCGGTGGAGAGTTCAGTGACGAGCTAA
<i>M. felifaucium</i>	ATCATTAGCTGATGGAAGATTTCATCGGCGCAGCTAA
<i>M. feliminutum</i>	AGTACTAAGTCTCTGGAAGAACTAGGCGGTAAAGTTAA
<i>M. felis</i>	ATTAGCTGATAGAGAACTTATCGGCGCAGCTAA
<i>M. gateae</i>	ATCATTAGTCGGTGGAGAGTTCAGTGACGAGCTAA
<i>M. leocaptivus</i>	ATGATTAGCTGATAGGAACCTATCGGCACAGCTAA
<i>M. leopharyngis</i>	ATCATTAGCTGATGGAAGAACTTCGCGCGCAGCTAA
<i>M. simbae</i>	ATCATTAGCTGATGGGAACCTATCGGCGCAGCTAA
Species	V6 region (E. coli bracketing nt 997 to 1030)
<i>M. arginini</i>	CCTTCGCAATGCTATAGAGATATAGCAG
<i>M. felifaucium</i>	AGTGG
<i>M. feliminutum</i>	ACTCGGCAATACTATAGAGATATAGTGG
<i>M. felis</i>	CTTCTGCAAGCTATAGAGATATAGTGG
<i>M. gateae</i>	CCTTCGCAATGCTATAGAGATATAGCGG
<i>M. leocaptivus</i>	CTTCCGCAAGCTATAGAGATATAGTTG
<i>M. leopharyngis</i>	CTTCTGCAAGCTATAGAGATATAGTGG
<i>M. simbae</i>	CTTCCGCAAGCTATAGAGATATAGTGG
Species	V7 region (E. coli bracketing nt 1114 to 1174)
<i>M. arginini</i>	CTATCTTTAGTTACTAACGAGTCATGTCGAGGACTCTAGAGATACGCTGGGTAACG
<i>M. felifaucium</i>	TTATCCTTAGTTACTACCAATNTAGTTGATGGACTCTAAGGAGACTNCCCGAGTAATCG
<i>M. feliminutum</i>	TTATTTGCTATTTGCCAGCATTCAGTTGGGAACATATAGTGAGACTGCCAGTGACAAACT
<i>M. felis</i>	TTGGTCCCTTAGTTAAATATTTCTAGGAGACTGCCCGAGTAATTTG
<i>M. gateae</i>	CTATCTTTAGTTACTAACGAGTCATGTCGAGGACTCTAGAGATACGCTGGGTAACCG
<i>M. leocaptivus</i>	TTTTCCTTTAGTTAAATTTTCTAGAGATNCTGCCAAGTAATTTG
<i>M. leopharyngis</i>	TTATCCTTTAGTTACTACCAATTAAGTTGAGGACTCTAAGGAGACTGCCGAGTAATCC
<i>M. simbae</i>	CTGTCTTTAGTTACTACCAATTTAGNGGAGACTCTAAGGAGACTGCCGACTAATCG
Species	V8 region (E. coli bracketing nt 1239 to 1298)
<i>M. arginini</i>	TGGTCGGTACAAAGAGAAGCAATATGGCGACATGGAGCAATCTCAAAAAGCCGATCT
<i>M. felifaucium</i>	TGGCCGGTACAAAGAGAAGCAAAACAGGCGACTGGGAGCAAACTCAAAAACCGGTCT
<i>M. feliminutum</i>	TGGTTGATACAAAGGGAAGCAAAAGCGTGAGCTGGAGCAAACTCCAGAAAAACAATCC
<i>M. felis</i>	TGGATGGTACAAAGAGAAGCAATACGGCGACGTNGAGCAAACTCAAAAACCATTTCT
<i>M. gateae</i>	TGGTCGGTACAAAGAGAAGCAATATGGCGACATGGAGCAATCTCAAAAAGCCGATCT
<i>M. leocaptivus</i>	TGGACGGTACAAAGAGAAGCAATATAGTGATATGGAGCAAACTCAAAAACCGTTCT
<i>M. leopharyngis</i>	TGGACAGTACAAAGAGAAGCAAAATGGTGACATCAAGCAAACTCAAAAACCTGTTCT
<i>M. simbae</i>	TGGCCGGTACACAGGACGACACTGGAGACAGAGCAAAACCAAAAAGCCGGTCT
Species	V9 region (E. coli bracketing nt 1408 to 1476)
<i>M. arginini</i>	CACCATGGGAGCTGGTAATACCAAGTCGGTTAGCTAACCTCGGAGCGACCGCTAAGGTAGGA
<i>M. felifaucium</i>	AACCATGGGAGCTGGTAATGCCCCAAGTCGGTTTATAAAGAACTGCCTAAGGCAGGA
<i>M. feliminutum</i>	AACCACGAAAGTTTATAATACCAAAACCGTGGTCTAACCGTAAGGAGGAGCCGCTAAGGTAGGG
<i>M. felis</i>	CACCATGGGAGCTGGTAATGCCCCAAGTCGGTTTCTTAACCTACGGAGACAACCTGCTAAGGCAGGCGCGT
<i>M. gateae</i>	CACCATGGGAGCTGGTAATACCAAGTCGGTTAGCTAACCTCGGAGCGACCGCTAAGGTAGGA
<i>M. leocaptivus</i>	CACCATGGGAGCTGGTAATGCCCCAAGTCGGTTTCTTAACCTACGGAGACAACCTGCTAAGGCAGGCGCGT
<i>M. leopharyngis</i>	AACCATGGGAGCTGGTAATGCCCCAAGTCGGTTTATAAAGAACTGCCTAAGGCAGGA
<i>M. simbae</i>	AACCATGGGAGCTGGTAATGCCCCAAGTCGGTTTATAAACAACCTGCCTAAGGCAGGA

FIG. 1. Compilation of feline mycoplasma 16S rRNA gene variable region nucleotide sequences. The variable regions were identified by rRNA secondary structure modeling (16); the V4 region is absent in prokaryotic 16S rRNA. Approximate bracketing nucleotides (nt) numbered according to the conventional system based on the 16S rRNA gene of *E. coli* are indicated.

binding, but nucleotide sequence analysis of the PCR-amplified 16S rRNA gene of each isolate revealed that only two of the five actually were *M. felis*. The sequence obtained from the other samples represented one mollicute species and did not match that of any other feline mycoplasma or any sequence currently in GenBank. The cross-reaction with anti-*M. felis* antisera may have been caused by shared immunodominant antigens. This observation may be important and of clinical significance because equine mollicute isolates, identified as *M. felis* by using an indirect immunofluorescence assay, are thought to be primary pathogens of respiratory disease of horses (27). Because mollicutes tend to be host specific or isolated from closely related hosts (for example, *M. pulmonis* from rats and mice and *Mycoplasma gallisepticum* and *Mycoplasma synoviae* from chickens and turkeys), the frequent isolation of *M. felis* from both cats and horses has always been intriguing. Independent species confirmation by 16S rRNA gene analysis of equine mollicutes may therefore be desirable.

As other examples, we obtained a mixed culture of mollicutes from oropharyngeal and tracheal swabs of a Florida panther (*Felis concolor coryi*, a legally protected endangered species). Five of nine isolates were identified as *M. gateae* by partial 16S rRNA gene nucleotide sequence determination, and four isolates represented yet another mollicute species whose 16S rRNA gene nucleotide sequence did not match that of any other feline mollicute or any sequence currently in GenBank. That sequence was most similar to that of *M. leocaptivus* (score, 0.94) (unpublished data). We also detected *M. felifaucium*, first isolated from the throat of a puma (10, 11), in nine gastric biopsies of cheetahs by PCR-based 16S rRNA gene nucleotide sequence analysis (unpublished data). Most veterinary diagnostic and research laboratories do not have reference antisera to a wide variety of mollicute species. The PCR-based sequence analysis was a rapid and cost-effective method for identifying clinical mollicute isolates from these unusual sources.

In lieu of nucleotide sequence analysis, the feline mycoplasmas examined in this study are easily distinguishable from each other by restriction endonuclease analysis of the PCR-amplified 16S rRNA gene. Use of this technique can result in considerable savings of time and expense because amplification and restriction analyses can be completed in a few days, in contrast to the nucleotide sequencing turnaround of 1 to 2 weeks. In the *M. fermentans* phylogenetic group, *M. felifaucium* has a unique restriction endonuclease *NheI* recognition site in the V1 variable region of the gene. There is a unique site for the rare endonuclease *SwaI* (5'-ATTTAAAT-3') in the V2 region of the gene of *M. felis*, a unique *BstXI* site in the V7 region of the gene of *M. leocaptivus*, and a unique *Eco57I* site between the V1 and V2 regions of the gene of *M. simbae*. The genes of *M. arginini* and *M. gateae* of the *M. hominis* phylogenetic group share a homologous *ApaLI* site not present in the 16S rRNA gene of the other feline mycoplasmas examined in this study, and *M. gateae* is recognizable by a *BstEII* site in the V7 region of the gene. There are nonhomologous recognition sequences for the restriction endonuclease *AseI* in the 16S rRNA gene of both *M. feliminutum* (between V3 and V5) and *M. leopharyngis* (in V2), and *M. feliminutum* is further distinguishable by an *AgeI* site in the V9 region of the gene.

Conclusions. We have positioned eight feline mycoplasmas in the *M. fermentans*, *M. hominis*, and *Acholeplasma* mollicute phylogenetic groups on the basis of 16S rRNA gene nucleotide sequence comparisons. A preference of these mollicutes for a feline host thus does not derive from their phylogenetic relationships. Mollicute species identification by PCR-based 16S

rRNA gene nucleotide sequence comparisons may offer specificity superior to identification by some immunological methods. Species-specific restriction endonuclease cleavage patterns of amplified gene segments can be used for preliminary species identification. A partial 16S rRNA gene nucleotide sequence can confirm species identification. This approach avoids problems associated with serological cross reactivity and antigenic variation.

ADDENDUM IN PROOF

The International Committee on Systematic Bacteriology has permitted attention to be drawn to errors in nomenclature or to errors in spelling. Prof. Hans G. Trüper has suggested to us that the name *Mycoplasma gateae* is illegitimate because the genitive of the medieval Latin word *gata* (female cat) would have been *gatae*, and there was no word for which *gateae* would have been a legitimate genitive. The name *Mycoplasma gateae* is used more frequently in the literature, including lists of bacterial names approved by the Committee, the ATCC's *Catalogue of Bacteria and Bacteriophages*, and now in GenBank, though two authors (9, 10) used *Mycoplasma gatae*.

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