Mycoplasma crocodyli sp. nov., a New Species from Crocodiles

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Organisms with the typical characteristics of mycoplasmas were isolated from joints and lungs of crocodiles. The results of growth inhibition tests and immunobinding assays showed that the 24 mycoplasma strains isolated were identical and distinct from previously described *Mycoplasma*, *Entomoplasma*, *Mesoplasma*, and *Acholeplasma* species. These organisms represent a new species, for which the name *Mycoplasma crocodyli* is proposed. *M. crocodyli* ferments glucose and maltose, does not produce films and spots, does not hydrolyze arginine, esculin, and urea, reduces tetrazolium chloride, and possesses phosphatase activity. It lyses and adsorbs bovine, ovine, and rabbit erythrocytes. Cholesterol or serum is required for growth. The optimum growth temperature is 37°C. The G+C content of the DNA is 27.6 mol%. This organism causes exudative polyarthritis in crocodiles. The type strain of *M. crocodyli* is strain MP145 (= ATCC 51981).

During outbreaks of exudative polyarthritis at five crocodile farms located in different parts of Zimbabwe, 24 mycoplasma strains were isolated from joints and lungs of diseased crocodiles (*Crocodylus niloticus*), and these strains appeared to be serologically identical as determined by growth inhibition tests (12). In this paper two of these strains, strains MP145^T and MP266, are characterized and compared with previously described *Mycoplasma*, *Entomoplasma*, *Mesoplasma*, and *Acholeplasma* species.

MATERIALS AND METHODS

Cultivation of mycoplasmas. The mycoplasma strains were isolated from synovial aspirates from living crocodiles and lung samples from dead animals in medium containing 37 g of brain heart infusion broth (Oxoid, Ltd., London, United Kingdom), 2 g of yeast extract (Oxoid), 8 ml of glycerol, 2.5 ml of thallium acetate (10%, wt/vol), 2 ml of ampicillin (5%, wt/vol), 10 ml of NAD (1%, wt/vol), and 700 ml of distilled water. This medium was also used to study glucose and mannose metabolism, arginine hydrolysis, tetrazolium chloride reduction, and phosphatase activity. After incubation at 37°C in a candle jar, the mycoplasmas were subcultured on solid medium containing 30 g of blood agar base no. 2 (Oxoid) instead of 37 g of brain heart infusion broth and 5 ml of glucose (10%, wt/vol). Additional passages and other investigations were performed with medium containing 19 g of heart infusion broth (or 30 g of heart infusion agar [Difco Laboratories, Detroit, Mich.] for solid medium), 5 g of yeast extract (Oxoid), 200 ml of heat-inactivated (56°C, 30 min) horse serum, 10⁶ IU of penicillin, and 800 ml of distilled water. The organisms were grown at 37°C in an atmosphere containing 5% CO₂. To determine the temperature range for growth and reproduction, the mycoplasmas were also incubated at 22, 25, 30, and 42°C.

Morphological studies. Colonies of the mycoplasmas were examined with a stereomicroscope (Leitz). The cellular morphology of the organisms was assessed by dark-field microscopy and transmission electron microscopy of sectioned organisms. Ultrathin sections of organisms were prepared as described previously (10).

Filtration studies. Cultures (after 24 h of incubation) were diluted 1:10 in liquid medium and filtered through membrane filters (Millipore Corp., Bedford, Mass.) with pore diameters of 220 and 450 nm. The numbers of CFU per milliliter in the filtrates were determined by plating the filtrates onto agar and were compared with the number of CFU per milliliter in an unfiltered culture dilution.

Reversion experiments. The organisms were subcultured 10 times by using liquid or solid medium lacking penicillin or thallium acetate and were incubated

at 37°C. Agar plates and fluid cultures were examined for alterations in the morphology of colonies and cells, respectively.

Sterol requirement. Cholesterol requirement studies were performed by the method described by Tully (19). Strains MP145^T and MP266 were inoculated into serum-free basal medium and into serum-free basal medium supplemented with 5 mg of bovine serum albumin per ml, 0.01 mg of palmitic acid per ml, and different concentrations of cholesterol (1.0, 5.0, 10.0, and 20.0 mg/ml). Growth of the mycoplasmas was determined by sedimenting the organisms (by centrifugation at 20,000 × g for 30 min) and determining the protein content in each cell pellet by the biuret method (Protein Assay ESL; Boehringer Mannheim GmbH, Mannheim, Germany).

Strains MP145^T and MP266 were also tested indirectly for sterol requirement by a paper disc inhibition method (8) by using a 1.5% (wt/vol) solution of digitonin (Sigma Chemical Co., St. Louis, Mo.) and 5, 10, and 20% (wt/vol) solutions of sodium polyanethanol sulfonate (Koch-Light Laboratories, Ltd., Copenbrook, United Kingdom). The widths of zones of growth inhibition were measured.

Biochemical tests. The crocodile mycoplasma strains were examined for metabolism of glucose and mannose; for hydrolysis of arginine, esculin, and urea; for reduction of tetrazolium chloride (aerobically and anaerobically); and for phosphatase activity. Film and spot production was tested on 10% egg yolk agar (1, 6, 14, 16, 18). Positive and negative controls were used for all tests. All negative tests were checked to make sure that the organisms were viable.

Hemolysis and hemadsorption. Strains MP145^T and MP266 were examined for hemolytic activity and hemadsorption by using bovine, ovine, and rabbit erythrocytes (9).

SDS-PAGE and Western blotting (immunoblotting). The procedures which we used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting have been described previously (17). Blots were treated with rabbit antisera (Table 1) diluted 1:1,000 as the primary antibodies and with peroxidase-conjugated goat antiserum to rabbit immunoglobulins (Nordic, Tilburg, The Netherlands) diluted 1:1,000 as the secondary antibodies and were developed with a solution containing 12 mg of 4-chloro-1-naphthol (Aldrich, Steinheim, Germany), 4 ml of methanol, 20 µl of H₂O₂ (30%), and 20 ml of phosphate-buffered saline.

DNA base composition. The guanine-plus-cytosine (G+C) contents of strains MP145^T and MP266 were determined by isopycnic centrifugation of three DNA batches (7).

Determination and analysis of partial 16S rRNA gene sequence. Strain MP145^T was harvested from a broth culture by centrifugation. A crude proteinase K digest of cells resuspended in SP4 culture medium was the template used for PCR amplification of the 16S rRNA gene (4). The amplification primers used were complementary to terminal sequences (sense strand nucleotides 11 to 30 [5'-AGAGTTTGATCCTGGCTCAGGA-3'] and anti-sense strand nucleotides 1512 to 1493 [5'-GGTAGGGATACCTTGTACGACT-3']) that are highly conserved in mollicutes (15). The optimum magnesium chloride concentration was 2.0 mM with each primer at a concentration of 1 µM, each deoxynucleoside triphosphate at a concentration of 200 µM, and 2.5 U of *Taq* DNA polymerase in a 50-µl reaction mixture. There were 50 cycles consisting 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. The sensitivity, as determined by visualization of 20 µl of unpurified 1.5-kb PCR product electrophoresed through ethidium bromide-

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TABLE 1. *Mycoplasma*, *Entomoplasma*, *Mesoplasma*, and *Acholeplasma* strains and antisera used in comparative serological tests with the crocodile mycoplasmas

Strain	Source(s) ^a	
	Mollicute	Antiserum
<i>Mycoplasma adleri</i> G-145 ^T	FCR	FCR
<i>M. agalactiae</i> PG2 ^T	IRC	IRC
<i>M. alkalescens</i> D12 ^T	NIH	NIH
<i>M. alvi</i> Ilsley ^T	IRC	IRC
<i>M. anatis</i> 1340 ^T	NIH	NIH, IMT
<i>M. anseris</i> 1219 ^T	VMR	VMR
<i>M. arginini</i> G230 ^T	NIH	NIH, IMT
<i>M. arthritis</i> PG6 ^T	NIH	NIH, IMT
<i>M. auris</i> U1A ^T	FCR	FCR
<i>M. bovis genitalium</i> PG11 ^T	NIH	NIH, IMT
<i>M. bovirhinis</i> PG43 ^T	NIH	NIH, IMT
<i>M. bovis</i> Donetta ^T	IRC	IRC, IMT
<i>M. bovovulvi</i> M165/69 ^T	IRC	IRC
<i>M. buccale</i> CH20247 ^T	NIH	NIH
<i>M. buteonis</i> Bb/T2g ^T	JMT	IMT
<i>M. californicum</i> ST6 ^T	IRC	IRC
<i>M. canadense</i> 275C ^T	IRC	IRC, IMT
<i>M. canis</i> PG14 ^T	NIH	NIH, IMT
<i>M. capricolum</i> subsp. <i>capricolum</i> California Kid ^T	IRC	IRC
<i>M. capricolum</i> subsp. <i>capri-pneumoniae</i> F38 ^T	IRC	IRC
<i>M. caviae</i> G122 ^T	IRC	NIH, IMT
<i>M. caviopharyngis</i> 117C ^T	FCR	FCR
<i>M. citelli</i> RG-2C ^T	IRC	IRC
<i>M. cloacale</i> 383 ^T	FCR	FCR
<i>M. collis</i> 58B ^T	FCR	FCR
<i>M. columbinasale</i> 694 ^T	IRC	IRC
<i>M. columbinum</i> MMP-1 ^T	IRC	IRC
<i>M. columborale</i> MMP-4 ^T	IRC	IRC
<i>M. conjunctivae</i> HRC581 ^T	IRC	NIH, IMT
<i>M. corogypsi</i> BV ^T	FCR	IMT, FCR
<i>M. cottleii</i> VIS ^T	FCR	FCR
<i>M. cricetuli</i> CH ^T	FCR	FCR
<i>M. cynos</i> H831 ^T	IRC	IRC, IMT
<i>M. dispar</i> 462/2 ^T	IRC	IRC, IMT
<i>M. edwardii</i> PG24 ^T	IRC	IRC, IMT
<i>M. elephantis</i> E42 ^T	MRC, FCR	IMT
<i>M. equigenitalium</i> T37 ^T	IMT	IMT
<i>M. equirhinis</i> M432/72 ^T	LIRA	LIRA, IMT
<i>M. falconis</i> H/T1 ^T	IMT	IMT
<i>M. fastidiosum</i> 4822 ^T	IRC	IRC
<i>M. faucium</i> DC333 ^T	NIH	NIH
<i>M. felifaucium</i> PU ^T	MRC	MRC
<i>M. feliminutum</i> Ben ^T	IRC	IRC
<i>M. felis</i> CO ^T	IRC	NIH, IMT
<i>M. fermentans</i> PG18 ^T	NIH	NIH, IMT
<i>M. flocculare</i> Ms42 ^T	SVS	SVS
<i>M. gallinaceum</i> DD ^T	NIH	NIH, IMT
<i>M. gallinarum</i> PG16 ^T	NIH	NIH
<i>M. gallisepticum</i> PG31 ^T	NIH	NIH, IMT
<i>M. gallopavonis</i> WR1 ^T	IRC	IRC
<i>M. gateae</i> CS ^T	IRC	IRC, IMT
<i>M. genitalium</i> G37 ^T	FCR	FCR
<i>M. glycophilum</i> 486 ^T	FCR	FCR
<i>M. gypis</i> B1/T1 ^T	IMT	IMT
<i>M. hominis</i> PG21 ^T	NIH	NIH
<i>M. hyopharyngis</i> H3-6BF ^T	FCR	FCR
<i>M. hyopneumoniae</i> J ^T	SVS	SVS, IMT
<i>M. hyorhinis</i> BTS7 ^T	NIH	NIH, CVM
<i>M. hyosynoviae</i> S16 ^T	CVM	CVM, IMT
<i>M. imitans</i> 4229 ^T	FCR	FCR
<i>M. indiensis</i> 3T ^T	FCR	FCR
<i>M. iners</i> PG30 ^T	NIH	NIH
<i>M. iowae</i> 695 ^T	IRC	IRC
<i>M. leocaptivus</i> 3L2 ^T	NCTC, FCR	NCTC, FCR
<i>M. leopharyngis</i> LL2 ^T	NCTC, FCR	NCTC, FCR
<i>M. lipofaciens</i> R171 ^T	FCR	FCR
<i>M. lipophilum</i> MaBy ^T	NIH	NIH
<i>M. maculosum</i> PG15 ^T	NIH	NIH

Continued

TABLE 1—Continued

Strain	Source(s) ^a	
	Mollicute	Antiserum
<i>M. meleagridis</i> 17529 ^T	IRC	IRC
<i>M. moatsii</i> MK405 ^T	IRC	IRC, IMT
<i>M. mobile</i> 163K ^T	IMT	IMT
<i>M. molaris</i> H542 ^T	IRC	IRC, IMT
<i>M. muris</i> RIII4 ^T	FCR	FCR
<i>M. mustelae</i> MX9 ^T	FCR	FCR
<i>M. mycoides</i> subsp. <i>mycoides</i> PG1 ^T	IRC	IRC
<i>M. mycoides</i> subsp. <i>capri</i> PG3 ^T	IRC	IRC
<i>M. neurolyticum</i> type A ^T	NIH	NIH, IMT
<i>M. opalescens</i> MH5408 ^T	IRC	IRC, IMT
<i>M. orale</i> CH19299 ^T	NIH	NIH
<i>M. ovipneumoniae</i> Y98 ^T	IRC	IRC, IMT
<i>M. oxoniensis</i> 128 ^T	FCR	FCR
<i>M. penetrans</i> GTU54 ^T	FCR	FCR
<i>M. phocacebrale</i> 1049 ^T	IMT	IMT
<i>M. phocarhinis</i> 852 ^T	IMT	IMT
<i>M. phocidae</i> 105 ^T	MAFC	MAFC
<i>M. pirum</i> HRC 70-159 ^T	FCR	FCR
<i>M. pneumoniae</i> FH ^T	NIH	NIH, IMT
<i>M. primatum</i> HRC292 ^T	NIH	NIH
<i>M. pullorum</i> CKK ^T	IRC	IRC
<i>M. pulmonis</i> PG34 ^T	NIH	NIH, IMT
<i>M. putrefaciens</i> KS-1 ^T	IRC	IRC, IMT
<i>M. salivarium</i> PG20 ^T	NIH	NIH
<i>M. simbae</i> LX ^T	NCTC, FCR	NCTC, FCR
<i>M. spermatophilum</i> AH 159 ^T	FCR	FCR
<i>M. spumans</i> PG13 ^T	NIH	NIH, IMT
<i>M. sualvi</i> Mayfield B ^T	GIRA	GIRA
<i>M. subdolum</i> TB ^T	IMT	IMT
<i>M. synoviae</i> WVU 1853 ^T	IRC	IRC
<i>M. testudinis</i> 01008 ^T	MRC	MRC
<i>M. verecundum</i> 107 ^T	IRC	IRC, IMT
<i>M. yeatsii</i> GIH ^T	FCR	FCR
<i>Entomoplasma ellychniae</i> ELCN-1 ^T	FCR	FCR
<i>E. lucivorax</i> PIPN-2 ^T	FCR	FCR
<i>E. luminosum</i> PIMN-1 ^T	FCR	FCR
<i>E. melaleuca</i> M1 ^T	FCR	FCR
<i>E. somniliux</i> PYAN-1 ^T	FCR	FCR
<i>Mesoplasma entomophilum</i> TAC ^T	FCR	FCR
<i>M. florum</i> L1 ^T	FCR	FCR
<i>M. lactucae</i> 831-C4 ^T	FCR	FCR
<i>M. seifferti</i> F7 ^T	FCR	FCR
<i>Acholeplasma axanthum</i> S-743 ^T	IRC	IRC, IMT
<i>A. caviogenitalium</i> GP3 ^T	FCR	FCR
<i>A. equifetale</i> C112 ^T	IMT	IMT
<i>A. granularum</i> BTS39 ^T	NIH	NIH, IMT
<i>A. hippikon</i> C1 ^T	IMT	IMT
<i>A. laidlawii</i> PG8 ^T	NIH	NIH, IMT
<i>A. modicum</i> PG49 ^T	IRC	IRC, IMT
<i>A. morum</i> 72-43 ^T	FCR	FCR
<i>A. multilocale</i> PN 525 ^T	MRC	MRC
<i>A. oculi</i> 19L ^T	IRC	IRC, IMT
<i>A. parvum</i> H23M ^T	FCR, VFW	FCR, VFW
Bovine serogroup 7 strain PG50	IRC	IRC

^a ATCC, American Type Culture Collection, Rockville, Md.; CVM, R. F. Ross, College of Veterinary Medicine, Iowa State University, Ames; FCR, J. G. Tully, Mycoplasma Section, Frederick Cancer Research Facility, Frederick, Md.; GIRA, R. M. Gourlay, Institute for Research on Animal Diseases, Compton, Newbury, Berkshire, England; IMT, H. Kirchhoff, Institut für Mikrobiologie und Tierseuchen, Tierärztliche Hochschule Hannover, Hannover, Germany; IRC, E. A. Freundt, Food and Agriculture Organization, World Health Organization, International Reference Centre for Animal Mycoplasmas, Aarhus, Denmark; LIRA, R. Lemcke, Institute for Research of Animal Diseases, Compton, Newbury, Berkshire, England; MAFC, H. L. Ruhne, Ministry of Agriculture and Food, Veterinary Laboratory Services, Guelph, Ontario, Canada; MRC, A. Hill, Medical Research Council Laboratories, Carshalton, Surrey, England; NCTC, National Collection of Type Cultures, London, England; NIH, M. F. Barile and J. G. Tully, National Institutes of Health, Bethesda, Md.; SVS, N. F. Friis, Statens Veterinære Serum Laboratorium, Copenhagen, Denmark; VFW, M. Ogata, Department of Veterinary Public Health, Azabu University, Fuchinobe Sagami-hara, Kanagawa, Japan; VMR, L. Stipkovits, Veterinary Medical Research Institute, Hungarian Academy of Science, Budapest, Hungary.

FIG. 1. (A) Typical fried-egg morphology of colonies of crocodile mycoplasma strain MP145^T on agar medium after incubation under aerobic conditions for 2 days. Bar = 50 μm. (B) Electron micrograph of an ultrathin section of crocodile mycoplasma strain MP145^T, showing the absence of a cell wall and the presence of a trilaminar membrane. Bar = 1 μm.

stained 0.8% (wt/vol) agarose gels, was 5 pg of template DNA or about 10^3 cells. The PCR products were purified by gel filtration spin chromatography and were quantified by spectrophotometry before automated fluorescent dideoxynucleotide sequence determination with an Applied Biosystems model 373A (version 1.2.1) instrument without molecular cloning. Both strands were sequenced completely by primer walking.

Previously determined *Mollicutes* 16S rRNA gene nucleotide sequences were obtained from the GenBank database by using the Basic Local Alignment Search Tool of the National Center for Biotechnology Information, National Institutes of Health. Pairwise nucleotide sequence similarity scores were computed by using University of Wisconsin Genetics Computer Group sequence analysis software with default gap and gap length weights. A score of 0 indicated that two sequences had only random sequence similarity, and a score of 1 indicated that sequences were identical (4).

Serological studies. Antisera were prepared as described by Morton and Roberts (13). A serological comparison of the crocodile strains with each other and a comparison of strains MP145^T and MP266 with members of previously described *Mollicutes* species were performed by using growth inhibition tests (5) and immunobinding assays with colonies transferred to nitrocellulose (11). The tests performed with strains MP145^T and MP266 were done in two directions; i.e., reference antisera were tested with strains MP145^T and MP266, and antisera against strains MP145^T and MP266 were tested with the type strains of previously described mycoplasmas. The antisera and type strains used for serological investigations are listed in Table 1.

Nucleotide sequence accession number. The 16S rRNA gene nucleotide sequence which we determined has been deposited in the GenBank database under accession no. U63137.

RESULTS AND DISCUSSION

Morphology and ultrastructure. Colonies of strains MP145^T and MP266 were visible on agar medium after 2 to 3 days of

incubation. The colonies had a typical fried-egg appearance (Fig. 1A). Ultrathin sections revealed the trilaminar structure of the cell membrane and the absence of a cell wall (Fig. 1B). The cells of the two strains investigated appeared to be mainly coccoid or round. No motility was observed when living strain MP145^T and MP266 cells were examined by dark-field microscopy.

Filtration characteristics. Filtration of a broth culture of strain MP145^T reduced the viable count from 9.8×10^8 CFU/ml in the original dilution to 6.5×10^6 CFU/ml in the 450-nm-pore-size membrane filtrate and to 3.0×10^5 CFU/ml in the 220-nm-pore-size membrane filtrate. For strain MP266 the 4.2×10^8 CFU/ml in the original dilution was reduced to 1.2×10^6 CFU/ml in the 450-nm-pore-size membrane filtrate and to 1.25×10^5 CFU/ml in the 220-nm-pore-size membrane filtrate.

Reversion studies. Strains MP145^T and MP266 and several other crocodile mycoplasma strains were tested for reversion to bacterial forms. No reversion was observed after serial subcultivation in broth or on agar lacking inhibitors.

Growth characteristics. The crocodile mycoplasma strains grew well in a CO₂-enriched atmosphere; the concentration in broth containing 20% horse serum or 27% swine serum was as high as 10^{12} CFU/ml, and the concentration in broth supplemented with crocodile serum was almost 1 log higher. The organisms grew well at temperatures between 25 and 42°C, and grew most rapidly at 37°C.

Sterol requirement. Strains MP145^T and MP266 required cholesterol for growth. The organisms could not be cultured in medium without cholesterol, but they grew in medium containing 5 mg of cholesterol per ml (2.2 mg of protein per ml), 10 mg of cholesterol per ml (2.5 mg of protein per ml), and 20 mg of cholesterol per ml (3.1 mg of protein per ml). The organisms were susceptible to digitonin and sodium polyanethol sulfonate, and the zones of growth inhibition were between 5 and 10 mm wide.

Biochemical tests. The biochemical activities of strains MP145^T and MP266 and several other crocodile mycoplasma strains were identical. All of the strains investigated fermented glucose and maltose, did not produce films and spots, did not hydrolyze arginine, esculin, and urea, reduced tetrazolium chloride (aerobically and anaerobically), and exhibited phosphatase activity. Strains MP145^T and MP266 lysed and adsorbed bovine, ovine, and rabbit erythrocytes.

DNA base composition. The G+C contents of strains MP145^T and MP266 were 27.6 and 27.7 mol%, respectively.

Partial 16S rRNA gene sequence. The 16S rRNA gene nucleotide sequence which we determined has been deposited in the GenBank database (U63137). Overall, the 16S rRNA gene nucleotide sequence similarity data placed strain MP145^T in the *Mycoplasma fermentans* phylogenetic clade, where its closest relative (similarity score, 0.98) was an isolate provisionally named "*M. lacertae*" (accession no. U56733) that was obtained from American alligators (3). The next closest relatives of strain MP145^T were the avian mycoplasmas *Mycoplasma corogypsi* (accession no. L08054) and *Mycoplasma sturni* (accession no. U22013), whose levels of sequence similarity were 0.91 and 0.90, respectively. For perspective, the level of sequence similarity between strain MP145^T and *M. fermentans* (accession no. M24289) was 0.87, and the level of sequence similarity between *M. fermentans* and *Lactobacillus casei* (accession no. M58815) was 0.72.

Serological investigations. The 24 crocodile mycoplasma strains reacted identically in the growth inhibition tests and immunobinding assays, indicating that they belong to the same species. In the immunobinding assays we observed weak reactions between strains MP145^T and MP266 and type strains *Mycoplasma arthritidis* PG6^T, *M. corogypsi* BV^T, *Mycoplasma elephantis* E42^T, *Mycoplasma flocculare* Ms42^T, *Mycoplasma glycyphilum* 486^T, *Mycoplasma leocaptivus* 3L2^T, *Mycoplasma leopharyngis* LL2^T, *Mycoplasma lipofaciens* R171^T, *Mycoplasma pneumoniae* FH^T, and *Mycoplasma spermatophilum* AH159^T in both directions. In metabolism inhibition tests strain MP145^T reacted with antiserum against *M. lipofaciens* R171^T (2.5-mm inhibition zone), and strain MP266 reacted with antisera against *M. flocculare* Ms42^T (2.5-mm inhibition zone) and *M. pneumoniae* FH^T (3.2-mm inhibition zone). No positive reactions were observed in the reverse tests. There were also no cross-reactions in growth inhibition and epiimmunofluorescence tests between strain MP145^T and the mycoplasma species isolated from American alligators (provisionally named "*M. lacertae*"), which exhibited the closest relationship to MP145^T in the 16S rRNA gene nucleotide sequence analysis.

Western blotting. The mycoplasma species that exhibited cross-reactions with strain MP145^T and/or strain MP266 were investigated by Western blotting by using antisera against strains MP145^T and MP266. In addition, reverse investigations were performed; i.e., strains MP145^T and MP266 were electrophoretically separated and after Western blotting were treated with antisera against the cross-reacting mycoplasma species. The antigen patterns obtained were quite different and differed markedly from the protein patterns of strains MP145^T and MP266. On the other hand, they clearly showed that the

FIG. 2. Western blot analysis of strain MP145^T (lane 1), *M. arthritidis* PG6^T (lane 2), *M. corogypsi* BV^T (lane 3), *M. elephantis* E42^T (lane 4), *M. glycyphilum* 486^T (lane 5), *M. leocaptivus* 3L2^T (lane 6), *M. leopharyngis* LL2^T (lane 7), *M. lipofaciens* R171^T (lane 8), *M. pneumoniae* FH^T (lane 9), and *M. spermatophilum* AH159^T (lane 10) performed with antiserum against strain MP145^T. MS-prestained SDS-PAGE molecular standards (Bio-Rad Laboratories, Hercules, Calif.) were used.

different mycoplasma species possess several common antigens. The reactions of the cross-reacting mycoplasma species with antiserum against strain MP145^T are shown in Fig. 2.

Pathogenicity. Mycoplasmas were isolated from crocodiles suffering from exudative polyarthritis. All sick animals consistently had swollen limb joints of the hind or fore limbs or both, as well as progressive lameness and paresis. The synovial structures in subacute cases contained mycoplasmas and excess turbid mucus which, at a later stage of the disease, became yellowish, inspissated, and sterile. Cellular changes in the joint capsules included edema, necrosis of the superficial layers of membrane, lymphocytic infiltration, and fibrosis. Evidence of pneumonia was observed in a few animals at necropsy. The disease occurred at five crocodile farms among rearing stocks with crocodiles 1 to 3 years old. No breeding stock was affected. The disease could be experimentally reproduced in crocodile yearlings by intracoelomic and intrapleural inoculation of strain MP266, and thus Henle-Koch's postulates were fulfilled. Goats were not susceptible to infection with strain MP266 administered intratracheally or intranasally (12).

Taxonomic assignment. The properties described above for strains MP145^T and MP266 fulfill the criteria (18) for species descriptions of members of the class *Mollicutes*. A cell wall is absent, and the cells can be filtered through 220- and 450-nm-pore-size membranes, fail to revert to walled bacteria when they are grown in antibiotic-free media, have low G+C contents, are resistant to penicillin, and produce typical fried-egg colonies on solid media. The growth requirement for sterol or serum, in conjunction with the lack of helicity, places these organisms in the order *Mycoplasmatales* and the family *Mycoplasmataceae*. The inability of the strains to hydrolyze urea mandates assignment to the genus *Mycoplasma*.

Strain MP145^T and the other crocodile mycoplasma strains isolated belong to the same species as they have identical biological characteristics and identical serological properties. The lack of serological relatedness of strains MP145^T and MP266 to other *Mollicutes* species demonstrates that they rep-

resent a previously unrecognized species, for which the name *Mycoplasma crocodyli* is proposed. The taxonomic description below summarizes the properties of this new species.

Description of *Mycoplasma crocodyli* sp. nov. *Mycoplasma crocodyli* (cro.co.dy'li. L. gen. n. *crocodyli*, of the crocodile). Cells lack true cell walls and are coccoid or round. Colonies on solid medium usually have a typical fried-egg appearance. Chemorganotroph. Ferments glucose and mannose. Arginine, esculin, and urea are not hydrolyzed. Reduces tetrazolium chloride. Does not produce films and spots on egg yolk agar. Possesses phosphatase activity. Lyses and adsorbs bovine, ovine, and rabbit erythrocytes. Cholesterol or serum is required for growth. The optimum growth temperature is 37°C. The G+C content of the DNA is 27.6 mol%. Strains of *M. crocodyli* were isolated from joints and lungs of crocodiles with exudative polyarthritis. Pathogenicity has been proved by experimental infection. The type strain is strain MP145 (= ATCC 51981), which originated from the joint of a diseased crocodile.

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