NOTES

Revised Taxonomy of the Class *Mollicutes*: Proposed Elevation of a Monophyletic Cluster of Arthropod-Associated Mollicutes to Ordinal Rank (*Entomoplasmatales* ord. nov.), with Provision for Familial Rank To Separate Species with Nonhelical Morphology (*Entomoplasmataceae* fam. nov.) from Helical Species (*Spiroplasmataceae*), and Emended Descriptions of the Order *Mycoplasmatales*, Family *Mycoplasmataceae*

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On the basis of recent phylogenetic studies of 47 species within the class *Mollicutes* and recent molecular and physiologic findings that distinguish a new group of non-sterol-requiring insect and plant mollicutes from *Acholeplasma* species, we propose a revised taxonomy of the class *Mollicutes*. Order I (*Mycoplasmatales*) is retained as described earlier (S. Razin and E. A. Freundt, p. 740–742, in N. R. Krieg and J. G. Holt, ed., *Bergey's Manual of Systematic Bacteriology*, vol. 1, 1984), with provision for a single family (*Mycoplasmataceae*) and two genera (*Mycoplasma* and *Ureaplasma*) for classification of sterol-requiring mollicutes primarily associated with vertebrates. Emended descriptions of the order and family for the above taxa are hereby included. Proposed order II (*Entomoplasmatales* ord. nov.) contains subtaxa for either nonhelical (*Entomoplasmataceae* fam. nov.) or helical (*Spiroplasmataceae*) mollicutes; the latter family is hereby transferred from the order *Mycoplasmatales*. The new order and family permit designation and classification of a monophyletic cluster of mollicute strains associated primarily with arthropods. It is proposed that nonhelical, sterol-requiring insect and plant mollicutes previously designated *Mycoplasma ellychniae* (Tully et al., 1989), *Mycoplasma melaleucae* (Tully et al., 1990), *Mycoplasma somnilux* (Williamson et al., 1990), *Mycoplasma luminosum* (Williamson et al., 1990), and *Mycoplasma lucivorax* (Williamson et al., 1990) be transferred to *Entomoplasma* gen. nov., family *Entomoplasmataceae* fam. nov., as *Entomoplasma ellychniae* comb. nov., *Entomoplasma melaleucae* comb. nov., *Entomoplasma somnilux* comb. nov., *Entomoplasma luminosum* comb. nov., and *Entomoplasma lucivorax* comb. nov. Furthermore, the genus *Mesoplasma* gen. nov. within the family *Entomoplasmataceae* fam. nov. is proposed for four non-sterol-requiring insect and plant mollicutes. This designation requires the transfer of *Acholeplasma florum* (McCoy et al., 1984), *Acholeplasma entomophilum* (Tully et al., 1988), *Acholeplasma seiffertii* (Bonnet et al., 1991), and *Mycoplasma lactucae* (Rose et al., 1990) to *Mesoplasma florum* comb. nov., *Mesoplasma entomophilum* comb. nov., *Mesoplasma seiffertii* comb. nov., and *Mesoplasma lactucae* comb. nov., respectively. Previously established orders *Acholeplasmatales* and *Anaeroplasmatales* within the class *Mollicutes* are designated orders III and IV, respectively, in the proposed revision. Classification and characteristics of lower taxa within these two orders remain as previously given. The proposal outlined here does not consider the large cluster of nonculturable, arthropod-associated, plant-pathogenic mollicutes ("mycoplasma-like organisms") recently shown to be more closely related to members of the order *Acholeplasmatales*.

The order *Mycoplasmatales*, first proposed in 1955 by Freundt (11), was later incorporated into the joint taxonomic proposals of Edward and Freundt in 1967 (9) to classify sterol-requiring, wall-less prokaryotes in the class *Mollicutes* (26). In these early endeavors, the single order encompassed a small cluster of about 12 serologically distinct

species. It soon became apparent that a number of newly described species in the genus *Mycoplasma* did not require sterols or cholesterol for growth, and subsequent proposals were made to separate these organisms into a new family (*Acholeplasmataceae*) and genus (*Acholeplasma*) within the order *Mycoplasmatales* (10, 39). By 1983, fundamental alterations in the perception of *Acholeplasma* ecology had occurred, including observations that the organisms were commensals in a variety of animal hosts and could be

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isolated from insects and plant surfaces (8, 20, 35, 39, 40). These and other major distinctions, particularly data showing that *Acholeplasma* species had twice the genome size (1,500 kbp) of *Mycoplasma* species, were used in a 1984 proposal that elevated the family *Acholeplasmataceae* to ordinal rank (order II, *Acholeplasmatales*) within the class *Mollicutes* (12). Later, a cluster of strictly anaerobic anaeroplasmas from the rumens of bovine and ovine hosts were raised to ordinal rank (order III, *Anaeroplasmatales*) within the class (29).

The taxonomic characterization and description of *Spiroplasma citri* in 1973 (32), the first helical, sterol-requiring mollicute of plant and/or arthropod origin, caused certain classification problems at higher taxonomic levels. The morphology of the organism was clearly different from that of all other known mollicutes, and while the helical organisms required cholesterol, their 1,500-kbp genomes were twice the size of those of other species of sterol-requiring mollicutes. Eventually, *S. citri* and other species in the genus *Spiroplasma* were elevated (34) to a family (*Spiroplasmataceae*) within the order *Mycoplasmatales*. Although many new spiroplasmas have been subsequently discovered (43, 53), all have been shown to be associated with arthropods.

While higher classification of the spiroplasmas may have been a minor dilemma, it soon became apparent that other newly isolated plant or insect mollicutes presented much more serious problems in classification. The tropical flower isolates described in 1979 by McCoy and associates (20) were the first to undergo extensive characterization. Three serologically related strains (LI, GF1, and PP2) appeared to possess most of the properties of acholeplasmas, including rapid and extensive growth in broth medium at a temperature optimum of about 30°C. Despite repeated testing, these strains could not be grown in medium devoid of cholesterol or serum. However, it was eventually shown that growth did occur in serum- or cholesterol-free media containing 0.04% Tween 80 (polyoxyethylene sorbitan) (40). Since these flower-associated strains apparently grew in the absence of serum or cholesterol, they were named *Acholeplasma florum* (18). Two additional serologically distinct species, again of insect or plant origin and with similar growth properties, were described more recently as *Acholeplasma entomophilum* (42) and *Acholeplasma seiffertii* (1).

The early studies of *A. florum* prompted efforts to understand the ecology of these plant-derived, nonhelical mollicutes. Subsequent studies of numerous nonhelical mollicutes, isolated by Clark and Whitcomb (4, 5) from insects collected over a wide geographical region in the United States, indicated that these organisms were common in the gut or hemolymph of these hosts. These observations not only provided the first evidence that nonhelical, non-sterol-requiring mollicutes occur in arthropods but offered a ready explanation for their presence on plant surfaces. From this collection of mollicutes, an additional 13 uncharacterized plant and insect isolates, representing at least seven putative species, have now been found to possess similar features and to show sustained growth in serum-free media only when supplemented with 0.04% Tween 80 (47).

While the response to Tween 80 suggested that the insect and plant isolates were distinct from classic acholeplasmas, the first substantial evidence of major differences came from a phylogenetic analysis of 47 mollicute species (or putative species) by Weisburg and colleagues (49). In this comparative study of 16S rRNA sequences, both *A. entomophilum* and *A. florum* were shown to belong to the so-called "spiro-

plasma/mycoides" group, a cluster of helical and nonhelical sterol-requiring organisms phylogenetically distinct not only from members of the orders *Acholeplasmatales* and *Anaeroplasmatales* but also from the vast majority of members of the family *Mycoplasmataceae*.

More recently, additional molecular distinctions between the non-sterol-requiring insect- or plant-derived strains and classic acholeplasmas have been documented. *A. florum* has been shown to possess a gene coding for one of the components (enzyme II) of a phosphoenolpyruvate-dependent sugar-phosphotransferase system and to utilize UGA as a tryptophan codon (21, 22). A functional phosphotransferase system(s) appears to be absent in *Acholeplasma* spp. and in some nonfermentative *Mycoplasma* species but is present in *Spiroplasma* spp. and fermentative *Mycoplasma* species (3, 37, 38). Likewise, the use of the UGA codon as a tryptophan signal is characteristic of *Mycoplasma* and *Spiroplasma* species (27, 54). In contrast, *Acholeplasma* species utilize the UGA codon as a stop signal (36). Major differences in the genome size among organisms in each of the two groups have also been disclosed recently. Data obtained by pulsed-field gel electrophoresis show that *A. florum* and other similar insect- or plant-derived strains have genome sizes in the range of 860 to 1,100 kbp (570 to 730 MDa) (2, 25). In contrast, classic *Acholeplasma* species have genomes of 1,500 to 1,600 kbp (2, 24, 28). Thus, genome size, UGA codon usage, presence of a phosphotransferase system enzyme II, and 16S rRNA sequences of the new insect and plant mollicutes clearly imply that they have greater affinities to members of the order *Mycoplasmatales* than to members of the order *Acholeplasmatales*.

The sterol-Tween 80 test system described initially for *A. florum* (18, 40) and subsequently applied to other insect and plant mollicutes (1, 42, 44, 45, 52) provides a consistent phenotypic marker for identification of strains that are capable of growing in the presence of 0.04% Tween 80. A simplified method for testing sterol requirements, including measuring growth promotion with Tween 80, has recently been proposed (31).

On the basis of the above observations, we offer the following proposals for revision in the taxonomy of the class *Mollicutes* (Table 1).

Proposal for *Entomoplasma* gen. nov. for sterol-requiring mollicutes of insect and/or plant origin. We propose that five species of sterol-requiring mollicutes of insect or plant origin currently assigned to the genus *Mycoplasma* be transferred to the genus *Entomoplasma* gen. nov. These include *Entomoplasma ellychniae* comb. nov. (Tully et al., 1989), *Entomoplasma melaleuciae* comb. nov. (Tully et al., 1990), *Entomoplasma somnilux* comb. nov. (Williamson et al., 1990), *Entomoplasma luminosum* comb. nov. (Williamson et al., 1990), and *Entomoplasma lucivorax* comb. nov. (Williamson et al., 1990).

The five species in the genus have guanine-plus-cytosine DNA contents of 27 to 29 mol% and genome sizes ranging from 790 to 1,140 kbp. The organisms require cholesterol or serum for growth and do not show sustained growth in serum-free or cholesterol-free media when supplemented with 0.01 to 0.04% Tween 80. The five species are serologically distinct from each other and from all currently recognized species in the genus *Mycoplasma*. Organisms currently being assigned to the new genus grow over a temperature range of 10 to 32°C, with optimum growth occurring at 30°C. Growth is not apparent at 37°C, the optimum growth temperature for most members of the genus *Mycoplasma*. All currently assigned species were isolated

TABLE 1. Proposed taxonomy and characteristics of members of the class *Mollicutes*

Classification	No. of recognized species	Guanine-plus-cytosine content (mol%)	Genome size (kbp)	Cholesterol requirement	Habitat	Other distinctive features
Order I, <i>Mycoplasmatales</i> , family I, <i>Mycoplasmataceae</i>						
Genus I, <i>Mycoplasma</i>	85	23–40	600–1,350	Yes	Humans, animals	Optimum growth usually at 37°C
Genus II, <i>Ureaplasma</i>	5	27–30	760–1,170	Yes	Humans, animals	Urea hydrolysis
Order II, <i>Entomoplasmatales</i>						
Family I, <i>Entomoplasmataceae</i>						
Genus I, <i>Entomoplasma</i>	5	27–29	790–1,140	Yes	Insects, plants	Optimum growth, 30°C
Genus II, <i>Mesoplasma</i>	4	27–30	870–1,100	No	Insects, plants	Optimum growth, 30°C; sustained growth in serum-free medium only with 0.04% Tween 80
Family II, <i>Spiroplasmataceae</i> , Genus I, <i>Spiroplasma</i>	11	25–30	940–2,200	Yes	Insects, plants	Helical filaments; optimum growth at 30–37°C
Order III, <i>Acholeplasmatales</i> , family I, <i>Acholeplasmataceae</i> , genus I, <i>Acholeplasma</i>	9	26–36	1,500–1,650	No	Animals, some plants and insects	Optimum growth at 30–37°C
Order IV, <i>Anaeroplasmatales</i> , family I, <i>Anaeroplasmataceae</i>						
Genus I, <i>Anaeroplasma</i>	4	29–34	1,500–1,600	Yes	Bovine or ovine rumen	Oxygen-sensitive anaerobes
Genus II, <i>Asteroleplasma</i>	1	40	1,500	No	Bovine or ovine rumen	Oxygen-sensitive anaerobes

from insects or plant surfaces where they were presumably deposited by insects. We propose the name *Entomoplasma* (En.to.mo'plas.ma. Gr. n. *entomon*, insect; Gr. neut. n. *plasma*, something formed or molded, a form; M.L. neut. n. *Entomoplasma*, name intended to show association with insects). The type species is *Entomoplasma ellychniae* (Tully, Rose, Hackett, Whitcomb, Carle, Bové, Colflesh, and Williamson, 1989).

The characteristics of the new genus and species are as described above and in previously published descriptions. Established type strains from earlier descriptions (*E. ellychniae* ELCN-1^T, *E. melaleuca* M1^T, *E. somnilux* PYAN-1^T, *E. luminosum* PIMN-1^T, and *E. lucivorax* PIPN-2^T) are retained.

Proposal for *Mesoplasma* gen. nov. for non-sterol-requiring mollicutes of insect and plant origin. Three non-sterol-requiring species of insect or plant origin currently assigned to the genus *Acholeplasma*, and a single species of plant origin (*Mycoplasma lactucae*) (30) recently shown to grow in 0.04% Tween 80-supplemented serum-free media (31), are hereby transferred to *Mesoplasma* gen. nov. These include *Mesoplasma florum* comb. nov. (McCoy et al., 1984), *Mesoplasma entomophilum* comb. nov. (Tully et al., 1988), *Mesoplasma seiffertii* comb. nov. (Bonnet et al., 1991), and *Mesoplasma lactucae* comb. nov. (Rose et al., 1990), with their respective type strains retained as previously established (*Mesoplasma florum* L1^T, *Mesoplasma entomophilum* TAC^T, *Mesoplasma seiffertii* F7^T, and *Mesoplasma lactucae* 831-C4^T).

The four species in the genus have guanine-plus-cytosine DNA contents of 27 to 30 mol% and genome sizes ranging from 860 to 1,100 kbp. Organisms utilize the UGA codon as a tryptophan signal. Neither serum nor cholesterol is required for growth, but organisms show sustained growth in

serum-free or cholesterol-free media supplemented with 0.04% Tween 80. Organisms grow over a temperature range of 18 to 37°C, with optimum growth occurring at 28 to 30°C. The five species are serologically distinct from each other and from all currently defined species in the genus *Mycoplasma* or the genus *Entomoplasma*, gen. nov. For these organisms, we propose the genus name *Mesoplasma* (Me.so'plas.ma. Gr. n. *meso*, middle; Gr. neut. n. *plasma*, something formed or molded, a form; M.L. neut. n. *Mesoplasma*, middle form, name intended to denote a middle position with respect to sterol or cholesterol requirement). The type species is *Mesoplasma florum* (McCoy, Basham, Tully, Rose, Carle, and Bové 1984). The characteristics of the new genus and species are as described above.

Elevation of *Entomoplasma* to family rank, *Entomoplasmataceae* fam. nov., with *Mesoplasma* gen. nov. as genus II. Recognition of the two proposed new genera for mollicutes of insect and plant origin and the current expectation that these hosts will continue to be a rich source of wall-less prokaryotes suggest that a formal classification at higher taxonomic levels of family and order would provide not only a logical identification scheme but one that would reflect current phylogenetic relationships (49).

Much discussion has taken place about the taxonomic significance of sterol requirements within the class *Mollicutes* (10, 26). The earlier elevation of the *acholeplasmas* to ordinal rank (12) would seem well justified now but from a phylogenetic or genetic standpoint rather than from that of sterol requirement. Growth in the absence of sterols has not proved to be synapomorphic for any mollicute taxon, because organisms with this characteristic occur in at least three phylogenetic groups (49). In recent proposals for taxonomy of the *anaeroplasmata*, other factors outweighed sterol requirement at the ordinal and family levels (29). The

clearly polyphyletic nature of mollicute sterol requirements is reminiscent of the status of genome size in the mollicutes. This character, once highly valued as a marker at higher taxon levels, was later found to be polyphyletic (49) and eventually proved to be variable within genera (2, 24). The decreased use of sterol requirement in the higher taxonomy of the mollicutes, the ambiguity in interpretation of tests with Tween 80, and the close phylogenetic position of sterol-requiring and non-sterol-requiring, nonhelical mollicutes from the insect and plant habitats argue against creation of new families on the basis of sterol requirements. Consequently, we propose that members of the sterol-requiring genus *Entomoplasma*, which cannot grow in the absence of sterols, and members of the genus *Mesoplasma*, which are able to grow in the presence of 0.04% Tween 80, should be placed in a single family. Accordingly, we propose (i) the elevation of the genus *Entomoplasma* to family rank, *Entomoplasmataceae*, and (ii) the inclusion of *Mesoplasma* as a second genus of the proposed new family.

Description of *Entomoplasmataceae*. (En.to.mo.plas.ma.ta'ce.ae. M.L. neut. n. *Entomoplasma*, type genus of the family; -aceae ending to denote a family; M.L. fem. pl. n. *Entomoplasmataceae*, the *Entomoplasma* family). Cells are usually coccoidal or occur as short, branched or unbranched, pleomorphic, nonhelical filaments. They are filterable through membranes with an average pore diameter of 220 to 450 nm; nonmotile; bounded by a single membrane, with no evidence of a cell wall; and facultatively anaerobic. Colonies on solid medium containing 0.8% Noble agar usually have a fried-egg appearance. The organisms are chemoorganotrophic. Acid is produced from glucose, with evidence of a phosphoenolpyruvate-dependent sugar transport system(s) in some members. Arginine and urea are not hydrolyzed. Colonies of some species may hemadsorb guinea pig erythrocytes. The organisms may or may not require serum or cholesterol for growth, with growth on serum-free broth medium occurring only in the presence of 0.04% Tween 80 (polyoxyethylene sorbitan). The temperature range for growth varies from 10 to 37°C, with the optimum usually at 30°C. The genome size ranges from 790 to 1,140 kbp, with the guanine-plus-cytosine content of the DNA varying from 27 to 30 mol%. The organisms utilize the UGA codon as a tryptophan signal.

Two genera are accepted in this family: *Entomoplasma* gen. nov., the type genus, and *Mesoplasma* gen. nov. Species of *Entomoplasma* require serum or cholesterol for growth. Species of *Mesoplasma* cannot grow in serum-free broth medium alone but show sustained growth in serum-free medium containing 0.04% Tween 80 (polyoxyethylene sorbitan) or in serum-containing medium.

Proposal for elevation of *Entomoplasmataceae* to ordinal rank (order II, *Entomoplasmatales*), with *Entomoplasmataceae* fam. nov. as family I and transfer of family *Spiroplasmataceae* from order I, *Mycoplasmatales*, as family II. The proposal to establish two new genera for mollicutes of insect and plant origin and to unite these organisms at a higher taxal level into a separate family still leaves some taxonomic problems for the insect- or plant-derived spiroplasmas unresolved. Currently, sterol-requiring, helical organisms in the genus *Spiroplasma* are classified within family II, *Spiroplasmataceae*, order I, *Mycoplasmatales*. Because 16S rRNA phylogenetic analysis (49) shows that the spiroplasma lineage gave rise to the genera *Entomoplasma* and *Mesoplasma*, these genera are most appropriately classified with spiroplasmas. In addition to the phylogenetic evidence, this decision is supported by the biological and ecological significance of mol-

licutes in these hosts and evidence that the numbers of mollicutes of these three genera found in insects may reach unprecedented numbers (4, 13, 41, 50).

It should be emphasized that the primary distinction being made concerns cultivable mollicutes that are consistently and evolutionarily associated with insects and plants, as contrasted with the possibly accidental isolation of true *Mycoplasma* species from plant hosts. In this regard, it should be noted that a strain identified serologically as an equine mycoplasma, *Mycoplasma equigenitalium*, was isolated from field plants in France (48). The isolate differed from most other known sterol-requiring mollicutes of insect or plant origin in having an optimum growth temperature of 37°C. In contrast to *Mycoplasma* species, several classic *Acholeplasma* species (*Acholeplasma axanthum* and *Acholeplasma oculi*) have been found frequently on plant surfaces (8, 39, 47). Finally, the "mycoplasma-like organisms" that cause many plant diseases (19), although clearly mollicutes (17), should not be easily confused with either entomoplasmas or mesoplasmas because they cannot be cultivated. Thus, characterization of any primary isolate of a nonhelical mollicute of plant or insect origin should first entail an analysis of sterol requirement and then serologic comparison with all established species in the genus *Entomoplasma* or *Mesoplasma*, depending on the organism's sterol requirement. If the results are negative, the isolate should then be tested against antisera to either *Acholeplasma* or *Mycoplasma* species, again depending on the results of the sterol test.

On the basis of these considerations, we believe it justified on phylogenetic and phenotypic grounds to propose a new order (*Entomoplasmatales* ord. nov.) within the class *Mollicutes* for separation of all established culturable nonacholeplasma mollicutes associated with insects and plants (Table 1). Two families are proposed within *Entomoplasmatales* ord. nov.: family *Entomoplasmataceae* fam. nov., as proposed above, would consist of two genera, *Entomoplasma* gen. nov. for sterol-requiring insect or plant mollicutes and *Mesoplasma* gen. nov. for those non-sterol-requiring organisms capable of sustained growth in serum-free medium supplemented with 0.04% Tween 80.

In addition, we propose the transfer of the family *Spiroplasmataceae* from family II within the *Mycoplasmatales* to family II of the *Entomoplasmatales*. The genus *Spiroplasma* would be retained as genus I within *Spiroplasmataceae*, and genus and family descriptions for these organisms would remain as previously proposed (34, 51). A comparison of the distinguishing properties among members of the order *Entomoplasmatales* is presented in Table 1.

Description of *Entomoplasmatales*, order II of class *Mollicutes* (Edward and Freundt 1967). *Entomoplasmatales* (En.to.mo.plas.ma.ta'les. M.L. neut. n. *Entomoplasma*, type genus of the family *Entomoplasmataceae*; -ales ending to denote an order; M.L. fem. pl. n. *Entomoplasmatales*, the *Entomoplasma* order). Cells may be helical or nonhelical. Helical cells are usually 100 to 200 nm in diameter and 3 to 5 µm in length during the logarithmic phase of growth. Cells are motile, with flexional and twisting movements and rotatory directional motility. Flagella, periplasmic fibrils, and cell wall structures are absent. Cells may also occur as nonmotile pleomorphic coccoidal forms (300 to 500 nm in diameter) or short, branched or unbranched filaments, bounded by a single membrane. Cells are filterable through membranes with an average pore diameter of 220 to 450 nm and are facultatively anaerobic. Nonhelical organisms usually form fried-egg-type colonies on solid medium containing

0.8% Noble agar. Helical organisms usually form diffuse colonies on 0.8% Noble agar, but fried-egg-type colonies can be seen on solid medium with 2.25% Noble agar. The cells are chemoorganotrophic. Acid is usually produced from glucose, with evidence that members in both families possess a phosphoenolpyruvate-dependent sugar phosphotransferase system(s). Arginine may or may not be hydrolyzed. There is no hydrolysis of urea. Colonies of some species may hemadsorb guinea pig erythrocytes. Cells may or may not require serum or cholesterol for growth, with growth on serum-free broth medium occurring only in the presence of 0.04% Tween 80 (polyoxyethylene sorbitan). The temperature range for growth varies from 10 to 43°C, with the optimum usually at 30 to 32°C. The genome size ranges from 790 to 2,200 kbp, with the guanine-plus-cytosine content of the DNA varying from 24 to 30 mol%. All organisms tested utilize the UGA codon as a tryptophan signal.

Emended description of *Mycoplasmataceae*, family I of *Mycoplasmatales* (Freundt 1955) Razin and Freundt 1984. Cells are nonhelical, varying from pleomorphic coccoidal forms 300 to 850 nm in diameter to short or long, branched or unbranched filaments 200 to 300 nm in width to 1,500 nm in length. Some have specialized terminal structures that mediate attachment to eukaryotic cells. Cells lack a cell wall and are bounded by a single plasma membrane. They are usually nonmotile, but some demonstrate gliding motility. The organisms are facultatively anaerobic, with some species showing growth only under more rigorous anaerobic (but not oxygen-free) environments. They possess a truncated flavin-terminated electron transport chain devoid of quinones and cytochromes. Fermentative species frequently have a phosphoenolpyruvate-dependent sugar-phosphotransferase enzyme system(s). Colonies are small (1 mm in diameter) to very small, varying in morphology from uniformly granular types to those with a fried-egg appearance. The organisms are chemoorganotrophs, using either sugars, arginine, or urea as a major energy source. Some species exhibit neither glucose fermentation nor arginine hydrolysis. The cells require cholesterol or a related sterol for growth. Temperature range for growth is 30 to 43°C, with the optimum usually at 37°C. Organisms are parasites and pathogens of a wide range of vertebrate hosts. The G+C content (in moles percent) of the DNA ranges from 23 to 40, and the genome size varies from 600 to 1,350 kbp. All species examined utilize UGA as a tryptophan codon. The type species is *Mycoplasma mycoides* (Borrel, Dujardin-Beaumetz, Jeantet, and Jouan 1910) Freundt 1955, 73.

Emended description of *Mycoplasmatales*, order I of class *Mollicutes* (Edward and Freundt 1967) Razin and Freundt 1984. The characteristics of the order are as described for the family.

The classification proposed herein permits mollicute taxonomy to be in close accord with phylogenetic concepts. Such a classification is highly desirable, since schemes that do not take phylogeny into account often encounter serious difficulties. Such difficulties surfaced in recent descriptions of new nonhelical mollicutes, in that insect- or plant-derived strains with the ability to grow without sterols, but in the presence of 0.04% Tween 80, were referred, of necessity, to the genus *Acholeplasma*. When the phylogenetic studies of Weisburg and colleagues (49) had been completed, it was evident that the insect and plant strains of non-sterol-requiring organisms belonged to an entirely different phylogenetic cluster than classic *Acholeplasmas*. It therefore came as no surprise that the insect and plant organisms differed from classical *Acholeplasmas* not only in their ability to grow

in Tween 80-supplemented media but also in their genome sizes, in their usage of the UGA codon, and in their possession of a component (enzyme II) of a phosphoenolpyruvate-dependent sugar transport system. Transfer of these organisms from *Acholeplasma* was therefore clearly mandated (15).

The most straightforward means of altering mollicute taxonomy to accommodate these new organisms is to adopt a classification scheme that is congruent with the phylogeny proposed by Weisburg and colleagues (49). In that study (Fig. 1), all cultivable arthropod-associated mollicutes (other than classical *Acholeplasmas*) clustered together. Ten *Spiroplasma* species, all from insects or ticks, clustered together to form a monophyletic group. A second monophyletic group, consisting of eight nonhelical species, branched from the *Spiroplasma* lineage. This cluster contained five insect and plant organisms. Our proposal retains the previous designation of the helical organisms as members of a family *Spiroplasmataceae* but proposes a new family, *Entomoplasmataceae*, to comprise the five nonhelical organisms, four similar organisms not studied phylogenetically, and a large number of putative species still under study. These organisms differ from members of the *Spiroplasmataceae* not only in lacking helicity but also in having smaller genomes. There is, at present, a conspicuous difference in the upper limits of temperature tolerance of the two families, since some *Spiroplasmas* grow at 41°C, or even at 43°C (16). Also, *Mesoplasma* species placed phylogenetically in this group have no sterol requirement for growth. All of the proposed members of the new family that were studied phylogenetically shared 22 nonrestrictive sequence signatures with members of the *Spiroplasma apis* clade (49).

We hope that the underlying metabolic or biophysical mechanisms involved in the ability of mesoplasmas to grow in the presence of Tween 80 will be elucidated in the near future. Analyses of batches of Tween 80 used for these studies have shown that the level of possible sterol contamination of the Tween 80 supplement was well below a substrate level (46). Although it is still possible that the contribution of Tween 80 to mesoplasma growth could involve its fatty acid components, supplementation with pure fatty acids such as oleic acid did not produce the desired effect. Growth promotion by the low levels of Tween 80 provided in our studies suggests that this detergent might function by structural or chemical alterations of the cell membrane, rather than by supplying some necessary fatty acid components.

At present, the main properties delineating members of the genus *Entomoplasma* are their sterol requirement (including failure to grow in the presence of Tween 80), an optimum growth temperature of 30°C, their relatively low temperature maxima, their nonhelicity, their small genome, and their association with arthropods. Their arthropod association forms a common link with the mesoplasmas and *Spiroplasmas* and provides a rational basis for their classification at the ordinal and family levels. Host association is a commonly used character in prokaryote taxonomy. Although most frequently used to classify organisms at the species level, it is not infrequently used at the genus level. The use of the character at the ordinal level is less common, but not unique, even in the class *Mollicutes*. The order *Anaeroplasmatales* is confined entirely to bovine and/or ovine hosts and is in fact confined to the ruminants of those species. The order *Mycoplasmatales*, as herein defined, is confined entirely to vertebrate animals. The coherence of these associations confirms the well-known principle that

FIG. 1. Phylogenetic basis for proposed amended classification. The dendrogram is modified from Weisburg et al. (49); branch lengths are diagrammatic. Numerals in the following refer to those in the dendrogram. The proposed genera *Entomoplasma* gen. nov., 7, and *Mesoplasma* gen. nov., 6, are placed in the family *Entomoplasmataceae* fam. nov., 4. Species of the genus *Spiroplasma*, 1, are assigned to the family *Spiroplasmataceae* and are placed with the family *Entomoplasmataceae* in the proposed order *Entomoplasmatales* ord. nov., 3. Species of the order *Mycoplasmatales*, 8 and 11, including the mycoides cluster, 5, are retained in the family *Mycoplasmataceae*, but these higher taxa are redescribed herein. The *Mycoplasma pneumoniae*, 10; *Mycoplasma hominis*, 12; and *Mycoplasma suis*, 13, clusters are as previously given (49). Although some species of *Mycoplasma* that have not been studied phylogenetically can be assigned to clusters on the basis of phenotypic markers, many cannot be so assigned. The genus *Ureaplasma*, 9, is most closely related to the *M. pneumoniae* cluster. Three species of *Mycoplasma*, including *Mycoplasma mycoides*, 5, are related phylogenetically to the family *Entomoplasmataceae* but cannot be transferred to the genus *Entomoplasma* because *Mycoplasma mycoides* is the type species of the genus *Mycoplasma* and of corresponding higher taxa. The polyphyly of the genus *Mycoplasma* is apparent from the detailed phylogenies of spiroplasmas and mycoplasmas (49). A primitive lineage of mollicutes includes the orders *Anaeroplasmatales*, 17, and *Acholeplasmatales*, 17. The genus *Asteroleplasma* has not been placed in this dendrogram because phylogenetic information concerning it was ambiguous (49). Recent phylogenetic studies (16) place the noncultivable "mycoplasma-like organisms" near the order *Acholeplasmatales*.

close association of parasites with their hosts creates evolutionary "dead ends," which make broad host transfers infrequent or impossible. The phylogenetic evidence (49) supporting retention of host affinities in the case of the mollicutes is overwhelming. All 15 of the arthropod-associated organisms clustered together, and 21 vertebrate *Mycoplasma* species clustered in a sister group. Because the phylogenetic rationale for the classification system proposed here is so robust, we anticipate that a number of additional phenotypic characters will emerge to assist in determination of emerging isolates.

From a phylogenetic point of view, the one anomaly created by the proposed classification is the retention of a group of three *Mycoplasma* species (*Mycoplasma mycoides*, *Mycoplasma capricolum*, and *Mycoplasma putrefaciens*) in the family *Mycoplasmataceae*. The phylogenetic studies (49) showed that this group is closely tied to the family *Entomoplasmataceae*. There have been a few reports of arthropod association involving members of this group (6, 7, 23, 33). Although there is no evidence at present that these mollicutes are natural inhabitants of the host arthropods, the suggestion of an arthropod link is intriguing. A host transfer from arthropods is the most likely route by which the mollicutes colonized vertebrates evolutionarily. From a taxonomic point of view, a phylogenetic classification of *Mycoplasma mycoides* and its close allies is impossible, since this

species is the type species for the genus and for its family and order. Also, because this species and some of its cohorts are of considerable importance in veterinary medicine, a name change at the generic level would be unwelcome. Furthermore, although serologic evidence suggests that other phenotypic characters might be found to separate the mycoides group from other *Mycoplasma* species, none are available at this time. Finally, the alternative of renaming all *Mycoplasma* species not assigned to the mycoides cluster, which could involve more than 85 species, would be as unthinkable as the renaming of *Mycoplasma mycoides*. It is, therefore, clear that the genus *Mycoplasma* must remain polyphyletic at this time (14, 15).

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