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 elychniae (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 Entomoplasmataceae fam. nov., family Entomoplasmataceae fam. nov., as Entomoplasmataceae comb. nov., Entomoplasmataceae melaleucae comb. nov., Entomoplasmataceae somnilux comb. nov., Entomoplasmataceae luminosum comb. nov., and Entomoplasmataceae 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 flororum (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 flororum 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
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, Acholeplastales) within the class Mollicutes (12). Later, a cluster of strictly anaerobic anaeroplasmata 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 (L1, 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. flororum 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 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. flororum were shown to belong to the so-called "spiroplasma/mycoides" group, a cluster of helical and nonhelical sterol-requiring organisms phylogenetically distinct not only from members of the orders Acholeplastales 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. flororum 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 stop 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. flororum 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 Acholeplastales.

The sterol-Tween 80 test system described initially for A. flororum (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).

Proposed for Entomoplasmatales 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 Entomoplasmatales gen. nov. These include Entomoplasmatales elychniae comb. nov. (Tully et al., 1989), Entomoplasmatales melaleucae comb. nov. (Tully et al., 1990), Entomoplasmatales somnilux comb. nov. (Williamson et al., 1990), Entomoplasmatales luminosum comb. nov. (Williamson et al., 1990), and Entomoplasmatales 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.
from insects or plant surfaces where they were presumably deposited by insects. We propose the name "Entomoplasma" (En.to.mo'pla.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-1T, E. melaleucae M1T, E. somnilux PYAN-1T, E. luminosum PIMN-1T, and E. lucivorax PIPN-2T) 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 published (Mesoplasma florum LT1T, Mesoplasma entomophilum TAC2T, Mesoplasma seiffertii F7T, and Mesoplasma lactucae 831-C4T).

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 (Mes.o'pla.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 anaeroplasmas, other factors outweighed sterol requirement at the ordinal and family levels (29).

<table>
<thead>
<tr>
<th>Classification</th>
<th>No. of recognized species</th>
<th>Guanine-plus-cytosine content (mol%)</th>
<th>Genome size (kbp)</th>
<th>Cholesterol requirement</th>
<th>Habitat</th>
<th>Other distinctive features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order I, Mycoplasmatales, family I, Mycoplasmataceae</td>
<td>85</td>
<td>23-40</td>
<td>600-1,350</td>
<td>Yes</td>
<td>Humans, animals</td>
<td>Optimum growth usually at 37°C</td>
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<tr>
<td>Genus I, Mycoplasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genus II, Ureaplasma</td>
<td>5</td>
<td>27-30</td>
<td>760-1,170</td>
<td>Yes</td>
<td>Humans, animals</td>
<td>Urea hydrolysis</td>
</tr>
<tr>
<td>Order II, Entomoplasmatales</td>
<td></td>
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<tr>
<td>Family I, Entomoplasmataceae</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Genus II, Mesoplasma</td>
<td>5</td>
<td>27-29</td>
<td>790-1,140</td>
<td>Yes</td>
<td>Insects, plants</td>
<td>Optimum growth, 30°C</td>
</tr>
<tr>
<td>Order III, Acholoplasmales, family I, Acholoplasmaeae, genus I, Acholoplasma</td>
<td>9</td>
<td>26-36</td>
<td>1,500-1,650</td>
<td>No</td>
<td>Animals, some plants and insects</td>
<td>Optimum growth at 30-37°C</td>
</tr>
<tr>
<td>Order IV, Anaeroplasmatales, family I, Anaeroplasmataceae</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Genus I, Anaeroplasma</td>
<td>4</td>
<td>29-34</td>
<td>1,500-1,600</td>
<td>Yes</td>
<td>Bovine or ovine rumen</td>
<td>Oxygen-sensitive anaerobes</td>
</tr>
<tr>
<td>Genus II, Asteroplasma</td>
<td>1</td>
<td>40</td>
<td>1,500</td>
<td>No</td>
<td>Bovine or ovine rumen</td>
<td>Oxygen-sensitive anaerobes</td>
</tr>
</tbody>
</table>
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 chemoorganotrophs. Acid is produced from glucose, with evidence of a phosphoenolpyruvate-dependent sugar transport system(s) in some members. Arginine and urea are not utilized. Glycogen is 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 genome varying from 37 to 41 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 taxon level into 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 mollicutes 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 occid.) 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 Entomoplasmatales; -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 rotary 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 Mycoplasmatales, family I of Mycoplasmatales (Freundt 1955) Razin and Freundt 1984. 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 1935, 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 rumens 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
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 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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