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The open-ended study of the International Working Group on Mycobacterial Taxonomy is an ongoing project to characterize slowly growing strains of mycobacteria that do not belong to well-established or thoroughly characterized species. In this fourth report we describe two numerical taxonomic clusters that represent subspecies or biovars of Mycobacterium simiae, one cluster that encompasses the erstwhile type strain of the presently invalid species "Mycobacterium paraffinicum," one cluster that is phenotypically very similar to Mycobacterium avium and Mycobacterium intracellulare but may be a separate genospecies, one cluster that appears to be phenotypically distinct from M. avium but reacts with a nucleic acid probe specific for M. avium, and three tentatively defined clusters in proximity to a cluster that encompasses the type strain of Mycobacterium malmoense. Of special practical interest is the fact that one of the latter three clusters is composed of clinically significant scotochromogenic bacteria that can be misidentified as the nonpathogenic organism Mycobacterium gordonae if insufficient biochemical tests are performed.

In their first cooperative studies, the members of the International Working Group on Mycobacterial Taxonomy (IWGMT) applied numerical taxonomic (NT) analysis methods to discrete and limited sets of mycobacteria to provide broad-based characterizations of clusters that corresponded to previously established species (5, 10, 12, 16, 26, 31). Subsequently, a cooperative open-ended study was initiated in which new strains of slowly growing mycobacteria were introduced on a continuing basis, and the data were analyzed at intervals (32–34). The purpose of this study of an expanding set of cultures was to characterize slowly growing mycobacterial strains that represented uncommonly encountered species that had not been represented in the first six studies, as well as to recognize clusters of previously unknown taxa.

A total of 38 new strains have been added to the open-ended study since the most recent report (34), and most of these fell into groups that exhibited phenotypic affinities to clusters that encompass the type strains of Mycobacterium simiae, M. intracellulare, M. avium, M. scrofulaceum, M. malmoense, and the presently invalid species "M. paraffinicum." As noted in the previous report (34), these clusters are phenotypically less clearly resolved from one another than clusters that represent other species of slowly growing mycobacteria, and in this study we analyzed the expanded data base for these organisms.

MATERIALS AND METHODS

Selection of strains. The criteria for introducing new cultures into this study have been described previously (32, 34).

The following 20 type strains of valid Mycobacterium species and the type strain of the presently invalid species "M. paraffinicum" (strain OES 90139 [from ATCC 12670]) were also included in this study: M. asiaticum OES 90126 (from TMC 803 [= ATCC 25276]), M. avium OES 90138 (from TMC 724 [= ATCC 25291]), M. bovis OES 90124 (from TMC 410 [= ATCC 19210]), M. farcinogenes OES 90127 (from TMC 805 [= NCTC 10953]), M. flavescens OES 90117 (from TMC 1541 [= ATCC 14474]), M. gasti OES 90136 (from TMC 1456 [= ATCC 15754]), M. gordoneae OES 90133 (from TMC 1324 [= ATCC 1412]), M. intracellulare OES 90135 (from TMC 1406 [= ATCC 13950]), M. kansasi OES 90130 (from TMC 1204 [= ATCC 12478]), M. malmoense OES 90125 (from TMC 802 [= ATCC 29571]), M. marinum OES 90129 (from TMC 1218 [= ATCC 9271]), M. nonchromogenicum OES 90118 (from TMC 1481 [= ATCC 19530]), M. scrofulaceum OES 90132 (from TMC 1323 [= ATCC 19981]), M. shimoidei OES 90013 (from Tsukamura 4796 [= ATCC 27962]), M. simiae OES 90128 (from TMC 1226 [= ATCC 25275]), M. szulgai OES 90134 (from TMC 1328 [= NCTC 10831]), M. terrae OES 90120 (from TMC 1450 [= ATCC 15755]), M. triviale OES 90119 (from TMC 1453 [= ATCC 23292]), M. tuberculosis OES 90123 (from TMC 102 [= ATCC 27294]), and M. xenopi OES 90137 (from TMC 1482 [= NCTC 10042]).

Editing and analysis of data. The criteria and editing programs for suppression of irrelevant, redundant, and incomplete data that were used in generating the NT matrices have been described previously (25, 32–34). Features were coded according to the RKC scheme of Rogosa et al. (15). NT analyses were based on simple matching coefficients, with sorting by unweighted average linkage (21, 25), and a table of feature frequencies was generated for selected clusters (24). The result ascribed to a given strain in a given...
test for both the NT analysis and tabulation of feature frequencies was the modal score (i.e., the result obtained for the strain by the majority of laboratories that performed that test) (35).

A number of the clusters identified during a preliminary NT analysis of the complete data set had been characterized in prior reports, and no significant changes were found in the sizes or compositions of these clusters in this analysis. These included the clusters designated clusters 1 through 6 in the most recent prior report (34) and corresponded to the species M. szulgai, M. triviale, M. shimoidei, M. gordonae, and M. asiaticum and an unnamed group. On the other hand, most of the 38 newly entered strains were members of or clustered in proximity to groups that corresponded to the poorly resolved clusters that were designated clusters 7 through 14 in that report. We decided to suppress the data for strains belonging to the clusters previously designated clusters 1 through 6 and to confine this analysis to the 21 marker type strains and strains that fell into or clustered in proximity to clusters that corresponded to the species M. intracellulare, M. avium, M. scrofulaceum, "M. paraffinicum," M. simiae, and M. malmoense.

**Taxonomic probability matrix.** A taxonomic probability matrix that was originally constructed to identify members of 14 species or biovars of slowly growing mycobacteria (35) was updated to encompass 19 species by using feature frequencies derived from recent analyses of data from this study and applied to the group of organisms described in this paper.

### RESULTS

After suppression of redundant and incomplete test data, as well as data for strains belonging to the clusters noted above, an NT analysis was conducted; this analysis was based on 100 features available for the remaining 130 strains. In reproducing the NT matrix for Fig. 1, we omitted the first five strains in the sequence, corresponding to cultures derived from the type strains of *M. flavescens*, *M. marinum*, *M. nonchromogenicum*, *M. triviale*, and *M. terrae*, and the last 11 strains, corresponding to the type strains of *M. asiaticum*, *M. gordonae*, *M. szulgai*, *M. kanssai*, *M. gastri*, *M. shimoidei*, *M. farcinogenes*, *M. xenopi*, *M. tuberculosi*, and *M. bovis* and one unknown strain, because they joined no cluster at matching scores of 80% or greater. However, all of the marker type strains were included in computations for Table 1.

After clusters were defined, mean intra- and intercluster matching scores (Table 2) and mean matching scores for each cluster with each of the marker type strains (Table 1) were calculated.

Feature frequencies were calculated for those properties that were used in the updated 19-species version of the taxonomic probability matrix (Table 3). The frequency of any feature for a given cluster is shown in Table 3 only if data were available from five or more strains belonging to that cluster.

We also used seroagglutination data for some of the test strains and the responses of extracts of some strains to probes based on DNA-RNA affinities specific for *M. avium* and for *M. intracellulare* (Gen-Probe, San Diego, Calif.) and/or cross-absorbed antibodies to M catalases specific for *M. asiaticum*, *M. gordonae*, *M. scrofulaceum*, *M. simiae*, and *M. bovis* and one unknown strain, because they joined no cluster at matching scores of 80% or greater. However, all of the marker type strains were included in computations for Table 1.

Cluster 1 (Fig. 1) consisted of nine strains with a mean internal matching score (MIMS) of 85% and mean matching scores of 82% with cluster 4 and 81% with clusters 2 and 5 (Table 1). Cluster 1 included the type strain of *M. simiae* (9) at a mean matching score of 87% (Table 1). Cluster 2 consisted of 11 strains with a MIMS of 85% and mean matching scores of 81% with clusters 1 and 5; it did not include any of the marker strains, but had mean matching scores of 84% with the type strain of *M. simiae*, 83% with the type strain of "*M. paraffinicum,*" and 82% with the type strain of...
FIG. 1. NT matrix of 114 strains of slowly growing mycobacteria. Species names indicate that the culture examined was derived from the type strain. Symbols that represent matching scores below 80% were not included to help visualize clusters.
strains in cluster 1 (strains OES 90006 and OES 90128, which was derived from the type strain of *M. simiae* and from nine of the strains in cluster 2 (strains OES 90007, OES 90008, OES 90010, OES 90012, OES 90021, OES 90022, OES 90024, OES 90025, and OES 90043) were tested against *M. avium*, *M. intracellulare*, and *M. scrofulaceum* in such key features as catalase activity, amidase activity, or photochromogenicity, whereas members of clusters 1 and 2 agglutinated as serovar Simiae 1, positive acid phosphatase reaction, whereas both *M. avium* and *M. intracellulare* are negative. Members of cluster 4 failed to grow at 45°C, whereas 86% of the strains in cluster 11 (*M. avium* did so. While none of the 19 strains in cluster 4 were identified in the taxonomic probability matrix, 8 of them exhibited combined ID scores with *M. avium* and *M. intracellulare* feature patterns greater than 0.99, placing them in the *M. avium-M. intracellulare* phenotypic complex. Strains OES 90239, OES 90242, OES 90236, OES 90230, OES 90248, and OES 90210 in cluster 4 may make up a subcluster that could equally well belong to cluster 6.

Eleven of the strains in cluster 4 were tested with nucleic acid probes specific for *M. avium* and for *M. intracellulare*, and all but one failed to react to either probe (Table 5). Strain OES 90245 reacted with the nucleic acid probe for *M. intracellulare* and agglutinated as serovar 12. The members of cluster 4 that did not react with either of the nucleic acid probes either failed to agglutinate with one of the *M. avium* complex typing sera or agglutinated as one of the higher-numbered serovars (e.g., serovar 24 or 26) (Table 4), which is in agreement with observations of Saito et al. (17).

Of the 19 strains in cluster 4, 1 was a clinically significant isolate obtained from lung tissue, 4 were obtained from sputum or bronchial washings and were of unknown significance, 3 were isolates obtained from AIDS patients, and the sources and significance of 11 strains were not known.

Cluster 5 consisted of four strains with a MIMS of 89% and a mean matching score of 83% with cluster 6 (Table 2). It included the type strain of *M. scrofulaceum* (20) (mean matching score, 89%) and had a mean matching score of 85% with the marker type strain of *M. paraffinicum*, which fell in cluster 6 (Table 1). Three of the strains in cluster 5, including the type strain, seroagglutinated as serovar 41, and one seroagglutinated as serovar 42 (Table 4); both of these serovars are generally ascribed to *M. scrofulaceum* (37).

Cluster 6 consisted of five strains with a MIMS of 89% and mean matching scores of 83% with clusters 5 and 8 (Table 2).
It included marker type strain OES 90139, which was derived from the type strain of the presently invalid species "M. paraffinicum," at a mean matching score of 90% and had mean matching scores of less than 84% with all of the other marker type strains (Table 1). Features that may be useful for differentiating strains in cluster 6 from M. scrofulaceum include growth at 42°C and urease activity (frequencies, 88 and 91% for M. scrofulaceum, respectively [35], and less than 1% for members of cluster 6) (Table 3). Most members of cluster 6 differed from M. simiae (clusters 1 and 2) by the nature of their pigmentation and by their urease reactions (Table 3). Two of the strains in cluster 6 agglutinated as serovar 42 (Table 4). These strains (strains OES 90101 and OES 90180) had mean matching scores of 88 and 90%, respectively, with cluster 6 and mean matching scores of only 83 and 82% with cluster 5; both strains failed to hydrolyze urea or grow at 42°C. All five strains in cluster 6 were below identification thresholds for both 16S and R scores with all of the species in the taxonomic probability matrix.

The marker type strain of "M. paraffinicum" was originally isolated from soil (4). A second strain in cluster 6,
strain OES 90097, was the cause of death from disseminated disease in an infant. Two other members of the cluster were isolated from sputum, but their significance is not known, and the source of the fifth strain is not known.

Cluster 7 consisted of 14 strains with a MIMS of 89% and mean matching scores of 84, 83, 82, and 81% with clusters 8, 9, 6, and 10, respectively (Table 2). It did not include any of the marker type strains and had mean matching scores of 83% with the type strain of *M. malmoense* and 80% or less with all of the other marker type strains. Most strains in cluster 7 were distinguished from the strains in cluster 8 by their scotochromogenicity and positive 10-day Tween hydrolysis reaction (Table 3). The major distinguishing features for clusters 7 and 10 (*M. malmoense*) were the pigmentation and urease reactions. The distinction between clusters 7 and 9 appeared to be based on partial differences in frequency distributions in a number of tests; the members of cluster 9 exhibited considerable variation in some key tests, and no individual feature provided definitive resolution. The positive reactions for Tween hydrolysis and low catalase activity differentiated members of this cluster from members of clusters 5 and 6. The only currently recognized species of slowly growing mycobacteria that is characterized as scotochromogenic and only weakly catalase positive is *M. xenopi*, and cluster 7 had a mean matching score of only 70% with the type strain of this species (Table 1). The distinguishing features for cluster 7 and *M. gordonae* included nicotinamide activity, pyrazinamidase activity, and semiquantitative catalase activity. One strain (strain OES 90151) was identified as *M. szulgai* in the taxonomic probability matrix, and another strain (strain OES 90196) was identified as *M. gordonae*. None of the remaining strains reached the iden-

### Table 4. Distribution of agglutinating serovars in NT clusters

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Species</th>
<th>Total no. of strains tested</th>
<th>Serovar simiae</th>
<th>Serovar avium complex</th>
<th>Serovar malmoense</th>
<th>Unknown</th>
<th>Spontaneous agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>An avium serovar</td>
<td>An intracellulare serovar</td>
<td>A scrofulaceum serovar</td>
<td>Other serovars</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>M. simiae</em></td>
<td>9</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>M. simiae</em></td>
<td>11</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td><em>M. intracellulare</em></td>
<td>4</td>
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<td></td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>Unknown</td>
<td>19</td>
<td>1</td>
<td></td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td><em>M. scrofulaceum</em></td>
<td>4</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>&quot;*M. paraffinicum&quot;&quot;</td>
<td>4</td>
<td>1</td>
<td></td>
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<td>Unknown</td>
<td>14</td>
<td>1</td>
<td></td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>Unknown</td>
<td>6</td>
<td>1</td>
<td></td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>Unknown</td>
<td>11</td>
<td>1</td>
<td></td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td><em>M. malmoense</em></td>
<td>12</td>
<td>7</td>
<td></td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>11</td>
<td><em>M. avium</em></td>
<td>7</td>
<td>6</td>
<td></td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>12</td>
<td><em>M. paraenulum</em> (?)</td>
<td>5</td>
<td>5</td>
<td></td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

* Serovars 1 through 6 and 8 through 11 were treated as avium serovars, serovars 7, 12 through 21, 23, and 25 were treated as intracellulare serovars, and serovars 41 through 43 were treated as scrofulaceum serovars.

* Strains that agglutinated with a specific typing serum but could not be confirmed by absorption of antibody were included in this category, along with strains that failed to agglutinate with any of the typing sera used.

* All six of these strains reacted as intracellulare serovar 18.

### Table 5. Distribution of positive reactions to semantide-based species-specific probes in NT clusters

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Species</th>
<th>Total no. of strains tested</th>
<th>No. of strains that reacted with avium probe</th>
<th>No. of strains that reacted with intracellulare probe</th>
<th>No. of strains having no reaction</th>
<th>Total no. of strains tested</th>
<th>No. of strains that reacted with scrofulaceum probe</th>
<th>No. of strains having no reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>M. simiae</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td><em>M. simiae</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td><em>M. intracellulare</em></td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Unknown</td>
<td>11</td>
<td>1</td>
<td>10</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td><em>M. scrofulaceum</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>&quot;*M. paraffinicum&quot;&quot;</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
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<tr>
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<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td><em>M. malmoense</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td><em>M. avium</em></td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
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<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* DNA probes for RNAs (Gen-Probe) specific for *M. avium* and for *M. intracellulare* were used.

* Cross-absorbed serum probes specific for seroprecipitation of M catalases of *M. simiae*, *M. scrofulaceum*, *M. asiaticum*, *M. gordonae*, and *M. szulgai* were used.
tification thresholds for either ID or R scores with any of the species in the taxonomic probability matrix.

None of the strains in cluster 7 belonged to any known agglutinating serovar (Table 4), and the single strain tested with nucelic acid probes failed to react (Table 5).

Cluster 7 included strains OES 90142, OES 90150, and OES 90151, which were derived from strains ATCC 15983, ATCC 23432, and ATCC 23433, respectively; these strains fell into a small but discrete cluster in the first published IWGMT study on scotochromogenic bacteria in 1971 (31). At that time it was suggested, but not formally proposed, that they might represent a new species. Two of these strains (strains ATCC 23432 [= AT5313A] and ATCC 23433 [= AT 507]) had been considered to be unique in a 1967 report (7) on a number of clinically significant opportunistic mycobacterial pathogens included in Protocol 6 of the Veterans Administration-Armed Forces Cooperative Study on Chemotherapy of Tuberculosis (7).

Of the 14 strains in cluster 7, 13 were isolated from human pulmonary secretions or tissues, and the source of the other was unknown. Seven were considered to be the cause of the source patients’ diseases, and the significance of the others was not known. Six of the seven whose geographic origins were known were from the southeastern United States, and the other was from California.

Cluster 8 consisted of six strains with a MIMS of 88% and mean matching scores of 84% with cluster 7 and 83% with clusters 4 and 6 (Table 2). It did not include any of the marker type strains and had mean matching scores of 84% with the type strain of M. paraffinicum and 81% with the type strain of M. simiae. The key phenotypic features that distinguished this cluster from cluster 7 are described above. The main differences in key feature frequencies between clusters 6 and 8 were pigmentation and catalase activity (Table 3). The distinction between clusters 4 and 8 appeared to be based on partial differences in frequency distributions in a number of tests; no individual feature provided definitive resolution. One strain in cluster 8 (strain OES 90056) exhibited mean matching scores of 88.7% with cluster 8 and 86.7% with both cluster 1 and cluster 7 and agglutinated as M. simiae serovar 1 (Table 4). It gave an ID score (0.962) which was slightly below the threshold for this species, but was within threshold R score limits, suggesting that it is an atypical strain of M. simiae. None of the other five strains reached the identification thresholds for either ID or R scores with any of the species in the taxonomic probability matrix, nor did they belong to any known agglutinating serovar (Table 4); two strains were tested with the DNA probes and failed to react (Table 5).

One strain in cluster 8 was isolated from lettuce: another was obtained from a gastric lavage specimen, but was not considered significant. The sources of the others were not known.

Cluster 9 consisted of 11 strains with a MIMS of 85% and mean matching scores of 83% with cluster 7, 82% with cluster 4, and 81% with cluster 8 (Table 2). It did not include any of the marker type strains and had mean matching scores of less than 80% with all of them. The difficulty in distinguishing strains of cluster 9 from strains of cluster 7 is described above. The major phenotypic differences between clusters 4 and 9 were hydrolysis of Tween 80 and resistance to 500 μg of hydroxyamine per ml; the main difference between clusters 8 and 9 was in Tween 80 hydrolysis. One strain (strain OES 90017) was identified as M. intracellulare in the taxonomic probability matrix. None of the other strains could be identified under both thresholds, although two gave ID scores with M. malmoense of greater than 0.999.

All of the strains in this cluster were tested for seroagglutination; six belonged to no known serovar, one reacted as serovar 18, one reacted as serovar Simiae 2, and three cross-reacted with sera for two M. intracellulare serovars (Table 4); one strain was tested with the DNA probes and failed to react (Table 5).

Five of the 11 strains in cluster 9 were isolated from sputum, and all were considered to be the cause of the patients’ diseases. One strain (strain OES 90160, serovar Simiae 2) was isolated from a clam, and the sources of the other five were unknown. Two of the clinically significant strains (strains OES 90187 and OES 90219) actually had higher mean matching scores with the strains in cluster 7 (86.5 and 86.7%) than with the strains in cluster 9, the cluster in which they appeared (85.0 and 84.3%, respectively). This suggested that cluster 9 is not properly circumscribed and that the interposition of cluster 8 between clusters 7 and 9 via the Calder effect (34) contributed to an erroneous interpretation of the NT matrix.

Cluster 10 consisted of 12 strains with a MIMS of 85% and mean matching scores of 81% with clusters 4, 7, and 8 (Table 2). This cluster included the type strain of M. malmoense (19) (mean matching score, 87%) and had matching scores of 79% or less with all of the other marker type strains included in the study. Nine of the strains were definitively identified as M. malmoense by the taxonomic probability matrix. Two others had ID scores greater than 0.999 with this species, but failed to pass the R score threshold by virtue of susceptibility to 1 μg of INH per ml and positive 68°C catalase activity in one case and positive aryl sulfatase activity and nitrate reduction in the other. The 12th strain (strain OES 90229) failed to reach either threshold; its highest ID score (0.988) was with M. simiae. It was recovered from a drinking trough at the zoo that housed the animals from which the strains in cluster 12 were isolated. The M. malmoense cluster was discussed in the previous report (34) and has been increased in size by only two strains since that report was prepared.

Cluster 11 consisted of eight strains with a MIMS of 89% and a mean matching score of 82% with cluster 4 (Table 2). This cluster included the type strain of M. avium (mean matching score, 84%) and had mean matching scores of 81 and 80% with the type strains of M. paraffinicum and M. simiae, respectively (Table 1). One of the strains exhibited spontaneous agglutination, and another was not tested, but the remaining six strains agglutinated as serovars 1, 2, 4, and 8 (Table 4), which are considered to belong to M. avium (2, 30). Five of these strains were examined with nucleic acid probes, and all reacted as M. avium (Table 5).

Cluster 12 consisted of four strains with a MIMS of 91% and mean matching scores of 80% or less with all of the other clusters (Table 2). It did not include any of the marker type strains and exhibited its highest matching score, only 79%, with the type strain of M. avium. Although three strains agglutinated spontaneously and the fourth could not be typed with any of the sera used (Table 4), all four strains in this cluster reacted as M. avium with the nucleic acid probes (Table 5). All four strains were isolated from tissue or stools of antelope suffering from paratuberculosis in the Antwerp, Belgium, zoo and so may represent a single source strain. This small cluster was omitted from Table 3, but behaved in key tests as M. avium. A few properties from this data set could be compared with features identified as characteristic of the recently proposed subspecies M. avium subsp. paratuberculosis and M. avium subsp. silvaticum (23). The
members of cluster 12 most closely resembled \textit{M. avium} subsp. \textit{silvaticum} in that they did not require mycobactin for growth and were not coccobacillary; however, they were resistant to 500 \textmu g of \textit{p}-nitrobenzoate per ml. in contrast to the responses attributed to both of the new subspecies. The disease in antelopes is comparable to the disease attributed to both \textit{M. avium} subsp. \textit{paratuberculosis} and \textit{M. avium} subsp. \textit{silvaticum} (23).

**DISCUSSION**

The group of slowly growing mycobacteria upon which we concentrated in this analysis is complex and difficult to resolve by using phenotypic characteristics alone. To the limited extent to which they have been performed on this set of strains, semantide-based studies have clarified the status of several named mycobacterial species and have offered direction for further studies.

Clusters 1 through 6, 8, 11, and 12 (Fig. 1) include taxa belonging to the \textit{M. avium} complex and the erstwhile ‘MAIS intermediate’ group (6, 33). These slowly growing mycobacteria do not hydrolyze Tween 80 include \textit{M. simiae}, \textit{M. intracellular}, \textit{M. avium}, \textit{M. scrofulaceum}, and ‘\textit{M. paraffinicum}’ and exhibit considerable intercluster overlap, with examples of both phenotypic convergence and phenotypic divergence. The apparent interposition into this series of taxa of clusters 7, 8, and 10, most strains of which hydrolyze Tween 80, and the separation of clusters with comparable or higher mean intercluster matching scores have been interpreted as a Calder effect (34).

The basis of the partial resolution of \textit{M. simiae} into clusters 1 and 2 (Fig. 1) is not clear. The results of recently published 16S rRNA sequence analyses (14) are consistent with the results of both DNA homology studies (2a) and catalase serology studies (28, 29) that demonstrated that \textit{M. simiae} is genetically distant from \textit{M. scrofulaceum}, \textit{M. intracellular}, and \textit{M. avium}. Baess and Magnusson (2a) demonstrated some divergence between serovars Simiae 1 and Simiae 2 on the basis of DNA hybridization data but at a level that was too low (27) to justify placing these serovars in separate species; this division appears to be reflected in the partial resolution of clusters 1 and 2 (Fig. 1), which corresponds to what may be both phenotypic divergence and genotypic divergence within the genus \textit{M. simiae}.

Despite the phenotypic similarity between strains of \textit{M. avium} and \textit{M. intracellular}, semantide-based studies have confirmed that these taxa represent phylogenetically distinct species (2, 17, 28, 30). The 16S rRNA sequence phylogenetic tree shows a fairly shallow-branched, but distinct separation of the two species (14). The emergence of a third phenotypically similar group, cluster 4 in this study, which includes a number of strains that did not react with nucleic acid probes for either \textit{M. avium} or \textit{M. intracellular}, further expands the scope of the so-called \textit{M. avium} complex. While IWGMT studies are being initiated to provide full DNA homology data and data from other semantide analyses, the data that are currently available suggest that cluster 4 represents a novel species in the \textit{M. avium} phenotypic complex. A decision to name this taxon a new species must also take into account the difficulty in defining phenotypic features to distinguish it from existing species (27). In any case, very similar and ubiquitous phenotypic patterns now appear to be associated with at least three different environmental genospecies, \textit{M. avium}, \textit{M. intracellular}, and the new cluster 4, suggesting that there was convergent evolution to an ecologically optimal state. On the other hand, both \textit{M. lepraemuriu}
parisons within cluster 6 and between the type strain and members of other species have been initiated by the IWGMT to determine whether "M. paraffinicum" should be revived as a distinct species or as a subspecies of some other taxon. The only instance of proven clinical significance of a strain in this cluster was strain OES 90097, which was isolated from autopsy tissue of an infant who died with disseminated mycobacterial disease. A sibling of this child also died several years before of a similar disease, suggesting that there was an inborn immunologic defect of the host rather than a special degree of virulence of the organism. These events occurred at least 10 years before the first descriptions of AIDS.

Cluster 7 is a fairly compact cluster that exhibits some overlap with clusters 8 and 9 and, to a lesser extent, with novel cluster 4 and cluster 10 (M. malmoense) (Fig. 1). This cluster has now grown to encompass 14 strains that do not agglutinate with serum to any recognized serovar. As noted above, some strains in cluster 9 also probably belong in cluster 7, further enlarging this significant group. In view of the low mean matching scores with all of the type strains, this cluster increasingly looks like a novel species, and the newly initiated IWGMT semantide-based cooperative study should establish whether the apparent phenotypic isolation of this cluster is reflected at the molecular level. The fact that at least one-half and probably more of the strains in this cluster were considered to be the cause of pulmonary disease imparts special importance to the resolution of the status of these organisms. Their scotochromogenicity, positive Tween hydrolysis, and lack of urease activity could lead to cultures of this group being incorrectly identified as the nonpathogenic organism M. gordonae if a semiquantitative catalase test were not performed in a diagnostic laboratory.

The failure to identify any phenotypic features that definitively distinguish members of cluster 8 from members of cluster 4 suggests that cluster 8 may be made up largely of outlying strains of the latter group. Further judgements must await semantide studies of members of these two clusters.

M. malmoense appears to represent a species that is distinct from other named species in terms of the results of both NT and semantide studies (8, 13, 29). Some members of clusters 7 and 9 were submitted as "M. malmoense-like," but the intercluster matching scores cast doubt on their relatedness to M. malmoense. Nevertheless, it is important that semantide-based techniques be applied to the resolution of these groups, especially since all three clusters include high proportions of clinically significant strains.

In the original proposal of the informal MAIS intermediate terminology (6), Hawkins discussed categories containing strains of scotochromogenic slow growers that do not hydrolyze Tween 80. The established species M. scrofulaceum yields over 45 mm of foam in the catalase test and is urease positive. The first intermediate category was simply that of scotochromogenic variants of M. avium, which had the low catalase activity and negative urease reaction characteristic of that species. The second intermediate category was rarely encountered and consisted of strains that had low catalase activity and no urease activity. The third, fairly common intermediate category had high catalase activity, but was urease negative. The last description conforms to strains in cluster 6, embracing "M. paraffinicum." Until a decision has been made as to whether to revive this species, this group of organisms can be referred to by an informal utility vernacular term, the "paraffinicum group," rather than continuing to link M. avium and M. intracellulare with the phenotypically dissimilar and unrelated species M. scrofulaceum in the MAIS terminology.

Although M. xenopi is well separated from M. avium and M. intracellulare as determined by NT, some key properties are shared by these organisms. The results of the recently published 16S rRNA sequence studies (14) strongly support the NT-based conclusions (12) that M. xenopi is a distinct species. Similarly, prior IWGMT reports (12, 34) defined three clusters representing M. terrae, M. nonchromogenicum, and M. triviale and two clusters representing M. gordonae and M. asiaticum, and the results of recent 16S rRNA sequence studies (14, 22) support the decision to treat all of these taxa as separate species.

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