Numerical Taxonomy of Rapidly Growing, Scotochromogenic Mycobacteria of the *Mycobacterium parafortuitum* Complex: *Mycobacterium austroafricanum* sp. nov. and *Mycobacterium diernhoferi* sp. nov., nom. rev.

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A numerical analysis of phenetic data collected from rapidly growing, scotochromogenic mycobacterial strains isolated from water in South Africa and from strains of the taxa *Mycobacterium parafortuitum*, *Mycobacterium aurum*, *Mycobacterium neoaurum*, and "*Mycobacterium diernhoferi*" indicated that all of these organisms belong to the *Mycobacterium parafortuitum* complex. Our results also indicated that each of the taxa mentioned above is worthy of species status within the complex. The name *Mycobacterium austroafricanum* sp. nov. is proposed for the South African isolates, and the characteristics of these isolates are described; the type strain is E9789-SA12441 (= ATCC 33464). The name *Mycobacterium diernhoferi* is revived for the organism described originally by Bönicke and Juhasz in 1965; the type strain of this species is strain 41001 (= ATCC 19340).

Three species of rapidly growing, scotochromogenic mycobacteria, *Mycobacterium parafortuitum* (16), *Mycobacterium aurum* (7), and *Mycobacterium neoaurum* (9), have not been clearly differentiated from each other in previous numerical classification schemes (5, 14) and have been considered members of a *Mycobacterium parafortuitum* complex. Saito et al. (5) showed that strains of "*Mycobacterium diernhoferi*" formed one cluster together with strains of *M. parafortuitum*. Based on this finding, "*M. diernhoferi*" was regarded as a synonym of *M. parafortuitum*. Recently, Kusunose et al. (M. Kusunose, E. Kusunose, I. Yano, S. Toriyama, H. Saito, and M. Tsukamura, *Kekkaku* 54:219–220, 1979) found that *M. parafortuitum* contains 54- to 60-carbon mycolic acids, whereas "*M. diernhoferi*" contains 68- to 76-carbon mycolic acids. Furthermore, a number of strains of rapidly growing, scotochromogenic mycobacteria have been isolated from water in South Africa. These strains seem to represent a new taxon.

The purpose of the present study was to examine the taxonomic relationships among the three species of the *M. parafortuitum* complex, five strains of "*M. diernhoferi*," and a cluster of 23 South African strains isolated from water.

MATERIALS AND METHODS

Bacterial strains and cultivation techniques. All strains of *M. parafortuitum*, *M. aurum*, and *M. neoaurum* were isolated in the laboratory of the National Chubu Hospital. These strains were maintained

at –20°C and were subcultured at 6-month intervals. Only the strains deposited in the American Type Culture Collection, Rockville, Md., are shown in Table 1. The type strains of all named species of rapidly growing, scotochromogenic mycobacteria were also included in this study (Table 1).

A total of 23 strains of rapidly growing, scotochromogenic mycobacteria were isolated from water in South Africa. These strains were isolated as follows. Samples of water were pretreated by adding 1.25% oxalic acid, which was neutralized after 10 min with 2% sodium hydroxide. After filtration, the filters were incubated at 37°C for 9 days on enriched Middlebrook 7H9 medium containing cycloheximide (2). This medium contained 4.7 g of Middlebrook 7H9 broth base (BBL Microbiology Systems), 1 mg of malachite green, 1 g of sodium propionate, 15 g of agar, 100 ml of Middlebrook OADC enrichment (Difco Laboratories), 25 mg of nalidixic acid, 25,000 U of penicillin, 50,000 U of mycostatin, 100 mg of cycloheximide, and 1,000 ml of distilled water.

A total of 107 biochemical or physiological characters were studied for each strain. Of these, 104 were used in a previous study (15), with methods described previously (10, 12). The three additional characters used were growth at 42°C, resistance to 5-fluorouracil (20 µg/ml) in Ogawa egg medium, and resistance to mitomycin C (5 µg/ml) in Ogawa egg medium. Growth at 42°C was assayed by incubating test strains at 42 ± 0.5°C for 7 days.

Numerical analysis. Numerical classification was carried out by using a method described previously (15). Clustering was made by the single-linkage method (6). According to Orchard and Goodfellow (4), single- and average-linkage methods give the same clusters.

In addition, a numerical analysis was also carried out to define the ranges of species (taxon), as follows. Ranges were measured by calculating $M \pm 2$ SD or $M \pm 1.7$ SD, where M is the mean matching coefficient (mean M value) for individual strains of each species relative to a hypothetical median organism pattern (HMO) (3) within that species or another species and SD is the standard deviation. The range $M \pm 2$ SD contains ca. 95% of the individual strains within a species (11). For example, if two species (taxa), species A and B, are distinct, the range for species A relative to the HMO of species A should be higher than the range for species B relative to the HMO of species A. There should be no overlap, or the overlap should occur in less than 5% of the strains within the proposed species. When we calculate $M - 2$ SD for species A relative to its own HMO, a range higher than the lower limit of the range should contain ca. 97.5% of the individual strains in species A. On the other hand, when we calculate $M + 2$ SD for species B relative to the HMO of species A, a range lower than the upper limit for the species should contain 97.5% of the strains in species B. Thus, if the lower limit of species A is higher than the higher limit of species B, the two species are regarded as distinctly separate and have either no overlap or an overlap of less than 2.5% of the individual strains within the species. If the lower limit of species A is measured as $M - 1.7$ SD, the higher limit of species B is measured as $M + 1.7$ SD, and the former limit is higher than the latter limit, the two species are regarded as separate. Again, there should be no overlap, or an overlap of less than 5% of the individual strains within each species (8).

RESULTS AND DISCUSSION

The results of our numerical analysis are shown in Fig. 1 as a dendrogram. Five clusters were observed at a matching coefficient (M value) level of 91%, although a few intermediate strains were present between the clusters. The first cluster was composed of the strains isolated from water from South Africa, and the remaining four clusters contained the type strains of *M. aurum*, *M. neoaurum*, "*M. diernhoferi*," and *M. parafortuitum*. Therefore, the latter four clusters were considered to be a complex of four distinct species, and the five groups could be combined into one large cluster at a level of 90%. Only one type strain of another species, *Mycobacterium rhodesiae*, was included in this large cluster.

The strains and species in the five clusters are shown in Table 1, whereas the biochemical and physiological properties of the groups are summarized in Table 2. The *M. parafortuitum* cluster was composed of two subgroups; one contained the type strain of the species, and the other contained three strains named by Saito et al. (5) as Kanazawa strains.

The *M. aurum* and *M. neoaurum* clusters each contained a number of strains labeled as other species, and these two species have been frequently confused with each other in identification techniques. This situation was clarified by our study. The major distinguishing features of these two species are summarized in Table 3.

FIG. 1. Dendrogram showing relationships among strains studied. See Table 1 for strain serial numbers.

TABLE 1. Mycobacterial strains used and cluster arrangement

Cluster	Serial no.	Laboratory no. ^a	Received as:	Source ^b	Habitat ^c
<i>M. austroafricanum</i>	1	E9789B	<i>M. austroafricanum</i> SA12441 ^T (= ATCC 33464 ^T)	1	Water, South Africa
	2	E9790B	<i>M. austroafricanum</i> SA12442 (= ATCC 33465)	1	Water, South Africa
	3	E9789A	<i>M. austroafricanum</i> SA12441 ^T (= ATCC 33464 ^T)	1	Water, South Africa
	4	E9790A	<i>M. austroafricanum</i> SA12442 (= ATCC 33465)	1	Water, South Africa
<i>M. aurum</i>	8	10005	<i>M. neoaurum</i> ATCC 25799	2	Soil, Japan
	9	10006	<i>M. neoaurum</i> ATCC 25800 ^d	2	Soil, Japan
	19	10004	<i>M. neoaurum</i> ATCC 25798	2	Soil, Japan
	23	10001	<i>M. neoaurum</i> ATCC 25791	2	Soil, Japan
	31	15012	<i>M. aurum</i> Lausanne 708	3	Soil, Japan
	32	15061	<i>M. aurum</i> ATCC 25792	2	Soil, Japan
	34	15035	<i>M. aurum</i> ATCC 25803	2	Soil, Japan
	37	15013	<i>M. aurum</i> ATCC 25793	2	Soil, Japan
	40	15006	<i>M. aurum</i> ATCC 23366 ^T (= NCTC 10437 ^T)	2	Soil, Japan
	41	15067	<i>M. aurum</i> ATCC 25794 ^e	2	Soil, Japan
	44	15022	<i>M. aurum</i> ATCC 27797 ^e	2	Soil, Japan
<i>M. neoaurum</i>	45	10003	<i>M. neoaurum</i> ATCC 25796	2	Soil, Japan
	47	10002	<i>M. neoaurum</i> ATCC 25795 ^T (= NCTC 10818 ^T)	2	Soil, Japan
	52	15001	<i>M. aurum</i> NCTC 10439	4	Soil, Japan
	53	15501	<i>M. aurum</i> ATCC 25790 (= NCTC 10440)	2	Soil, Japan
	63	15509	<i>M. aurum</i> ATCC 25801	2	Soil, Japan
"M. diernhoferi"	65	41001	"M. diernhoferi" ATCC 19340 ^T	2	Soil, Germany
	66	41003	"M. diernhoferi" ATCC 19341	2	Soil, Germany
	67	41004	"M. diernhoferi" ATCC 19344	2	Soil, Germany
	68	41006	"M. diernhoferi" ATCC 25959	2	Soil, Germany
	69	41005	"M. diernhoferi" ATCC 25958	2	Soil, Germany
<i>M. parafortuitum</i>	74	16001	<i>M. parafortuitum</i> ATCC 19687 (= NCTC 10410)	2	Soil, Japan
	75	16002	<i>M. parafortuitum</i> ATCC 19686 ^T (= NCTC 10411 ^T)	2	Soil, Japan
	76	16003	<i>M. parafortuitum</i> ATCC 19688	2	Soil, Japan
	82	16008	<i>M. parafortuitum</i> ATCC 25808 ^f	2	Soil, Japan
	83	16010	<i>M. parafortuitum</i> ATCC 25810 ^f	2	Soil, Japan
	84	16011	<i>M. parafortuitum</i> ATCC 25811 ^f	2	Soil, Japan
	85	16033	<i>M. parafortuitum</i> ATCC 25807	2	Soil, Japan
Others	86	16007	<i>M. parafortuitum</i> ^g	5	Sputum, humans
	87	16041	<i>M. parafortuitum</i> ^g	5	Sputum, humans
	88	16044	<i>M. parafortuitum</i> ^g	5	Sputum, humans
	89	02002	<i>M. rhodesiae</i> ATCC 27024 ^T	2	Sputum, humans
	90	16009	<i>M. parafortuitum</i> ^g		
	92	01039	<i>Mycobacterium thermoresistibile</i> ATCC 19527 ^T	2	Soil, Japan
	93	03001	<i>Mycobacterium gadium</i> ATCC 27726 ^T	6	Sputum, humans
	94	21007	<i>Mycobacterium vaccae</i> ATCC 15483 ^T	2	Soil, Germany
	95	48013	<i>Mycobacterium chubuense</i> ATCC 27278 ^T	2	Soil, Japan
	99	18112	<i>Mycobacterium fortuitum</i> ATCC 6841 ^T	2	Skin lesion, human

TABLE 1—Continued

Cluster	Serial no.	Laboratory no. ^a	Received as:	Source ^b	Habitat ^c
	100	22012	<i>Mycobacterium chelonae</i> subsp. <i>abscessus</i> ATCC 19977 ^T	2	Gluteal abscess
	101	29505	<i>Mycobacterium duvalii</i> NCTC 358 ^T	7	?
	102	33001	<i>Mycobacterium flavescens</i> ATCC 14474 ^T	2	Guinea pig
	103	19054	<i>Mycobacterium chelonae</i> subsp. <i>chelonae</i> NCTC 946 ^T	7	?
	104	E8757	<i>Mycobacterium komossense</i> ATCC 33013 ^T	8	Sphagnum vegetation
	105	49005	<i>Mycobacterium aichiense</i> ATCC 27280 ^T	2	Soil, Japan
	106	E8761	<i>Mycobacterium sphagnii</i> ATCC 33027 ^T	8	Sphagnum vegetation
	107	14025	<i>Mycobacterium phlei</i> ATCC 11758 ^T	2	?
	108	29006	<i>Mycobacterium gilvum</i> NCTC 10742 ^T	7	?
	109	47503	<i>Mycobacterium tokaiense</i> ATCC 27282 ^T	2	Soil, Japan
	110	47001	<i>Mycobacterium obuense</i> ATCC 27023 ^T	2	Sputum, humans
	111	90012	<i>Mycobacterium agri</i> ATCC 27406 ^T	2	Soil, Japan
	112	17027	<i>Mycobacterium smegmatis</i> ATCC 14468	2	?
	113	31002	<i>Mycobacterium chitae</i> ATCC 19627 ^T	2	Soil, Japan
	114	18256	<i>Mycobacterium smegalense</i> NCTC 10956 ^T	9	?

^a The suffix A indicates before lyophilization and the suffix B indicates after lyophilization.

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^c All 23 strains of *M. austroafricanum* were isolated in South Africa, and all strains of *M. parafortuitum*, *M. aurum*, and *M. neoaurum* were isolated in Japan.

^d *M. parafortuitum* according to Saito et al. (5).

^e Outside the *M. aurum* cluster.

^f Kanazawa strains according to Saito et al. (5).

^g Outside the *M. parafortuitum* cluster.

In the study of Saito et al. (5) strains of "*M. diernhoferi*" formed a cluster together with strains of *M. parafortuitum*, and thus the former name was regarded as a synonym of *M. parafortuitum*. However, based on our study, we believe that these two organisms can be differentiated by a number of markers (Table 4).

In a previous study (14), in which 80 test characters were examined, three species (*M. parafortuitum*, *M. aurum*, and *M. neoaurum*) were somewhat difficult to differentiate from each other. Our results based on the 107 characters described here provide a more adequate differentiation of these three species. Although Saito et al. (5) used some additional markers (177 tests), their study contained a number of

linked characters (since participants carried out various tests independently), and inclusion of linked characters is known to disturb the formation of clear-cut clusters (13). In our study, we omitted linked characters where possible, and we believe that our results show a better separation of the three mycobacterial clusters.

To confirm the distinctions described above, we performed a numerical analysis by using the ranges of species as measured by the expression $M \pm 1.7$ SD. Table 5 shows the results of a numerical analysis of the relationships among *M. parafortuitum*, *M. aurum*, and *M. neoaurum*. By estimating M values relative to the HMO of *M. parafortuitum*, *M. neoaurum* could be differentiated from *M. parafortuitum* (as the lower

TABLE 2. Biological and physiological characteristics of strains examined

Character ^a	% of strains showing positive reaction				
	<i>M. austro-africanum</i> (n = 23)	<i>M. aurum</i> (n = 35)	<i>M. neo-aurum</i> (n = 19)	<i>M. para-fortuitum</i> (n = 12)	<i>"M. diern-hoferi"</i> (n = 5)
Strongly acid-fast	100	100	95	100 (100) ^b	100
Weakly or partially acid-fast	100	100	100	100 (100)	100
Permanent mycelium	0	0	0	0 (0)	0
Temporary mycelium	0	0	0	0 (0)	0
Long rods (>7 µm long)	0	0	0	0 (0)	20
Intermediate rods (3 to 6 µm long)	13	91	79	58 (20)	40
Short rods (<2 µm long)	100	86	89	100 (100)	100
Cross barring	0	0	0	0 (0)	0
Cord formation	0	0	0	0 (0)	0
Rough colonies	0	17	0	92 (80)	0
Pigmentation of colonies in the dark	96	100	100	0 (0)	0
Photochromogenicity	0	0	0	50 (100)	0
Growth at 28°C	100	100	100	100 (100)	100
Growth at 37°C	100	100	100	100 (100)	100
Growth at 42°C	0	0	26	42 (100)	0
Growth at 45°C	0	0	0	0 (0)	0
Growth at 52°C	0	0	0	0 (0)	0
Growth after 3 days	100	100	100	100 (100)	100
Resistance to 0.2% <i>p</i> -aminosalicylate	100	100	100	100 (100)	100
<i>p</i> -Aminosalicylate degradation	0	0	0	0 (0)	0
Resistance to NH ₂ OH · HCl (125 µg/ml)	100	89	100	75 (100)	100
Resistance to NH ₂ OH · HCl (250 µg/ml)	65	51	95	17 (40)	0
Resistance to NH ₂ OH · HCl (500 µg/ml)	0	6	84	0 (0)	0
Growth on Sauton agar medium	100	100	100	100 (100)	100
Growth on Sauton agar containing 0.1% sodium salicylate	100	29	84	50 (100)	80
Degradation of salicylate	0	0	0	0 (0)	0
Tolerance to 0.1% picric acid (Sauton agar)	100	100	100	100 (100)	100
Tolerance to 0.2% picric acid (Sauton agar)	100	100	100	100 (100)	100
Arylsulfatase (3 days)	100	14	89	25 (40)	0
Arylsulfatase (14 days)	100	29	100	75 (100)	60
Resistance to 1 µg of thiophene-2-carboxylic acid hydrazide per ml	100	100	100	100 (100)	100
Resistance to 0.05% sodium salicylate	100	100	100	100 (100)	100
Resistance to ethambutol (5 µg/ml)	0	3	100	0 (0)	0
Tolerance to 0.1% NaNO ₂ (Sauton agar)	100	100	100	100 (100)	100
Tolerance to 0.2% NaNO ₂ (Sauton agar)	96	83	100	83 (100)	100
Growth on Sauton agar containing 1% Tween 80	100	100	100	100 (100)	100
Resistance to 0.5 mg of <i>p</i> -nitrobenzoic acid per ml	100	97	100	58 (100)	100
Resistance to rifampin (25 µg/ml)	100	89	100	50 (100)	100
Niacin production	0	0	0	0 (0)	0
Tween hydrolysis after 7 days	61	29	63	0 (0)	0
Tween hydrolysis after 14 days	61	40	63	0 (0)	0
Catalase (foam height >45 mm)	13	14	16	25 (40)	0
α-Esterase	39	14	11	8 (0)	0
β-Esterase	100	94	100	50 (80)	100
β-Galactosidase	0	9	5	42 (60)	0
Acid phosphatase	0	0	0	0 (0)	60
Nitrate reduction after 6 h	100	17	21	67 (20)	100
Nitrate reduction after 24 h	100	17	74	67 (20)	100
Acetamidase	0	71	100	50 (100)	100
Benzamidase	0	0	16	0 (0)	0
Urease	100	97	100	100 (100)	100
Isonicotinamidase	0	0	0	0 (0)	0
Nicotinamidase	100	74	100	100 (100)	100

TABLE 2—Continued

Character ^a	% of strains showing positive reaction				
	<i>M. austro-africanum</i> (n = 23)	<i>M. aurum</i> (n = 35)	<i>M. neo-aurum</i> (n = 19)	<i>M. para-fortuitum</i> (n = 12)	" <i>M. diernhoferi</i> " (n = 5)
Pyrazinamidase	100	91	100	100 (100)	100
Salicylamidase	0	0	0	0 (0)	0
Allantoinase	0	71	100	17 (20)	80
Succinamidase	0	0	0	0 (0)	0
Glutamate as N and C sources	100	100	100	100 (100)	100
Serine as N and C sources	0	0	0	0 (0)	0
Glucosamine as N and C sources	13	97	100	83 (100)	80
Acetamide as N and C sources	4	74	100	0 (0)	0
Benzamide as N and C sources	100	77	100	0 (0)	100
Monoethanolamine as N and C sources					
Trimethylene diamine as N and C sources	9	100	100	100 (100)	100
Glucose as C source (glutamate N)	100	100	100	100 (100)	100
Acetate as C source (glutamate N)	100	100	100	100 (100)	100
Succinate as C source (glutamate N)	100	100	100	100 (100)	100
Pyruvate as C source (glutamate N)	100	100	100	100 (100)	100
Acetate as C source	100	100	100	100 (100)	100
Citrate as C source	100	9	89	42 (80)	40
Succinate as C source	100	100	100	58 (100)	20
Malate as C source	100	100	100	58 (100)	0
Pyruvate as C source	100	100	100	100 (100)	100
Benzoate as C source	0	0	0	8 (0)	0
Malonate as C source	0	3	26	8 (0)	0
Fumarate as C source	100	100	100	50 (100)	0
Glucose as C source	100	100	100	100 (100)	100
Fructose as C source	100	100	100	100 (100)	100
Sucrose as C source	100	17	5	58 (100)	0
Ethanol as C source	100	91	100	92 (100)	100
n-Propanol as C source	100	100	100	100 (100)	100
Propylene glycol as C source	48	89	84	50 (100)	0
1,3-Butylene glycol as C source	100	43	68	0 (0)	0
1,4-Butylene glycol as C source	0	3	0	0 (0)	0
2,3-Butylene glycol as C source	96	69	63	50 (40)	0
n-Butanol as C source	100	86	100	92 (80)	100
Isobutanol as C source	100	86	100	92 (80)	100
Acid from glucose	100	54	100	0 (0)	100
Acid from mannose	9	23	100	0 (0)	100
Mannose as C source	9	100	100	100 (100)	100
Galactose as C source	0	20	42	0 (0)	0
Arabinose as C source	30	89	68	42 (80)	100
Xylose as C source	100	97	68	42 (80)	100
Rhamnose as C source	0	94	100	0 (0)	0
Trehalose as C source	0	46	100	25 (0)	0
Inositol as C source	100	80	100	8 (0)	100
Mannitol as C source	96	94	100	50 (100)	100
Sorbitol as C source	0	11	0	8 (0)	0
Acetamide as N source	100	91	100	92 (100)	100
Benzamide as N source	0	0	84	8 (20)	0
Urea as N source	100	74	100	92 (100)	100
Pyrazinamide as N source	100	54	100	92 (100)	100
Nicotinamide as N source	100	40	100	92 (100)	60
Nitrate as N source	100	49	100	92 (100)	80
Nitrite as N source	0	3	89	50 (20)	0
Resistance to 5-fluorouracil (20 µg/ml)	0	0	0	50 (100)	20
Resistance to mitomycin (5 µg/ml)	0	0	0	0 (0)	0

^a Unless noted otherwise, resistance tests were performed in Ogawa egg medium, and tests for the utilization of carbohydrates were carried out by using ammoniacal nitrogen. Tests for the utilization of nitrogen sources were made by using glycerol as the carbon source.

^b The values in parentheses indicate the percentages of strains showing positive reactions in the *M. parafortuitum* cluster at a 92% level (*M. parafortuitum sensu stricto*; n = 5).

TABLE 3. Differentiation between strains of *M. aurum* and strains of *M. neoaurum*

Character	% Of strains showing positive reaction	
	<i>M. aurum</i> (n = 35)	<i>M. neoaurum</i> (n = 19)
Arylsulfatase (3 days)	14	89
Arylsulfatase (14 days)	29	100
Resistance to ethambutol (5 µg/ml)	3	100
Nitrate reduction (24 h)	17	74
Citrate as C source	9	89
Rhamnose as C source	94	0
Benzamide as N source	0	84
Nitrite as N source	3	89

TABLE 4. Differentiation between strains of *M. parafortuitum* and strains of "*M. diernhoferi*"

Character	% Of strains showing positive reaction	
	<i>M. parafortuitum</i> ^a	" <i>M. diernhoferi</i> "
Photochromogenicity	100	0
Growth at 42°C	100	0
β-Galactosidase	60	0
Monoethanolamine as N and C sources	0	100
Malate as C source	100	0
Fumarate as C source	100	0
Sucrose as C source	100	0
Propylene glycol as C source	100	0
Inositol as C source	0	100
Resistance to 5-fluorouracil (20 µg/ml)	100	0

^a Strains forming one cluster at a level of 92%.

limit of the range for *M. parafortuitum* was 81.0% and the higher limit of the range for *M. neoaurum* was 78.4%). On the other hand, *M. aurum* could not be differentiated from *M. parafortuitum*, since there was a considerable overlap between the ranges for these two species. Conversely, when M values relative to the HMO of *M. aurum* were analyzed, *M. parafortuitum*

could be differentiated from *M. aurum*, whereas *M. neoaurum* was not clearly separated from *M. aurum*. Finally, when M values relative to the HMO of *M. neoaurum* were determined, both *M. parafortuitum* and *M. aurum* were differentiated from *M. neoaurum*. In view of the results described above, we believe that the three clusters can be regarded as three distinct species.

The type strain of *M. rhodesiae* was in the large cluster of strains in the *M. parafortuitum* complex (Fig. 1). The M values of the *M. rhodesiae* type strain (ATCC 27024) relative to the HMOs of the organisms examined were as follows: *M. parafortuitum*, 78%; *M. aurum*, 82%; *M. neoaurum*, 81%; "*M. diernhoferi*," 80%; South African strains, 84%. All of these M values were outside the ranges estimated for the species described above (Tables 5 to 7) and our results confirm the previous finding (15) that indicated the distinct nature of *M. rhodesiae*.

The status of the South African isolates (*Mycobacterium austroafricanum*) was also examined by numerical analysis (Table 6). When we estimated the M values for individual strains of each species relative to their own HMOs and to the HMOs of four other groups, this group of strains isolated from water showed the highest mean M value and the smallest standard deviation, indicating that this group is very homogeneous. When we compared the M values for these South African isolates with the HMOs of various species of the *M. parafortuitum* complex, the strains isolated from water were clearly differentiated from *M. aurum* and *M. neoaurum*, but they could not be separated from *M. parafortuitum*. In a reciprocal analysis (comparing M values of other groups with the HMO of the South African isolates), the South African isolates were distinct from all species of the *M. parafortuitum* complex. When we analyzed the situation with respect to strains of "*M. diernhoferi*," we found that this group is also distinct from other mycobacteria in the *M. parafortuitum* complex (Table 7).

In previous works, Saito et al. (5) and Tsukamura and Mizuno (14) did not believe that there was sufficient evidence to establish individual species for *M. parafortuitum*, *M. aurum*, and *M.*

TABLE 5. Numerical analysis of relationships among *M. parafortuitum*, *M. aurum*, and *M. neoaurum*

HMO relative to which M values were determined	M ± SD (%) ^a		
	<i>M. parafortuitum</i> (n = 12)	<i>M. aurum</i> (n = 35)	<i>N. neoaurum</i> (n = 19)
<i>M. parafortuitum</i>	88.0 ± 4.11	79.1 ± 4.50	77.7 ± 1.19
<i>M. aurum</i>	76.5 ± 3.06	90.8 ± 4.57	83.0 ± 2.38
<i>M. neoaurum</i>	72.9 ± 5.63	82.3 ± 3.70	95.0 ± 2.13

^a M is the mean M value for the individual strains of each species, and SD is the standard deviation.

TABLE 6. Numerical analysis of relationships among South African strains isolated from water, "*M. diernhoferi*," *M. parafortuitum*, *M. aurum*, and *M. neoaurum*

HMO relative to which M values were determined	M $\pm$ SD (%) ^a				
	South African isolates (n = 23)	<i>M. parafortuitum</i> (n = 12)	<i>M. aurum</i> (n = 35)	<i>M. neoaurum</i> (n = 19)	" <i>M. diernhoferi</i> " (n = 5)
South African isolates	97.3 $\pm$ 1.71	76.4 $\pm$ 3.40	78.9 $\pm$ 4.46	82.1 $\pm$ 2.41	81.0 $\pm$ 1.22
<i>M. parafortuitum</i>	81.5 $\pm$ 1.74	88.0 $\pm$ 4.11			
<i>M. aurum</i>	80.2 $\pm$ 1.72		90.8 $\pm$ 4.57		
<i>M. neoaurum</i>	83.8 $\pm$ 1.66			95.0 $\pm$ 2.13	
" <i>M. diernhoferi</i> "	82.0 $\pm$ 1.91				97.2 $\pm$ 1.79

^a See Table 5, footnote a.

neoaurum, and so these three groups of organisms were regarded as parts of the *M. parafortuitum* complex. However, we believe that these three groups can be differentiated from each other and that they are sufficiently distinct to warrant status as *Mycobacterium* species. Our findings also confirm that there are a number of similarities among the individual species, so there is still some justification for retaining the designation *M. parafortuitum* complex. In addition, our results indicate that both "*M. diernhoferi*" and the South African strains isolated from water belong to the *M. parafortuitum* complex, although members of each of these two groups could be distinguished from named species within the complex. We believe that these two clusters of mycobacteria are also worthy of species status.

It is somewhat unusual that no common biochemical or physiological characteristic was observed among the five groups in the complex (Table 2). This finding differs from the situation observed in all other *Mycobacterium* complexes, including the slowly growing mycobacteria, the *Mycobacterium tuberculosis* complex, the *Mycobacterium avium* complex, and the *Mycobacterium nonchromogenicum* complex, where some common characters (12; unpublished data) are apparent.

In our study, the South African strains isolated from water were shown to be distinct from all

named species of rapidly growing, scotochromogenic mycobacteria, and we propose that members of this group be named *Mycobacterium austroafricanum* sp. nov. The basic biological and physiological characteristics of this species are summarized in Table 2. The following is a description of the species.

Mycobacterium austroafricanum (aus tro af ri ca num. of South Africa). Acid-fast, rod-shaped cells 2 to 6 μ m long and 0.5 μ m wide. Forms mucoid, yellowish pigmented colonies in the dark, showing stronger pigmentation after exposure to light. Grows within 3 days. Grows at 28 and 37°C, but not at 42°C. Resistant to NH₂OH · HCl at a concentration of 125 μ g/ml, but susceptible to NH₂OH · HCl at a concentration of 250 μ g/ml. Susceptible to isoniazid (10 μ g/ml) and ethambutol (5 μ g/ml); resistant to 5% NaCl, *p*-nitrobenzoic acid (0.5 mg/ml), rifampin (25 μ g/ml), and thiophene-2-carboxylic acid hydrazide (1 μ g/ml). Grows on Sauton agar medium containing either 0.1% sodium salicylate, 0.1% NaNO₂, or 0.2% picric acid.

Niacin is not produced. Catalase activity (semiquantitative) negative, α -esterase negative, β -esterase positive, β -galactosidase negative, acid phosphatase negative, Tween 80 hydrolysis (after 14 days) negative, and arylsulfatase (after 3 days) positive. Nitrate reduced to nitrite (after 6 h). Nicotinamidase and pyrazinamidase positive, but negative for benzamidase, isonicotina-

TABLE 7. Numerical analysis of relationships among "*M. diernhoferi*," *M. parafortuitum*, *M. aurum*, *M. neoaurum*, and South African strains isolated from water

HMO relative to which M values were determined	M $\pm$ SD (%) ^a				
	" <i>M. diernhoferi</i> " (n = 5)	<i>M. parafortuitum</i> (n = 12)	<i>M. aurum</i> (n = 35)	<i>M. neoaurum</i> (n = 19)	South African isolates (n = 23)
" <i>M. diernhoferi</i> "	97.2 $\pm$ 1.79	80.9 $\pm$ 2.27	80.7 $\pm$ 2.43	80.5 $\pm$ 1.71	82.0 $\pm$ 1.91
<i>M. parafortuitum</i>	81.6 $\pm$ 1.52	88.0 $\pm$ 4.11			
<i>M. aurum</i>	85.8 $\pm$ 1.30		90.8 $\pm$ 4.57		
<i>M. neoaurum</i>	80.6 $\pm$ 2.41			95.0 $\pm$ 2.13	
South African isolates	81.0 $\pm$ 1.73				97.3 $\pm$ 1.71

^a See Table 5, footnote a.

midase, salicylamidase, allantoinase, and succinamidase.

Glutamate and monoethanolamine are utilized as simultaneous nitrogen and carbon sources, but serine, glucosamine, acetamide, benzamide, and trimethylene diamine are not utilized as such sources; acetate, citrate, succinate, malate, pyruvate, or fumarate is utilized as a sole source of carbon in the presence of ammoniacal nitrogen, but benzoate and malonate are not utilized as carbon sources. The following carbohydrates are utilized as sole sources of carbon in the presence of ammoniacal nitrogen: glucose, fructose, sucrose, ethanol, *n*-propanol, *n*-butanol, isobutanol, 1,3-butylene glycol, 2,3-butylene glycol, and xylose. The following carbohydrates are not utilized as sole sources of carbon: propylene glycol, 1,4-butylene glycol, mannose, galactose, arabinose, rhamnose, trehalose, and sorbitol. Inositol and mannitol are usually utilized as sole sources of carbon.

Source: water from South Africa.

Type strain: E9789-SA12441 (= ATCC 33464).

Previously, "*M. diernhoferi*" was regarded as a synonym of *M. parafortuitum* (3). However, in the present study, we established that organisms in this group could be differentiated from *M. parafortuitum*. Furthermore, Kusunose et al. (Kekkaku 54:219–220, 1979) recently reported that *M. parafortuitum* contains 54- to 60-carbon mycolic acids, whereas "*M. diernhoferi*" strains contain 68- to 76-carbon mycolic acids. In view of these findings, we believe that the name "*M. diernhoferi*" should be revived for the organisms described originally by Bönicke and Juhász (1) in 1965. The species description is as follows.

Mycobacterium diernhoferi (diern hó fer i. of Diernhof, who originally isolated the organisms). Acid-fast, rod-shaped cells 2 to 6 μ m long and 0.5 μ m wide. Grows within 3 days. Forms white, smooth colonies. Non-photochromogenic. Grows at 28 and 37°C, but does not grow at 42°C. Resistant to $\text{NH}_2\text{OH} \cdot \text{HCl}$ at a concentration of 125 $\mu\text{g/ml}$, but susceptible to $\text{NH}_2\text{OH} \cdot \text{HCl}$ at a concentration of 250 $\mu\text{g/ml}$. Resistant to isoniazid (10 $\mu\text{g/ml}$), 5% NaCl, thiophene-2-carboxylic acid hydrazide (1 $\mu\text{g/ml}$), rifampin (25 $\mu\text{g/ml}$), and *p*-nitrobenzoic acid (0.5 mg/ml); susceptible to ethambutol (5 $\mu\text{g/ml}$). Tolerant of 0.2% picric acid and 0.1% NaNO_2 in Sauton agar medium. Tween 80 not hydrolyzed after 14 days. Catalase (semiquantitative) negative, α -esterase negative, β -esterase positive, and β -galactosidase negative. Nitrate reduced to nitrite after 6 h. Acetamidase, urease, nicotiamidase, pyrazinamidase, and allantoinase positive, but negative for benzamidase, isonicotinamidase, salicylamidase, and succina-

midase. Niacin is not produced. Glutamate, glucosamine, monoethanolamine, and trimethylene diamine are utilized as simultaneous nitrogen and carbon sources, but serine, acetamide, and benzamide are not utilized as such sources; acetate and pyruvate are utilized as sole sources of carbon in the presence of ammoniacal nitrogen, but citrate, succinate, malate, benzoate, malonate, and fumarate are not utilized as such sources. Glucose, fructose, mannose, arabinose, xylose, inositol, and mannitol are utilized as sole sources of carbon, but sucrose, galactose, rhamnose, trehalose, and sorbitol are not utilized as such sources. Ethanol, *n*-propanol, *n*-butanol, and isobutanol are utilized as sole sources of carbon, but propylene glycol, and 1,3-, 1,4- 2,3-butylene glycols are not utilized as such sources.

Source: soil in a cattle field.

Type strain: 41001 (= ATCC 19340).

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