

## Phylogenetic relationships among the *Chromatiaceae*, their taxonomic reclassification and description of the new genera *Allochromatium*, *Halochromatium*, *Isochromatium*, *Marichromatium*, *Thiococcus*, *Thiohalocapsa* and *Thermochromatium*

Johannes F. Imhoff, Jörg Süling and Ralf Petri

Author for correspondence: Johannes F. Imhoff. Tel: +49 431 697 3850. Fax: +49 431 565876.  
e-mail: jimhoff@ifm.uni-kiel.de

Institut für Meereskunde  
an der Universität Kiel,  
Abteilung Marine  
Mikrobiologie,  
Düsternbrooker Weg 20,  
D-24105 Kiel, Germany

Sequences of the 16S rDNA from all available type strains of *Chromatium* species have been determined and were compared to those of other *Chromatiaceae*, a few selected *Ectothiorhodospiraceae* and *Escherichia coli*. The clear separation of *Ectothiorhodospiraceae* and *Chromatiaceae* is confirmed. Most significantly the sequence comparison revealed a genetic divergence between *Chromatium* species originated from freshwater sources and those of truly marine and halophilic nature. Major phylogenetic branches of the *Chromatiaceae* contain (i) marine and halophilic species, (ii) freshwater *Chromatium* species together with *Thiocystis* species and (iii) species of the genera *Thiocapsa* and *Amoebobacter* as recently reclassified [Guyoneaud, R. & 6 other authors (1998). *Int J Syst Bacteriol* 48, 957–964], namely *Thiocapsa roseopersicina*, *Thiocapsa pendens* (formerly *Amoebobacter pendens*), *Thiocapsa rosea* (formerly *Amoebobacter roseus*), *Amoebobacter purpureus* and *Thiolamproyum pedioforme* (formerly *Amoebobacter pedioformis*). The genetic relationships between the species and groups are not in congruence with the current classification of the *Chromatiaceae* and a reclassification is proposed on the basis of 16S rDNA sequence similarity supported by selected phenotypic properties. The proposed changes include the transfers of *Chromatium minus* and *Chromatium violascens* to *Thiocystis minor* comb. nov. and *Thiocystis violascens* comb. nov., of *Chromatium vinosum*, *Chromatium minutissimum* and *Chromatium warmingii* to the new genus *Allochromatium* as *Allochromatium vinosum* comb. nov., *Allochromatium minutissimum* comb. nov., and *Allochromatium warmingii* comb. nov., of *Chromatium tepidum* to the new genus *Thermochromatium* as *Thermochromatium tepidum* comb. nov., of *Chromatium salexigens* and *Chromatium glycolicum* to the new genus *Halochromatium* as *Halochromatium salexigens* comb. nov. and *Halochromatium glycolicum* comb. nov., of *Chromatium gracile* and *Chromatium purpuratum* to the new genus as *Marichromatium gracile* comb. nov. and *Marichromatium purpuratum* comb. nov., of *Thiocapsa pfennigii* to *Thiococcus pfennigii* gen. nom. rev., of *Thiocapsa halophila* to the new genus *Thiohalocapsa* as *Thiohalocapsa halophila* comb. nov., and of *Chromatium buderi* to the new genus *Isochromatium* as *Isochromatium buderi* comb. nov.

**Keywords:** phylogeny, *Chromatiaceae*, taxonomic reclassification

## INTRODUCTION

Historically, the taxonomy of phototrophic purple sulfur bacteria, has been based on easily recognizable phenotypic properties (see Pfennig & Trüper, 1974). The traditional taxonomic system of these bacteria is not phylogenetically oriented. It is primarily of practical relevance, and inconsistencies between the taxonomic treatment and genetic relationships of the purple sulfur bacteria have been discussed previously (Gibson *et al.*, 1979; Fowler *et al.*, 1984; Stackebrandt *et al.*, 1984; Trüper, 1987). Indeed, phylogenetic information became available only with the establishment of sequencing techniques for proteins and nucleic acids on the basis of sequences of cytochrome *c* (Ambler *et al.*, 1979; Dickerson, 1980) and oligonucleotides obtained from 16S rRNA digestion with specific endonucleases (Gibson *et al.*, 1979; Fowler *et al.*, 1984; Stackebrandt *et al.*, 1984), later by sequences of the 16S rRNA gene. It was obvious already from the early investigations on the genetic relationships that phototrophic bacteria are found in several major branches of a sequence-based phylogenetic bacterial system (see Woese, 1987), in part separated from and in part intermixed with non-phototrophic bacteria. Among the phototrophic purple bacteria, separate phylogenetic lines are represented by the purple sulfur bacteria (*Chromatiaceae* and *Ectothiorhodospiraceae*) in the  $\gamma$ -*Proteobacteria* and by the purple non-sulfur bacteria in the  $\alpha$ -*Proteobacteria* and the  $\beta$ -*Proteobacteria*. First surveys on the relationships of purple sulfur bacteria have been made by comparison of oligonucleotide catalogues (Fowler *et al.*, 1984; Stackebrandt *et al.*, 1984). More recently, sequence analyses and comparisons lead to the separation of the *Ectothiorhodospiraceae* into two genera that can also be recognized by their salt responses and other phenotypic properties (Imhoff & Süling, 1996). 16S rDNA sequences of only a few species of the *Chromatiaceae* were available until recently (DeWeerd *et al.*, 1990; Wahlund *et al.*, 1991; Caumette *et al.*, 1997; Guyoneaud *et al.*, 1997, 1998). In the present work 16S rDNA sequences of all available type strains of *Chromatium* species are compared to related purple sulfur bacteria and used to characterize their genetic relationships. On this basis and with the additional support of phenotypic properties a reclassification of these bacteria is proposed. A positive correlation between differences in several phenotypic properties to similarities of 16S rDNA sequences is discussed and in particular these properties are considered to be of importance for the classification of these bacteria.

## METHODS

**Source and culture of bacterial strains.** All strains of purple sulfur bacteria used for this study are listed in Table 1, which in addition shows the previously used and the newly proposed names, the original strain designations, the DSM numbers when available and the EMBL accession numbers for their 16S rDNA sequences. Cultures of all strains are maintained in our laboratory (see Table 1).

Strains were cultivated in synthetic medium according to Pfennig & Trüper (1992). The final pH was 7.2. For marine and halophilic strains, 1–10% NaCl was added to the medium, as required. Pure cultures were grown in 100 ml screw-capped bottles completely filled with synthetic medium and incubated in the light.

**PCR amplification and 16S rDNA sequencing and analysis.** Cell material for 16S rDNA sequencing was taken from 1–2 ml of well-grown liquid cultures. DNA was extracted and purified by using the QIAGEN genomic DNA buffer set. Recombinant Taq polymerase was used for PCR (Mullis & Faloona, 1987) which was started with the following primers: 5'-GTTTGATCCTGGCTCAG-3' and 5'-TACC-TTGTTACGACTTCA-3' (positions 11–27 and 1489–1506, respectively, according to the *Escherichia coli* 16S rRNA numbering of the International Union of Biochemistry). The PCR products were purified by using the QIAquick PCR purification kit. Sequences were obtained by cycle sequencing with the SequiTherm sequencing kit (Biozym) and the chain-termination reaction (Sanger *et al.*, 1977) using an automated laser fluorescence sequencer (Pharmacia). Sequences were aligned using the CLUSTAL W program (Thompson *et al.*, 1994). The alignment length was from positions 29 to 1381 according to the *Escherichia coli* numbering (including gaps 1400 positions). The distance matrix was calculated on the basis of the algorithm of Jukes & Cantor (1969) with the DNADIST program within the PHYLIP package (Felsenstein, 1989). The FITCH program in the PHYLIP package fitted a tree to the evolutionary distances.

**Nucleotide sequence accession numbers.** The 16S rDNA sequences determined in this study have been deposited in the EMBL database under accession numbers given in Table 1. Accession numbers of additional 16S rDNA sequences of *Chromatiaceae* that were published previously are also contained in Table 1. Further sequences (accession nos in parentheses) from *Ectothiorhodospiraceae* and *Escherichia coli* (K02555) were available from the EMBL database: *Ectothiorhodospira mobilis* strain DSM 237<sup>T</sup> (X93481), *Ectothiorhodospira haloalkaliphila* strain ATCC 51935<sup>T</sup> (X93479), *Ectothiorhodospira shaposhnikovii* strain DSM 239 (X93480), *Halorhodospira halophila* strain DSM 244<sup>T</sup> (M26630), and *Halorhodospira halochloris* DSM 1059<sup>T</sup> (M59152) (see Imhoff & Süling, 1996).

## RESULTS

The relationships of the investigated type strains of *Chromatium* species and related purple sulfur bacteria based on almost complete sequences of the 16S rDNA is shown in a distance matrix with similarity values and  $K_{nuc}$  values (Table 2) and a phylogenetic tree, based on the data from this distance matrix (Fig. 1).

It is quite apparent from their genetic relationships that a major deeply branching cluster with marine and halophilic *Chromatiaceae* species is clearly separated from another branch with predominantly freshwater species. Within this latter branch, one cluster contains *Thiocapsa* and *Amoebobacter* species according to Guyoneaud *et al.* (1998), while another one includes freshwater *Chromatium* species together with known *Thiocystis* species.

The first branch consists of *Chromatium* species which have been isolated from marine and hypersaline environments and which specifically and character-

**Table 1.** Species and strains of *Chromatiaceae* compared in this studyType strains are denoted by <sup>T</sup>; NN, not available in this collection.

Previous name	New name	DSM no.	Other designation(s)	Authors' no.	EMBL no.
<i>Chromatium okenii</i>	Unchanged	169 <sup>T</sup>	1111	6010	Y12376
<i>Chromatium vinosum</i>	<i>Allochromatium vinosum</i>	180 <sup>T</sup>	D	5110	M26629
<i>Chromatium minutissimum</i>	<i>Allochromatium minutissimum</i>	1376 <sup>T</sup>	MSU	5310	Y12369
<i>Chromatium warmingii</i>	<i>Allochromatium warmingii</i>	173 <sup>T</sup>	6512	5810	Y12365
<i>Chromatium tepidum</i>	<i>Thermochromatium tepidum</i>	3771 <sup>T</sup>	MC	NN	M59150
<i>Chromatium minus</i>	<i>Thiocystis minor</i>	178 <sup>T</sup>	1211	5710	Y12372
<i>Chromatium violascens</i>	<i>Thiocystis violascens</i>	168 <sup>T</sup>	6111	5410	AJ224438
<i>Chromatium purpuratum</i>	<i>Marichromatium purpuratum</i>	1591 <sup>T</sup>	984	5500	AJ224439
<i>Chromatium gracile</i>	<i>Marichromatium gracile</i>	203 <sup>T</sup>	8611	5210	X93473
<i>Chromatium buderi</i>	<i>Isochromatium buderi</i>	176 <sup>T</sup>	8111	5610	AJ224430
<i>Chromatium salexigens</i>	<i>Halochromatium salexigens</i>	4395 <sup>T</sup>	SG3201	6310	X98597
<i>Chromatium glycolicum</i>	<i>Halochromatium glycolicum</i>	11080 <sup>T</sup>	SL3201	6410	X93472
<i>Thiocapsa pfennigii</i>	<i>Thiococcus pfennigii</i>	226	8013	4250	Y12373
<i>Thiocapsa halophila</i>	<i>Thiohalocapsa halophila</i>	6210 <sup>T</sup>	SG3202	4270	AJ002796
<i>Thiorhodovibrio winogradskyi</i>	Unchanged	6702 <sup>T</sup>	06511	4910	Y12368
<i>Thiocystis violacea</i>	Unchanged	207 <sup>T</sup>	2711	4340	Y11315
<i>Thiocystis gelatinosa</i>	Unchanged	215 <sup>T</sup>	2611	4310	Y11317
<i>Rhabdochromatium marinum</i>	Unchanged	5261 <sup>T</sup>	8315	6510	X84316
<i>Thiorhodococcus minus</i>	<i>Thiorhodococcus minor</i> *	11518 <sup>T</sup>	CE2203	6680	Y11316
<i>Amoebobacter purpureus</i>	Unchanged	4197 <sup>T</sup>	ThSchl2	4450	Y12366
<i>Thiocapsa roseopersicina</i>	Unchanged	217 <sup>T</sup>	1711	4210	Y12364
<i>Amoebobacter pendens</i>	<i>Thiocapsa pendens</i> †	236 <sup>T</sup>	1314	4410	AJ002797

\* Corrected epithet.

† See Guyoneaud *et al.* (1998).

istically require various concentrations of salt for growth. *Chromatium salexigens* and *Chromatium glycolicum* form one line. *Chromatium gracile* and *Chromatium purpuratum* form a second line together with an isolate that has been first described as '*Rhodobacter marinus*' (Burgess *et al.*, 1994; 16S rDNA sequence of this strain as personal communication of T. Matsunaga). Also, *Thiocapsa pfennigii* and *Thiocapsa halophila* belong to this cluster and therefore certainly can no longer be classified as *Thiocapsa* species (Guyoneaud *et al.*, 1998). In addition, *Thiorhodovibrio winogradskyi*, *Rhabdochromatium marinum*, *Thiorhodococcus minor* and *Chromatium buderi* form well-separated lines within this cluster (Fig. 1).

The second branch mostly contains species that originate from freshwater habitats and is significantly distinct from the branch of truly marine and halophilic species. On the basis of the sequence data, two major clusters can be recognized, one with *Chromatium* and *Thiocystis* species, the other with *Thiocapsa* and *Amoebobacter* species, including *Thiolamprovum pedioforme* (formerly *Amoebobacter pedioformis*).

In the first cluster *Chromatium warmingii*, *Chromatium vinosum* and *Chromatium minutissimum* form one

subcluster to which *Chromatium tepidum* is distantly related. A second subcluster is represented by *Chromatium minus*, *Thiocystis violacea*, *Thiocystis gelatinosa* and *Chromatium violascens*. *Chromatium okenii*, the type species of the genus *Chromatium*, is only distantly related to other bacteria of this cluster. *Chromatium weissei*, which is phenotypically quite similar to *Chromatium okenii*, also has a number of significant positions in its 16S rDNA sequence in common specifically with *Chromatium okenii*, as could be delineated from the comparison of its oligonucleotide catalogue (Fowler *et al.*, 1984) with the sequence of *Chromatium okenii*. A pure culture of *Chromatium weissei* is at present not available and therefore the comparison of its complete sequence was not possible.

The second cluster of this branch showed some distant relationships to the freshwater *Chromatium* species. In this cluster are *Thiolamprovum pedioforme* (formerly *Amoebobacter pedioformis*), *Amoebobacter purpureus*, *Thiocapsa pendens* (formerly *Amoebobacter pendens*), *Thiocapsa rosea* (formerly *Amoebobacter roseus*) and *Thiocapsa roseopersicina* (Fig. 1; Guyoneaud *et al.*, 1998). Although most of these species characteristically are found in freshwater habitats, a number of

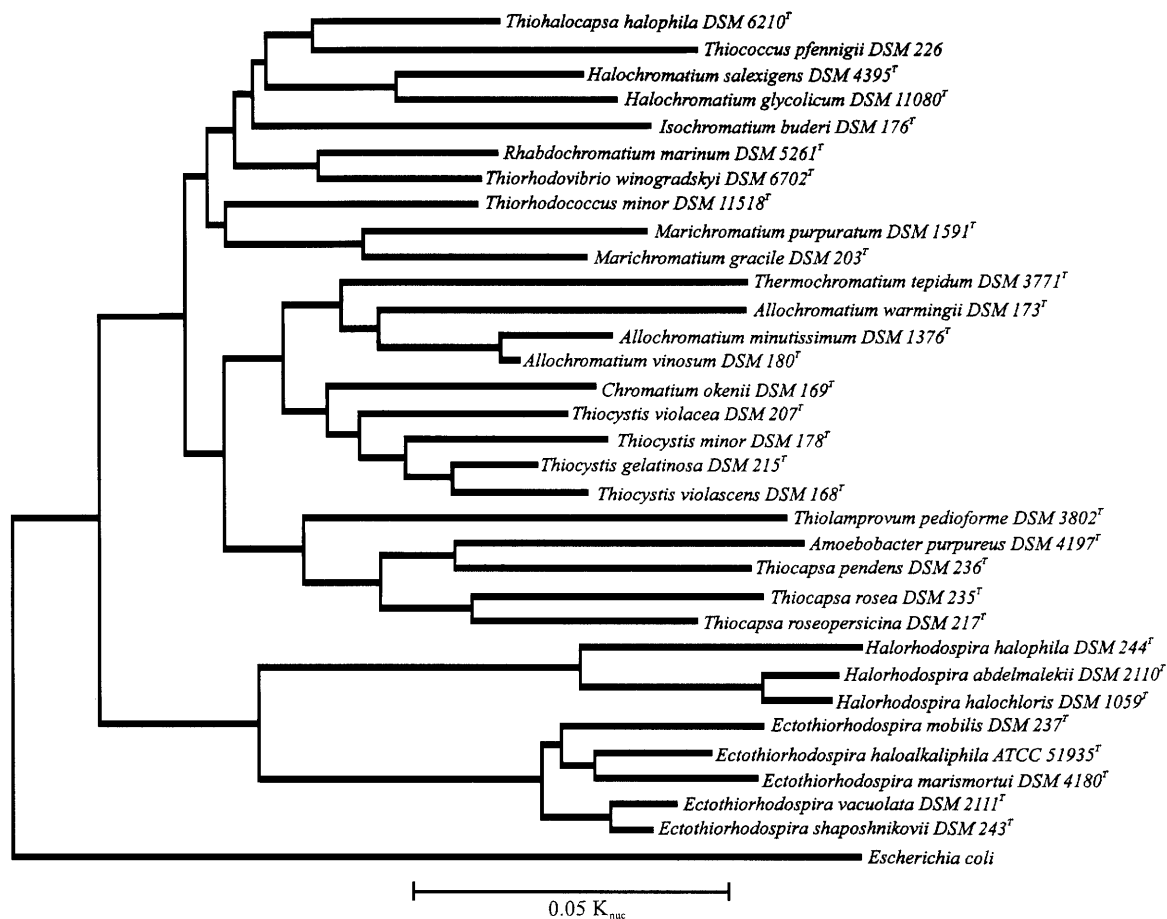
**Table 2.** Levels of 16S rDNA sequence similarity and evolutionary distances of presently recognized *Chromatium* species with other phototrophic purple sulfur bacteria and *Escherichia coli* as reference species

The values on the upper right are the uncorrected sequence similarities (%), and the values on the lower left are  $K_{\text{mut}}$  values corrected for multiple base change by the methods of Jukes & Cantor (1969). Organisms: 1, *Marichromatium purpuratum* DSM 1591<sup>T</sup>; 2, *Marichromatium gracile* DSM 203<sup>T</sup>; 3, *Halochromatium glycolicum* DSM 11080<sup>T</sup>; 4, *Halochromatium salexigens* DSM 4395<sup>T</sup>; 5, *Isochromatium buderi* DSM 176<sup>T</sup>; 6, *Thiococcus pfennigii* DSM 226; 7, *Thiohalocapsa halophila* DSM 6210<sup>T</sup>; 8, *Thiocapsa roseopersicina* DSM 217<sup>T</sup>; 9, *Thiocapsa rosea* DSM 235<sup>T</sup>; 10, *Thiolamproyum pedioforme* DSM 3802<sup>T</sup>; 11, *Thiocystis violascens* DSM 168<sup>T</sup>; 12, *Thiocystis gelatinosa* DSM 215<sup>T</sup>; 13, *Thiocystis minor* DSM 178<sup>T</sup>; 14, *Thiocystis violacea* DSM 207<sup>T</sup>; 15, *Chromatium okenii* DSM 169<sup>T</sup>; 16, *Allochromatium vinosum* DSM 180<sup>T</sup>; 17, *Allochromatium minutissimum* DSM 1376<sup>T</sup>; 18, *Allochromatium warmingii* DSM 173<sup>T</sup>; 19, *Thermochromatium tepidum* DSM 3771<sup>T</sup>; 20, *Thiocapsa pendens* DSM 236<sup>T</sup>; 21, *Amoebobacter purpureus* DSM 4197<sup>T</sup>; 22, *Thiorhodovibrio winogradskyi* DSM 6702<sup>T</sup>; 23, *Rhabdochromatium marinum* DSM 5261<sup>T</sup>; 24, *Thiorhodococcus minor* DSM 11518<sup>T</sup>; 25, *Escherichia coli*; 26, *Ectothiorhodospira shaposhnikovii* DSM 243<sup>T</sup>; 27, *Ectothiorhodospira vacuolata* DSM 2111<sup>T</sup>; 28, *Ectothiorhodospira marismortui* DSM 4180<sup>T</sup>; 29, *Ectothiorhodospira haloalkaliphila* ATCC 51935<sup>T</sup>; 30, *Ectothiorhodospira mobilis* DSM 237<sup>T</sup>; 31, *Halorhodospira halochloris* DSM 1059<sup>T</sup>; 32, *Halorhodospira abdelmalekii* DSM 2110<sup>T</sup>; 33, *Halorhodospira halophila* DSM 244<sup>T</sup>.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1		94.07	90.30	91.27	89.32	90.50	92.24	89.34	87.85	87.43	89.53	90.83	89.88	90.77	90.46	91.10	90.14	89.05	87.03	87.57	87.78	92.04	91.05	92.37	83.70	87.39	86.95	86.30	87.31	86.21	84.90	85.08	85.57
2	0.062		91.41	91.89	90.69	90.43	92.23	90.03	88.39	87.40	90.91	91.39	90.31	90.69	90.90	92.13	91.48	88.39	87.94	88.71	89.36	92.09	90.98	92.78	84.20	88.68	88.02	87.54	88.30	87.65	85.45	85.55	86.04
3	0.104	0.091		95.19	91.03	91.08	93.08	89.10	88.97	88.78	89.62	90.44	89.88	90.66	90.35	91.16	90.25	87.69	88.40	87.53	88.94	92.85	92.22	92.54	84.24	88.59	87.99	87.80	88.16	87.42	85.86	85.86	86.50
4	0.093	0.086	0.050		91.49	90.98	93.82	88.98	88.66	88.44	90.13	91.28	90.26	90.46	90.69	91.34	90.45	88.20	88.76	88.30	89.69	93.23	92.38	92.02	85.41	89.35	88.92	88.74	88.38	87.93	86.24	86.53	86.31
5	0.115	0.099	0.096	0.090		90.61	92.65	88.14	87.97	89.80	90.69	91.05	90.28	91.01	91.28	91.22	90.55	88.53	88.97	87.85	88.51	91.45	92.27	90.38	83.55	86.48	86.03	86.38	85.58	85.25	84.80	85.21	85.30
6	0.102	0.102	0.095	0.096	0.100		93.35	88.83	88.54	88.03	88.90	90.18	89.50	89.63	89.86	90.44	89.78	87.06	86.95	88.65	87.91	91.72	91.37	90.18	84.15	86.42	86.20	86.56	85.96	85.30	86.16	85.65	85.72
7	0.082	0.082	0.073	0.064	0.077	0.070		90.07	89.61	90.30	90.54	91.61	90.51	91.31	91.87	92.77	91.87	89.27	88.51	90.35	90.26	94.52	94.24	93.38	85.67	89.34	89.12	88.41	87.85	87.73	86.98	86.97	86.95
8	0.115	0.107	0.118	0.119	0.129	0.121	0.107		93.99	91.13	91.26	90.89	91.08	89.63	90.08	91.24	90.18	87.87	89.02	93.28	89.75	91.28	90.70	89.90	84.24	86.61	85.98	85.69	85.23	85.32	85.60	85.18	85.33
9	0.133	0.126	0.119	0.123	0.131	0.124	0.112	0.063		90.44	90.08	90.46	89.90	90.30	90.32	90.17	89.28	88.16	88.99	92.10	87.89	89.58	89.77	90.29	82.62	85.89	85.34	85.69	85.18	84.74	84.44	84.06	84.86
10	0.138	0.138	0.122	0.126	0.110	0.130	0.104	0.094	0.102		89.10	89.40	88.58	90.12	88.79	89.31	88.50	87.52	88.46	87.69	86.34	89.69	90.68	90.94	83.16	85.97	85.55	84.98	85.47	84.76	83.82	83.90	83.73
11	0.113	0.097	0.112	0.106	0.099	0.120	0.101	0.093	0.106	0.118		97.38	95.42	94.77	94.29	93.49	92.93	91.87	91.08	90.17	89.69	91.82	91.76	92.04	84.11	88.28	88.03	87.90	88.08	86.99	86.15	86.13	86.86
12	0.098	0.091	0.102	0.093	0.095	0.105	0.089	0.097	0.102	0.114	0.027		96.13	95.42	94.44	94.60	93.37	91.20	91.20	91.05	90.16	92.28	92.32	92.53	85.32	88.95	88.97	88.56	87.80	87.71	86.70	86.41	86.54
13	0.109	0.104	0.109	0.104	0.104	0.113	0.101	0.095	0.108	0.124	0.047	0.040		94.84	93.75	93.28	92.43	91.85	91.13	90.44	89.04	90.88	92.01	91.71	83.79	87.52	87.43	86.84	87.47	86.90	85.83	86.02	86.38
14	0.098	0.099	0.100	0.102	0.096	0.112	0.092	0.112	0.104	0.106	0.054	0.047	0.053		93.28	94.08	92.86	91.02	92.35	90.21	89.93	92.12	92.93	92.36	84.55	88.34	88.22	87.46	86.29	86.82	87.15	86.93	85.36
15	0.102	0.097	0.103	0.099	0.093	0.109	0.086	0.106	0.104	0.121	0.059	0.058	0.065	0.070		92.99	92.33	90.55	90.39	91.03	89.69	92.01	91.18	91.75	83.68	88.19	88.22	87.58	87.16	86.84	85.76	86.05	85.88
16	0.095	0.083	0.094	0.092	0.093	0.102	0.076	0.093	0.105	0.115	0.068	0.056	0.070	0.062	0.074		98.40	93.79	93.04	88.89	89.41	92.53	92.03	93.74	85.36	89.44	89.05	88.16	88.06	88.39	87.56	87.29	86.60

Table 2 (cont.)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
17	0.106	0.090	0.104	0.102	0.101	0.110	0.086	0.105	0.116	0.125	0.074	0.069	0.080	0.075	0.081	0.016		93.32	91.66	88.46	89.08	90.81	90.77	92.22	84.37	88.04	87.81	87.10	86.56	86.82	85.92	86.15	85.64
18	0.118	0.126	0.134	0.128	0.124	0.142	0.116	0.132	0.129	0.136	0.086	0.094	0.086	0.096	0.101	0.065	0.070		91.54	88.07	88.20	89.35	89.32	89.63	82.88	87.65	87.47	86.70	86.43	86.60	85.49	85.61	86.03
19	0.142	0.131	0.126	0.122	0.119	0.143	0.125	0.119	0.119	0.125	0.095	0.094	0.094	0.081	0.103	0.073	0.088	0.090		87.61	86.53	89.62	90.12	90.25	82.92	87.24	87.07	86.59	86.60	86.85	86.20	86.13	85.01
20	0.136	0.122	0.136	0.127	0.133	0.123	0.103	0.070	0.083	0.134	0.105	0.095	0.102	0.105	0.096	0.120	0.125	0.130	0.135		92.57	90.56	90.39	90.58	81.88	86.02	85.52	85.62	85.47	84.95	83.96	83.62	83.55
21	0.133	0.115	0.120	0.111	0.125	0.132	0.104	0.110	0.132	0.151	0.111	0.105	0.118	0.108	0.111	0.114	0.118	0.128	0.148	0.078		90.01	89.75	90.32	81.78	85.82	85.97	85.30	85.48	85.10	83.52	83.63	83.95
22	0.084	0.084	0.075	0.071	0.091	0.088	0.057	0.093	0.112	0.111	0.087	0.081	0.097	0.083	0.084	0.079	0.098	0.115	0.112	0.101	0.107		95.96	94.08	85.42	89.38	88.78	88.33	88.76	87.94	88.91	88.17	88.15
23	0.095	0.096	0.082	0.080	0.082	0.092	0.060	0.099	0.110	0.100	0.087	0.081	0.084	0.074	0.094	0.084	0.098	0.115	0.106	0.103	0.110	0.042		93.65	86.22	89.40	88.91	88.08	88.33	88.24	87.72	87.89	87.04
24	0.080	0.076	0.079	0.084	0.103	0.105	0.069	0.108	0.104	0.097	0.084	0.079	0.088	0.081	0.087	0.065	0.082	0.112	0.104	0.101	0.104	0.062	0.066		84.18	89.45	89.04	87.87	87.49	87.57	87.22	86.97	86.83
25	0.184	0.177	0.177	0.162	0.186	0.178	0.159	0.177	0.198	0.191	0.179	0.163	0.183	0.173	0.184	0.163	0.175	0.194	0.194	0.207	0.209	0.162	0.152	0.178		84.07	83.97	83.20	81.86	82.59	82.39	82.50	81.55
26	0.138	0.123	0.124	0.115	0.149	0.150	0.115	0.147	0.152	0.155	0.127	0.120	0.137	0.127	0.129	0.114	0.130	0.135	0.140	0.155	0.157	0.115	0.114	0.114	0.179		98.75	95.66	96.98	95.92	89.14	89.61	88.18
27	0.143	0.131	0.131	0.120	0.155	0.153	0.118	0.155	0.156	0.160	0.130	0.119	0.138	0.128	0.128	0.118	0.133	0.137	0.142	0.161	0.155	0.122	0.120	0.118	0.180	0.013		95.72	96.59	95.75	88.76	88.80	88.08
28	0.151	0.136	0.133	0.122	0.150	0.148	0.126	0.159	0.163	0.168	0.132	0.124	0.145	0.137	0.136	0.129	0.142	0.146	0.148	0.160	0.164	0.127	0.130	0.132	0.190	0.045	0.044		96.72	95.32	87.55	86.97	87.44
29	0.139	0.127	0.129	0.126	0.160	0.155	0.133	0.164	0.165	0.162	0.130	0.133	0.137	0.151	0.141	0.130	0.148	0.150	0.148	0.162	0.161	0.122	0.127	0.137	0.208	0.031	0.035	0.034		95.84	88.26	88.19	87.98
30	0.152	0.135	0.138	0.132	0.164	0.164	0.134	0.163	0.171	0.170	0.143	0.134	0.144	0.145	0.145	0.126	0.145	0.148	0.145	0.168	0.166	0.131	0.128	0.136	0.198	0.042	0.044	0.048	0.043		89.23	89.36	86.98
31	0.169	0.162	0.157	0.152	0.170	0.153	0.143	0.160	0.174	0.182	0.153	0.146	0.157	0.141	0.158	0.136	0.156	0.161	0.152	0.180	0.186	0.120	0.134	0.140	0.201	0.117	0.122	0.136	0.128	0.116		98.26	93.92
32	0.166	0.161	0.157	0.148	0.165	0.159	0.143	0.165	0.179	0.181	0.153	0.150	0.155	0.144	0.154	0.139	0.153	0.160	0.153	0.185	0.185	0.129	0.132	0.143	0.199	0.112	0.121	0.143	0.129	0.115	0.018		93.60
33	0.160	0.154	0.149	0.151	0.164	0.158	0.143	0.163	0.169	0.183	0.144	0.148	0.150	0.151	0.156	0.148	0.159	0.155	0.167	0.186	0.181	0.129	0.142	0.145	0.212	0.129	0.130	0.137	0.131	0.143	0.063	0.067	



**Fig. 1.** Phylogenetic tree showing the relationships of the investigated type strains of *Chromatium* species and related purple sulfur bacteria. Derived from the distance matrix (Table 2) and calculated as indicated in Methods.

isolates from coastal environments do grow in the presence of low concentrations of salt but do not require salt for growth (R. Guyoneaud & P. Caumette, personal communication).

**DISCUSSION**

The available 16S rDNA sequence information of *Ectothiorhodospiraceae* (Imhoff & Siling, 1996) and *Chromatiaceae* species (DeWeerd *et al.*, 1990; Wahlund *et al.*, 1991; Caumette *et al.*, 1997; Guyoneaud *et al.*, 1997, 1998; and this work) support the separation of these two families (Imhoff, 1984a) and at the same time demonstrate the inconsistency of the present taxonomic classification of the *Chromatiaceae* with their genetic relationships. Most of the morphological and physiological properties, upon which the current taxonomy is based, have to be regarded as being of minor importance in support of a genetically oriented taxonomic classification, while others, so far much less considered properties, supposedly are of higher significance in this regard. The differential characteristics of the genera and species of the *Chromatiaceae* are summarized in Table 3, and are discussed below.

**Morphological properties**

The principal classification schemes of *Chromatiaceae* genera use cell form, motility by flagella and presence of gas vesicles as key properties (Imhoff & Truper, 1982; Pfennig & Truper, 1974, 1989). Though it would be an exaggeration to state that these properties have no taxonomic meaning, it is clear that they are insufficient taxonomic characteristics and not sufficient to decide on the assignment of bacteria to a particular genus. Non-motile spherical purple sulfur bacteria without gas vesicles, for example, had been united within the genus *Thiocapsa*. However, genetic relationships indicate that two of the three known species (*Thiocapsa halophila* and *Thiocapsa pfennigii*) are not at all related to the type species of this genus, which is *Thiocapsa roseopersicina*. On the other hand, some of the spherical cells containing gas vesicles (former *Amoebobacter* species) are now also recognized as members of this genus, indicating that the presence of gas vesicles should no longer be a genus determinative property either. Nonetheless, one of the major phylogenetic clusters of *Chromatiaceae* is constituted out of spherical cells without flagellar movement and with or without gas vesicles (Fig. 1; Guyoneaud *et al.*, 1998).

**Table 3.** Differential characteristics of genera and species of the *Chromatiaceae* family

Genus and new species name	Motile	Gas vesicles	Cell form and size ( $\mu\text{m}$ )	Optimum salinity (%)	$T_{\text{opt}}$ ( $^{\circ}\text{C}$ )	Vitamins required	G + C content (mol %)	Chemoautotrophy
<b><i>Halochromatium</i></b>	Yes	No	Rod					
<i>Halochromatium salexigens</i>			2.0–2.5	10	20–30	B <sub>12</sub>	64.6	Yes
<i>Halochromatium glycolicum</i>			0.8–1.0	2.5–6.0	30–35	None	66.3	Yes
<b><i>Thiohalocapsa</i></b>	No	No	Sphere					
<i>Thiohalocapsa halophila</i>			1.5–2.5	5–7	20–30	B <sub>12</sub>	65.9–66.6	Yes
<b><i>Thiococcus</i></b>	No	No	Sphere					
<i>Thiococcus pfennigii</i>			1.2–1.5	1–2	20–35	None	69.4–69.9	No
<b><i>Rhabdochromatium</i></b>	Yes	No	Rod					
<i>Rhabdochromatium marinum</i>			1.5–1.7	1.5–5	30	None	60.4	No
<b><i>Isochromatium</i></b>	Yes	No	Rod					
<i>Isochromatium buderi</i>			3.5–4.5	1–3	25–30	B <sub>12</sub>	62.2–62.8	No
<b><i>Thiorhodococcus</i></b>	Yes	No	Sphere					
<i>Thiorhodococcus minor</i>			1.0–2.0	2	30–35	None	66.9	Yes
<b><i>Marichromatium</i></b>	Yes	No	Rod					
<i>Marichromatium gracile</i>			1.0–1.3	2–3	30–35	None	68.9–70.4	Yes
<i>Marichromatium purpuratum</i>			1.2–1.7	2–7	25–30	o	68.9	o
<b><i>Thiorhodovibrio</i></b>	Yes	No	Spiral					
<i>Thiorhodovibrio winogradskyi</i>			0.8–1.4	2–3	33	None	61.0–62.4	Yes
<b><i>Allochromatium</i></b>	Yes	No	Rod					
<i>Allochromatium vinosum</i>			2.0	o	30–35	None	61.3–66.3	Yes
<i>Allochromatium minutissimum</i>			1.0–1.2	None	30–35	None	63.7	Yes
<i>Allochromatium warmingii</i>			3.5–4.0	None	25–30	B <sub>12</sub>	55.1–60.2	No
<b><i>Thermochromatium</i></b>	Yes	No	Rod					
<i>Thermochromatium tepidum</i>			1.0–2.0	None	48–50	None	61.5	No
<b><i>Chromatium</i></b>	Yes	No	Rod					
<i>Chromatium okenii</i>			4.5–6.0	None	25–30	B <sub>12</sub>	48.0–50.0	No
<i>Chromatium weissei</i>			3.5–4.0	None	25–30	B <sub>12</sub>	48.0–50.0	No
<b><i>Thiocystis</i></b>	Yes	No	Sphere/rod					
<i>Thiocystis gelatinosa</i>			3.0	None	30	None	61.3	Yes
<i>Thiocystis minor</i>			2.0	None	30	None	62.2	Yes
<i>Thiocystis violacea</i>			2.5–3.5	o	25–35	None	62.8–67.9	Yes
<i>Thiocystis violascens</i>			2.0	o	30–35	None	61.8–64.3	Yes
<b><i>Thiocapsa</i></b>	No	No	Sphere					
<i>Thiocapsa roseopersicina</i>			1.2–3.0	o	20–35	None	63.3–66.3	Yes
<i>Thiocapsa rosea</i>			2.0–3.0	None	20–35	B <sub>12</sub>	64.3	Yes
<i>Thiocapsa pendens</i>			1.5–2.5	None	20–35	B <sub>12</sub>	65.3	No
<b><i>Thiolamprovum</i></b>	No	Yes	Sphere					
<i>Thiolamprovum pedioforme</i>			2.0	None	37	(B <sub>12</sub> )	65.5	Yes
<b><i>Amoebobacter</i></b>	No	Yes	Sphere					
<i>Amoebobacter purpureus</i>			1.9–2.3	None	23–25	o	63.5	Yes
<b><i>Thiodictyon</i></b>	No	Yes	Rod					
<i>Thiodictyon elegans</i>			1.5–2.0	None	20–25	None	65.3	No
<i>Thiodictyon bacillosum</i>			1.5–2.0	None	20–30	None	66.3	No
<b><i>Lamprobacter</i></b>	Yes	Yes	Rod					
<i>Lamprobacter modestohalophilus</i>			2.0–2.5	1–2	25–27	B <sub>12</sub>	64.0	Yes
<b><i>Lamprocystis</i></b>	Yes	Yes	Sphere					
<i>Lamprocystis roseopersicina</i>			3.0–3.5	None	20–30	None	63.8	No
<b><i>Thiospirillum</i></b>	Yes	No	Spiral					
<i>Thiospirillum jenense</i>			2.5–4.5	None	20–25	B <sub>12</sub>	45.5	No
<b><i>Thiopedia</i></b>	No	Yes	Sphere					
<i>Thiopedia rosea</i>			1.0–2.0	None	20	B <sub>12</sub>	62.5–63.5	No

### Physiological properties

Physiological properties always have been important characteristics in taxonomic considerations, although their taxonomic significance is ambiguous. On the one hand, physiological properties of the *Chromatiaceae* are quite conservative in regard to the use of sulfur sources and the restricted use of organic carbon sources. All species are able to grow photoautotrophically under anoxic conditions in the light using sulfide or elemental sulfur as an electron donor. On the other hand, we distinguish two major physiological groups, versatile and specialized species. The specialized species depend on strictly anoxic conditions and are obligately phototrophic. Sulfide is required, thiosulfate and hydrogen are not used as electron donors. Only acetate and pyruvate (or propionate) are photoassimilated in the presence of sulfide and CO<sub>2</sub>. These bacteria do not grow with organic electron donors and chemotrophic growth is not possible. None of these species assimilates sulfate as a sulfur source. Among these species are *Chromatium okenii*, *Chromatium weissei*, *Chromatium warmingii*, *Chromatium buderi*, *Thiospirillum jenense* and *Thiocapsa pfennigii*. The versatile species photoassimilate a greater number of organic substrates. Most of them are able to grow with sulfate and in the absence of reduced sulfur sources with organic substrates as electron donors for photosynthesis. Some species even grow as chemoautotrophs, and a few species can grow chemoheterotrophically in addition (Gorlenko, 1974; Kondratieva *et al.*, 1976; Kampf & Pfennig, 1980, 1986). Among these species are several small-celled *Chromatium* species, *Thiocystis violacea*, *Thiocapsa roseopersicina*, *Thiocapsa rosea* (formerly *Amoebobacter roseus*), *Thiocapsa pendens* (formerly *Amoebobacter pendens*) and *Lamprobacter modestohalophilus*.

Although some specialized species, such as *Chromatium okenii*, *Chromatium buderi* and *Thiocapsa pfennigii* form well-separated genetic lines, others like *Chromatium warmingii* are moderately related to the versatile *Chromatium vinosum*, justifying their inclusion into one and the same genus. Therefore, at present the versatility versus specialization of the species appears not to be a good taxonomic criterion.

### Salt relationships

Though salt dependence has been used already in the past to distinguish between species and even genera, the phylogenetic and taxonomic importance of this property has so far not been fully recognized. Major phylogenetic branches within the *Chromatiaceae* can be distinguished on the basis of their salt requirement (Fig. 1, Table 2), one exclusively containing marine and halophilic species, the other primarily freshwater isolates. This implies principally that a separate evolutionary development has occurred in the marine and in the freshwater environments and that therefore the salt response should be considered as an important

taxonomic criterion. The salt requirement apparently is of general taxonomic importance in phototrophic bacteria and already has been considered in the taxonomy of purple non-sulfur bacteria and *Ectothiorhodospiraceae*. It proved to be of relevance to distinguish the genera *Halorhodospira* and *Ectothiorhodospira* (Imhoff & Siling, 1996), *Rhodovulum* and *Rhodobacter* (Hiraishi & Ueda, 1994) and also genera of the spiral-shaped purple non-sulfur  $\alpha$ -*Proteobacteria*, the former *Rhodospirillum* species (Imhoff *et al.*, 1998). Furthermore, phylogenetic relationships of the *Chlorobiaceae* indicate that salt relationships may be a useful criterion also for these bacteria (Overmann & Tuschak, 1997).

Genetic relationships of the *Chromatiaceae* (Fig. 1, Table 2) revealed the obvious divergence of freshwater and saltwater bacteria. In particular the so far known genera *Chromatium* and *Thiocapsa* have representatives in both of these phylogenetic branches. As a consequence thereof, the salt relation should be considered as property for characterization of 'genetically defined' genera. Thus, both the genetic relationships and the salt responses enable differentiation of the halophilic *Chromatium* species *Chromatium salexigens* and *Chromatium glycolicum* from the marine species *Chromatium gracile* and *Chromatium purpuratum*, and from the freshwater species of *Chromatium vinosum* and relatives. Although salt relationships obviously are important properties for the taxonomic characterization of *Chromatiaceae*, in a few species the congruence between their phylogenetic position and their salt response requires further attention. Species such as *Chromatium vinosum* and *Thiocapsa roseopersicina* have been reported to be found in freshwater, brackish water and marine habitats. In regard to *Thiocapsa roseopersicina*, sequence relationships indicate that a number of strains isolated from the marine environment and assigned to this species could be distinguished at the species level (Guyoneaud *et al.*, 1998). These bacteria behave indifferently to low salt concentrations and may be poorly adapted to the marine environment, being halotolerant but not halophilic. In regard to *Chromatium vinosum* the situation is unclear at present. Large differences in the G+C content of the DNA of strains assigned to this species (61.3–66.3 mol%) may indicate genetic heterogeneity. So far, clear proof of identity of various isolates from marine habitats assigned to *Chromatium vinosum* on the species level is lacking. Further studies will be needed to determine whether differences in the G+C content are correlated with the occurrence of these bacteria in marine or freshwater environments.

### DNA base ratio

The DNA base ratio, expressed as the G+C content, which always has been regarded as a taxonomically important parameter, is gaining new attention in view of a genetically oriented taxonomic system. It has been found to be highly variable in some of the known *Chromatiaceae* genera (e.g. values from 48.0 to



70.4 mol% G + C in the genus *Chromatium*). However, it is assumed that to a large extent the intrageneric and also the intraspecies variability represents the non-phylogenetic nature of the current taxonomic system and misclassification of strains, respectively. A more natural system would be expected to group bacteria together that have much less variation in their G + C content. Indeed, genetically closely related species of the *Chromatiaceae* in general have a quite narrow range of their G + C content, which is approximately 63–66 mol% in the *Thiocapsa/Amoebobacter* cluster, between 61–64 mol% in the proposed *Thiocystis* group (with the exception of a few strains of *Thiocystis violacea*), at 69–70 mol% in the proposed *Marichromatium* species and at 64–66 mol% in the proposed *Halochromatium* species. In the true *Chromatium* species it is as low as 48–50 mol%. A few species, *Chromatium vinosum* (61.3–66.3 mol%), *Chromatium warmingii* (55.1–60.2 mol%), and *Thiocystis violacea* (62.8–67.9 mol%) have surprisingly high intraspecies variation of their G + C content. Further studies are needed to clarify whether analytical problems have caused these variations or misclassification of some strains is indicated. Variation at the intraspecies level of more than 5 and up to 10 mol% G + C content of the DNA is by far beyond an acceptable level of natural variation within bacteria as closely related as to justify their classification into one and the same species.

#### Temperature response

The temperature response of many species is not well-resolved and in most cases probably of minor significance for taxonomic purposes. However, as far as the temperature extremes are concerned, specific adaptation mechanisms are involved, specific environmental niches are inhabited and a different evolutionary background can be expected. Therefore, it is very well indicated to taxonomically consider the successful adaptation to the extremes of temperature. The only species of the *Chromatiaceae* so far known, which exhibits a very high optimum temperature response is *Chromatium tepidum* (optimum at 48–50 °C), which also is genetically distinct from other species within the cluster of freshwater species.

#### Molecular parameters

Though only scattered information is available on fatty acid and quinone composition of *Chromatiaceae*, it appears that these components, which are well-accepted tools for identification and taxonomic differentiation of phototrophic bacteria (Imhoff, 1984b, 1991; Imhoff & Bias-Imhoff, 1995; Thiemann & Imhoff, 1996), show minor variation and are of low significance for taxonomic differentiation within the *Chromatiaceae* (Imhoff & Bias-Imhoff, 1995). However, the polar lipid composition, that has been analysed so far only in several freshwater species, despite surprisingly similar patterns in genetically related species, shows significant variation between

groups of species (J. F. Imhoff, unpublished results). For example, *Chromatium vinosum* and *Chromatium warmingii*, two species of the proposed new genus *Allochromatium*, contain the same lipids. Their lipid composition is different from a second group of species, the proposed members of the genus *Thiocystis* (*Chromatium minus*, *Chromatium violascens* and the two known *Thiocystis* species), which also show uniformity in this property. In contrast, the lipid composition of *Thiocapsa pfennigii* is different from all other so far analysed phototrophic purple bacteria (J. F. Imhoff, unpublished results). Thus, the pattern of polar lipid composition, as far as known, is congruent with the sequence-based similarity of these bacteria and may well turn out to be a significant property to distinguish genetically related groups of *Chromatiaceae* in the future. More analytical data are required to support this concept.

Despite these first attempts to define phenotypic properties that are congruent with the genetic relationships of the purple sulfur bacteria, the search for more of such properties must be continued and those properties that are of relevance must be analysed in all known species. The differentiation of the *Chromatiaceae* species on the basis of some diagnostic properties is shown in Table 3.

#### Proposed taxonomic changes

On the basis of their genetic relationships and in consideration of the preceding discussion on the relevance of various phenotypic properties, the following taxonomic changes are proposed:

- (1) *Chromatium minus* and *Chromatium violascens* are most closely related to *Thiocystis gelatinosa*, but different from this bacterium at the species level. Therefore, we propose to transfer these species to the genus *Thiocystis* with the new names *Thiocystis minor* comb. nov. and *Thiocystis violascens* comb. nov., respectively.
- (2) *Chromatium vinosum*, *Chromatium minutissimum* and *Chromatium warmingii* are closely related species that should be placed into a new genus, because of their genetic distance from *Chromatium okenii*. Because *Chromatium vinosum* is among the best-studied purple sulfur bacteria and the name *Chromatium* is very much associated with this species, we propose the name *Allochromatium* gen. nov. ('the other *Chromatium*') for the new genus, with *Allochromatium vinosum* comb. nov. as the type species and *Allochromatium minutissimum* comb. nov. and *Allochromatium warmingii* comb. nov. as additional species of the genus.
- (3) Because *Chromatium tepidum* quite obviously can not be retained within the genus *Chromatium* and it is neither specifically related to *Thiocystis* nor to *Allochromatium*, it should also be placed in a new genus. The 16S rDNA sequence data justify this conclusion. *Chromatium tepidum* is the only thermophilic isolate of the purple sulfur bacteria described so

far. Although it is not well-established whether and to what extent the temperature response is of phylogenetic and taxonomic significance, we regard the thermophilic nature and high temperature optimum of this species as a valid criterion for genus differentiation and propose *Thermochromatium* gen. nov. as the new genus name and *Thermochromatium tepidum* comb. nov. as the new name for this species.

(4) *Chromatium salexigens* and *Chromatium glycolicum* shall be transferred to the new genus *Halochromatium* gen. nov. because of their extended salt dependence and salt tolerance, which is the highest within the *Chromatiaceae*, with *Halochromatium salexigens* comb. nov. as the type species and *Halochromatium glycolicum* comb. nov. as an additional species.

(5) *Chromatium gracile* and *Chromatium purpuratum* are transferred to the new genus *Marichromatium*. The name is proposed according to the marine origin and the moderate salt dependence that is characteristically found in typical marine bacteria. The new names *Marichromatium gracile* comb. nov. and *Marichromatium purpuratum* comb. nov. are proposed for these two species. *Marichromatium gracile* is the proposed type species.

(6) By its intracytoplasmic membrane system and its pigment composition, *Thiocapsa pfennigii* is unique among the family *Chromatiaceae* (Eimhjellen *et al.*, 1967; Eimhjellen, 1970). Certainly, it can no longer be regarded as a species of the genus *Thiocapsa*, because it is significantly different from this genus on the basis of the available genetic sequence information and belongs to the cluster of the halophilic *Chromatiaceae* species. Its phenotypic traits together with the results on its genetic relationships to other *Chromatiaceae* led us to consider this species as a separate genus. According to Rule 33c of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992), we propose to revive the genus name *Thiococcus* Eimhjellen *et al.* 1967 and to transfer *Thiocapsa pfennigii* to *Thiococcus pfennigii* gen. nom. rev., comb. nov. The name *Thiococcus*, originally used by Eimhjellen *et al.* (1967) to describe this bacterium, was not validly published and is illegitimate and therefore was not included into the Approved Lists of Bacterial Names.

(7) Phylogenetically, the bacterium described as *Thiocapsa halophila* is not related to the genus *Thiocapsa* and is most similar on the basis of its 16S rDNA sequence to *Thiocapsa pfennigii*, *Chromatium salexigens* and *Chromatium glycolicum* (Fig. 1). Because of significant genotypic and phenotypic properties to these bacteria, it should be recognized as a separate and new genus. *Thiohalocapsa* gen. nov. is proposed as the new genus name and *Thiocapsa halophila* is transferred to the new genus as *Thiohalocapsa halophila* comb. nov.

(8) *Chromatium buderi* is a marine species of the *Chromatiaceae*. However, according to its 16S rDNA sequence it is dissimilar to any of the other described

species of this branch to a degree that suggests its recognition as a separate genus for which the name *Isochromatium* is proposed. *Chromatium buderi* is transferred to this new genus as *Isochromatium buderi* comb. nov.

#### Emended description of the genus *Chromatium* Perty 1852, 174<sup>AL</sup>

*Chromatium* (Chro.ma'ti.um. Gr. n. *chroma* colour; M.L. neut. n. *Chromatium* the one which is coloured).

Cells are straight or slightly curved rods, more than 3 µm wide, motile by a polar tuft of flagella. Cells occur singly or in pairs. Multiply by binary fission. Gram-negative. Contain internal photosynthetic membrane systems of vesicular type in which the photosynthetic pigments bacteriochlorophyll *a* and carotenoids are located. Obligately phototrophic, strictly anaerobic, require sulfide-reduced media. Under anoxic conditions capable of photolithoautotrophic growth with sulfide or elemental sulfur as electron donor. Elemental sulfur is formed as an intermediate oxidation product and stored in the form of highly refractile globules inside the cells. Sulfate is the ultimate oxidation product. In the presence of sulfide and bicarbonate, able to photoassimilate only a few simple organic compounds such as acetate and pyruvate. Assimilatory sulfate reduction is lacking. Vitamin B<sub>12</sub> is required. Mesophilic with optimal growth temperatures of 20–30 °C, freshwater bacteria without a distinct requirement for salt. Habitat: ditches, ponds and lakes with stagnant freshwater containing hydrogen sulfide and exposed to light. The G+C content of the DNA is 48.0–50.0 mol% (Bd). Type species is *Chromatium okenii* (Ehrenberg 1838, Perty 1852, 174<sup>AL</sup>). Most likely also *Chromatium weissei* is a species of this genus.

#### Emended description of the genus *Thiocystis* Winogradsky 1888, 60<sup>AL</sup>

*Thiocystis* (Thi.o.cys'tis. Gr. n. *thios* sulfur; Gr. n. *cystis* the bladder, a bag; M.L. fem. n. *Thiocystis* sulfur bag).

Cells are rod-shaped or ovoid to spherical, before cell division often diplococcus-shaped. Cells occur singly or in pairs, may grow in irregular aggregates surrounded by slime. Multiply by binary fission, motile by means of flagella. Gram-negative. Contains internal photosynthetic membrane systems of vesicular type in which the photosynthetic pigments bacteriochlorophyll *a* and carotenoids are located. Under anoxic conditions, cells are capable of photolithoautotrophic growth with sulfide or sulfur as electron donor, thiosulfate may also be used. During sulfide oxidation, globules of elemental sulfur are transiently stored inside the cells. The final oxidation product is sulfate. In the presence of sulfide and bicarbonate, several simple organic substrates are photoassimilated. Facultatively chemoautotrophic or mixotrophic under micro-oxic to oxic conditions. Mesophilic bacteria

with good growth at 25–35 °C, pH 6.5–7.6. Principally freshwater bacteria, several of which may be found also in brackish and marine environments. Habitat: anoxic sulfide-containing water and mud of freshwater ponds and lakes as well as brackish water and marine environments exposed to light. The G+C content of the DNA is 61.3–67.9 mol% (Bd). Type species is *Thiocystis violacea* Winogradsky 1888, 65<sup>AL</sup>. Other species are *Thiocystis gelatinosa*, *Thiocystis minor* and *Thiocystis violascens*.

#### Description of *Thiocystis minor* comb. nov.

*Thiocystis minor* (*Chromatium minus* Winogradsky 1888, 99<sup>AL</sup>) (mi'nor. L. fem. comp. adj. *minor* less, smaller). The description is the same as that for *Chromatium minus* (Winogradsky, 1888; Pfennig & Trüper, 1989).

#### Description of *Thiocystis violascens* comb. nov.

*Thiocystis violascens* (*Chromatium violascens* Perty 1852, 174<sup>AL</sup>) (vi.o.las'cens. L. part. *violascens* becoming violet). The description is the same as that for *Chromatium violascens* (Perty, 1852; Pfennig & Trüper, 1989).

#### Description of *Thiococcus* gen. nom. rev.

*Thiococcus* (Thi.o.coc'cus. Gr. n. *thios* sulfur; L. masc. n. *coccus* sphere; M.L. masc. n. *Thiococcus* sphere with sulfur). The genus name *Thiococcus* was proposed for this bacterium by Eimhjellen *et al.* (1967). However, this name has never been validly published and the organism was validly named *Thiocapsa pfennigii* (Eimhjellen, 1970; Approved Lists of Bacterial Names, 1980). The name *Thiococcus* is revived according to Rule 33c of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992).

Cells are spherical with diplococcus-shaped division stages. Multiply by binary fission. Gram-negative. Internal photosynthetic membrane system consisting of bundles of ribbonlike branched tubes continuous with the cytoplasmic membrane. Individual cells colourless, colour of cell suspensions yellowish to orange-brown. Photosynthetic pigments are bacteriochlorophyll *b* and carotenoids. Obligately phototrophic and strictly anaerobic. Capable of photolithoautotrophic growth with sulfide and sulfur as electron donor. Elemental sulfur globules are transiently stored inside the cells, final oxidation product is sulfate. In the presence of sulfide and bicarbonate organic substrates are photoassimilated. Good growth at 20–35 °C, pH 6.5–7.5. Salt is required for optimum growth. Habitat: brackish and marine waters and sediments containing hydrogen sulfide and exposed to the light. The G+C content of the DNA is 69.4–69.9 mol% (Bd). Type species is *Thiococcus pfennigii*.

#### Description of *Thiococcus pfennigii* comb. nov.

*Thiococcus pfennigii* (*Thiocapsa pfennigii* Eimhjellen 1970, 193<sup>AL</sup>) (pfen.nig'i.i. M.L. gen. n. *pfennigii* of Pfennig; named after Norbert Pfennig, a German microbiologist). The description is the same as that for *Thiocapsa pfennigii* (Eimhjellen, 1970; Pfennig & Trüper, 1989).

#### Description of *Thiohalocapsa* gen. nov.

*Thiohalocapsa* (Thi.o.ha.lo.cap'sa. Gr. n. *thios* sulfur; Gr. gen. n. *halos* of the salt; L. fem. n. *capsa* capsule; M.L. fem. n. *Thiohalocapsa* the sulfur capsule of the salt).

Cells are spherical, non-motile; multiply by binary fission. Individual cell surrounded by a strong slime capsule; cell aggregates may be formed. Gram-negative. Internal photosynthetic membrane system of vesicular type containing the photosynthetic pigments bacteriochlorophyll *a* and carotenoids. Under anoxic conditions capable of photoautotrophic growth with sulfide, elemental sulfur and possibly other electron donors. During oxidation of sulfide, globules of elemental sulfur are transiently stored inside the cells, final oxidation product is sulfate. Assimilatory sulfate reduction lacking. Chemoautotrophic or chemorganotrophic growth possible under micro-oxic to oxic conditions in the dark. Vitamin B<sub>12</sub> is required. Mesophilic bacteria with good growth at 20–30 °C, pH 6.5–7.5. Elevated concentrations of sodium chloride are required for growth. Habitat: sulfide-containing waters and sediments of saltern and other coastal habitats with elevated salt concentrations and exposed to light. The G+C content of the DNA is 65.9–66.6 mol% (Bd). Type species is *Thiohalocapsa halophila*.

#### Description of *Thiohalocapsa halophila* comb. nov.

*Thiohalocapsa halophila* (*Thiocapsa halophila* Caumette *et al.* 1991) (ha.lo'phi.la. Gr. n. *hals* salt; Gr. adj. *philos* loving; M.L. fem. adj. *halophila* salt-loving). The description is the same as that for *Thiocapsa halophila* (Caumette *et al.*, 1991).

#### Description of *Halochromatium* gen. nov.

*Halochromatium* (Ha.lo.chro.ma'ti.um. Gr. gen. n. *halos* of the salt; *Chromatium* a genus name; M.L. neut. n. *Halochromatium* the *Chromatium* of the salt).

Cells are straight to slightly curved rods, multiply by binary fission; motile by polar flagella. Cells occur singly or in pairs. Gram-negative. Contain internal photosynthetic membrane systems of vesicular type in which the photosynthetic pigments bacteriochlorophyll *a* and carotenoids are located. Under anoxic conditions in the light capable of photolithoautotrophic growth with sulfide or elemental sulfur as electron donor. Elemental sulfur is formed as an intermediate oxidation product and stored in the form of highly refractile globules inside the cells.

Sulfate is the ultimate oxidation product. Hydrogen may be used as electron donor. In the presence of sulfide and bicarbonate organic substrates can be photoassimilated. Chemolithotrophic and chemo-organotrophic under micro-oxic conditions in the dark. Not capable of assimilatory sulfate reduction. Mesophilic with optimal growth temperatures of 20–35 °C, optimum pH 7.2–7.6. Elevated salt concentrations are required for growth. Habitat: reduced sediments in salinas, microbial mats in hypersaline environments, and coastal lagoons with elevated salt concentrations, which are exposed to light. The G+C content of the DNA is 64.6–66.3 mol% (Bd, HPLC). Type species is *Halochromatium salexigens*. An additional species is *Halochromatium glycolicum*.

**Description of *Halochromatium salexigens* comb. nov.**

*Halochromatium salexigens* (*Chromatium salexigens* Caumette *et al.* 1988) (sal.ex'i.gens. L. n. *sal* salt; L. v. *exigo* demanding, to demand; M.L. part. adj. *salessigens* salt-demanding). The description is the same as that for *Chromatium salexigens* (Caumette *et al.*, 1988).

**Description of *Halochromatium glycolicum* comb. nov.**

*Halochromatium glycolicum* (*Chromatium glycolicum* Caumette *et al.* 1997) (gly.co'li.cum. M.L. neut. adj. *glycolicum* related to glycolate as a substrate). The description is the same as that for *Chromatium glycolicum* (Caumette *et al.*, 1997).

**Description of *Marichromatium* gen. nov.**

*Marichromatium* (Ma.ri.chro.ma'ti.um. L. gen. n. *maris* of the sea; *Chromatium* a genus name; M.L. neut. n. *Marichromatium* the *Chromatium* of the sea, the truly marine *Chromatium*).

Cells are straight to slightly curved rods, multiply by binary fission, motile by polar flagella, occur singly or in pairs or may stick together and form clumps. Gram-negative. Contain internal photosynthetic membrane systems of vesicular type in which the photosynthetic pigments bacteriochlorophyll *a* and carotenoids are located. Capable of photolithoautotrophic growth with sulfide or elemental sulfur as electron donor under anoxic conditions in the light. Elemental sulfur is formed as an intermediate oxidation product and stored in the form of highly refractile globules inside the cells. Sulfate is the ultimate oxidation product. Molecular hydrogen may be used as electron donor. In the presence of sulfide and bicarbonate, organic substrates can be photoassimilated. Chemolithotrophic and chemo-organotrophic growth may be possible under micro-oxic conditions in the dark. Mesophilic bacteria with optimal growth temperatures of 25–35 °C, pH 6.5–7.6. Optimum growth at salt concentrations typical of marine bacteria. Habitat: anoxic marine sediments and stagnant waters con-

taining hydrogen sulfide and exposed to light, marine sponges and other marine invertebrates. The G+C content of the DNA is 68.9–70.4 mol% (Bd). Type species is *Marichromatium gracile*. An additional species is *Marichromatium purpuratum*.

**Description of *Marichromatium gracile* comb. nov.**

*Marichromatium gracile* (*Chromatium gracile* Strzeszewski 1913, 321<sup>Al</sup>) (gra'ci.le. L. neut. adj. *gracile* thin, slender). The description is the same as that for *Chromatium gracile* (Strzeszewski, 1913; Pfennig & Trüper, 1989).

**Description of *Marichromatium purpuratum* comb. nov.**

*Marichromatium purpuratum* (*Chromatium purpuratum* Imhoff and Trüper 1980b, 601<sup>Vp</sup>) (pur.pur.a'tum. L. neut. adj. *purpuratum* dressed in purple). The description is the same as that for *Chromatium purpuratum* (Imhoff & Trüper, 1980a).

**Description of *Thermochromatium* gen. nov.**

*Thermochromatium* (Ther.mo.chro.ma'ti.um. Gr. adj. *thermos* hot; *Chromatium* a genus name; M.L. neut. n. *Thermochromatium* the hot *Chromatium*).

Cells are straight to slightly curved rods. Multiply by binary fission, motile most likely by polar flagella. Gram-negative. Contain internal photosynthetic membrane systems of vesicular type in which the photosynthetic pigments bacteriochlorophyll *a* and carotenoids are located. Obligately phototrophic, photolithoautotrophic growth with sulfide or elemental sulfur as electron donor under anoxic conditions in the light. Elemental sulfur is formed as an intermediate oxidation product and stored inside the cells. Sulfate is the ultimate oxidation product. In the presence of sulfide and bicarbonate, organic substrates can be photoassimilated. Thermophilic bacteria with optimal growth temperatures at 48–50 °C, pH optimum is 7.0. Freshwater bacteria without a requirement for significant levels of salt. Habitat: sulfide-containing hot springs of neutral to alkaline pH. The G+C content of the DNA is 60–63 mol% (Bd). Type species is *Thermochromatium tepidum*.

**Description of *Thermochromatium tepidum* comb. nov.**

*Thermochromatium tepidum* (*Chromatium tepidum* Madigan 1986) (te'pi.dum. L. neut. adj. *tepidum* lukewarm). The description is the same as that for *Chromatium tepidum* (Madigan, 1986).

**Description of *Allochromatium* gen. nov.**

*Allochromatium* (Al.lo.chro.ma'ti.um. Gr. adj. *allos* the other; *Chromatium* a genus name; M.L. neut. n. *Allochromatium* the other *Chromatium*).

Cells are straight to slightly curved rods. Multiply by binary fission, motile by polar flagella. Cells occur singly or in pairs. Gram-negative. Contain internal photosynthetic membrane systems of vesicular type in which the photosynthetic pigments bacteriochlorophyll *a* and carotenoids are located. Photo-lithoautotrophic growth with sulfide or elemental sulfur as electron donor under anoxic conditions in the light. Elemental sulfur is formed as an intermediate oxidation product and stored in the form of highly refractile globules inside the cells. Sulfate is the ultimate oxidation product. Molecular hydrogen may be used as electron donor. In the presence of sulfide and bicarbonate organic substrates can be photo-assimilated, potentially mixotrophic. Mesophilic bacteria with optimal growth temperatures of 25–35 °C, pH range 6.5–7.6. No distinct requirement for salt, except for strains that originate from the marine and brackish water environment and may tolerate or require low concentrations of salt. Habitat: ditches, ponds and lakes with stagnant freshwater containing hydrogen sulfide and exposed to light; also sewage lagoons, estuaries and salt marshes. The G + C content of the DNA is 55.1–66.3 mol% (Bd). Type species is *Allochromatium vinosum*. Other species are *Allochromatium minutissimum* and *Allochromatium warmingii*.

#### Description of *Allochromatium vinosum* comb. nov.

*Allochromatium vinosum* (*Monas vinosus* Ehrenberg 1838, 11; *Chromatium vinosum* Winogradsky 1888, 99<sup>AL</sup>) (vi.no'sum. L. neut. adj. *vinosum* full of wine). The description is the same as that for *Chromatium vinosum* (Winogradsky, 1888; Pfennig & Trüper, 1989).

#### Description of *Allochromatium minutissimum* comb. nov.

*Allochromatium minutissimum* (*Chromatium minutissimum* Winogradsky 1888, 100<sup>AL</sup>) (mi.nu.tis'si.mum. L. neut. sup. adj. *minutissimum* very small, smallest). The description is the same as that for *Chromatium minutissimum* (Winogradsky, 1888; Pfennig & Trüper, 1989).

#### Description of *Allochromatium warmingii* comb. nov.

*Allochromatium warmingii* (*Monas warmingii* Cohn 1875, 167; *Chromatium warmingii* Migula 1900, 1048<sup>AL</sup>) (war.min'gi.i. M.L. gen. n. *warmingii* of Warming; named after E. Warming, a Danish botanist). The description is the same as that for *Chromatium warmingii* (Migula, 1900; Pfennig & Trüper, 1989).

#### Description of *Isochromatium* gen. nov.

*Isochromatium* (I.so.chro.ma'ti.um. Gr. adj. *isos* equal, similar; *Chromatium* a genus name; M.L. neut. n.

*Isochromatium* the similar *Chromatium*, the bacterium similar to *Chromatium*).

Cells are straight to slightly curved rods, multiply by binary fission, motile by a polar tuft of flagella. Gram-negative. Contain internal photosynthetic membrane systems of vesicular type in which the photosynthetic pigments bacteriochlorophyll *a* and carotenoids are located. Obligately phototrophic and strictly anaerobic. Under anoxic conditions in the light capable of photolithoautotrophic growth with sulfide or elemental sulfur as electron donor. Elemental sulfur is formed as an intermediate oxidation product and stored in the form of highly refractile globules inside the cells. Sulfate is the ultimate oxidation product. In the presence of sulfide and bicarbonate organic substrates can be photoassimilated. Mesophilic bacteria with optimal growth temperatures of 25–35 °C, pH range 6.5–7.6. Salt is required for growth at concentrations typical for marine bacteria. Habitat: estuarine salt flats and salt marshes. The G + C content of the DNA is 62.2–62.8 mol% (Bd). Type species is *Isochromatium buderi*.

#### Description of *Isochromatium buderi* comb. nov.

*Isochromatium buderi* (*Chromatium buderi* Trüper and Jannasch 1968, 364<sup>AL</sup>) (bu'der.i. M.L. gen. n. *buderi* of Buder; named after J. Buder, a German plant physiologist). The description is the same as that for *Chromatium buderi* (Trüper & Jannasch, 1968).

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