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Description of *Labrenzia alexandrii* gen. nov., sp. nov., a new *Alphaproteobacterium* containing bacteriochlorophyll *a*, and consequences for the taxonomy of the genus *Stappia*

Hanno Biebl [1¶], Rüdiger Pukall [2], Heinrich Lünsdorf [1], Stefan Schulz[3], Martin Allgaier[1*], Brian J. Tindall [2] and Irene Wagner-Döbler [1]

[1]Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany

[2] DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

[3]Technical University of Braunschweig, Institut of Organic Chemistry, Braunschweig, Germany

Correspondence: Irene Wagner-Döbler, e-mail iwd@gbf.de

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* Present address: Leibniz-Institut für Gewässerökologie und Binnenfischerei (IGB), Alte Fischerhütte 2, 16775 Stechlin-Neuglobsow, Germany
¶ Present address: Heimstättenweg 10, 38126 Braunschweig, Germany

Summary

A slightly pink-coloured strain was isolated from single cells of the marine dinoflagellate *Alexandrium lusitanicum* and was found to contain the genes coding for two proteins of the photosynthetic reaction centre, *pufL* and *pufM.* 16S rRNA gene sequence analysis revealed that it belonged to the α-2 subgroup of the *Proteobacteria* and was most closely related to *Stappia aggregata* (97.7 % similarity), *S. alba* (98.0 %) and *S. marina* (98.0 %). Dark-grown cells of DFL-11^T contained small amounts of bacteriochlorophyll *a* (bchl *a*) and a carotenoid. They were rods 0.5-0.7 x 0.9-3.0 µm in size and motile by means of a single, subpolarly inserted flagellum. The strain was strictly aerobic and used a wide range of organic carbon sources, including fatty acids, tricarboxylic acid cycle intermediates and sugars. Biotin and thiamine were required as growth factors. Growth was obtained at sea salt concentrations between 1 and 10 %, a pH between 6 and 9.2 and a temperature up to 33 °C (optimum 26 °C). Nitrate was not reduced and indole was not produced from tryptophan. DFL11^T was resistant to potassium tellurite and transformed it to elemental tellurium. The major respiratory lipoquinone was ubiquinone 10 (Q10). The polar lipids comprised phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylcholine, an unidentified aminolipid, and the glycolipid sulphoquinovosyldiacylglyceride (SQDG). The fatty acids comprised 16:1ω7c, 16:0, 18:1ω7c, 18:0, 11-methyl-18:1ω6t, 11-methyl-20:1ω6t, 20:1ω7c, 22:0, 22:1 and the hydroxy fatty acids 3-OH 14:0, 3-OH 16:0 (ester linked), 3-OH 18:0, 3-OH 20:1 and 3-OH 20:0, all of which are amide linked. The G+C value was 56 %. Comparative analysis of α-2 subgroup 16S rRNA gene sequences showed

45 that the type species *S. stellulata* is only distantly related to *S. aggregata* (95.3 %).
46 Based on the combination of the 16S rDNA data, a detailed chemotaxonomic study
47 and the biochemical and physiological properties of members of the genera *Stappia*,
48 *Pannonibacter*, and *Roseibium* we propose transferring *S. aggregata, S. alba, S.*
49 *marina* and the new isolate DFL-11^T to a new genus. We propose the genus name
50 *Labrenzia*, with the type strain being represented by a novel species, *Labrenzia al-*
51 *exandrii* sp. nov., deposited as DSM 17067^T and NCIMB 14079^T. The *pufLM* genes
52 of the photosynthesis reaction center were shown to be present in some, but not all
53 species of the new genus *Labrenzia*, and they were identified for the first time in *S.*
54 *stellulata*. Additionally it was necessary to provide emended descriptions for the
55 genera *Pannonibacter*, *Roseibium*, and *Stappia*, in accord with data collected in this
56 study.
57

Introduction

More than 40 aerobic bacterial species have been described to date that synthesize bacteriochlorophyll *a* and are usually referred to as aerobic anoxygenic phototrophs (Yurkov & Beatty, 1998; Rathgeber *et al.,* 2004; Wagner-Döbler & Biebl, 2006). The majority of them belong to the α-3 and α-4 subgroups of the *Proteobacteria* including members of the typical marine genera *Roseobacter* (α-3) and *Erythrobacter* (α-4), although early reports also centred on members of the genus *Methylobacterium* (see Harashima *et al.,* 1989 for a review). A smaller group of four more or less extremophilic genera were assigned to the α-1 subgroup. Within the α-2 group, aerobic anoxygenic phototrophs were, until recently, represented only by members of the genus *Roseibium,* the strains of which were isolated from biological material from Shark Bay in West Australia (Shiba *et al.*, 1991; Nishimura *et al.*, 1994; Suzuki *et al.*, 2000).

During our search for aerobic marine bacteria that contained genes coding for the photosynthetic reaction centre, *pufL* and *pufM,* we obtained two groups of aerobic anoxygenic phototrophs that belonged to the α-2 subgroup of the *Proteobacteria* according to their 16S rRNA gene sequences (Allgaier *et al.*, 2003). One group was found to be closely related to members of the genera *Ahrensia* and *Hoeflea* and has been described (Biebl *et al.*, 2006). The other group, presently consisting of only one strain, was found to be related to *Roseibium* and *Stappia* and is characterized in the present study.

Methods

Cultivation. Two media were used for culturing the strains, both prepared with 20 g sea salts (Sigma) per litre: A complex medium containing 3 g Bacto peptone (Difco) and 0.5 g yeast extract (Difco) per litre for general use, and a mineral medium for substrate tests containing 0.3 g $(NH_4)_2SO_4$, 0.1 g KH_2PO_4, 1 ml trace element solution SL12 (Pfennig & Trüper, 1992), a suitable carbon source and 0.1 g yeast extract per litre or a vitamin solution (Biebl *et al.*, 2005). The pH was adjusted to 7.5 using 0.5 M H_2SO_4. Cultures were incubated at 30 °C in the dark if not otherwise indicated. Growth was measured turbidometrically at 650 nm. Influence of light and oxygen was tested in soft agar tubes (1 % agar) using the mineral medium with either acetate or glucose as substrates. Incubation was under aerobic and anaerobic conditions in the dark as well as in the light. A purple sulfur bacterium (*Rhodobacter veldkampii* DSM 11550) was used for comparison.

Type strains of reference taxa included *Stappia stellulata* (DSM 5886^T), *S. aggregata* (DSM 13394^T), *S. marina* (DSM 17023^T), *S. alba* (DSM 18320^T), *Roseibium denhamense* (JCM 10543^T), *R. hamelinense* (JCM 10544^T) and *Pannonibacter phragmitetus* (DSM 14782^T).

Electron microscopy. Cells in the mid-logarithmic growth phase were adsorbed onto carbon-formvar foils for 1 min. The cells were washed once with water, blotted and air-dried. They were shadow-cast at 15° elevation with platinum-carbon and analyzed with an energy-filtered transmission electron microscope (CEM902, Zeiss,

104 Oberkochem, Germany) as described by Golyshina *et al*. (2000). Cells were also
105 embedded and processed for sections as described by Yakimov *et al.* (1998).
106
107 **Physiological and biochemical tests.** Tests that required liquid cultures were
108 performed in 22.5 ml metal-capped test tubes containing 5 ml of medium. Sigma
109 sea salts were used for determination of the salt requirement in the complex me-
110 dium in concentrations up to 10.5 %. The temperature range for growth was deter-
111 mined using a gradient shaking incubator (Toyo Kogaku Sangyo Co. Ltd., Tokyo)
112 that allowed the temperature to be adjusted between 15 and 45 °C at intervals of 3 °C.
113 The pH range was tested at intervals of 0.5 units between pH 5.0 and 9.5 (initial
114 pH). Growth was measured at an early stage of the growth phase before the pH was
115 appreciably changed by growth. Carbon sources for substrate tests were supplied at
116 a concentration of 1 g/L. Requirement for vitamins was determined in mixtures of 7
117 vitamins (Biebl *et al*., 2005) in which one was omitted. The precultures were grown
118 in a medium without any growth factors. Degradation of polymers was tested on
119 agar plates using the complex medium. Starch was added at a concentration of 2
120 g/L, alginate at 7.5 g/L, Tween 80 (lipase test) and gelatine at 4 g/L. Starch
121 degradation was demonstrated with Lugol‘s solution, alginate degradation by clear
122 zones around the colonies, degradation of Tween 80 by the formation of the
123 insoluble calcium salts, and gelatine liquefaction by precipitation of undigested
124 gelatine with saturated ammonium sulfate solution. Nitrate reduction capacity was
125 checked in the 22.5 ml tubes with 10 ml of mineral medium supplemented with 0.4 g
126 $NaNO_3$ and 0.5 g yeast extract per litre. Nitrogen formation was demonstrated using
127 Durham tubes, formation of nitrite and consumption of nitrate with Merckoquant test
128 sticks (Merck). Presence of catalase, oxidase and indole formation was determined

(Merck). Presence of catalase, oxidase and indole formation was determined according to Gerhardt *et al.* (1981). The reaction to tellurite was tested either in complex medium or in the mineral medium using Na-acetate (1.37 g/L) as a carbon source. Analytical grade K_2TeO_4 was added aseptically to the autoclaved media from a sterile stock solution. Due to its limited solubility in seawater medium, in particular in acetate medium, K_2TeO_4 was added only up to a final concentration of 1 g/L. The media were then adjusted to pH 7.6 with sterile 1N NaOH and dispensed aseptically into sterile metal-capped test tubes in aliquots of 5 ml. Cultures were incubated at 30°C on a rotary shaker. Tellurium formation was recognized by black-brown to jet-black colouration of the culture.

Photosynthetic pigments. The photosynthetic pigments were extracted from 30 ml culture grown in complex medium in the dark. Bacterial cells were harvested by centrifugation and pigments were extracted with acetone-methanol (7:2). The absorption spectrum was recorded in a Shimadzu UV-3000 double beam spectrophotometer. Bacteriochlorophyll *a* (bchl *a*) absorption was measured at 772 nm after 1 h of incubation at room temperature in absolute darkness. An extinction coefficient of 75 mmol/1 cm (Clayton, 1963) was used for the calculation of bchl *a* concentration.

Fatty acids, respiratory lipoquinones and polar lipids. Lipoquinones and polar lipids were extracted and separated according to the methods described by Tindall (1990). The fatty acid composition was determined by the method described by Labrenz *et al.* (1999). The unusual fatty acids 11-methyl-18:1ω6t and 13-methyl-20:1ω6t were characterized by performing several microderivatizations on the

152 methyl ester extracts obtained by acidic methanolysis and GC/MS analysis. Hydro-
153 genation with Pd/C furnished saturated methyl esters carrying a methyl group at po-
154 sitions 11 or 13, respectively (Francke *et al*., 1989). Double bond positions were de-
155 termined by the formation of dimethyl disulfide adducts, showing double bonds at C-
156 12 or C-14 (Scribe *et al.,* 1988). Alternatively, formation of 3-pyridinemethanol was
157 performed, but the resulting mass spectra proved to be disappointing. They did not
158 allow unequivocal determination of the localization of the double bonds in the methyl
159 branched compounds (Harvey 1982). The configuration of the double bonds of 11-
160 methyl-18:1ω6t and the bishomolog (*E*)-13-methyleicosa-14-enoic acid (13-methyl-
161 20:1ω6t) were determined by comparison of gas chromatographic retention behav-
162 ior with published values of synthesized (*E*)- and (*Z*)-diastereomers of 11Me-18:1ω6
163 (Carballeira *et al*., 1998). While the (*E*)-diastereomer elutes slightly after 18:0 on an
164 apolar phase, the (*Z*)-diastereomer elutes before this acid. The 13-methyl-20:1ω6
165 present in the strains eluted slightly after 20:0, indicating the (*E*)-configuration of the
166 double bond.
167
168 **16S rRNA sequences and phylogenetic inferences**. DNA extraction, amplification
169 and sequencing of the 16S rRNA has been described (Allgaier *et al*., 2003). The
170 sequence was manually aligned and compared with published sequences from the
171 DSMZ 16S rDNA database, including sequences available from the Ribosomal Data
172 Project (Maidak *et al*., 2001) and EMBL. Manual alignment was constructed with the
173 BioEdit program (Hall, 1999) and used for calculating the distance matrix with the
174 DNAdist program. A phylogenetic dendrogram was inferred using the Neighbour-

175 Joining method contained in the PHYLIP package (Felsenstein, 1993). Bootstrap
176 analysis was based on 1000 re-samplings.
177
178 **Determination of the G+C content and amplification of the *pufLM* genes.** The G+C
179 content of the DNA was determined by HPLC (Mesbah *et al.*, 1989). Amplification of
180 the *pufLM* genes was performed as described (Allgaier *et al.,* 2003).
181

Results and Discussion

Morphological and physiological features.

Strain DFL-11^T was isolated from single, washed cells of the dinoflagellate *Alexandrium lusitanicum* using the culture ME207 of the Biological Institute of Helgoland (Germany). The cells were plated on agar prepared with tenfold diluted Marine Broth 2216 (Difco). After three weeks of incubation at room temperature under a natural dark-light regime, slightly pink colonies appeared that were transferred to undiluted Marine Agar 2216 and purified. One isolate, DFL-11^T, was maintained and further investigated.

The cells of strain DFL-11^T were rods of 0.5 - 0.7 x 0.9 – 3.0 µm size. Unequal ends in the longer cells were often observed (Fig.1). Aggregates, often star-shaped, occurred frequently and have been found in many taxa within the *Alphaproteobacteria*. The cells were motile by means of a single, subpolarly inserted flagellum (Fig. 2 and Suppl. Fig. 1). Ultrathin-sections showed a typical Gram-negative cell wall (Fig.2, inset). Colonies on Marine Agar 2216 appeared beige to slightly pink, almost transparent and smooth with an entire margin.

When grown in peptone / yeast extract medium at least 1% of artificial sea salt (Sigma) was required. No growth occurred at 0.5 % sea salts. The cultures had a broad growth optimum between 1 and 7 % sea salts. At 10 % sea salts, the onset of growth was delayed. Growth was found at a temperature between 15 and 34 °C, with an optimum at 26 °C. Below 20 °C, growth was very slow, and above 35 °C, no

205 growth occurred. The pH optimum was between pH 7.0 and 8.5, and slow growth
206 occurred down to pH 6.0 and up to pH 9.2. In mineral medium supplemented with
207 0.1 g/L yeast extract strain DFL-11^T utilized all organic carbon sources tested (see
208 species description) except for methanol, ethanol and glycerol. Yeast extract could
209 be replaced by the addition of biotin and thiamine (10 and 50 µg/L respectively).
210 Gelatine was hydrolysed by the strain, but not Tween 80 (lipase), starch or alginate.
211 Nitrate was not reduced. No growth occurred under anaerobic conditions in the light
212 when acetate or glucose was the substrate. Glucose was not fermented. The strain
213 was positive for catalase and oxidase and did not form indole from tryptophan.
214 Addition of 1 g/L sodium thiosulfate resulted in a higher yield of cell mass (22 % in a
215 mineral medium with 1 g/L acetate and 15 % in a peptone-based medium) suggest-
216 ing that oxidation of this sulfur compound provides additional energy for growth. In
217 contrast to the majority of Gram-negative bacteria, DFL-11^T is only moderately sen-
218 sitive to the rare earth salt potassium tellurite. Growth inhibition at 0.05 g/L tellurite
219 was 65 % in peptone medium and 35 % in mineral medium, and increased to 75 %
220 at 0.5 g/L tellurite in peptone medium (60 % in mineral medium). Elemental tellurium
221 was formed in small amounts in mineral medium, but not in peptone medium. This
222 mode of reaction to tellurite is similar to that described for *Roseococcus thiosulfato-*
223 *philus* by Yurkov *et al.* (1996).
224
225 **Photosynthetic pigments in strain DFL-11^T**
226
227 Using specific primers it had been shown that strain DFL-11 contained the photo-
228 synthetic reaction centre genes *pufL* and *pufM,* suggesting that the complete photo-

229 synthetic apparatus might be present. However, bacteriochlorophyll *a* (bchl *a*) could
230 not be detected initially (Allgaier *et al.*, 2003). Meanwhile, using higher amounts of
231 cell mass, bchl *a* was clearly demonstrated after extraction with acetone-methanol
232 (7:2). The bchl *a* content per cell mass was low in peptone-based medium (0.3
233 nmol/mg protein), but in the same range as found for other weakly pigmented aero-
234 bic bacteriochlorophyll producing bacteria (Sato, 1978; Yurkov *et al.*, 1993). The
235 absorption spectrum of the acetone-methanol extract showed the typical infra-red
236 peak of bchl *a* at 772 nm (Fig. 3). Absorption between 420 and 550 nm is due to a
237 carotenoid, probably spheroidenone as inferred from comparison with the absorp-
238 tion spectrum of *Dinoroseobacter shibae,* where this carotenoid has been identified
239 (Biebl *et al.*, 2005). Due to the low bchl *a* content it was not possible to obtain an
240 adequate *in vivo* absorption spectrum with the existing methods used to reduce light
241 scattering of the cells. However, infrared maxima were seen at about 800 and 865
242 nm.
243
244 **Presence of the *pufLM* genes of the photosynthetic reaction centre in *Stappia***
245 **and *Pannonibacter* species**
246
247 In order to obtain a better overview of the presence of the *pufLM* genes of the pho-
248 tosynthetic reaction centre we used specific primers and obtained a PCR product of
249 the correct length for DFL-11^T and S. *stellulata*, while no such product was found in
250 *S. aggregata* and *P. phragmitetus* (Fig. 5). The absence of the *pufLM* genes can be
251 interpreted as the complete lack of the ability to synthesize the photosynthetic reac-
252 tion centre, however their presence requires more cautious interpretation. Given the

253 fact that the presence of bchl *a* has not been described for *S. stellulata*, we ana-
254 lyzed acetone extracts of the cells photometrically and obtained a very tiny peak at
255 the expected wavelength, which shows that the cells are able to produce bchl *a,* al-
256 beit at low concentrations. Thus, their photosynthetic reaction centre can be as-
257 sumed to be functional. It has been shown previously for *Roseovarius tolerans* that
258 several strains of this species isolated from different depths were variable with re-
259 spect to the presence of bchl *a*, hence the genus name (Labrenz *et al*., 1999). How-
260 ever, PCR showed that the *pufLM* genes were present in all of these strains, and
261 some showed production of bchl *a* after several years of maintenance in culture
262 (Labrenz *et al.,* 1999, Allgaier *et al.,* 2003). Similarly, in *Hoeflea phototrophica*, in
263 spite of the presence of the *pufLM* genes, bchl *a* was initially not detected, but later
264 found in experiments at low nutrient levels and decreased salt concentrations (Biebl
265 *et al*., 2006). Thus, the expression of the photosynthetic reaction centre genes
266 seems to be highly dependent on environmental parameters in these aerobic bacte-
267 riochlorophyll *a* producing bacteria. It is presently unclear if the low amounts of bchl
268 *a* seen in DFL-11^T under standard cultivation conditions are representative of the
269 expression level in the natural marine environment. It has been shown in chemostat
270 culture of this strain - in which normally only trace amounts of bchl were detected -
271 that starvation periods in connection with illumination caused a drastic increase in
272 bchl production after nutrient supply and darkness were resumed (Biebl & Wagner-
273 Döbler, 2006). However, even under environmental stress the bchl *a* level was still
274 low in comparison to the levels found in species such as *Roseobacter denitrificans*,
275 where bacteriochlorophyll is clearly visible during the extraction of lipid material
276 (Tindall, unpublished), and *Erythromicrobium hydrolyticum* (Yurkov & van Gemer-

277 den,1993). The metabolic significance of this low level of bchl content is an open
278 question. However, slight energetic advantages (i.e. the ability to harvest light en-
279 ergy, even if not used for photoautotrophic growth) might play an important role un-
280 der the selective pressure of the open ocean environment.
281
282 The ability to synthesize the intact photosynthetic reaction center (based on the de-
283 tection of bchl *a* or of the *pufLM* genes) can vary between species that show a high
284 degree of 16S rRNA gene sequence similarity. In the group under consideration
285 here (see Table 1 and Fig. 5), bchl *a* is present in *S. marina* and DFL-11^T, but ab-
286 sent in *S. aggregata* and *S. alba.* Due to the high degree of similarity of these organ-
287 isms with respect to fatty acids, polar lipids, and morphological and physiological
288 characteristics, together with 16S rRNA gene sequence similarity values which lie
289 between 97.7 % and 99.1 %, we do not think that it is warranted to create new gen-
290 era based mainly/solely on the presence or absence (of trace amounts) of bchl *a*.
291 We encountered a similar case in the two species of the genus *Hoeflea*. *H. photo-*
292 *trophica* contained bchl *a*, and *H. marina* did not, although their 16S rRNA similarity
293 was 98.4 % and they were virtually identical in their chemical composition (Biebl *et*
294 *al.,* 2006). The *pufLM* genes coding for the photosynthetic reaction centre proteins
295 have been found on linear plasmids, leading to speculation that they may be trans-
296 ferred horizontally to related species (Pradella *et al.,* 2004). The taxonomic signifi-
297 cance of their presence should therefore be viewed in perspective with other traits.
298 Additional studies of more strains and species may support or refute our current
299 taxonomic hypothesis.
300

Respiratory lipoquinones, polar lipids and fatty acids

All strains produced ubiquinones, a characteristic feature of the majority of the *Alpha-*, *Beta-* and *Gammaproteobacteria*. The predominance of ubiquinone 10 as the single respiratory lipoquinone is a feature of the majority of the members of the *Alphaproteobacteria*.

Thin layer chromatograms of extracts of DFL-11^T showed the presence of phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylmonomethylethanolamine (PMME), sulphoquinovosyldiacylglyceride (SQDG) and an unidentified aminolipid (AL) (Fig. 6). A very similar pattern was found in the two *Roseibium* species, *S. aggregata*, *S. marina* and *S. alba*. In some of the strains, the presence of PE could not be unambiguously distinguished from possible slight tailing effects from the lipid which runs above it, PMME. PMME is known to arise as a result of methylation of PE, indicating that this latter lipid must be synthesized, even if it could not be unambiguously detected. Furthermore, it is not known whether growth conditions/growth phase may also affect the relative composition of PE and PMME. *P. phragmitetus* and *S. stellulata* could clearly be differentiated from the other strains studied since SQDG was not present, providing support for their classification as separate genera. The presence of the phospholipids PG, DPG, PC, PE and PMME is a feature typical of certain subgroups within the *Alphaproteobacteria*. The taxonomic significance of the polar lipid pattern including the presence/absence of lipids (e.g. SQDG or additional amino lipids) must be interpreted together with the fatty acid patterns.

325

326 The fatty acid composition was analysed for DFL-11^T in comparison to *S. stellulata*,
327 *S. aggregata*, *S. alba, S. marina, P. phragmitetus* and the two *Roseibium* species
328 (Table 2 and Supplementary Table A). In all species tested, 18:1ω7c was the main
329 component (49 to 60 %), which is true for virtually all members of the *Alphaproteo-*
330 *bacteria*, a fact usually missed in the majority of species descriptions relating to
331 members of this major evolutionary group. All strains contained 3-OH 14:0, an
332 ester linked fatty acid, probably located in the lipopolysaccharide (and not de-
333 rived from the polar lipid fraction). This fatty acid has also been reported in
334 members of the *Agrobacterium*/*Rhizobium*/*Ensifer* (formerly the genus *Si-*
335 *norhizobium*)/*Mesorhizobium* group (Tighe *et al.,* 2000; Quan *et al.,* 2005).
336 Additional 3-OH fatty acids included 3-OH 16:0, 3-OH 18:0, 3-OH 18:1, and 3-
337 OH 20:0, all of which appeared to be amide linked. The distribution of these 3-
338 OH fatty acids was such that 3-OH 16:0 appeared to be present in strain DFL-
339 11^T, *S. stellulata* and *P. phragmitetus*. Strain DFL-11^T was the only strain in
340 which the 3-OH 16:0 fatty acid appeared to be ester linked, like the 3-OH 14:0.
341 3-OH 18:0 was present in all strains examined (albeit in trace amounts in
342 strain DFL-11^T). 3-0H 18:1 was present in *S. stellulata* and *P. phragmitetus*
343 (trace amounts). All strains contained 3-OH derivatives of a C-20 fatty acid. In
344 most cases both the 3-OH 20:0 and 3-OH 20:1 derivatives were present, the
345 exceptions being *S. stellulata* (only 3-OH 20:0) and *P. phragmitetus* (only 3-
346 OH 20:1). In addition the majority of strains contained a 20:1ω7c fatty acid,

347 although the level in *P. phragmitetus* was very low and it was below the level
348 of detection in *S. stellulata.* It is interesting to note that 20:1ω7c has also been
349 reported in members of the genus *Nesiotobacter* (Donachie *et al.,* 2006), but is
350 apparently absent in members of the genus *Pseudovibrio* (Fukunaga *et al.,*
351 2006). These results taken together with the polar lipid patterns provided un-
352 ambiguous evidence for the chemical heterogeneity within the genus *Stappia*
353 as currently defined and indicated that the chemotaxonomy is a valuable pa-
354 rameter in delineating taxa within this group of organisms.
355
356 **New compound**
357
358 The unsaturated branched longchain fatty acid 11-methyl 18:ω6t has been previ-
359 ously identified in bacteria (Rotani *et al.,* 2005; Caballeira *et al.,* 1997; Couderc
360 1995; Kerger *et al.,* 1986). The bishomolog (*E*)-13-methyleicosa-14-enoic acid (11-
361 methyl 20:1ω6t), which was present in *S. aggregata* (3 %) and *R. denhamense* (2
362 %), has to the best of our knowledge not been reported before from nature.
363
364 **Taxonomic position of strain DFL-11^T and reorganization of the genus *Stappia***
365
366 Fig. 4 shows the taxonomic position of strain DFL-11^T as revealed by neighbour join-
367 ing analysis of the 16S rRNA gene sequence alignment. High similarity was found
368 with *S. aggregata* ATCC 25650^T (= IAM 12614^T = DSM 13394^T), *S. alba* DSM
369 18320^T *and S. marina* DSM 17023^T (97.7 %, 98.0 % and 98.0 %, respectively), and

somewhat lower similarity to *R. denhamense* JCM 10543^T (96.1 %) and *R. hamelinense* JCM 10544^T (97.1 %). A lower degree of relatedness was exhibited with the non-bchl-containing species from a soda lake, *P. phragmitetus* (Borsodi *et al.*, 2003) (95.0 % similarity) and still less with *S. stellulata* (94.3 %). DFL-11^T is distinct from the recently described marine genera *Nesiotobacter* and *Pseudovibrio* (Shieh *et al.,* 2004; Donachie *et al.,* 2006). The adjacent group of the α-2 *Proteobacteria* around *Mesorhizobium* was clearly separated (about 90 % similarity). The 16S rDNA sequence data clearly support the separation of DFL-11^T, *S. alba*, *S. marina* and *S. aggregata* from *S. stellulata*, the type species of the genus *Stappia.*

In Table 1 the morphological and physiological traits of DFL-11^T investigated are compared with those of the described *Stappia* and *Roseibium* species and with *Pannonibacter*. Differences exist with respect to flagellation, polar lipid distribution, occurrence of the photosynthetic pigments, reduction of nitrate, indole production, requirement for NaCl, and G+C content. Members of the genus *Roseibium* are characterized by peritrichous flagellation, while in all other species one or several flagella are inserted at the cell poles. They are also different from all other tested species through their ability to produce indole from tryptophan. The G+C content of the members of the genus Roseibiium is in the upper range for the group, i.e. 58 – 63 %, surpassed only by *Pannonibacte*r (65 %). By contrast, the G+C content of DFL-11^T is the lowest among the investigated species (56 %). Strain DFL-11^T also differs from members of the genus *Roseibium* (and all described *Stappia* species) since it is unable to reduce nitrate. The *coxL* genes have been identified in DFL-11^T (Gary King, pers. comm.) and in all *Stappia* species tested so far (King 2003); it is not known if they are present in *Roseibium* species. Photosynthetic pigments are

394 present in members of the genus *Roseibium* and have been demonstrated to be
395 present in small amounts in two of the four described *Stappia* species. In *S. marina*,
396 only the presence of the *pufLM* genes was detected.
397 The chemical composition of all the strains examined indicated that many of the fea-
398 tures present were typical of members of the *Alphaproteobacteria* (i.e. presence of
399 Q10 and the dominance of 18:1ω7c (+11,12- cyclopropane 19:0)), whereas other
400 features allowed finer differentiation, in particular the presence/absence of SQDG
401 among the polar lipids as well as the distribution and nature of the linkage of the 3-
402 OH fatty acids. In summary, one of the problems that we have encountered is the
403 fact that in the past polar lipid composition has not always been taken into consid-
404 eration when examining the chemotaxonomy of organisms within the *Roseibium-*
405 *Stappia* group. In addition, the analysis of the fatty acid composition of the strains
406 has not always been as comprehensive as in the studies undertaken here. In par-
407 ticular the longer chain 3-OH fatty acids have not been reported (although probably
408 present in the samples). In the case of the 3-OH 20:1 and 3-OH 20:0 the standard
409 identification system offered by MIDI does not include them, to date, in the peak
410 naming table. The relevance of the way the 3-OH fatty acids are linked to their par-
411 ent molecules (i.e. ester or amide linked) is an additional feature that allows differen-
412 tiation, although it is rarely used. Further studies are needed in order to determine
413 the nature of the parent molecule which gives rise to the amide linked 3-OH fatty
414 acids. The results also indicate that a thorough study of the chemotaxonomy of
415 novel taxa, either within or closely related to this group, is essential in any future
416 taxonomic study and is in line with recommendations made by two *ad hoc* subcom-
417 mittees (Wayne *et al*., 1987; Murray *et al*., 1990).

418

419 Based on these traits the close relationship of strain DFL-11^T to *S. aggregata* as
420 well as to *S. alba* and *S. marina* appears obvious. *S. stellulata* is only distantly re-
421 lated to *S. aggregata* (95.3 %), justifying the placement of the former group in a
422 separate genus. *S. stellulata* is the type species (Stapp & Klösel,1954) which must
423 be retained in the genus, and the circumscription emended, while the species *S.*
424 *aggregata*, *S. alba*, *S. marina* and DFL-11^T do not fit within that emended circum-
425 scription of the genus *Stappia* and must be placed in a different genus for which the
426 name *Labrenzia* is proposed. Accordingly strain DFL-11^T constitutes a new species
427 of this genus, to be designated *Labrenzia alexandrii*, and also serves as the type
428 species.
429 The description of *S. stellulata* must be extended with respect to the presence of the
430 *pufLM* and *coxL* genes, the ability to produce small amounts of bchl *a*, the lack of
431 SQDG, and the fatty acid composition. The new genus *Labrenzia* is differentiated
432 from the genus *Roseibium* through its flagellation, slightly lower G+C content, pres-
433 ence of the *coxL* genes, requirement for NaCl and lack of indole production, as well
434 as in the details of the chemotaxonomy. Bchl *a* may be present in small amounts.
435 The genus *Pannonibacter* must also be emended in order to cater for the presence
436 of phosphatidylcholine (not phosphatidylserine) and in details of the fatty acid com-
437 position.
438 We do not consider the presence or absence of trace amounts of bacteriochloro-
439 phyll or the presence/absence of the *pufL/M* genes to be a primary taxonomic
440 marker at the genus level in the organisms under study here, although we do not
441 dispute their potential role in the biology of the organisms concerned.

Emended description of the genus *Pannonibacter* Borsodi *et al.* 2003

In addition to the criteria given by Borsodi *et al.* (2003) the genus circumscription should be emended as follows. The polar lipid composition comprises phosphatidylglycerol, diphosphatidylglycerol, monomethylphosphatidylethanolamine, phosphatidyllcholine, an amino lipid and an unidentified lipid running close to the amino lipid and monomethylphosphatidylethanolamine. Phosphatidylethanolamine was not detected, but it is a precursor of monomethylphosphatidylethanolamine. Neither phosphatidylserine nor the glycolipid SQDG are present. The fatty acids comprise 16:1ω7c, 16:0, 18:1ω7c, 18:0, 11-methyl-18:1ω6t, 20:1ω7c, 22:0 and the hydroxy fatty acids 3-OH 14:0 (ester linked), 3-OH 16:0, 3-0H 18:1, 3-OH 18:0 and 3-OH 20:1, all of which are amide linked.

The type of the genus is *Pannonibacter phragmitetus* and it is the only species contained within this genus at present. The type strain C6/19 is deposited as DSM 14782^T and NCAIM B02025^T.

Emended description of the genus *Stappia* Uchino *et al.* 1999

The description of the genus *Stappia* is as given by Uchino *et al.* (1998) with the following additions: The polar lipid composition comprises phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, monomethylphosphatidylethanolamine, phosphatidyllcholine, and amino lipid. The glycolipid SQDG is absent. The fatty acids comprise 16:1ω7c, 16:0, 18:1ω7c, 18:0, 11-methyl-18:1ω6t, 20:1ω7c, 22:0, 22:1 and the hydroxy fatty acids 3-OH 14:0 (ester linked), 3-OH 16:0, 3-0H 18:1, 3-OH 18:0 and 3-OH 20:0, all of which are amide linked. The 3-OH

18:0 predominates over the 3-OH 18:1. As a result of the current emendation, the genus comprises a single species, *Stappia stellulata*, which is also the type species of the genus. Additonal features of the type strain: The *pufLM* genes of the photosynthesis reaction center and the *coxL* genes for oxidation of carbon monoxide are present. Acetone extracts of dark grown cells show a small peak at 772 nm, indicating the presence of bchl *a*.

The type strain is deposited as ATCC 15215^T, DSM 5886^T, CIP 105977^T and NBRC 15764^T.

Emended description of the genus *Roseibium* Suzuki *et al.* 2000

In addition to the criteria given by Suzuki *et al.* (2000) the genus circumscription should be emended as follows. The polar lipid composition comprises phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, monomethylphosphatidylethanolamine, phosphatidyllcholine, the glycolipid SQDG and amino lipid. The fatty acids comprise 16:0, 18:1ω7c, 18:0, 20:1ω7c and the hydroxy fatty acids 3-OH 14:0 (ester linked), 3-OH 18:0, 3-OH 20:0 and 3-OH 20:1, all of which are amide linked. The level of 20:1ω7c is usually in the range 7-11%. 11-methyl-18:1ω6t is produced by all species of the genus, and 11-methyl-20:1ω6t by some strains. The genus comprises *Roseibium denhamense* and *Roseibium hamelinense,* the former being the type species.

Emended description of the species *Roseibium denhamense* Suzuki *et al.* 2000.

The description is the same as that given by Suzuki *et al.* (2000) with the additions

that the polar lipid composition is consistent with that of the emended genus circumscription. The fatty acids comprise 16:1ω7c, 16:0, 18:1ω7c, 18:0, 11-methyl-18:1ω6t, 11-methyl-20:1ω6t, cyclopropane 19ω7, 20:1ω7c, 11-methyl-20:1ω6t, 22:1 and the hydroxy fatty acids 3-OH 14:0 (ester linked), 3-OH 18:0, 3-OH 20:1 and 3-OH 20:0, all of which are amide linked.

The type strain Och 254 is deposited as ATCC BAA-251^T, CIP 107047^T, JCM 10543^T and NBRC 16782^T.

Emended description of the species *Roseibium hamelinense* Suzuki *et al.* 2000

The description is the same as that given by Suzuki *et al.* (2000) with the additions that the polar lipid composition is consistent with that of the emended genus circumscription. The fatty acids comprise 16:0, 18:1ω7c, 18:0, 11-methyl-18:1ω6t, 11-methyl-20:1ω6t, 20:1ω7c and the hydroxy fatty acids 3-OH 14:0 (ester linked), 3-OH 18:0, 3-OH 20:1 and 3-OH 20:0, all of which are amide linked.

The type strain Och 368 is deposited as ATCC BAA-252^T, CIP 107048^T, JCM 10544^T and NBRC 16783^T.

Description of *Labrenzia* gen. nov.

Labrenzia (Lab.ren'zi.a, N.L. fem. noun derived from the name Labrenz honoring Dr. Matthias Labrenz, a German marine microbiologist who described many interesting bacterial isolates from hypersaline Ekho Lake, Antarctica, including three new genera of aerobic anoxygenic phototrophs, using a polyphasic approach).

513

514 Cells are Gram-negative rods. They are motile by means of one or several polarly
515 inserted flagella. Colonies are white to cream, but may become pink if incubated in
516 the dark at appropriate conditions. Ability to produce bchl *a* in small amounts may
517 be present. NaCl is required for growth. Optimum salinity range is 1 – 10 %. Opti-
518 mum pH is 7.0 – 8.5. They may be able to reduce nitrate to nitrite or to N_2. Growth is
519 chemoheterotrophic, non fermentative, under aerobic or anaerobic conditions. In-
520 dole is not produced. The major respiratory lipoquinone is Q10. The polar lipid com-
521 position comprises phosphatidylglycerol, diphosphatidylglycerol, phosphatidyletha-
522 nolamine, monomethylphosphatidylethanolamine, phosphatidyllcholine, the glycol-
523 ipid SQDG and an amino lipid. The fatty acids comprise 16:0, 18:1ω7c, 18:0, 11-
524 methyl-18:1ω6t, 20:1ω7c and the hydroxy fatty acids 3-OH 14:0, 3-OH 18:0 and 3-
525 OH 20:0, all of which are amide linked. The level of 20:1ω7c is usually in the range
526 5-10%. G+C content is 56 – 60 mol%.
527 At present the genus comprises four species, *L. alba*, *L. marina*, *L. aggregata* and
528 *L. alexandrii*, which also serves as the type species.
529

530 **Description of *Labrenzia alexandrii* sp. nov.**

531 *Labrenzia alexandrii* (a.le.xan'dri.i N.L. gen. n. *alexandrii*, of Alexandrium, the genus
532 name of the dinoflagellate *Alexandrium lusitanicum*, the source of isolation of the
533 type strain).
534 Gram-negative rods of 0.5-0.7 x 0.9-3.0 µm size, motile by means of a single sub-
535 polarly inserted flagellum. Cell ends often unequal. Star-shaped aggregates occur.
536 Colonies on Marine Agar 2216 (Difco) beige to slightly pink, almost transparent,

smooth and with entire margin. Strictly aerobic, non-fermentative heterotroph. Growth occurs within a salinity of 1 to 10 %, a temperature range of 15 to 34 °C (optimum 26 °C) and a pH between 6.0 and 9.2 (optimum 7.0 to 8.5). The type strain uses acetate, butyrate, succinate, fumarate, malate, citrate, glutamate, pyruvate, glucose and fructose, but not methanol, ethanol and glycerol. Biotin and thiamine are required as growth factors. Gelatine is hydrolyzed, but not starch, alginate or Tween 80. Nitrate is not reduced. Indole is not produced from tryptophan. Dark-grown cells contain small amounts of bacteriochlorophyll *a* and a carotenoid. Cells are weakly resistant to potassium tellurite. The chemical composition of the cells is consistent with that of the genus circumscription. In addition, cells contain 3-OH 16:0 (ester linked) as well as the following fatty acids: 16:0, 18:1w9c, 20:0, cyclo 21:0, 3-OH 20:1 and 22:1. The G+C content of the type strain is 56.1 %.

The type strain DFL-11^T (deposited as DSM 17067^T, NCIMB 14079^T) was isolated from cultured cells of the marine dinoflagellate *Alexandrium lusitanicum*.

Description of *Labrenzia aggregata* comb. nov.

Basonym: *Stappia aggregata* (ex Ahrens 1968) Uchino *et al.* 1999

The description is the same as that for *Stappia aggregata* (Uchino *et al.,* 1998) with the addition that the polar lipid composition is consistent with that of the genus circumscription. The fatty acids comprise 16:1ω7c, 16:0, 18:1ω7c, 18:0, 11-methyl-18:1ω6t, 20:1ω7c, 20:0 (trace amounts) and the hydroxy fatty acids 3-OH 14:0 (ester linked), 3-OH 18:0, 3-OH 20:1 and 3-OH 20:0, all of which are amide linked. The level of 20:1ω7c is usually in the range 5-6%.

The type strain B1 is deposited as ATCC 2560^T, DSMZ 13394^T, NBRC 16684^T,

561 LMG 122^T and NCIMB 2208^T.

562

563 **Description of *Labrenzia marina* comb. nov.**

564 Basonym: *Stappia marina* Kim *et al.* 2006

565 The description is the same as that given by Kim *et al.* (2006) with the addition that
566 the polar lipid composition is consistent with that of the genus circumscription. The
567 fatty acids comprise 16:1ω7c, 16:0, 18:1ω7c, 18:0, 11-methyl-18:1ω6t, 11-methyl-
568 20:1ω6t, cyclopropane 19ω7, 20:1ω7c, 11-methyl-20:1ω6t and the hydroxy fatty
569 acids 3-OH 14:0 (ester linked), 3-OH 18:0, 3-OH 20:1 and 3-OH 20:0, all of which
570 are amide linked.

571 The type strain mano 18 is deposited as DSM 17023^T and KCTC 12288^T.

572

573 **Description of *Labrenzia alba* comb. nov.**

574 Basonym: *Stappia alba* Pujalte *et al.* 2006

575 The description is the same as that given by Pujalte *et al.* (2005), with the additions
576 that the respiratory lipoquinone and polar lipid composition is consistent with that of
577 the genus circumscription. The fatty acids comprise 16:1ω7c, 16:0, 18:1ω7c, 18:0,
578 11-methyl-18:1ω6t, 11-methyl-20:1ω6t, 20:1ω7c, 20:0, 11-methyl-20:1ω6t and the
579 hydroxy fatty acids, 3-OH 14:0 (ester linked), 3-OH 18:0, and 3-OH 20:0, all of which
580 are amide linked.

581 The type strain 50M6 is deposited as DSM 18380^T, CECT 5095^T, CIP 108402^T.

582

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789 **Legends to the figures**

790

791 **Fig. 1.** Phase contrast photomicrograph of DFL-11^T cells mounted on agar. The
792 culture was grown in marine peptone medium. Unequal ends are highlighted by ar-
793 rows.

794

795 **Fig. 2.** Electron micrograph of *Labrenzia alexandrii* DFL-11^T. The shadow-casted
796 preparation shows the bacterium to be subpolarly flagellated, as is obvious from the
797 flattened cell body, which clearly shows the insertion (ins; a detailed view is shown
798 in the lower inset) of an individual flagellum (fl) in a dividing cell (s = septum). The
799 arrowhead at the bottom indicates the shadowing direction. The sectioned cell wall
800 (upper inset) shows Gram-negative architecture, as is indicated by the outer mem-
801 brane (om), murein (m), and cytoplasmic membrane (cm). Scale bars are indicated.

802

803 **Fig. 3.** Absorption spectrum of an acetone-methanol extract (7:2) of strain DFL-11^T.
804 A pellet from 165 ml dark-grown culture was extracted with1.5 ml solvent.

805

806 **Fig. 4.** Neighbour joining dendrogram of 16S rRNA gene relatedness showing the
807 phylogenetic position of *Labrenzia alexandrii* DFL-11^T within the *Alphaproteobacte-*
808 *ria*. Bootstrap values greater than 70 % confidence are shown at branching points
809 (percentage of 1000 replicates). The scale bar represents 1 substitution per 100
810 nucleotides. Sequence accession numbers are given in parentheses.

811

812 **Fig. 5.** Amplification by PCR of the *pufLM* genes (1.5 kb) of the photosynthesis
813 reaction centre in strain DFL-11^T, related strains, and described aerobic anoxygenic

814 phototrophs using *pufLM* specific primers. The identity of the amplified band has
815 been confirmed by sequencing for DFL-11^T, *Roseobacter litoralis* and *R. denitrifi-*
816 *cans* (Allgaier *et al.,* 2003). M molecular marker (GeneRuler 1 kb), 1, *Pannonibac-*
817 *ter phragmitetus* DSM 14782^T; 2, *Stappia aggregata* DSM 13394^T; 3, *S. stellulata*
818 DSM 5886^T; 4, *R. litoralis* DSM 6996^T; 5, *R. denitrificans* DSM 7001^T; 6, DFL-11^T; 7,
819 H_2O control.
820
821 **Figure 6.** Polar lipids of DFL-11^T and related species. 1, DFL-11^T; 2, *Stappia aggre-*
822 *gata* DSM 13394^T (Uchino *et al*., 1998; Rüger & Höfle, 1992); 3, *S. marina* DSM
823 17023^T (Kim *et al.,* 2006), 4, *S. alba* DSM 18320^T (Pujalte *et al.,* 2005); 5, *S. stellu-*
824 *lata* DSM 5886^T (Uchino *et al*., 1998; Rüger & Höfle, 1992); 6, *Pannonibacter*
825 *phragmitetus* DSM14782^T (Borsodi *et al*., 2003); 7, *Roseibium denhamense* JCM
826 10543^T (Suzuki *et al.,* 2000); 8, *R. hamelinense* JCM 10544^T (Suzuki *et al*., 2000).
827 AL aminolipid; DPG diphosphatidglycerol; PG phosphatodylglycerol; PC phosphati-
828 dylcholine; PE phosphatidylethanolamine; PMME phosphatidylmonomethyletha-
829 nolamine; SQDG sulphoquinovosyldiacylglyceride; ? unidentified compound.

Table 1. Differential chraracteristics of strain DFL-11^T compared to *Stappia, Pannonibacter* and *Roseibium* species.
Species: 1, DFL-11^T; 2, *S. aggregata* DSM 13394^T (Uchino *et al.,* 1998; Rüger & Höfle, 1992); 3, *S. marina* DSM 17023^T (Kim *et al*., 2006); 4, *S. alba* DSM 18320^T (Pujalte *et al*., 2005); 5, *S. stellulata* DSM 5886^T (Uchino *et al.,* 1998 ; Rüger & Höfle, 1992); 6, *P. phragmitetus* DSM 14782^T (Borsodi *et al*., 2003); 7, *R. denhamense* JCM 10543^T (Suzuki *et al*., 2000); 8, *R. hamelinense* JCM 10544^T (Suzuki *et al.,* 2000). + growth or positive reaction, (+) weak reaction, n.t. not tested.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
cell shape	rods, uneven ends	large rods, joined together	club-shaped	large rods	rods, star-shaped	long rods, star shaped	rods	rods
flagella	monotrichous	oligotrichous	monotrichous	monotrichous	monotrichous	monotrichous	peritrichous	peritrichous
surface cultures	faint pink	white to cream	n.t.	opaque	white to brown	beige	pink	pink
bchl *a in vivo* peak (nm)	801, 865	-	n.t.	-	(+)	-	804, 863	804, 863
***pufLM* genes**	+	-	+	n.t.	+	-	+	+
***coxL* genes**	+	+	+	n.t.	+	n.t.	n.t.	n.t.
sea salt range (%)	1 - 10	0.3 - 6	3 - 6	1 - 8	0.3 - 6	0 - 5	0.5 - 7.5	0 - 10
requirement for NaCl	+	+	+	+	+	-	+	-
reduction of nitrate to nitrite	-	+	+	+	+	+	+	+
reduction of nitrate to N_2	-	+	-	+	+	+	-	-
indole production	-	-	-	-	-	-	+	+
polar lipid SQDG	+	+	+	+	-	-	+	+
polar lipid PMME	+	+	+	+	+	+	+	+
G+C content (mol %)	56	59	60	n.t.	59	65	58 - 60	59 - 63
16S rRNA similarity to DFL-11 (%)	100.0	97.7	98.0	98.0	94.3	95.0	96.1	97.1

Table 2. Cellular fatty acids of DFL-11^T and related species as percent of all recorded acids. 1, DFL-11^T; 2, *S. aggregata* DSM 13394^T (Uchino *et al*., 1998; Rüger & Höfle, 1992); 3, *S. marina* DSM 17023^T (Kim *et al.,* 2006), 4, *S. alba* DSM 18320^T (Pujalte *et al.,* 2005); 5, *S. stellulata* DSM 5886^T (Uchino *et al*., 1998; Rüger & Höfle, 1992); 6, *P. phragmitetus* DSM14782^T (Borsodi *et al*., 2003); 7, *R. denhamense* JCM 10543^T (Suzuki *et al.,* 2000); 8, *R. hamelinense* JCM 10544^T (Suzuki *et al*., 2000). M1 shows ester-linked fatty acids, while M2 shows ester- and amide-linked fatty acids. Values given in bold face indicate taxonomically important fatty acids.

	1		**2**		**3**		**4**		**5**		**6**		**7**		**8**	
	M1	**M2**	**M1**	**M2**	**M1**	**M2**	**M1**	**M2**	**M1**	**M2**	**M1**	**M2**	**M1**	**M2**	**M1**	**M2**
unidentified 1	1.0	0.7	0.6	0.6	0.8	0.7	0.7	0.8	1.2	0.6	1.0	0.6	0.9	1.1	1.0	0.7
unidentified 2	1.0	0.5	0.8	0.5	0.5	0.5	0.5	0.4	1.0	0.6	1.5	0.7	0.9		0.7	0.4
unidentified 3		0.3		0.3			0.3	0.3				0.3			0.5	0.3
3-OH 14:0	**3.4**	**2.1**	**2.4**	**2.2**	**2.4**	**2.2**	**2.3**	**2.4**	**4.5**	**2.7**	**3.8**	**2.6**	**3.9**	**2.0**	**3.8**	**2.3**
16:1w7c			0.6	0.3	5.7	5.5			0.8	0.5		0.3				
16:0		0.3	0.6	0.7	1.2	1.2	0.4	0.3	8.1	6.6	5.1	4.5	0.7	0.5	0.5	0.5
3-OH 16:0	**1.5**	**1.2**								**0.6**		**1.1**				
unidentified 4									0.9	0.7						
18:1w9c	1.0	1.0														
18:1w7c	**71.0**	**66.0**	**76.6**	**63.0**	**40.7**	**38.9**	**64.0**	**62.3**	**62.4**	**55.6**	**75.5**	**67.0**	**68.7**	**60.4**	**64.4**	**59.0**
18:0	**6.5**	**7.5**	**4.8**	**5.6**	**7.7**	**7.5**	**7.2**	**7.0**	**1.8**	**1.8**	**1.7**	**1.7**	**7.0**	**8.2**	**13.6**	**15.6**
11-methyl 18:w6t	**3.7**	**4.1**	**5.4**	**15.2**	**12.8**	**12.3**	**11.3**	**11.0**	**15.8**	**17.8**	**10.4**	**10.7**	**6.9**	**7.7**	**8.5**	**9.1**
unidentified 5		0.3	0.6	0.3			0.5	0.5		0.8		1.0			0.5	0.6
unidentified 6									1.2	0.4						
cyclo 19:0					**19.2**	**18.3**			**2.4**	**2.6**						
3-OH 18:1										**0.5**		**1.6**				
3-OH 18:0		**0.8**		**2.3**		**2.6**		**1.0**		**4.7**		**3.0**		**1.8**		**1.5**
unidentified 7											0.9	0.7				
20.1w7c	**9.1**	**10.4**	**5.9**	**4.7**	**5.8**	**5.5**	**8.7**	**8.7**				**0.5**	**7.9**	**10.5**	**5.7**	**6.6**
unidentified 8		0.4														
20:0		0.7		0.3			0.8	1.0		0.6			1.6	2.4		0.5
11-methyl 20:1w6t							**3.1**	**3.2**					**1.5**	**2.2**		
unidentified 3			1.7	1.8												
cyclo 21:0	**1.7**	**2.0**			**3.1**	**3.0**										
unidentified 9												0.4			0.7	0.4
3-OH 20:1		**0.4**		**1.2**		**1.3**						**1.9**		**0.5**		**1.4**
3-OH 20:0		**0.5**		**1.1**		**0.6**		**1.0**		**0.7**				**2.1**		**1.1**
22:1		0.7								0.3				0.6		
22:0										0.8		1.3				
unidentified 10										1.1						

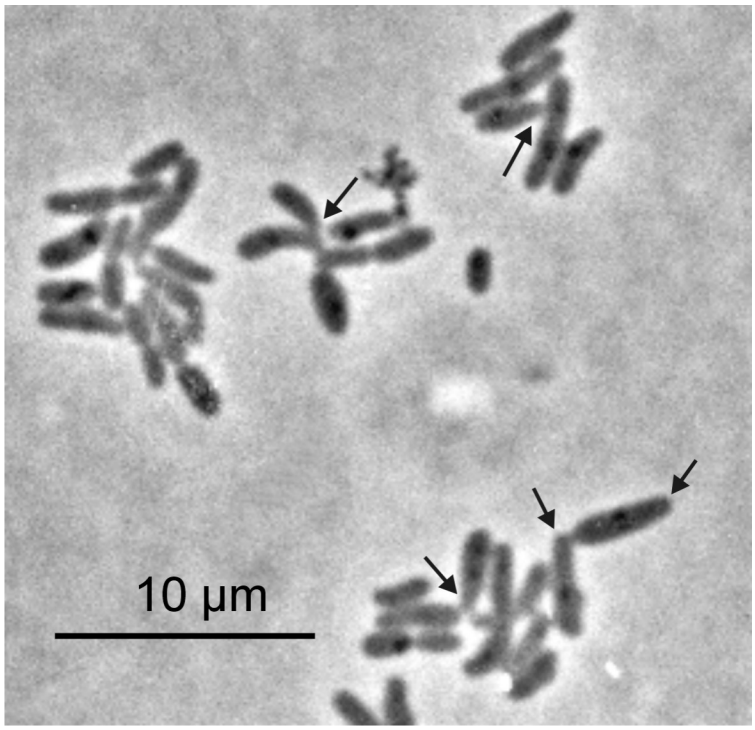


Fig. 1

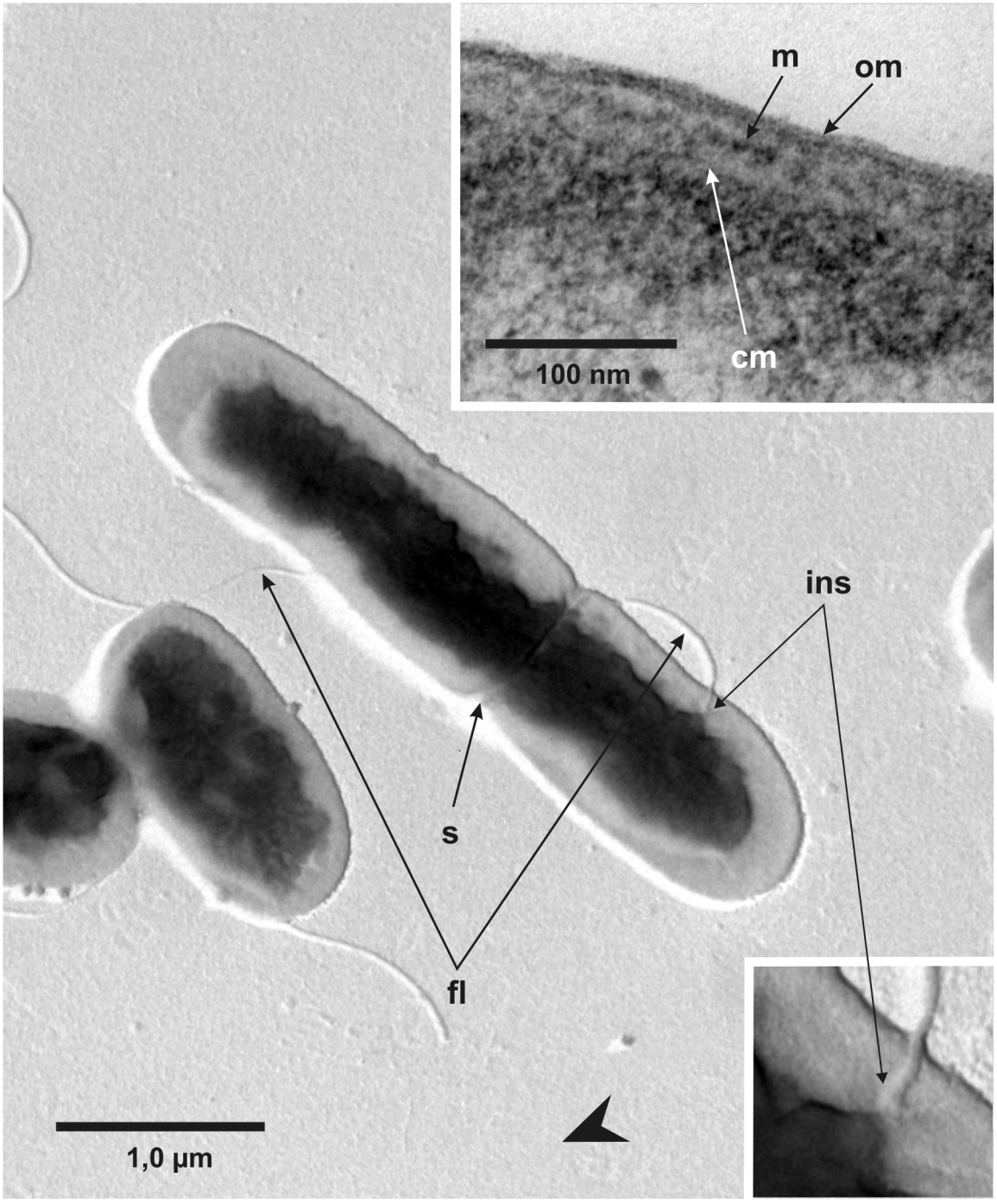


Fig. 2

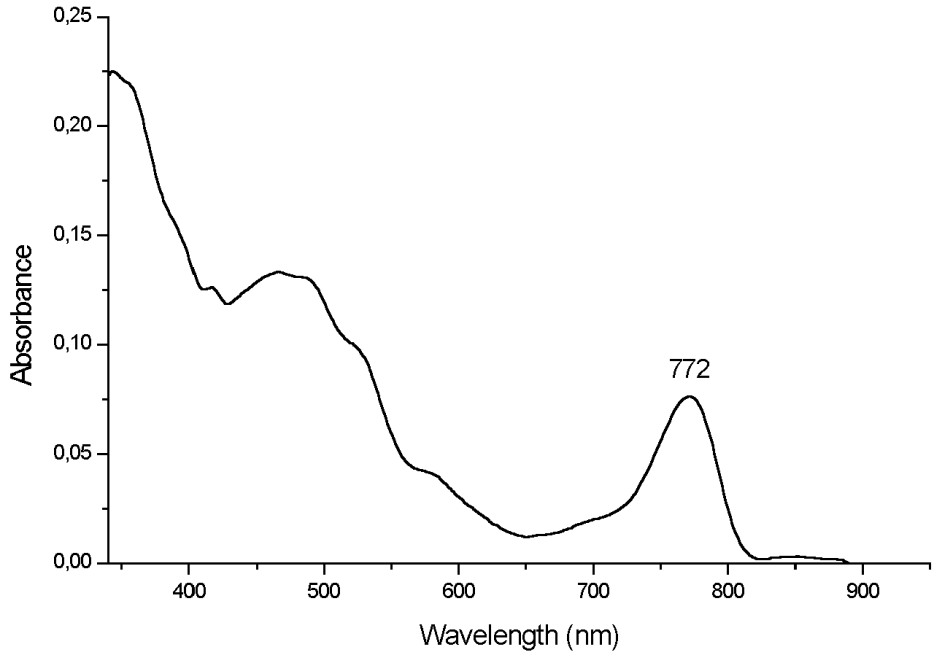
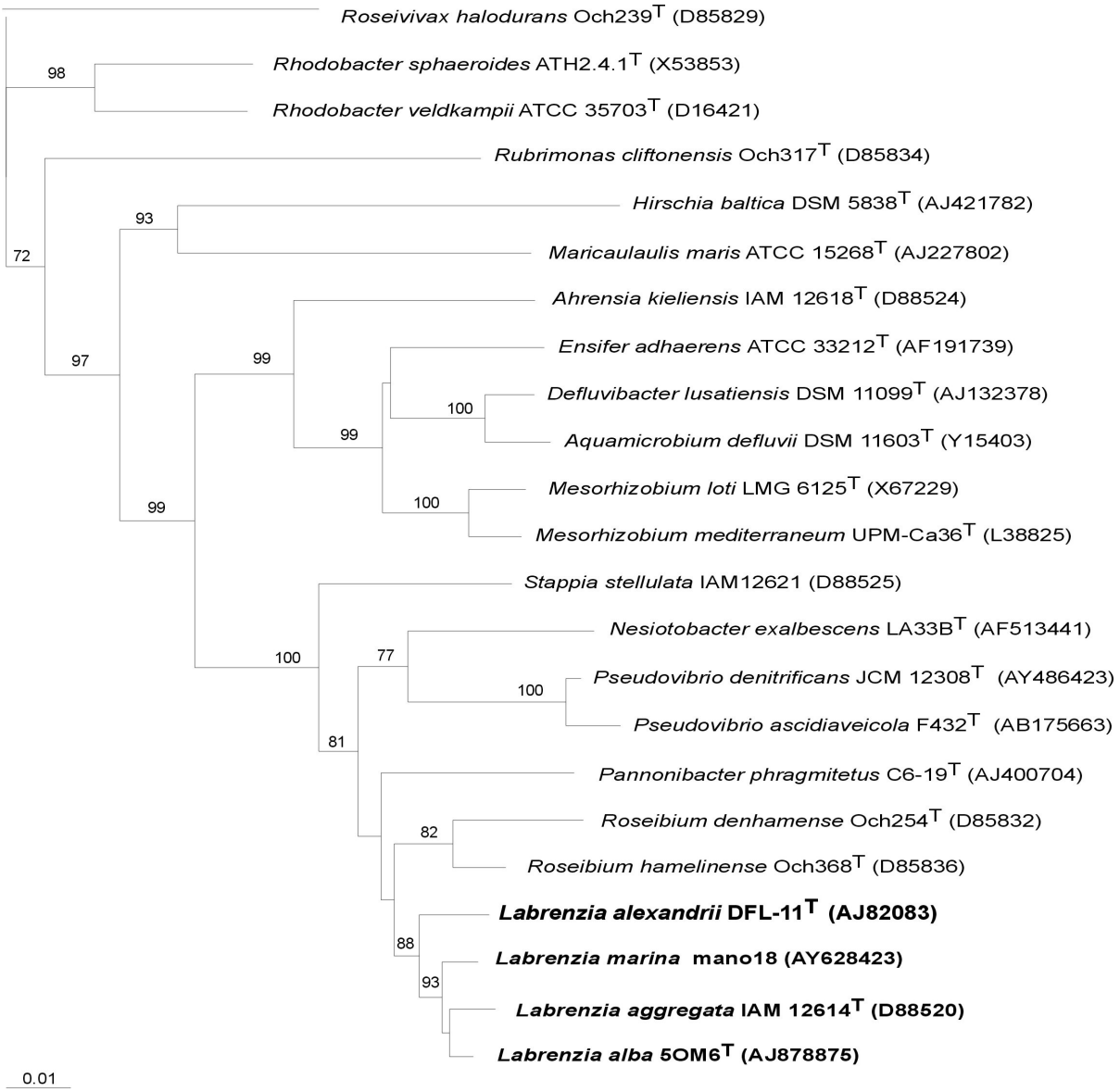

0,25
0,20
0,15
0,10
0,05
0,00
Absorbance
772
400
500
600
700
800
900
Wavelength (nm)

Fig. 3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Rubrimonas cliftonensis Och317T (D85834)
Hirschia baltica DSM 5838T (AJ421782)
93
72
Maricaulaulis maris ATCC 15268T (AJ227802)
Ahrensia kieliensis IAM 12618T (D88524)
97
99
Ensifer adhaerens ATCC 33212T (AF191739)
Defluvibacter lusatiensis DSM 11099T (AJ132378)
100
99
Aquamicrobium defluvii DSM 11603T (Y15403)
Mesorhizobium loti LMG 6125T (X67229)
99
100
Mesorhizobium mediterraneum UPM-Ca36T (L38825)
Stappia stellulata IAM12621 (D88525)
Nesiotobacter exalbescens LA33BT (AF513441)
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77
Pseudovibrio denitrificans JCM 12308T (AY486423)
100
Pseudovibrio ascidiaveicola F432T (AB175663)
81
Pannonibacter phragmitetus C6-19T (AJ400704)
Roseibium denhamense Och254T (D85832)
82
Roseibium hamelinense Och368T (D85836)
Labrenzia alexandrii DFL-11T (AJ82083)
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Fig. 4

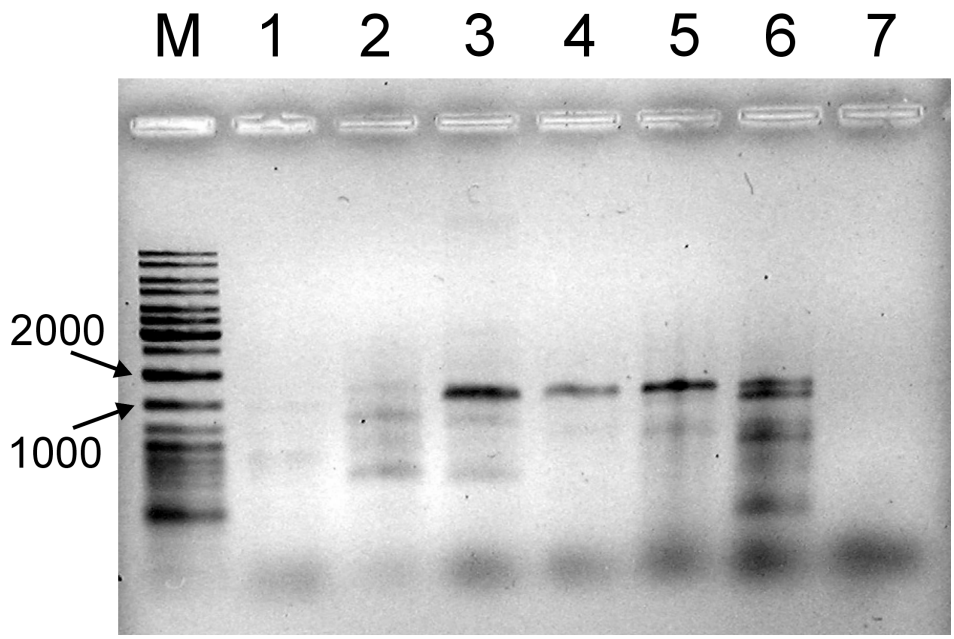
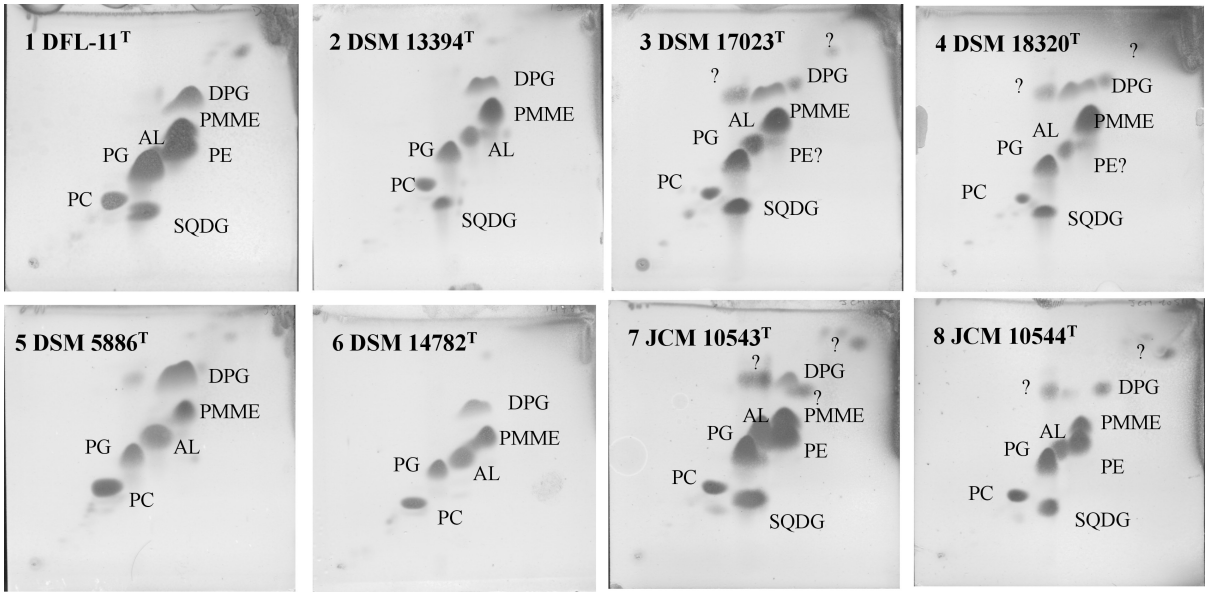


Fig. 5

Figure 6


1 DFL-11T
DPG
PMME
AL
PG
PE
PC
SQDG
2 DSM 13394T
DPG
PMME
PG
AL
PC
SQDG
3 DSM 17023T
?
?
DPG
AL
PMME
PG
PE?
PC
SQDG
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DPG
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PMME
PG
PE
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SQDG
8 JCM 10544T
?
?
DPG
AL
PMME
PG
PE
PC
SQDG

Supplementary Table A. Similarity matrix of almost complete 16S rRNA sequences of DFL-11^T and related species. See Fig. 4 for sequence references.

1	DFL-11^T								
2	*S.alba*	98.0							
3	*S.marina*	98.0	99.1						
4	*S.aggregata*	97.7	98.9	98.5					
5	*S. stellulata*	94.3	94.6	94.7	95.3				
6	*Pannonibacter phragmitetus*	95.0	95.6	95.7	95.7	93.8			
7	*Roseibium denhamense*	96.1	95.4	95.7	95.8	92.6	94.0		
8	*Roseibium hamelinense*	97.1	97.2	97.3	96.7	93.8	94.9	97.2	
		1	2	3	4	5	6	7	8

Supplementary Fig. 1

Shadow-casted preparation of *Labrenzia alexandrii* DFL-11^{T} showing the subpolar insertion of the flagellum. The flagellar hook is enlarged in the inset.