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1 *Hellea balneolensis* gen. nov., sp. nov.,
2 a novel prosthecate alphaproteobacterium from the Mediterranean Sea

3
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17
18 Running title: *Hellea balneolensis* gen. nov. sp. nov.

19
20 Category: New taxa, *Proteobacteria*

21
22 Footnote: The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain
23 26III/A02/215^T is AY576758.

24 The graph showing the effect of temperature on the maximum growth rate (μ_{max}) of strain
25 26III/A02/215^T (Fig. S1) is available in IJSEM online.

26
27 **A novel aerobic, heterotrophic, prosthecate bacterium designated 26III/A02/215^T, was isolated from**
28 **surface waters of the north-western Mediterranean sea. Cells were Gram-negative, straight to slightly**
29 **curved rods, forming red colonies on agar plates. The strain grew at 15-37°C inclusive (optimum:**

30 30°C), and optimally at seawater salinity. Growth on organic acids, amino-acids and complex organic
31 substrates was observed. The fatty acids (> 5%) detected in strain 26III/A02/215^T were C_{17:1}ω6c,
32 C_{18:1}ω7c, and C_{17:0}. The lipid pattern indicated the presence of phosphatidylglycerol,
33 glucuronopyranosyldiglyceride, monoglycosyldiglyceride, an unidentified glycolipid and three
34 unidentified phospholipids. Phosphatidylethanolamine and diphosphatidylglycerol were absent.
35 Ubiquinone Q10 was the only respiratory lipoquinone. The G+C content of the genomic DNA was
36 46.8 mol%.

37 Comparative 16S rRNA gene sequence analysis indicated that strain 26III/A02/215^T belonged to the
38 *Hyphomonas-Hirschia-Robiginitomaculum* branch of the order *Caulobacterales*. This affiliation was
39 consistent with the results of polar lipid analyses. Among this group, the novel isolate was most closely
40 related to *Robiginitomaculum antarcticum* (93.9% 16S rDNA sequence similarity). On the basis of
41 genotypic, chemotaxonomic and phenotypic distinctness, we propose a novel genus, *Hellea* gen. nov.,
42 with *Hellea balneolensis* sp. nov. as the type species. The type strain is 26III/A02/215^T (= DSM 19091^T
43 = CIP 109500^T = OOB 269^T).

44
45 The phylum *Proteobacteria* is one of the 24 phyla of the domain *Bacteria*, described in *Bergey's Manual of*
46 *Systematic Bacteriology*, 2nd edn (Garrity & Holt, 2001). To date, more than 200 genera have been
47 described, making this phylum one of the largest bacterial phyla. Representative members of this group are
48 widely distributed in nature and are physiologically and metabolically diverse. The phylum *Proteobacteria*
49 is currently divided into 5 classes, called the *Alphaproteobacteria*, *Betaproteobacteria*,
50 *Gammaproteobacteria*, *Deltaproteobacteria* and *Epsilonproteobacteria*, all of which have been defined
51 exclusively on the basis of 16S rRNA gene sequence analysis (Garrity & Holt, 2001). At present, the class
52 *Alphaproteobacteria* Garrity *et al.* 2006 (Validation List 107, Garrity *et al.*, 2005a) is composed of seven
53 orders: *Caulobacterales* Henrici & Johnson, 1935 (Henrici & Johnson, 1935), *Kordiimonadales* Kwon *et al.*
54 2005 (Kwon *et al.*, 2005), *Rhodobacterales* Garrity *et al.* 2006 (Validation List 107, Garrity *et al.*, 2005b)
55 *Rhodospirillales* Pfenning & Trüper, 1971 (Pfenning & Trüper, 1971), *Rickettsiales* Gieszczykiewicz, 1939
56 (Gieszczykiewicz, 1939), *Rhizobiales* Kuykendall 2006 (Validation List 107, Kuykendall, 2005) and

57 *Sphingomonadales* Yabuuchi & Kosako, 2006 (Validation List 107, Yabuuchi & Kosako, 2005). Marine
58 species make up more than half of the *Alphaproteobacteria* described to date.

59 Some confusion is being caused at present by the different taxonomic placement of the “stalked” bacteria.
60 While Lee *et al.* (2005) place the members of the genera *Hyphomonas*, *Oceanicaulis*, *Hirschia*, and
61 *Maricaulis* in a new family, the *Hyphomonadaceae*, within the order *Caulobacterales* (which includes
62 members of the family *Rhodobacteraceae*), Garrity *et al.* (2005a) have placed members of these genera
63 within the family *Rhodobacteraceae*, within the order *Rhodobacterales*, leaving the members of the family
64 *Caulobacteraceae* within the order *Caulobacterales*. This situation is particularly unsatisfactory since use of
65 the name of the order *Caulobacterales* alone does not give unambiguous information on which taxa are to be
66 included within it. Furthermore, Lee *et al.* (2005) dealt with the taxonomy of the family *Rhodobacteraceae*
67 before the name was validly published (Validation List 107). Paradoxically Lee *et al.* (2005) created a new
68 family, the family *Hyphomonadaceae*, with the type defined as the genus *Hyphomonas*, a taxon specifically
69 included in the taxon proposed by Garrity *et al.* (2005b) as the family *Rhodobacteraceae*. Based on the
70 principle of priority, the family proposed by Garrity *et al.* (2005b) must be named after the earliest validly
71 published family name, which is the family *Hyphomonadaceae*. The family name *Rhodobacteraceae* Garrity
72 *et al.* 2006 may only be used if specifically defined to exclude the type genus of the family
73 *Hyphomonadaceae*. It should be noted that members of the genera *Woodsholea* (Abraham *et al.*, 2004) and
74 *Robiginitomaculum* (Lee *et al.*, 2007) should be included in the family *Hyphomonadaceae* Lee *et al.* 2007.

75 The members of the families *Hyphomonadaceae* and *Caulobacteraceae* contain organisms that share the
76 particular feature of being appendaged (Poindexter, 1981; Abraham *et al.*, 1999; Weiner *et al.*, 2000;
77 Strömpl *et al.*, 2003). As indicated by their vernacular name (in Latin, *caulis* means stalk), these
78 ‘caulobacteria’ bear one or several stalks, so-called prosthecae. These stalks are cytoplasm extrusions that
79 undoubtedly play a role in attachment. As a result, they increase significantly the surface to volume ratio of
80 the cells. Consequently, they have often been interpreted as an evolutionary adaptation to life in oligotrophic
81 waters. Most genera of the families *Hyphomonadaceae* and *Caulobacteraceae* (i.e. members of the genera
82 *Hyphomonas*, *Caulobacter*, *Asticcacaulis*, *Phenyllobacterium*, *Hirschia*, *Robiginitomaculum*, *Woodsholea*,
83 *Maricaulis*, *Oceanicaulis* and *Brevundimonas*) are widely distributed in marine environments (Anast &

84 Smit, 1988), and especially (but not exclusively) in oligotrophic waters. They are believed to play an
85 important role in the mineralization of dissolved organic matter (Abraham *et al.*, 1999).

86

87 In this study, a novel marine caulobacterium is described. Based on the results of a polyphasic taxonomic
88 analysis, the strain 26III/A02/215^T represents a novel species and genus, *Hellea balneolensis* gen. nov., sp.
89 nov.

90

91 In September 2001, coastal waters were collected in the bay of Banyuls-sur-mer (42°29'N, 3°08'E), in the
92 Mediterranean Sea, France. A sea sample from the surface microlayer was spread on marine agar 2216 (MA;
93 Difco) plate, and then incubated at 25°C. After 2 weeks, a red-coloured colony was picked, purified by
94 repeated streaking on MA plates, and referenced as strain 26III/A02/215^T (Agogué *et al.*, 2005). Stock
95 cultures were stored at -80°C in marine broth 2216 (MB; Difco) supplemented with 5% (v/v) DMSO or
96 35% (v/v) glycerol, until characterization.

97

98 Both strands of the almost complete 16S rRNA gene (1412 bp) of the strain were sequenced from one single
99 colony, as described elsewhere (Agogué *et al.*, 2005). This sequence was compared to those in available
100 databases by use of the BLAST program (Altschul *et al.*, 1990) and then aligned to its nearest neighbours
101 using the CLUSTALX program (Thompson *et al.*, 1997). Alignments were refined manually using the
102 SEAVIEW program (Galtier *et al.*, 1996). Phylogenetic trees were constructed by the PHYLIP (PHYlogeny
103 Inference Package) version 3.63 software (<http://evolution.genetics.washington.edu/phylip/getme.html>) on
104 the basis of evolutionary distance (neighbour-joining method with Jukes and Cantor corrections) (Saitou and
105 Nei, 1987) and maximum likelihood (Felsenstein, 1981). The robustness of the inferred topologies was
106 assessed by bootstrap analyses based on 1000 bootstrap resamplings for the neighbour-joining and 100
107 replications for the maximum likelihood method (Felsenstein, 1985). The 16S rRNA gene-based analysis
108 located the strain 26III/A02/215^T within the class *Alphaproteobacteria*, in the bacterial domain. The results
109 of different phylogenetic reconstructions performed with different treeing algorithms located the novel
110 isolate within the *Hyphomonas-Hirschia-Robiginitomaculum* branch, amongst the marine caulobacteria of
111 family *Hyphomonadaceae* (Lee *et al.*, 2005), order *Caulobacterales* (Fig. 1). Within this branch, the novel

112 isolate clustered with the recently described genus *Robiginitomaculum* (Lee *et al.*, 2007) sharing 93.9% 16S
113 rDNA sequence similarity with the only species of this genus. The level of 16S rRNA gene sequence
114 similarity between strain 26III/A02/215^T and representative of the genera *Hyphomonas* and *Hirschia* ranged
115 from 89 to 92%.

116 The DNA G+C content was determined, by the Identification Service of the DSMZ (Deutsche Sammlung
117 von Mikroorganismen und Zellkulturen GmbH, Braunschweig
118 Germany), by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989). The G+C
119 content of strain 26III/A02/215^T was 46.8 mol%. Thus, it differed by more than 10 mol% from the DNA
120 G+C content of its closest relative *Robiginitomaculum antarcticum* (60.3 mol%). Clearly, this large
121 difference in the DNA base ratio, together with the 16S rDNA level of similarity, suggest that strain
122 26III/A02/215^T belongs to a novel genus (Rosselló-Mora and Amann, 2000).

123

124 Colonies on MA were circular, smooth, brilliant, convex, with an entire edge and intensely pigmented brick-
125 red. After 1 week incubation, colonies were about 1 mm in diameter. Morphological characteristics of the
126 cells were determined by light microscopy (Olympus AX70) and by transmission electron microscopy
127 (Hitachi H-7500) after negative staining with uranyl acetate (Raguénès *et al.*, 1997). Briefly, cells of strain
128 26III/A02/215^T were Gram-negative, thin straight to curved rods bearing one polar stalk (Fig. 2). Cells
129 bearing several lateral stalks were occasionally observed. Mid-exponential phase cells were 2.70-5.60 µm in
130 length (mean 3.58 ± 0.88 µm, n=15), 0.28-0.48 µm in width (mean 0.42 ± 0.06 µm, n=15), some of which
131 produced a stalk(s). When present, the stalk was more generally cylindrical and extended centrally along the
132 cell axis from one pole. This stalk showed constriction sites distributed equally all along the tube, but which
133 not corresponded to a compartmentalization. This type of constriction has been observed previously in
134 *Oceanicaulis alexandrii* (Strömpl *et al.*, 2003). Stalked-cells were non-motile, while non-stalked cells were
135 motile by means of a polar flagellum. Cells divided by budding.

136

137 In order to analyse respiratory quinones and polar lipids, strain 26III/A02/215^T was grown for 3 days on MB
138 medium at 30°C, and checked for purity. Initial analyses of the polar lipids and respiratory quinones were
139 carried out by the Identification Service, DSMZ, Braunschweig, Germany. Ubiquinone (Q10) was

140 determined to be the sole respiratory quinone. The thin layer chromatogram obtained with cell extracts from
141 the novel isolate was very characteristic (Fig. 3). The polar lipid pattern showed the presence of
142 phosphatidylglycerol (PG), monoglycosyldiglyceride (MGDG), glucuronopyranosyldiglyceride (GUDG),
143 one unidentified glycolipid (GI) and three phospholipids (PL1, PL2, PL3). The presence of the polar lipids
144 monoglycosyldiglyceride (MGDG) and glucuronopyranosyldiglyceride (GUDG) appears to be a
145 characteristic signature for other members of the families *Hyphomonadaceae* and *Caulobacteraceae*,
146 together with the absence of phosphatidylethanolamine, phosphatidylcholine and diphosphatidylglycerol.
147 The presence of two unidentified phospholipids PL2 and PL3, together with an unidentified glycolipid
148 appeared to be a characteristic feature of the lipid pattern of this taxon. The determination of the whole-cell
149 fatty acid composition was performed on cultures grown at 30°C for 72h on marine agar 2216. The analysis
150 was carried out at the DSMZ according to the standard protocol of the Microbial Identification System
151 (MIDI Inc., Del. USA, 2001). Extracts were analysed using a Hewlett Packard model HP6890A gas
152 chromatograph equipped with a flame-ionization detector as described by Kämpfer & Kroppenstedt (1996).
153 Results are summarized in Table 1. The fatty acids in strain 26III/A02/215^T comprised C_{16:0}, C_{17:0}, C_{18:0},
154 C_{19:0}, C_{17:1}ω8c, C_{17:1}ω6c, C_{18:1}ω9c, C_{18:1}ω7c, C_{20:1}ω7c, 3-OH C_{10:0}, 3-OH C_{11:0}, 3-OH C_{12:1}, 2-OH C_{18:1}, TBSA
155 10-methyl C_{18:0}, Summed feature 3, Summed feature 7. The presence of C_{18:1}ω7c, together with Q10 is
156 typical of the vast majority of taxa within the *Alphaproteobacteria*. Although the polar lipid composition of
157 the recently described *Robiginitomaculum antarcticum* was not reported, there were clear differences in the
158 fatty acid patterns, in particular the distribution of the 3-hydroxy fatty acids, which are probably derived
159 from lipopolysaccharide. A number of recent publications are also not complete with regard to the
160 chemotaxonomic data. In papers on the genera *Oceanicaulis* (Strömpl *et al.*, 2003), *Woodsholea* (Abraham
161 *et al.*, 2004) and some *Maricaulis* (Abraham *et al.*, 2002) species, the quinone composition has not been
162 reported. In the case of *Oceanicaulis*, 3-OH fatty acids are not reported, probably because only fatty acids
163 from extracted lipids have been reported. Reports on the polar lipid composition may be incomplete because
164 emphasis has been placed on the presence of phosphate and sulfonic acid containing lipids (Strömpl *et al.*,
165 2003, see also Abraham *et al.*, 1997). The glycolipids that are otherwise characteristic for this evolutionary
166 group are not mentioned.

167

168 Unless stated otherwise, physiological characterization was carried out aerobically in marine broth medium
169 (MB 2216; Difco), in triplicate, and cell suspension incubated with agitation in the dark. Growth was
170 routinely monitored by measuring the increase in optical density at 600 nm using a spectrophotometer. Cell
171 numbers were determined by flow cytometry (Marie *et al.*, 2000) in order to calculate calibration curves
172 'Cell numbers = f(OD₆₀₀)'. Growth rates were calculated using linear regression analysis from five to nine
173 points along the logarithmic portions of the resulting growth curves. Growth temperature was tested over the
174 range 9-44°C (i.e. 9, 15, 20, 25, 30, 33, 37, 44°C). The novel isolate was found to be mesophilic, growing at
175 15-37°C; optimal growth yields occurred at 30°C (see Supplementary Fig. S1 in IJSEM online). The
176 optimum pH for growth was tested at 30°C in buffered MB medium and was found to be around pH 6.0-8.0.
177 Salt tolerance was tested at 30°C in MB medium prepared with various concentrations of NaCl (0.02, 0.5, 1,
178 2, 3, 4, 5, 6, 7 and 9% w/v). Results indicated that the strain was a general typical marine-type halophile.
179 Growth was observed in media containing 0.02% (w/v) to 5% (w/v) NaCl, but it was better in media
180 containing half- to full-strength seawater salinity. The optimal NaCl concentration for growth was around
181 3% (w/v) NaCl.

182

183 Strain 26III/A02/215^T was found to be aerobic. Conventional phenotypic tests including those for oxidase,
184 catalase, tween esterase and nitrate reductase were performed according to standard methods (Smibert &
185 Krieg, 1994). The results are given in Table 2. Biochemical tests were performed at 30°C using api®ZYM
186 (bioMérieux) and Biolog GN2 microplates (Oxoid). These tests were inoculated with cells grown on MA
187 plates, swabbed from the surface of the agar plates and then suspended in ASW ½ (diluted artificial
188 seawater) to the density specified by the manufacturer. Supplementary biochemical tests were also
189 performed using api®20NE strips (bioMérieux), following the manufacturer's instructions. The data
190 obtained are given in Table 2. Testing for oxidation of carbon sources with Biolog GN2 plates indicated that
191 the strain was able to oxidize a wide range of organic acids and amino acids. To confirm these results and to
192 test for the capability of the strain to catabolize different substrates as sole carbon and energy source, with
193 oxygen as a terminal electron acceptor, the strain was grown aerobically, in the dark, on a mineral medium
194 supplemented with one substrate. The defined medium (modified from Widdel *et al.* 2004) had the
195 following composition (l⁻¹): phosphate buffer, 30 mM; NaCl 20 g, MgCl₂.6H₂O 3 g, CaCl₂.2H₂O 1.0 g,

196 NH₄Cl 0.3 g, KCl 0.5 g, Na₂SO₄ 3 g, NaNO₃ 1 g; trace element solution, 1 ml; selenite-tungstate solution, 1
197 ml; vitamin solution, 1 ml. The strain was found to grow heterotrophically on a wide range of substrates. It
198 catabolized organic acids, amino acids, and complex substrates for energy and growth (Table 2). The
199 carbohydrates tested were unable to support growth when provided alone in the medium.

200 Antibiotic sensitivity tests were performed by using susceptibility discs (Biorad) or filter-paper discs
201 impregnated with different antibiotics. Discs were placed on MA plates spread with a culture of the isolate
202 and were then incubated at 30°C for one week. Susceptibility was scored as positive at zone diameters above
203 10 mm. The results are summarized in Table 2.

204
205 During the course of this work, we also have had cause to re-examine the taxonomy of members of the
206 families *Hyphomonadaceae* and *Caulobacteraceae*. The placement of members of the genera *Hyphomonas*,
207 *Hirschia*, *Maricaulis*, and *Oceanicaulis*, in the family *Rhodobacteraceae* (Garrity *et al.* 2005a) has been
208 called into question by Lee *et al.* (2005). Independent work on the genome of *Hyphomonas neptunium* has
209 indicated that the 16S rDNA sequence based interpretation may be prone to error (Badger *et al.*, 2005;
210 Badger *et al.*, 2006). This conclusion is also in accord with the chemical composition reported for members
211 of these genera, which share a number of distinctive similarities with members of the genera *Caulobacter*,
212 *Brevundimonas*, *Asticcacaulis* and *Phenylobacterium*. Similarly, extensive chemotaxonomic work on
213 additional taxa within the family *Rhodobacteraceae*, as defined by Garrity *et al.* (2005a) would also indicate
214 inconsistencies (Biebl *et al.*, 2005a, 2005b, 2006, 2007; Martens *et al.*, 2006; Labrenz *et al.*, 1999, 2000)
215 with the proposal of Lee *et al.* (2005) to unite members of the families *Rhodobacteraceae* (as defined by Lee
216 *et al.* (2005)), *Hyphomonadaceae* and *Caulobacteraceae* (as defined by Lee *et al.*, 2005 and Garrity *et al.*,
217 2005a). Clearly the family *Hyphomonadaceae* should comprise the genera *Hirschia*, *Hyphomonas*,
218 *Maricaulis*, *Oceanicaulis*, *Woodsholea*, *Robiginitomaculum*, and the new taxon proposed here. It is
219 interesting to note that this family may be subdivided into two groups, one with cells that divide by budding,
220 the other by binary fission. In addition, there is some evidence that there may also be a correlation between
221 the two groups and the polar lipid patterns, although additional work is needed to test this hypothesis. When
222 such work is completed it would be appropriate to emend the description and circumscription of the family
223 *Hyphomonadaceae* (Lee *et al.* 2005) in the light of chemotaxonomic data, bringing it into line with

224 recommendations dating back to the *ad hoc* committee reports of Wayne *et al.* (1987) and Murray *et al.*
225 (1990). A similar treatment of the family *Caulobacteraceae* would be appropriate, which comprises the
226 genera *Caulobacter*, *Brevundimonas*, *Asticcacaulis* and *Phenylobacterium*. The order *Caulobacterales*
227 should also be restricted to include only the members of the families *Caulobacteraceae* and
228 *Hyphomonadaceae* and emended accordingly. A further consequence would be that the members of the
229 family *Rhodobacteraceae* as defined by Lee *et al.* (2005), should be formally assigned to a family that
230 excludes the type of the family *Hyphomonadaceae*. Based on published chemotaxonomic data it would also
231 be prudent to test whether members of that taxon should be further divided into several families and all
232 included in the order *Rhodobacterales*.

233
234 Briefly, the results of our genotypic, chemotaxonomic, morphological and physiological investigations,
235 together with the phylogenetic analyses, revealed that strain 26III/A02/215^T is distinct from other members
236 of the family *Hyphomonadaceae*. The main characteristics differentiating the novel isolate from its closest
237 phylogenetic neighbours are summarized in Table 2. In brief, the novel taxon can be distinguished from all
238 its closest relatives, with the exception of members of the genus *Hirschia*, by its significantly lower G+C
239 content. The fatty acid composition and polar lipid composition represent other distinctive criteria between
240 the new taxon and other members of the family *Hyphomonadaceae*. Although much emphasis is put on the
241 “major fatty acids” in the majority of recent taxonomic papers we emphasise here, the fact that the large
242 amounts of 18:1 ω 7c (together with the presence of Q10) only indicate that this genus is a member of the
243 *Alphaproteobacteria* and cannot be described as “characteristic” of this, or any other genus. On the contrary
244 the sum of chemotaxonomic data, not only clearly place it within the family *Hyphomonadaceae*, order
245 *Caulobacterales*, but also provides a unique signature for with taxon within these higher taxa. In terms of
246 other phenotypic features, differences in morphological characteristics such as the fine structure of the stalk,
247 its position, the flagellation of the cells, the colonial pigmentation and the mode of division of the cells can
248 also be use to distinguish the novel isolate from members of the genera *Robiginitomaculum*, *Hyphomonas*,
249 *Hirschia*, *Woodsholea*, *Oceanicaulis* and *Maricaulis* (Table 2).

250 In conclusion, on the basis of the phylogenetic position and of genotypic, chemotaxonomic and
251 physiological, biochemical and morphological differences, we propose that the isolate 26III/A02/215^T

252 should be assigned as the type strain of a novel genus and species, for which the name *Hellea balneolensis*
253 gen. nov., sp. nov. is proposed.

254

255 **Description of *Hellea* gen. nov.**

256 *Hellea* (He.lle'a. L. fem. n. *Helle* a sea goddess in Greek mythology; N. L. fem. n. *Hellea*, named after *Helle* in
257 reference to the marine origin of the strain). Cells are Gram-negative, non-spore forming, rod-shaped to vibrioid, and
258 dimorphic: usually, they possess one polar stalk (prostheca) and are non-motile or / they are non-stalked and motile by
259 means of a polar flagellum. Aerobic and heterotrophic. Mesophilic. Neutrophilic. Grows best at salt concentrations close
260 to marine salinity. The predominant quinone is Q10. Polar lipids comprise glucuronopyranosyldiglyceride,
261 monoglycosyldiglyceride, phosphatidylglycerol, and unidentified glycolipid and phospholipids. Fatty acids comprise
262 C_{16:0}, C_{17:0}, C_{18:0}, C_{19:0}, C_{17:1}ω8c, C_{17:1}ω6c, C_{18:1}ω9c, C_{18:1}ω7c, C_{20:1}ω7c, 3-OH C_{10:0}, 3-OH C_{11:0}, 3-OH C_{12:1}, 2-OH
263 C_{18:1}, TBSA 10-methyl C_{18:0}, Summed feature 3, Summed feature 7 (percentage compositions are given in Table 1). The
264 G+C content of the DNA is close to 47 mol%. The genus *Hellea* belongs to the class *Alphaproteobacteria*, order
265 *Caulobacterales*, family *Hyphomonadaceae*, showing a distant relatedness to prosthecate bacteria of marine origin,
266 namely members of the genera *Hyphomonas*, *Robiginitomaculum*, *Hirschia*, *Woodsholea*, *Maricaulis* and *Oceanicaulis*.
267 The type species is *Hellea balneolensis*. This is the only species within the genus.

268

269 **Description of *Hellea balneolensis* sp. nov.**

270 *Hellea balneolensis* (bal.ne'o.len.sis. M. L. n. *Balneola*, the ancient name of Banyuls-sur-mer; N. L. fem. adj.
271 *balneolensis*, pertaining to *Balneola* from where the strain was isolated). In addition to the characters described for the
272 genus, the species is characterised by the following properties. Colonies on MA medium are round, convex, brilliant and
273 pigmented a brick red colour. Optimal growth occurs at 30°C, with a growth range from 15 to 37°C. pH optimum is
274 close to neutrality. Grows at NaCl concentrations from 0.02% to 5% (w/v), with a clear optimum at 3% (w/v) NaCl.
275 Growth occurs on acetate, citrate, propionate, pyruvate, succinate, aspartate, glutamate, L-alanine, L-asparagine, L-
276 histidine, L-proline, casamino acids, peptone, tryptone, yeast extract and D-mannitol. Substrates positive in Biolog GN2
277 plates are all the substrates cited above and as well as *cis*-aconitic acid, D-glucuronic acid, β-hydroxybutyric acid, γ-
278 hydroxy butyric acid, α-ketoglutaric acid, methyl-pyruvate, quinic acid, urocanic acid, L-pyroglutamic acid, hydroxyl-L-
279 proline, putrescine, *n*-acetyl glucosamine, D-arabitol, *m*-inositol and xylitol. Does not reduce NO₃⁻. Catalase positive,
280 oxidase negative. Tween 40 and tween 80 hydrolysis activities are positive.

281 The G+C content is 46.8 mol%.

282 The type strain, 26III/A02/215^T (DSM 19091^T, CIP 109500^T = OOB 269^T), was isolated from the surface microlayer of
283 coastal waters, in the bay of Banyuls-sur-mer, north-western Mediterranean sea, France (42°29'N, 3°08'E).

284

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296

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435

436 **TABLES and FIGURES**

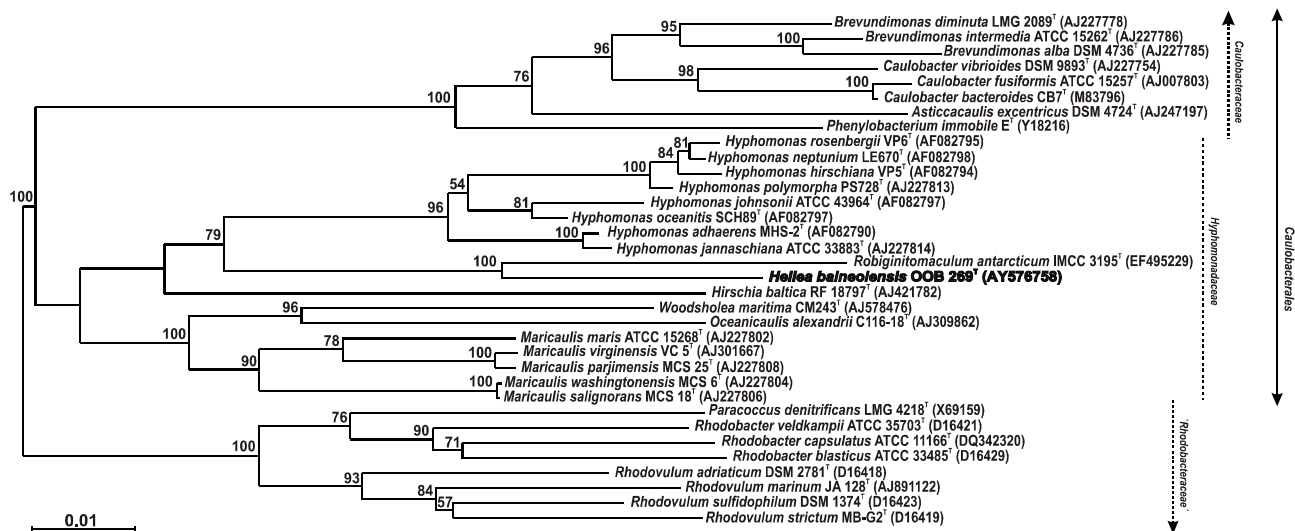
437 **Table 1. Whole cell fatty acid profile of strain 26III/A02/215 cultivated on marine agar.**

438 Values are percentages of total fatty acids. The nomenclature is as follows: the first number indicates the
 439 number of carbon atoms in the molecule. The prefixes 'iso', 'OH' and 'cyclo' indicate isobranched, hydroxy
 440 or cyclic fatty acids. The second number following the colon indicates the number of double bonds present.
 441 The position of the double bond is indicated by the carbon atom position starting from the methyl (ω) end of
 442 the molecule. The suffix *c* indicates the *cis* isomer. Summed feature contain one or more of each fatty acid.
 443 Summed features: **3**, C_{16:1} ω 7*c* and/or 2-OH iso-C_{15:0}; **7**, C_{19:0} cyclo ω 10*c*/C_{19:1} ω 6*c* and/or unknown ECL
 444 18.846. ECL, equivalent chain length. TBSA, tuberculostearic acid (10-methyloctadecanoic acid). Major
 445 fatty acids are indicated by bold values. Only 62% of the fatty acid peaks could be assigned to the fatty acids
 446 listed in the peak naming table of the MIS database (MIS, Microbial identification System; MIDI, Del.
 447 USA). Unknown ECL that were detected are: 16.760, 17.608, 18.116, 18.585, 18.797 and 19.347.
 448

Fatty acid	Strain 26III/A02/215 ^T grown on marine agar
Saturated fatty acids	
C _{16:0}	0.98
C_{17:0}	5.63
C _{18:0}	1.81
C _{19:0}	0.79
Monounsaturated fatty acids	
C _{17:1} ω 8 <i>c</i>	2.18
C_{17:1}ω6<i>c</i>	6.60
C _{18:1} ω 9 <i>c</i>	1.06
C_{18:1}ω7<i>c</i>	67.22
C _{20:1} ω 7 <i>c</i>	0.68
Hydroxy fatty acids	
2-OH C _{18:1}	2.51
3-OH C _{10:0}	2.23
3-OH C _{11:0}	0.92
3-OH C _{12:1}	1.23
Methyl substituted fatty acid	
TBSA 10-methyl C _{18:0}	1.39
Summed features	
Summed feature 3	0.66
Summed feature 7	4.11

449

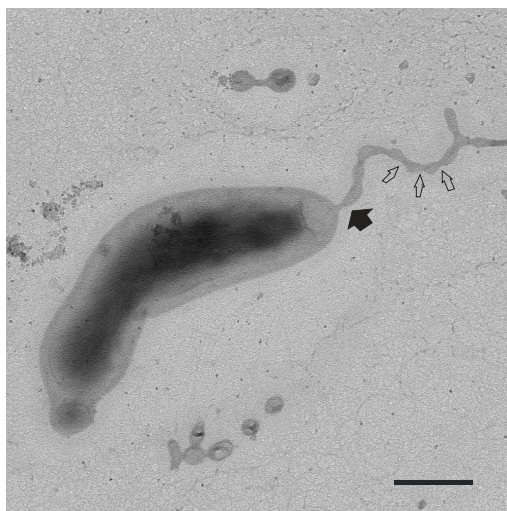
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453 **Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain**
 454 **26III/A02/215^T within the order *Caulobacterales* (as outlined in this article), class *Alphaproteobacteria*.**
 455 The alignment was performed with 16S rDNA sequences of related species. Sequence data of reference
 456 strains were obtained from the GenBank/EMBL and/or RDP databases. Accession numbers are indicated in
 457 parentheses. The topology shown corresponds to an unrooted tree obtained by the neighbour-joining
 458 algorithm, established using the PHYLIP package. Bootstrap values (from 1000 replicates) are indicated at
 459 the branch nodes. The positioning of the novel isolate was confirmed by the maximum likelihood method.
 460 The scale bar indicates 1.0 nt substitutions per 100 nt.

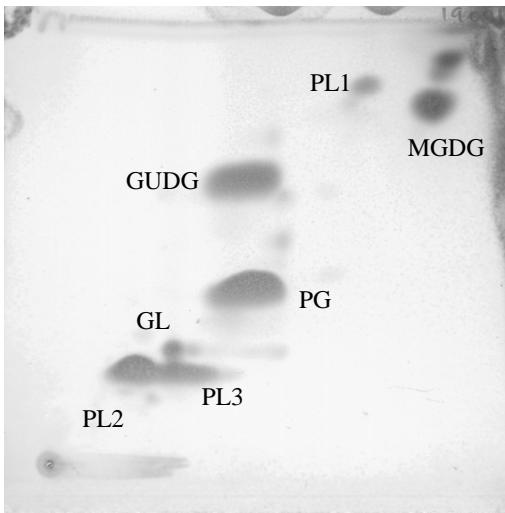
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463

464 **Fig. 2. Transmission electron micrograph of a budding cell of strain 26III/A02/215^T negatively stained**
 465 **with uranyl acetate.** It can be observed that the products of the cell division are unequal (simple budding).
 466 The cell bears a polar stalk (black arrow) which is an open ring regularly constricted on its length (open
 467 arrows). Bar, 0.5 μ m.

468



469

470 **Fig. 3. Polar lipids of strain 26III/A02/215^T.** Legend: PG, phosphatidylglycerol; PL1, PL2, PL3,
 471 phospholipids; GL, unidentified glycolipid; MGDG, monoglycosyldiglyceride; GUDG,
 472 glucuronopyranosyldiglyceride.

473

474 **Table 1. Phenotypic and genotypic characteristics of strain 26III/A02/215^T.** Legend: +, positive; —,
 475 negative; W, weakly positive; ND, not determined; VS, very susceptible (diameter of inhibition zone > 20
 476 mm); S, susceptible (diameter of inhibition zone: 10-20 mm).

477

Characteristic	Strain 26III/A02/215 ^T
Temperature range for growth (°C) – [Optimum]	15-37 [30]
NaCl range for growth (%) – [Optimum]	0.02-5 [3]
Catalase activity	+
Oxidase activity	—
Hydrolysis of tween 40	+
Hydrolysis of tween 80	+
<i>API ZYM</i> / <i>API 20NE</i>	
Alkaline phosphatase	+
Esterase	+
Esterase lipase	+
Naphtol-AS-BI-phosphohydrolase	+
β-galactosidase	+
Urease activity	—
Nitrate reductase activity	—
Hydrolysis of aesculin (β-glucosidase)	+
Hydrolysis of gelatin	—
Glucose fermentation	—
<i>Oxidation of (Biolog) / Utilization as sole carbon and energy source (minimal mineral medium)</i>	
Acetic acid	+ / +
<i>cis</i> -aconitic acid	+ / ND
Citric acid	+ / +
D-Glucuronic acid	+ / ND
β-Hydroxy butyric acid	+ / ND
γ-Hydroxy butyric acid	+ / ND
α-keto glutaric acid	+ / ND
Propionic acid	+ / +
Pyruvic acid	+ / +
Methyl-pyruvate	+ / ND
Quinic acid	+ / ND
Succinic acid	+ / +
Urocanic acid	+ / ND
L-aspartic acid	+ / +
L-glutamic acid	+ / +
L-pyroglutamic acid	+ / ND

L-alanine	+ / +
L-asparagine	+ / +
L-histidine	+ / +
Hydroxy-L-proline	+ / ND
L-proline	+ / +
Putrescine	+ / ND
Casamino acids	ND / +
Peptone	ND / +
Tryptone	ND / +
Yeast extract	ND / +
n-acetyl glucosamine	+ / ND
D-arabitol	+ / ND
<i>m</i> -inositol	+ / ND
D-mannitol	+ / +
xylitol	+ / ND
Dextrin	w / ND
D-mannose	w / ND
D-cellobiose	w / ND
α -D-glucose	w / w
Starch	w / w
<i>Susceptibility to :</i>	
Ciprofloxacin (100 μ g per disc)	VS
Oxacillin (5 μ g per disc)	S
Penicillin (6 μ g per disc)	S
Rifampicin (100 μ g per disc)	VS
Tetracyclin (100 μ g per disc)	S
Vancomycin (100 μ g per disc)	VS
DNA G+C content (mol%)	46.8

479 **Table 2. Characteristics differentiating *Hellea* from the related genera of the family *Hyphomonadaceae*.**

480

Characteristic	Genus						
	<i>Hellea</i>	<i>Robiginitomaculum</i> [#]	<i>Hyphomonas</i> [*]	<i>Hirschia</i> [†]	<i>Oceanicaulis</i> [‡]	<i>Maricaulis</i> [§]	<i>Woodsholea</i> [#]
Colony colour	Brick red	Rusty-orange	Grey or colourless may produce a water soluble brown/red-brown pigment	Yellow	colourless	colourless	colourless
Prostheca(e)	One, polar	One, polar	One to two, polar	One to two, polar	One, polar	One, polar	One, polar
Stalk cross wall	+	-	-	-	+	-	+
Mode of division	Budding	Binary fission	Budding	Budding	Binary fission	Binary fission	Binary fission
Flagellation	Monotrichous, polar	Absent	One to three, polar	Monotrichous, polar	Monotrichous, polar	Monotrichous, polar	Monotrichous, polar
Nitrate reduction	-	+	+	-	+	±	-
Growth at 6% NaCl	-	-	V	ND	+	±	+
Polar lipid(s) [#]	PG, MGDG, GUDG, GL, PL ₁ , PL ₂ , PL ₃	ND	MGDG, GUDG, PG, Tau	MGDG, GUDG, PG, GL ⁵	(PG), SQDG [MGDG, GUDG ¹]	PG, SQDG, Tau, MGDG, GUDG	SQDG, Tau, MGDG, GUDG
Major fatty acids	<u>3-OH C_{10:0a}</u> , <u>3-OH C_{12:1a}</u> , <u>3-OH C_{11:0a}</u> , C _{17:1ω6c} , C _{17:1ω8c} , C _{18:1ω7c} , C _{17:0}	<u>3-OH C_{9:0a}</u> , <u>3-OH C_{10:0a}</u> , <u>3-OH C_{11:0a}</u> , C _{15:1ω8c} , C _{15:1ω6c} , C _{15:0} , C _{16:0} , C _{16:1ω9c} , C _{16:1ω7c} , C _{17:1ω8c} , C _{17:0} , C _{17:1ω6c} , C _{18:0} , C _{18:1ω7c} , C _{18:1ω9c}	<u>3-OH C_{12:0a}</u> , <u>3-OH C_{12:1}</u> , (C _{15:0}) C _{16:0} , C _{17:1ω8³, (C_{17:1} ω6), C_{17:0}, C_{18:1ω7}, 11-Me-C_{18:1ω6}, C_{19:1ω8}}	<u>3-OH C_{12:0a}</u> , <u>3-OH C_{14:1}</u> , C _{16:1ω11c} , C _{16:1ω7c} , C _{16:0} , C _{18:0} , C _{18:1ω7c} , C _{18:2ω7}	<u>3-OH FAME^{2*}</u> , C _{16:0} , C _{17:1ω6⁴, C_{17:0}, C_{18:1ω7}, C_{18:0}, 7-Me- C_{18:1ω6}, C_{19:0}}	<u>3-OH iso C_{11:0}</u> , C _{16:0} , C _{17:0} , C _{16:1ω7c*} , C _{17:0} , iso C _{17:0} , iso C _{17:1ω9c} , C _{17:1ω6c} , C _{17:1ω8c} , C _{18:1ω7c*} , C _{18:1ω9c}	<u>3-OH C_{12:0}</u> , C _{16:0} , C _{17:0} , C _{18:0} , C _{18:1ω7c}
Quinones	Q10	ND	Q10 or Q11	Q10	ND	Q10	ND
DNA G+C content (mol%)	47	60	57-64	45-47	61-62	62-65	65

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[¶]Data from Lee *et al.* (2007)

^{*}Data from Weiner *et al.* 1985, 2000; Moore *et al.*, 1984

[†]Data from Schlesner *et al.* (1990)

[‡]Data from Strömpl *et al.* (2000)

[§]Data from Abraham *et al.* (2002), Sittig and Hirsch (1992)

[#]Data from Abraham *et al.* (2004)

¹ The lipids MGDG and GUDG are not specifically mentioned, but the lipid fraction containing them was not investigated.

² Hydroxylated fatty acids not mentioned, but only the fatty acids from a polar lipid fraction was examined

³ Fatty acid nomenclature used in the original paper was the Δ nomenclature – this has been converted to the ω nomenclature in this table. Cis- or trans- isomers not specified

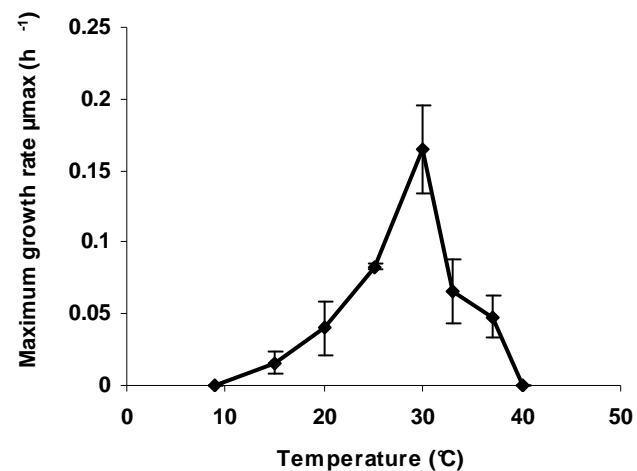
⁴ Cis- or trans- isomers not specified

⁵ Tindall, unpublished

Hydroxy fatty acids (which probably originate for the lipopolysaccharide are underlined)

‡ PG, Phosphatidylglycerol; MGDG, monoglycosyldiglyceride; SQDG, 1,2-diacyl-3-*O*-sulfoquinovosylglycerol; GUDG, glucuronopyranosyldiglyceride; SQDG, sulfo-quinovosyl diacylglycerol; Tau, 1,2-diacyl-3- α -D-glucuropyranosyl-*sn*-glycerol taurineamide; PL, unidentified phospholipid; GL, unidentified glycolipids.

Legend: V, variable; ND Not determined



516 **Fig. S1. Effect of temperature on the maximum growth rate of strain 26III/A02/215^T.** The strain was grown in MB medium. Growth rates were calculated by
517 performing linear regression analysis along the logarithmic part of the growth curves.