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1 **Genomic and phylogenomic insights into the family *Streptomycetaceae***
2 **lead to the proposal of six novel genera**

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24 **Keywords** Actinomycetota · genome-based phylogeny · genome metrics · genome-based
25 taxonomy · Streptomycetales

26 **Abbreviations** AAI – Average Amino acid Identity · dDDH – Digital DNA-DNA hybridization ·
27 ANI – Average Nucleotide Identity · POCP – Percentage of conserved proteins

28 The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and genome sequence data for
29 novel combinations in the present study are as follows: *Actinacidiphila acididurans* KK5PA1^T
30 (MW201570 and GCA_016918855); *Actinacidiphila alni* CGMCC 4.3510^T (DQ460470 and
31 GCA_900112845); *Actinacidiphila bryophytorum* DSM 42138^T (KF923804 and
32 GCA_016916835); *Actinacidiphila epipremni* PRB2-1^T (LC333557 and GCA_012034175);
33 *Actinacidiphila glaucinigra* FXJ14^T (AB249964 and GCA_900188405); *Actinacidiphila*
34 *guanduensis* CGMCC 4.2022^T (AY876942 and GCA_900103985); *Actinacidiphila yanglinensis*
35 CGMCC 4.2023^T (AY876940 and GCA_900107965); *Actinacidiphila yeochonensis* CN732^T
36 (AF101415 and GCA_000745345); *Actinacidiphila oryziradicis* NEAU-C40^T (MK245982 and
37 GCA_005047355); *Actinacidiphila paucisporea* CGMCC 4.2025^T (AY876943 and
38 GCA_900142575); *Actinacidiphila rubida* CGMCC 4.2026^T (AY876941 and GCA_900110255);
39 *Actinacidiphila soli* LAM7114^T (MF784350 and GCA_003999195); *Mangrovactinospora*
40 *gilvigrisea* MUSC 26^T (KJ632660 and GCA_001879105); *Peterkaempfera bronchialis* DSM

41 106435^T (MK089584 and GCF_003258605); *Peterkaempferia griseoplana* NRRL B-3064^T
42 (AB184138 and GCA_001418575); *Phaeacidiphilus oryzae* S16-07^T (AB894335 and
43 GCA_017676365); *Streptantibioticus cattleyicolor* NBRC 14057^T (FQ859185 and
44 GCA_000240165); *Wenjunlia tyrosinilytica* CGMCC 4.7201^T (KM000839 and
45 GCA_014646055); and *Wenjunlia vitaminophila* ATCC 31673^T (AB184589 and
46 GCA_001445835)(see also Table S1 and Table S5).

47 **ABSTRACT**

48 **The family *Streptomycetaceae* is a large and diverse family within the phylum *Actinomycetota*.**
49 **The members of the family are known for their ability to produce medically important**
50 **secondary metabolites, notably antibiotics. In this study, 19 type strains showing low 16S**
51 **rRNA gene similarity (<97.3%) to other members of the family *Streptomycetaceae* were**
52 **identified and their high genetic diversity was reflected in a phylogenomic analysis using**
53 **conserved universal proteins. This analysis resulted in the identification of six distinct genus-**
54 **level clades, with two separated from the genus *Streptacidiphilus* and four separated from**
55 **the genus *Streptomyces*. Compared to members of *Streptacidiphilus* and *Streptomyces*,**
56 **average amino acid identity (AAI) analysis of the new genera identified gave values within**
57 **the range of 63.9 - 71.3 %, as has been previously observed for comparisons of related but**
58 **distinct bacterial genera. The whole-genome phylogeny was reconstructed using PhyloPhlAn**
59 **3.0 based on an optimized subset of conserved universal proteins, AAI and percentage of**
60 **conserved proteins (POCP) analyses indicated that these phylogenetically distinct taxa may**
61 **be assigned to six novel genera, namely *Actinacidiphila* gen. nov., *Mangrovactinospora* gen. nov.,**

62 *Peterkaempfera* gen. nov., *Phaeacidiphilus* gen. nov., *Streptantibioticus* gen. nov. and
63 *Wenjunlia* gen. nov.

64 DATA SUMMARY

65 All genome sequences used in this work were previously published, and the 16S rRNA gene and
66 whole genome assembly accession are provided in Dataset Table S1. One supplementary figure
67 and four supplementary datasets as excel files are included in the online version of this article. All
68 the raw data (List of conserved universal marker proteins in *Streptomycetaceae*, AAI values and
69 POCP values) used to generate the figures presented in this manuscript are available through
70 figshare at <https://doi.org/10.6084/m9.figshare.20339073.v1> [1].

71 INTRODUCTION

72 The family *Streptomycetaceae* Waksman and Henrici 1943 (Approved Lists 1980) emend. Nououi
73 *et al.* 2018 [2, 3] currently contains six genera, which themselves contain more than 750 species
74 with a validly published and correct name (<https://lpsn.dsmz.de/family/streptomycetaceae>). The
75 family was first described by Waksman and Henrici [2] and has recently been placed within the
76 sub-order *Streptomycineae* (Stackebrandt *et al.* 1997) emend. Zhi *et al.* 2009 [4, 5] of the class
77 *Actinomycetia* [6]. Members of the family *Streptomycetaceae* are Gram-positive, aerobic
78 organisms lacking mycolic acids and form extensively branched substrate mycelium, with
79 generally non-septate hyphae that rarely fragment into conidia [2, 7]. In 1982, *Kitasatospora* was
80 added to the family *Streptomycetaceae* (with *Kitasatospora setae* as the type species) but members
81 of the genus were later reclassified into *Streptomyces* [8, 9]. However, *Kitasatospora* was
82 reestablished based on the exclusive presence of galactose and differences in the diamino acids of
83 the cell walls of members of the genus compared to *Streptomyces* [10]. Subsequently the genera

84 *Streptacidiphilus* and *Allostreptomyces* were added to the family *Streptomycetaceae* [11, 12].
85 Recently, a genome-based approach has further clarified the taxonomy within the family, allowing
86 the demarcation of two novel genera, *Embleya* and *Yinghuangia* [3]. Thus, the family
87 *Streptomycetaceae* currently contains six genera i.e., *Allostreptomyces*, *Embleya*, *Kitasatospora*,
88 *Streptacidiphilus*, *Streptomyces*, and *Yinghuangia* [2, 3, 8, 11-13], although Salam *et al.* [6] have
89 proposed placement of *Allostreptomyces* in *Allostreptomycetaceae* fam. nov.

90 Initially, the taxonomy of *Streptomycetaceae* was mainly based on the morphological
91 characteristics [2]. However, later ‘polyphasic’ studies based on phenotypic and single-gene
92 phylogenetic analyses have been unable to provide a well-resolved phylogeny within this family
93 [14, 15]. Labeda *et al.* [16] showed 16S rRNA gene sequences could be used to demonstrate the
94 species diversity within the family *Streptomycetaceae*, defining 130 statistically supported clades,
95 several unsupported clusters, and additional single species lineages. The phylogenetic resolution
96 within the family was significantly improved by the development of a multi-locus sequence
97 analysis (MLSA) scheme using the *atpD*, *gyrB*, *rpoB*, *recA*, and *trpB* housekeeping genes [17].
98 This analysis supported the phylogenetic distinctiveness of the closely related genera
99 *Kitasatospora* and *Streptacidiphilus* and the transfer of nine *Streptomyces* species into the genus
100 *Kitasatospora*. Further clarification was achieved by the presentation of an emended description
101 of the genus *Kitasatospora* and the reclassification of *Streptomyces indigoferus* and *Streptomyces*
102 *xanthocidicus* into the genus [3].

103 Taxonomic studies based on genomic metrics have gained momentum as a promising approach
104 for delineation of genera and species related to and within *Streptomycetaceae* [5, 13, 15, 18-20].
105 Genome sequence-derived parameters such as digital DNA-DNA hybridization (dDDH), average

106 nucleotide identity (ANI), average amino acid identity (AAI) are now routinely used for taxonomic
107 delineation especially at the species and genus levels [21, 22]. Furthermore, phylogenomic trees
108 based on core proteins surpass MLSA performed with a few housekeeping genes for higher
109 taxonomic placements. Hence, the present study was designed to elucidate the evolutionary
110 relationships of taxa belonging to the family *Streptomycetaceae* using genome sequence data and
111 to assign the appropriate taxonomic rank to taxa identified as needing reclassification.

112 **METHODS**

113 **16S rRNA gene and conserved protein based phylogenetic analysis**

114 To understand the evolutionary relationships between the members of *Streptomycetaceae*, a
115 phylogenetic tree was reconstructed using the 16S rRNA gene sequences from 456 type strains
116 belonging to *Streptomycetaceae* (Table S1) using to the sequence accessions provided by the List
117 of Prokaryotic names with Standing in Nomenclature (<https://psn.dsmz.de/family/streptomycetaceae>) [23].
118 The sequences were aligned using SINA 1.2.11 [24] and positions containing gaps were then
119 removed using sequence alignment editor BioEdit 7.2. The 'phangorn' v. 2.6.3 R package was used
120 to construct a neighbor-joining (NJ) and maximum likelihood (ML) trees [25]. The ModelTest
121 function was used to select the GTR+G+I as the best-fitted model according to the Akaike
122 information criterion, which was used to reconstruct a phylogenetic tree using the ML method with
123 the NJ tree as a starting point. A protocol with 500 non-parametric bootstraps was then calculated
124 using the bootstrap.pml function. The tree was rooted using *Acidotherrmus cellulolyticus* ATCC
125 43068 as outgroup. The R packages 'phytools' [26] v.0.7-70 and 'ggtree' [27] v.2.4.2 were used to
126 view and edit the tree. The 16S rRNA gene sequence similarity value of two sequences was

127 calculated using the algorithm of Myers and Miller [28], in the pairwise nucleotide sequence
128 alignment tool (<http://www.ezbiocloud.net/tools/pairAlign>).

129 To extend the result of 16S rRNA gene analysis, a whole genome-based phylogenetic analysis was
130 carried out for various taxa of *Streptomycetaceae*. Among the total 456 strains available with
131 whole-genome sequence, 34 strains were not included due to their genome containing frameshifted
132 proteins, fragmented assembly, anomalous assembly and mislabeling that leads to low quality of
133 sequences based on BUSCO scores (refer Table S1). Therefore, genomic data for 422 taxa of the
134 *Streptomycetaceae* alone were retrieved from NCBI (February 2020), and their genome assembly
135 was confirmed using the EzBioCloud database [29] and finally subjected to genome analysis
136 (Table S1). A phylogenetic tree was constructed from the protein sequences of the entire dataset
137 using PhyloPhlAn 3.0 [30]. PhyloPhlAn constructs highly robust maximum-likelihood
138 phylogenetic trees from a protein sequence alignment generated by concatenating computationally
139 selected subset of amino acid sequences from 400 conserved universal proteins [30, 31]. The
140 algorithm found 379 of these marker proteins in our dataset (Table S2) and constructed the
141 phylogenetic tree from an amino acid alignment 36710 aa in length.

142 **Analyses of genomic metrics**

143 Genome distances in the form of dDDH values were calculated by the Genome-to-Genome
144 Distance Calculator version 2.1 [32]. The ANI was calculated based on the BLASTn method for
145 close relatives using EDGAR v2.3 [33]. The pairwise average AAI was calculated using the AAI
146 workflow with default settings in CompareM package v0.0.23 (<https://github.com/dparks1134/CompareM>)
147 for selected representatives from each clade.

148 The percentage of conserved proteins (POCP) between two genomes was calculated as $[(C1 +$
149 $C2)/(T1 + T2)] \times 100\%$, where C1 and C2 represent the conserved number of proteins in the two
150 genomes being compared, respectively, and T1 and T2 represent the total number of proteins in
151 the two genomes being compared, respectively. Conserved proteins were identified using BLASTp
152 match with an e-value of less than $1e-5$, sequence identity of more than 40%, and an alignable
153 region of the query protein sequence of more than 50%, as previously recommended [34].

154 RESULTS AND DISCUSSION

155 To study the taxonomic relationships within the family *Streptomycetaceae*, we first reconstructed
156 a 16S rRNA gene phylogeny with most of the *Allostreptomyces*, *Embleya*, *Kitasatospora*,
157 *Streptacidiphilus*, *Streptomyces* and *Yinghuangia* type strains described so far (Fig. S1). The 16S
158 rRNA gene sequence alignment using SINA produced 1,144 nucleotide with no gaps. Of these,
159 331 (28.9%) sites were variable. The bootstrap support values for the 16S rRNA gene based trees
160 reconstructed were low in many branches, which reflects the reduced resolution when attempting
161 to discriminate between a large group of strains that share highly similar 16S rRNA gene
162 sequences, as is the case for members of *Streptomyces* [35]. In these analyses, *Allostreptomyces*,
163 *Embleya* and *Yinghuangia* were resolved from *Streptomyces*, whereas *Kitasatospora* and
164 *Streptacidiphilus* were recovered within *Streptomyces*. Whilst *Kitasatospora* species were
165 recovered in a single cluster, the analyses showed both *Streptomyces* and *Streptacidiphilus* to be
166 polyphyletic. We note that the taxonomic position of *Allostreptomyces* with regard to *Streptomyces*
167 (Fig. 1 versus Fig. S1) should be addressed in further studies.

168 In order to provide further taxonomic resolution for the family *Streptomycetaceae*, a phylogenomic
169 analysis based on conserved universal proteins within the *Streptomycetaceae* was carried out. This

170 resolved members of the genera *Streptacidiphilus* and *Streptomyces* into several well-supported
171 lineages (Fig. 1). Three lineages were identified within the genus *Streptacidiphilus*. Seven species
172 (*Streptacidiphilus anmyonensis*, *Streptacidiphilus carbonis*, *Streptacidiphilus jiangxiensis*,
173 *Streptacidiphilus melanogenes*, *Streptacidiphilus neutrinimicus*, *Streptacidiphilus pinicola* and
174 *Streptacidiphilus rugosus*) clustered with the type species, *Streptacidiphilus albus* i.e., form
175 *Streptacidiphilus sensu stricto*. The second lineage, which formed a sister clade to *Kitasatospora*,
176 consisted of *Streptacidiphilus griseoplanus*, a recently reclassified *Streptomyces* that produces
177 grey mycelium, and *Streptacidiphilus bronchialis*, a ciprofloxacin-resistant bacterium producing
178 aerial mycelium of different colors depending on the growth medium [36]. Their 16S rRNA gene
179 sequences exhibit 98.8 % similarity and on phylogenetic analysis formed a cluster with high
180 bootstrap support. The earlier MLSA analysis of Labeda *et al.* [17] also placed *S. griseoplanus*
181 outside of *Streptacidiphilus sensu stricto* and suggested that this species might belong to a novel
182 genus, whilst Nououi *et al.* [36] discussed characteristics of *S. bronchialis* indicative of a ‘fuzzy’
183 species with a hybrid of genomic and chemotaxonomic features from *Streptacidiphilus* and
184 *Streptomyces*. The conserved protein-based phylogeny presented here (Fig. 1) clearly shows that
185 these taxa are phylogenetically more closely related to *Kitasatospora* than *Streptacidiphilus sensu*
186 *stricto*. The coherent grouping of these two species is also evident through the visualization of the
187 AAI data (Fig. 2). Furthermore, both these type strains share 81.3 % AAI with one another but
188 share 67.8– 70.0% (mean: 68.41%) AAI with other *Streptacidiphilus* species (Table 1, Fig. 2 and
189 Table S3) i.e., below the ~70-74% AAI threshold for comparisons of different genera [37, 38].
190 When the AAI values for the type strains of these two species were determined in comparison with
191 the members of *Kitasatospora* (Table 1, Fig. 2, and Table S3), a slightly higher mean value of 70.7
192 % (range 69.2 – 72.8 %) was obtained, supporting the suggestion that these two species may be a

193 sister clade of *Kitasatospora*. The AAI values strongly suggest that these two species should not
194 be placed in *Kitasatospora*. Likewise, the POCP values of these two species to each other (56.4%,
195 Fig. 3) are also notably higher than those of the two species to *Kitasatospora* type strains (45.3-
196 52.4%, average 48.7%) and to other *Streptacidiphilus sensu stricto* species (43.9 – 48.7%, average
197 46.2%, excluding *Streptacidiphilus oryzae* – see below), consistent with both belonging to a genus
198 distinct from either *Streptacidiphilus* or *Kitasatospora*. Notably, these two species were not
199 recovered within *Streptacidiphilus sensu stricto* in the TYGS-generated phylogenomic analysis of
200 Malik *et al.* [39] and have also been placed within a novel genus in the Genome Taxonomy
201 Database (GTDB, release 07-RS207, April 2022) [40], which classifies them as ‘g_Streptomyces_D’
202 within the family *Streptomycetaceae* (https://gtdb.ecogenomic.org/tree?r=f__Streptomycetaceae). The
203 phylogenomic analysis of Li *et al.* [13] also noted that the *S. bronchialis* type strain is more closely
204 related to *Kitasatospora* than *Streptacidiphilus* and may belong to a novel genus. Cumulatively,
205 these data support the reclassification of these two species into a novel genus within the family
206 *Streptomycetaceae*, for which the name *Peterkaempfera* gen. nov. is proposed, with
207 *Peterkaempfera griseoplanus* as the type species. Phenotypically, members of this genus possessed
208 galactose, ribose, and traces of mannose in the whole-cell hydrolysates, with MK-9 (H₆) as a
209 menaquinone (Table 2).

210 The third cluster distinguished within *Streptacidiphilus* contains the type strain of
211 *Streptacidiphilus oryzae*, an acidophilic actinomycete (which grows between pH 3.0 to 6.5), strains
212 of which were originally recovered from the acidic soil of a rice paddy in Thailand [41]. When its
213 16S rRNA gene was analyzed, the phylogenetically closest member was found to be *S.*
214 *griseoplanus* with 97.4 % similarity, and these two taxa were originally grouped in ‘Cluster 54’ in
215 the comprehensive 16S rRNA gene phylogenetic analysis of Labeda *et al.* [16]. However, *S. oryzae*

216 was recovered with *Streptacidiphilus* species in the subsequent MLSA analysis [17]. In the present
217 study, the distinctness of this cluster is reflected in the conserved protein-based phylogenomic
218 analysis (Fig. 1). The AAI analysis showed that *S. oryzae* shares 67.3 – 68.6% (mean: 67.8%) with
219 other *Streptacidiphilus sensu stricto* species (Table 1; Table S3 and Fig. 2), again below the AAI
220 threshold of ~70-74% that can be used to delineate genera. When this strain was compared with
221 members of *Kitasatospora* (Table S3 and Fig. 2), the mean AAI was 66.2% (range 65.7-66.7%).
222 POCP values for this species compared to other *Streptacidiphilus* species were 46.4 – 51.2% (Fig.
223 3, mean 48.8%) consistent with the type strain belonging to a genus distinct from *Streptacidiphilus*.
224 Moreover, this species was not recovered within *Streptacidiphilus sensu stricto* in the analyses of
225 Nouioui *et al.* and Malik *et al.* [24, 39], and the species has also been placed within a novel genus
226 in the GTDB 07-RS207 classification, which classifies it as genus ‘g_Streptacidiphilus_A’ within
227 the family *Streptomycetaceae* (https://gtdb.ecogenomic.org/tree?r=g_Streptacidiphilus_A). In
228 addition, distinguishing phenotypic properties detected are that the diagnostic sugars in the whole-
229 cell hydrolysates include galactose, glucose, mannose, and ribose as previously noted by Wang *et*
230 *al* [41] and that the cell wall contains LL- & *meso*-A₂pm as diamino acid (Table 2). Therefore,
231 these polyphasic data support the proposal of a novel genus, *Phaeacidiphilus* gen. nov., to
232 accommodate *Streptacidiphilus oryzae* as the type species.

233 The core genome analysed using conserved universal proteins also identified unique
234 lineages at the periphery of the genus *Streptomyces* (Fig. 1). One early branch, supported by 100%
235 bootstrap support, contains “*Streptomyces cattleya*”, a single species lineage, separated (with 81%
236 bootstrap support) from a group of eleven species. “*S. cattleya*” has received study as a source of
237 secondary metabolites, notably including the antibiotic thienamycin and fluorinated compounds
238 [42-45] but has not been formally described as a species [17]. The mean AAI between the type

239 strain of this species and reference *Streptomyces* spp. was 70.0% (Table 1; range 69.0-71.1, Table
240 S3 and Fig. 2), and the POCP value with the type strain of the genus, *Streptomyces albus* subsp.
241 *albus*, is 52.7% (Fig. 3). Although this value is slightly greater than the originally proposed 50%
242 POCP threshold for delineating genera [34], numerous studies have suggested this threshold is too
243 stringent [47-49]. “*S. cattleya*” is recognized as phylogenetically divergent from *Streptomyces* [17,
244 46] and was placed in a novel genus (g_Streptomyces_C) in the 06-RS202 release of the GTDB,
245 although we note that in the later release 07-RS207 it is placed in *Streptomyces*. Based on these
246 cumulative phylogenomic, AAI and POCP data presented here, we propose that “*S. cattleya*” is
247 classified as the type species of *Streptantibioticus* gen. nov. However, in order to correct the
248 malformed species epithet in “*S. cattleya*” we here name the type species *Streptantibioticus*
249 *cattleyicolor*. The MLSA study of Labeda *et al.* [17] also suggests that *Streptomyces ferralitis* [50]
250 may be a member of this genus and a 16S rRNA gene phylogeny analysis indicated *Streptomyces*
251 *rubrisoli*, a neutrotolerant, acidophilic bacteria recovered from red soil [51] was also present within
252 this clade, but this needs to be confirmed by further analysis.

253 A closely related group of eleven species are the type strains of *Streptomyces acididurans*,
254 *Streptomyces alni*, *Streptomyces bryophytorum*, *Streptomyces glauciniger*, *Streptomyces*
255 *guanduensis*, *Streptomyces oryziradicis*, *Streptomyces paucisporeus*, *Streptomyces rubidus*,
256 “*Streptomyces soli*” *Streptomyces yanglinensis* and *Streptomyces yeochonensis*. The members of
257 this clade showed a low 16S rRNA gene similarity range (96.3- 97.4 %) to “*S. cattleya*” and to
258 *Streptomyces sensu stricto* species (ranging between 96.1- 97.3 %). When the genomes of these
259 eleven species were subjected to AAI analysis, the organisms within this clade share 70.6–87.2 %
260 (mean: 74.9 %) AAI with one another, but only share 67.2–71.3 % (mean: 69.0 %) AAI with the
261 reference *Streptomyces* species, which is reflected in the AAI data visualized as a heat map (Fig.

262 2 and Table S3). These AAI values are thus consistent with the ~70-74% AAI threshold for
263 comparisons of different genera [37, 38]. We also note that our earlier study [20] shows AAI values
264 between the genus *Carbonactinospora* in the family *Carbonactinosporaceae* and members of
265 *Streptomyces* to be ~55-60% and so using a higher threshold for genus delineation within
266 *Streptomycetaceae* seems reasonable. POCP values among this group of 11 type strains were 41.8-
267 71.2% (mean: 54.2%) whereas values compared to the *Streptomyces* reference strains were 38.9-
268 62.7 (mean: 47.3%, Table 1, Fig 3, and Table S4). Seven among these 11 taxa were placed in a
269 novel genus (g_Streptomyces_B) in the 06-RS202 release of the GTDB, although we note that in
270 the later 07-RS207 release they were returned to *Streptomyces*. In contrast *S. glauciniger*, *S.*
271 *rubidus*, *S. yanglinensis*, and *S. yeochonensis* were well separated from *Streptomyces sensu stricto*
272 in the phylogenomic analysis by Malik *et al.* [39] and the nine of these species included in the
273 phylogenomic analysis of Chantavorakit *et al.* [52] formed a coherent grouping separated from the
274 *Streptomyces* reference strains. Likewise, the recently described *Streptomyces epipremni* also
275 clustered with members of this group in phylogenomic analyses [53]. Cumulatively, these data
276 support the conclusion that these 12 species should be classified in a separate genus, for which we
277 propose the name *Actinacidiphila* gen. nov., with *Actinacidiphila yeochonensis* comb. nov. as the
278 type species. The MLSA analysis of Labeda *et al.* [17] suggests that *Streptomyces cocklensis* may
279 also be a member of this genus but this needs to be confirmed by further analysis.

280 A single lineage containing the type strain of “*Streptomyces gilvigriseus*” [54] showed a
281 low 16S rRNA gene sequence similarity of 96.2 % with its apparent nearest relative, *Streptomyces*
282 *qinglanensis* (with which it shares ANI, dDDH and AAI values of 74.8, 20.8, and 64.1 %, respectively). This lineage branch formed a sister clade to *Embleya*, branching before
283 *Streptomyces* in the conserved protein phylogeny (Fig. 1). The AAI analysis shows that “*S.*
284 *Streptomyces* in the conserved protein phylogeny (Fig. 1). The AAI analysis shows that “*S.*

285 *gilvigriseus*” shares 63.9 – 64.9% (mean: 64.5 %, Table 1) AAI with the reference *Streptomyces*
286 species (Fig. 3 and Table S3) i.e., below the AAI threshold of ~70-74% than can be used to
287 delineate genera. Likewise, POCP data show <55.5% values (average 44.0%) to all *Streptomyces*
288 species included (Fig. 3), again suggesting this species belongs to a separate genus. This species
289 has also been placed in a novel genus (g_Streptomyces_G) by the GTDB 07-RS207 classification
290 of *Streptomycetaceae* (https://gtdb.ecogenomic.org/tree?r=g_Streptomyces_G). The phylogenetic
291 analyses, combined with the low AAI values, support the proposal of a novel genus
292 *Mangrovactinospora* to accommodate “*S. gilvigriseus*”.

293 Similar to “*S. gilvigriseus*”, an early branch distinct from *Streptomyces* within the
294 conserved protein-based tree (Fig. 1) was recovered containing the type strain of *Streptomyces*
295 *vitaminophilus* [55, 56]. This species was also found to form a clade in the 16S rRNA gene tree
296 and in conserved protein-based tree with *Streptomyces tyrosinilyticus* (Fig. S1 and Fig. 1) and a
297 close relationship to *S. tyrosinilyticus* was previously reported by Zhao *et al.* [57]. In addition, the
298 mean AAI values of *S. vitaminophilus* and *S. tyrosinilyticus* and the phylogenetically closely related
299 taxa in the core genome tree i.e. “*S. cattleya*” and “*S. gilvigriseus*” are 69.6 and 65.0%, respectively
300 (Fig. 2 and Table S3) i.e. below the ~70-74% threshold for delineating genera [37, 38]. Both *S.*
301 *vitaminophilus* and *S. tyrosinilyticus* have been placed in a novel genus (g_Streptomyces_A) by the GTDB
302 07-RS207 classification of *Streptomycetaceae* (https://gtdb.ecogenomic.org/tree?r=g_Streptomyces_A),
303 and they share 73.6% AAI (Fig 2 and Table S3) i.e. on the boundary used here for genus
304 delineation. Although a POCP value of 49.5% was calculated between *S. vitaminophilus* and *S.*
305 *tyrosinilyticus* (Fig. 3), for now it seems prudent to classify them in the same genus based on the
306 AAI and GTDB data. *S. vitaminophilus* was originally named *Actinosporangium vitaminophilum*
307 [55] before transfer to the genus *Streptomyces* based on physiological properties and certain

308 chemotaxonomic characteristics (cell wall type, type-II phospholipid pattern, presence of hexa-
309 and octa-hydrogenated menaquinones [56]. The name *Actinosporangium*, with the type species of
310 *Actinosporangium violaceum* corrig. Krassilnikov and Yuan (Approved Lists 1980) [58], remains
311 validly published even though the type species is currently classified in the genus *Streptomyces*
312 [56]. Therefore, it is necessary to propose a new genus to accommodate these two species, for
313 which we propose the name *Wenjunlia*, with *Wenjunlia vitaminophilum* comb. nov. as the type
314 species. No genome is available for *Streptomyces capparidis* but 16S rRNA gene analysis is noted
315 to suggest a close relationship with *S. vitaminophilus* [59] and thus the taxonomic placement of
316 this species needs further investigation.

317 **CONCLUSION**

318 In conclusion, from phylogenomic and AAI analyses, combined with 16S rRNA gene analysis and
319 synthesis of data from other studies (notably GTDB), it is clearly evident that several taxa within
320 the family *Streptomycetaceae* require reclassification at the genus level. Cumulatively, the data
321 support the proposal of two novel genera containing species previously classified within
322 *Streptacidiphilus* and four genera containing species previously classified in *Streptomyces*; none
323 of these taxa can be placed in *Embleya* or *Yinghuangia* (Fig. 1 and Fig. S1). The descriptions of
324 these genera are provided herewith, whilst key phenotypic properties are summarized in Table 2
325 and further details are in the literature cited for each original description of the taxa reclassified.

326 **TAXONOMIC CONSEQUENCES:**

327 **DESCRIPTION OF *ACTINACIDIPHILA* GEN. NOV.**

328 *Actinacidiphila* (Ac.tin.a.ci.di'phi.la. Gr. fem. n. *actis*, *actinos*, a ray; N.L. neut. n. *acidum*, acid;
329 Gr. masc. adj. *philos*, loving; N.L. fem. adj. *Actinacidiphila*, an acid-loving actinomycete).

330 Aerobic, Gram-positive, non-motile, acidophilic to neutrotolerant, sporulating streptomycetes that
331 form the branched substrate and aerial hyphae. Mesophilic. The cell wall contains LL-
332 diaminopimelic acid. Contains diphosphatidylglycerol, phosphatidylethanolamine,
333 phosphatidylinositol, and phosphatidylinositol mannosides as major polar lipids. Hexa- and octa-
334 hydrogenated menaquinones with nine isoprene units [MK-9 (H₆,H₈)] are the predominant
335 menaquinones. The genomic DNA G+C content ranges from 72-74 %. The genus can be separated
336 from *Streptomyces* based on phylogenomic analyses. The type species is *Actinacidiphila*
337 *yeochonensis*.

338 **DESCRIPTION OF *ACTINACIDIPHILA ACIDIDURANS* COMB. NOV.**

339 *Actinacidiphila acididurans* (a.ci.di.du'rans. L. neut. adj. *acidum*, acid; L. pres. part. *durans*,
340 resisting; N.L. part. adj. *acididurans*, acid-resisting).

341 Basonym: *Streptomyces acididurans* Chantavorakit *et al.* 2021

342 The description is the same as that given for *Streptomyces acididurans* [52]. The species is
343 assigned to the genus based on phylogenomic analysis. The G+C content of the type strain genome
344 is 72.3 % and its approximate genome size is 9.68 Mbp (GenBank accession number
345 GCA_016918855). The GenBank accession number of the 16S rRNA gene sequence of the type
346 strain is MW201570. The type strain is KK5PA1^T (= NBRC 114802^T= TBRC 13094^T).

347 **DESCRIPTION OF *ACTINACIDIPHILA ALNI* COMB. NOV.**

348 *Actinacidiphila alni* (al'ni. L. gen. fem. n. *alni*, of the alder, referring to the isolation of the type
349 strain from *Alnus nepalensis*).

350 Basonym: *Streptomyces alni* Liu *et al.* 2009 emend. Nouioui *et al.* 2018.

351 The description is the same as that given for *Streptomyces alni* [60]. The species is assigned to the
352 genus based on phylogenomic analysis. The G+C content of the type strain genome is 72.1 % and
353 its approximate genome size is 8.27 Mbp (GenBank accession number GCA_900112845). The
354 GenBank accession number of the 16S rRNA gene sequence of the type strain is DQ460470. The
355 type strain is D65^T (= CGMCC 4.3510^T =DSM 42036^T = JCM 16122^T =NRRL B-24611^T).

356 **DESCRIPTION OF *ACTINACIDIPHILA BRYOPHYTORUM* COMB. NOV.**

357 *Actinacidiphila bryophytorum* (bry.o.phy.to'rum. N.L. gen. neut. pl. n. *bryophytorum*, of
358 Bryophyta, referring to the isolation of the type strain from a member of the phylum Bryophyta)

359 Basonym: *Streptomyces bryophytorum* Li *et al.* 2016

360 The description is the same as that given for *Streptomyces bryophytorum* [61]. The species is
361 assigned to the genus based on phylogenomic analysis. The G+C content of the type strain genome
362 is 72.5 % and its approximate genome size is 8.05 Mbp (GenBank accession number
363 GCA_016916835). The GenBank accession number of the 16S rRNA gene sequence of the type
364 strain is KF923804. The type strain is NEAU-HZ10^T (= CGMCC 4.7151^T = DSM 42138^T).

365 **DESCRIPTION OF *ACTINACIDIPHILA EPIPREMNI* COMB. NOV.**

366 *Actinacidiphila epipremni* (e.pi.prem'ni. N.L. gen. n. *epipremni*, of *Epipremnum*, referring to the
367 generic name of *Epipremnum aureum* from which the strain is isolated).

368 Basonym: *Streptomyces epipremni* Duangupama *et al.* 2022

369 The description is the same as that given for *Streptomyces epipremni* [53]. The species is assigned
370 to the genus based on phylogenomic analysis. The G+C content of the type strain genome is 73.6
371 % and its approximate genome size is 8.2 Mbp (GenBank accession number GCA_012034175).
372 The GenBank accession number of the 16S rRNA gene sequence of the type strain is LC333557.
373 The type strain is PRB2-1^T (= NBRC 113169^T = TBRC 7642^T).

374 **DESCRIPTION OF *ACTINACIDIPHILA GLAUCINIGRA* COMB. NOV.**

375 *Actinacidiphila glaucinigra* (glau.ci.ni'gra L. masc. adj. *glaucus*, greenish grey; L. masc.
376 adj. *niger*, black; N.L. fem. adj. *glaucinigra*, greenish-black, referring to the color of the colony
377 reverse on modified Bennett's agar).

378 Basonym: *Streptomyces glauciniger* Huang *et al.* 2004.

379 The description is the same as that given for *Streptomyces glauciniger* [62]. The species is
380 assigned to the genus based on phylogenomic analysis. The G+C content of the type strain genome
381 is 72.3 % and its approximate genome size is 9.81 Mbp (GenBank accession number
382 GCA_900188405). The GenBank accession number of the 16S rRNA gene sequence of the type
383 strain is AB249964. The type strain is FXJ14^T (=AS 4.1858^T = DSM 41867^T = JCM 12278^T
384 = LMG 22082^T = NBRC 100913^T).

385 **DESCRIPTION OF *ACTINACIDIPHILA GUANDUENSIS* COMB. NOV.**

386 *Actinacidiphila guanduensis* (gu.an.du.en'sis. N.L. fem. adj. *guanduensis*, of or belonging to
387 Guandu, the source of the soil from which the type strain was isolated).

388 Basonym: *Streptomyces guanduensis* Xu *et al.* 2006.

389 The description is the same as that given for *Streptomyces guanduensis* [63]. The species is
390 assigned to the genus based on phylogenomic analysis. The G+C content of the type strain genome
391 is 73.1 % and its approximate genome size is 8.22 Mbp (GenBank accession number
392 GCA_900103985). The GenBank accession number of the 16S rRNA gene sequence of the type
393 strain is AY876942. The type strain is 701^T (= CGMCC 4.2022^T = DSM 41944^T = JCM 13274^T
394 = NBRC 102070^T)

395 **DESCRIPTION OF *ACTINACIDIPHILA ORYZIRADICIS* COMB. NOV.**

396 *Actinacidiphila oryziradicis* (o.ry.zi.ra'di.cis. L. fem. n. *oryza*, rice; L. fem. n. *radix* (gen. *radicis*),
397 root; N.L. gen. fem. n. *oryziradicis*, of the rice root)

398 Basonym: *Streptomyces oryziradicis* Li *et al.* 2020

399 The description is the same as that given for *Streptomyces oryziradicis* [65]. The species is
400 assigned to the genus based on phylogenomic analysis. The G+C content of the type strain genome
401 is 69.5 % and its approximate genome size is 11.5 Mbp (GenBank accession number
402 GCA_005047355). The GenBank accession number of the 16S rRNA gene sequence of the type
403 strain is MK245982. The type strain is NEAU-C40^T (=DSM 107943^T =CCTCC AA 2018038^T).

404 **DESCRIPTION OF *ACTINACIDIPHILA PAUCISPOREA* COMB. NOV.**

405 *Actinacidiphila paucisporea* (pau.ci.spo're.a. L. masc. adj. *paucus*, few; N.L. masc. adj. *sporeus*,
406 spored; N.L. fem. adj. *paucisporea*, few spored, forming few spores).

407 Basonym: *Streptomyces paucisporeus* Xu *et al.* 2006 emend. Nouioui *et al.* 2018.

408 The description is the same as that given for *Streptomyces paucisporeus* [63]. The species is
409 assigned to the genus based on phylogenomic analysis. The G+C content of the type strain genome
410 is 72.2 % and its approximate genome size is 8.16 Mbp (GenBank accession number
411 GCA_900142575). The GenBank accession number of the 16S rRNA gene sequence of the type
412 strain is AY876943. The type strain is 1413^T (= CGMCC 4.2025^T = DSM 41946^T = JCM 13276^T
413 = NBRC 102072^T).

414 **DESCRIPTION OF *ACTINACIDIPHILA RUBIDA* COMB. NOV.**

415 *Actinacidiphila rubida* (ru'bi.da. L. fem. adj. *rubida*, dark red).

416 Basonym: *Streptomyces rubidus* Xu *et al.* 2006 emend. Nouioui *et al.* 2018.

417 The description is the same as that given for *Streptomyces rubidus* [63]. The species is assigned
418 to the genus based on phylogenomic analysis. The G+C content of the type strain genome is 72.9
419 % and its approximate genome size is 9.01 Mbp (GenBank accession number GCA_900110255).
420 The GenBank accession number of the 16S rRNA gene sequence of the type strain is AY876941.
421 The type strain is 13c15^T (= CGMCC 4.2026^T = DSM 41947^T = JCM 13277^T = NBRC 102073^T).

422 **DESCRIPTION OF *ACTINACIDIPHILA SOLI* SP. NOV.**

423 *Actinacidiphila soli* (so'li. L. gen. n. *soli* of soil).

424 The description is the same as that given for "*Streptomyces soli*" [64]. The species is assigned to
425 the genus based on phylogenomic analysis. The G+C content of the type strain genome is 69.95
426 % and its approximate genome size is 9.40 Mbp (GenBank accession number GCA_003999195).
427 The GenBank accession number of the 16S rRNA gene sequence of the type strain is MF784350.
428 The type strain is LAM7114^T (= CGMCC 4.7581^T = JCM 32822^T).

429 **DESCRIPTION OF *ACTINACIDIPHILA YANGLINENSIS* COMB. NOV.**

430 *Actinacidiphila yanglinensis* (yang.lin.en'sis. N.L. fem. adj. *yanglinensis*, of or belonging to
431 Yanglin, the source of the soil from which the type strain was isolated).

432 Basonym: *Streptomyces yanglinensis* Xu *et al.* 2006 emend. Nouioui *et al.* 2018.

433 The description is the same as that given for *Streptomyces yanglinensis* [63]. The species is
434 assigned to the genus based on phylogenomic analysis. The G+C content of the type strain genome
435 is 72.6 % and its approximate genome size is 9.59 Mbp (GenBank accession is GCA_900107965).
436 The GenBank accession number of the 16S rRNA gene sequence of the type strain is AY876940.
437 The type strain is 1307^T (= CGMCC 4.2023^T = DSM 41945^T = JCM 13275^T = NBRC 102071^T).

438 **DESCRIPTION OF *ACTINACIDIPHILA YEOCHONENSIS* COMB. NOV.**

439 *Actinacidiphila yeochonensis* (ye.o.chon.en'sis. N.L. fem. adj. *yeochonensis*, of Yeochon, a
440 province in Korea, referring to the place where the organism was first isolated).

441 Basonym: *Streptomyces yeochonensis* Kim *et al.* 2004 emend. Nouioui *et al.* 2018.

442 The description is the same as that given for *Streptomyces yeochonensis* [66]. The species is
443 assigned to the genus based on phylogenomic analysis. The G+C content of the type strain genome
444 is 73.6 % and its approximate genome size is 7.82 Mbp (GenBank accession number
445 GCA_000745345). The GenBank accession number of the 16S rRNA gene sequence of the type
446 strain is AF101415. The type strain is CN 732^T (= DSM 41868^T = IMSNU 50114^T = JCM 12366^T
447 = KCTC 9926^T = NBRC 100782^T = NRRL B-24245^T).

448 **DESCRIPTION OF *MANGROVACTINOSPORA* GEN. NOV.**

449 *Mangrovactinospora* (Man.grov.ac.ti.no.spo'ra. N.L. neut. n. *mangrovum*, a mangrove; Gr. fem.
450 n. *actis*, *actinos*, a ray; Gr. fem. n. *spora*, a seed and, in biology, a spore; N.L. fem.
451 n. *Mangrovactinospora*, a mangrove actinomycete with spiny spores).

452 Gram-stain positive mesophilic actinomycete. Forms substrate and aerial mycelium. The cell wall
453 peptidoglycan contains LL-diaminopimelic acid. The major polar lipids include
454 diphosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, hydroxy
455 phosphatidylethanolamine, phosphatidyl methyl ethanolamine and hydroxy phosphatidyl methyl
456 ethanolamine, and the major fatty acids are anteiso-C_{15:0}, iso-C_{16:0}, iso-C_{15:0} and anteiso-C_{17:0}. The
457 menaquinones are MK-9 (H₈) and MK-9(H₆). The cell wall sugars include galactose, glucose,
458 mannose, ribose, and rhamnose. The genomic DNA G+C content is around 73 %. The genus can
459 be distinguished from *Streptomyces* based on phylogenomic analyses. The type species is
460 *Mangrovactinospora gilvigriseus*.

461 **DESCRIPTION OF *MANGROVACTINOSPORA GILVIGRISEA* SP. NOV.**

462 *Mangrovactinospora gilvigrisea* (gil.vi.gri'se.a. L. masc. adj. *gilvus*, yellow; L. masc. adj. *griseus*,
463 grey; N.L. fem. adj. *gilvigrisea*, yellow-grey, referring to the color of the mycelium).

464 The description is the same as that given for "*Streptomyces gilvigriseus*" [54]. The species is
465 assigned to the genus based on phylogenomic analysis. The G+C content of the type strain genome
466 is 73 % and its approximate genome size is 5.21 Mbp (GenBank accession number
467 GCA_001879105). The GenBank accession number of the 16S rRNA gene sequence of the type
468 strain is KJ632660. The type strain is MUSC 26^T (=DSM 42173^T = MCCC 1K00504^T = NBRC
469 110931^T).

470 **DESCRIPTION OF *PETERKAEMPFERA* GEN. NOV.**

471 *Peterkaempfera* (Pe.ter.kaemp'fe.ra. N.L. fem. gen. n. *Peterkaempfera*, named in recognition of
472 the contribution of Peter Kämpfer to the systematics of actinomycetes).

473 Aerobic, mesophilic Gram-stain positive, non-acid-fast, non-motile, streptomycetes producing
474 branched mycelium and aerial hyphae. The cell wall peptidoglycan contains LL-diaminopimelic
475 acid as the diagnostic diamino acid and glucose, mannose and ribose are present in whole-cell
476 hydrolysates; the major menaquinones are MK-9 (H₈) or MK-9 (H₆). The polar lipid profile
477 contains diphosphatidyl glycerol, phosphatidylethanolamine, phosphatidylinositol, and other
478 lipids; the major fatty acids are anteiso-C_{15:0} and iso-C_{16:0}. The genomic DNA G+C content is
479 around 72 %. The genus can be separated from *Streptacidiphilus* based on phylogenomic analyses.
480 The type species is *Peterkaempfera griseoplanus*.

481 **DESCRIPTION OF *PETERKAEMPFERA BRONCHIALIS* COMB. NOV.**

482 *Peterkaempfera bronchialis* (bron.chi.a'lis. L. pl. n. *bronchia* the bronchial tubes; L. fem. suff. -
483 *alis* suffix used with the sense of pertaining to; N.L. fem. adj. *bronchialis*, pertaining to the
484 bronchial tubes).

485 Basonym: *Streptacidiphilus bronchialis* Nouioui *et al.* 2019.

486 The description is the same as that given for *Streptacidiphilus bronchialis* [36]. The species is
487 assigned to the genus based on phylogenomic analysis. The G+C content of the type strain genome
488 is 72.6 % and its approximate genome size is 7.01 Mbp (GenBank accession number
489 GCA_003258605). The GenBank accession number of the 16S rRNA gene sequence of the type
490 strain is MK089584. The type strain is 15-057A^T (=DSM 106435^T = ATCC BAA-2934^T).

491 **DESCRIPTION OF *PETERKAEMPFERA GRISEOPLANA* COMB. NOV.**

492 *Peterkaempfera griseoplana* (gri.se.o.pla'na. L. masc. adj. *griseus*, grey; L. masc. adj. *planus*, flat,
493 level; N.L. fem. adj. *griseoplana* flat, grey, referring to the restricted, flat, planar growth and
494 greyish spore color in masse of the organism).

495 Basonym: *Streptacidiphilus griseoplanus* (Backus *et al.* 1957) Nouioui *et al.* 2019.

496 The description is the same as that given for *Streptacidiphilus griseoplanus* [36, 67, 68]. The
497 species is assigned to the genus based on phylogenomic analysis. The G+C content of the type
498 strain genome is 72.5 % and its approximate genome size is 8.25 Mbp (GenBank accession number
499 GCA_001418575). The GenBank accession number of the 16S rRNA gene sequence of the type
500 strain is AB184138. The type strain is DSM 40009^T (=NBRC 12779^T = ISP 5009^T = RIA 1046^T =
501 NBRC 12779^T = CBS 505.68^T = IFO 12779^T = ATCC 19766^T = AS 4.1868^T).

502 **DESCRIPTION OF *PHAEACIDIPHILUS* GEN. NOV.**

503 *Phaeacidiphilus* (Phae.a.ci.di'phi.lus. Gr. masc. adj. *phaeos*, grey, brown; L. neut. n. *acidum*,
504 acid; Gr. masc. adj. *philos*, loving; N.L. masc. adj. *Phaeacidiphilus*, brown-colored substrate
505 mycelium producing, acid-loving).

506 Aerobic, mesophilic, and acidophilic Gram-stain positive, non-acid alcohol fast staining
507 actinomycetes. Spores are born on aerial hyphae. The diamino acid of the cell wall peptidoglycan
508 is LL-diaminopimelic acid, although minor amounts of the meso-isomer may be present. Whole-
509 cell hydrolysates contain galactose, glucose, mannose, and ribose. Contains hexa- and octa-
510 hydrogenated menaquinones with nine isoprene units. The major polar lipids are diphosphatidyl
511 glycerol, phosphatidyl ethanolamine, phosphatidyl inositol and phosphatidyl inositol mannosides.
512 The genus can be distinguished from the genus *Streptacidiphilus* based on phylogenomic analyses.
513 The type species is *Phaeacidiphilus oryzae*.

514 **DESCRIPTION OF *PHAEACIDIPHILUS ORYZAE* COMB. NOV.**

515 *Phaeacidiphilus oryzae* (o.ry'zae. L. gen. fem. n. *oryzae*, of rice, denoting the isolation of the
516 strains from a rice field).

517 Basonym: *Streptacidiphilus oryzae* Wang *et al.* 2006 emend. Nouioui *et al.* 2018.

518 The description is the same as that given for *Streptacidiphilus oryzae* [41]. The species is assigned
519 to the genus based on phylogenomic analysis. The G+C content of the type strain genome is 73.4
520 % and its approximate genome size is 7.81 Mbp (GenBank accession number GCA_000744815).
521 The GenBank accession number of the 16S rRNA gene sequence of the type strain is AB894335.
522 The type strain is TH49^T (=CGMCC 4.2012^T = DSM 45098^T = JCM 13271^T).

523 **DESCRIPTION OF *STREPTANTIBIOTICUS* GEN. NOV.**

524 *Streptantibioticus* (Strept.an.ti.bi.o'ti.cus. Gr. masc. adj. *streptos*, pliant, twisted; N.L. masc.
525 adj. *antibioticus*, against life, antibiotic; N.L. masc. n. *Streptantibioticus*, a streptomycete that
526 produces antibiotics).

527 Aerobic, mesophilic Gram-stain positive non-acid fast staining sporulating actinomycetes.
528 Sporophores are produced on the aerial mycelium. The genomic DNA G+C content is around 73
529 %. The genus can be distinguished from *Streptomyces* based on phylogenomic analyses. The type
530 species is *Streptantibioticus cattleyicolor*.

531 **DESCRIPTION OF *STREPTANTIBIOTICUS CATTLEYICOLOR* SP. NOV.**

532 *Streptantibioticus cattleyicolor* (catt.ley.i'co.lor. N.L. fem. n. *Cattleya*, an orchid genus; L. masc.
533 n. *color*, colour; N.L. masc. adj. *cattleyicolor*, *Cattleya*-coloured, orchid white).

534 The description is the same as that given for "*Streptomyces cattleya*" [42, 43]. The species epithet
535 reflects the original intention of Kahan *et al.* [43] that "*S. cattleya*" should be named in recognition
536 of its distinctive orchid-white colored aerial mycelium but corrected for the Latinisation of the
537 name. Further characteristics are given in the genome description [43]. The species is assigned to
538 the genus based on phylogenomic analysis. The type strain is notable for the production of the
539 antibiotic thienamycin and the fluorinated antibiotic 4-fluorothreonine when cultivated in the
540 presence of fluorine. The G+C content of the type strain genome is 73 % and its approximate
541 genome size is 8.10 Mbp including one linear plasmid (GenBank accession number
542 GCA_000240165). The GenBank accession number of the 16S rRNA gene sequence of the type

543 strain is FQ859185. The type strain is NBRC 14057^T (MA4297^T = ATCC 35852^T = DSM 46488^T
544 = JCM 4925^T = NCIMB 11928^T = NRRL 8057^T).

545 **DESCRIPTION OF *WENJUNLIA* GEN. NOV.**

546 *Wenjunlia* (Wen.jun.li'a. N.L. fem. n. *Wenjunlia*, named in recognition of the contribution of
547 Professor Wen-Jun Li to the systematics of actinomycetes).

548 Cells are Gram-stain positive, aerobic, mesophilic, and can form non-motile spores. Spores are
549 formed on the aerial mycelium. The major menaquinones are MK-9(H₆) and MK-9(H₈). LL-
550 diaminopimelic acid is present in the cell wall. The major phospholipids include diphosphatidyl
551 glycerol, phosphatidyl ethanolamine, phosphatidyl inositol, and phosphatidylinositol
552 mannosides. The genomic DNA G+C content is around 72 %. The genus can be distinguished
553 from *Streptomyces* based on phylogenomic analyses. The type species is *Wenjunlia*
554 *vitaminophilus*.

555 **DESCRIPTION OF *WENJUNLIA VITAMINOPHILA* COMB. NOV.**

556 *Wenjunlia vitaminophila* (N.L. neut. n. *vitaminum*, vitamin; N.L. masc. adj. *philus*, friend, loving;
557 from Gr. masc. adj. *philos*, on; N.L. fem. adj. *vitaminophila*, vitamin-loving).

558 Basonym: *Streptomyces vitaminophilus* corrig. (Shomura *et al.* 1983) Goodfellow *et al.* 1986
559 emend. Nouioui *et al.* 2018.

560 The description is the same as that given for *Streptomyces vitaminophilus* [24, 55, 56]. The species
561 is assigned to the genus based on phylogenomic analysis. The G+C content of the type strain
562 genome is 72 % and its approximate genome size is 6.55 Mbp (GenBank accession number

563 GCA_001445835). The GenBank accession number of the 16S rRNA gene sequence of the type
564 strain is AB184589. The type strain is SF 2080^T (=ATCC 31673^T = DSM 41686^T = IFO 14294^T
565 = JCM 6054^T = NBRC 14294^T = NRRL B-16933^T).

566 **DESCRIPTION OF *WENJUNLIA TYROSINILYTICA* COMB NOV.**

567 *Wenjunlia tyrosinilytica* (ty.ro.si.ni.ly'ti.ca. N.L. neut. n. *tyrosinum*, tyrosine; Gr. masc.
568 adj. *lytikos* able to dissolve; N.L. fem. adj. *tyrosinilytica*, tyrosine dissolving).

569 Basonym: *Streptomyces tyrosinilyticus* Zhao *et al.* 2015

570 The description is the same as that given for *Streptomyces tyrosinilyticus* [57]. The species is
571 assigned to the genus based on phylogenomic analysis. The G+C content of the type strain genome
572 is 71 % and its approximate genome size is 8.49 Mbp (GenBank accession number
573 GCA_014646055). The GenBank accession number of the 16S rRNA gene sequence of the type
574 strain is KM000839. The type strain is NEAU-Jh3-20^T (= CGMCC4.7201^T = DSM 42170^T).

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580 **Conflicts of interest**

581 The authors declare that there are no conflicts of interest.

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823 **Table 1.** Mean POCP and AAI values between representative *Streptomyces* and other validly described genera; and newly proposed
 824 genera (bold) derived from *Streptomyces* and *Streptacidiphilus*.

Sl. No	Taxa	¹ POCP (%)										
		1	2	3	4	5	6	7	8	9	10	11
1	<i>Allostreptomyces</i>		50.2 ± 2.8	45.8 ± 2.7	43.0 ± 1.7	44.9 ± 1.1	40.4 ± 0.8	42.1	36.5 ± 0.4	36.0	45.8 ± 0.3	47.5
2	<i>Streptomyces</i> *	71.6 ± 1.9		47.3 ± 4.2	43.9 ± 2.0	46.2 ± 2.6	41.2 ± 1.6	45.0 ± 1.5	37.4	44.0 ± 2.8	47.5 ± 7.0	52.0 ± 2.4
3	<i>Actinacidiphila</i> gen. nov.	69.2 ± 0.7	69.0 ± 1.02		44.1 ± 2.8	47.5 ± 2.7	44.0 ± 2.4	46.0 ± 2.4	36.6 ± 1.8	42.5 ± 4.0	46.6 ± 2.0	48.1 ± 3.4
4	<i>Kitasatospora</i>	64.7 ± 0.3	64.8 ± 0.6	65.3 ± 0.5		48.7 ± 1.7	47.0 ± 1.5	44.3 ± 1.1	38.7 ± 1.2	36.7 ± 0.9	42.8 ± 1.4	45.7 ± 1.0
5	<i>Peterkaempferia</i> gen. nov.	66.6 ± 0.5	66.7 ± 0.6	67.5 ± 0.5	70.7 ± 0.8		46.3 ± 1.0	48.4	37.2 ± 0.9	39.3 ± 2.1	46.3 ± 2.4	47.3 ± 1.7
6	<i>Streptacidiphilus</i>	64.7 ± 0.3	64.7 ± 0.5	65.3 ± 0.5	67.1 ± 0.6	68.4 ± 0.6		48.8 ± 1.4	36.3 ± 0.6	36.0 ± 1.0	40.8 ± 2.1	44.3 ± 0.9
7	<i>Phaeacidiphilus</i> gen. nov.	65.4	65.2 ± 0.3	65.3 ± 0.3	66.2 ± 0.4	68.3 ± 0.10	67.8 ± 0.4		36.5 ± 0.6	41.0	43.4 ± 0.1	48.4
8	<i>Embleya</i>	62.0 ± 0.1	61.9 ± 0.5	61.9 ± 0.4	61.9 ± 0.3	63.2 ± 0.3	61.5 ± 0.4	61.3 ± 0.2		29.4 ± 0.4	37.6 ± 1.1	38.3 ± 0.4
9	<i>Mangrovactinospora</i> gen. nov.	64.1	64.5 ± 0.4	64.3 ± 0.3	63.6 ± 0.3	65.1 ± 0.4	63.6 ± 0.19	64.7	61.4 ± 0.2		48.4 ± 1.3	39.8
10	<i>Wenjunlia</i> gen. nov.	68.4 ± 0.5	68.6 ± 1.0	68.6 ± 0.9	65.7 ± 0.5	68.0 ± 0.5	65.5 ± 0.46	65.5 ± 0.2	63.1 ± 0.2	65.0 ± 0.18		49.0 ± 1.6
11	<i>Streptantibioticus</i> gen. nov.	69.8	70.0 ± 0.8	70.8 ± 0.7	65.7 ± 0.5	67.8 ± 0.35	65.6 ± 0.34	66.1	62.5 ± 0.2	65.2	69.6 ± 0.6	

²AAI (%)

825 * Average of the following members from each clade of *Streptomyces* genus cluster- *Streptomyces fragilis* NBRC 12862, *Streptomyces platensis*
 826 ATCC 23948, *Streptomyces griseocarneus* JCM 4580, *Streptomyces megaspores* NRRL B 16372, *Streptomyces albus* subsp. *albus* NRRL B 1811,
 827 and *Streptomyces albus* subsp. *chlorinus* NRRL B 24108; ¹POCP- Percentage of Conserved Protein; ²AAI – Average Amino-acid Identity, Values
 828 are mean ± standard deviation (SD).

829 **Table 2.** Morphological, physiological, and chemotaxonomic characteristics of currently available and newly proposed genera of
 830 *Streptomycetaceae* family^a

Genera / Characteristic	Long chains of spores formed on aerial hyphae	pH range for growth (Optimal range)	Temperature range (optimum)	Diagnostic sugars in whole organism hydrolysates ^c	Isomer(s) of diaminopimelic acids in whole-organism hydrolysates	Fatty acid pattern	Predominant phospholipids ^e	Major menaquinones ^f	G + C content of DNA (%)
Newly proposed genera									
<i>Actinacidiphila</i> gen. nov.	+	4.3–7.3 (5.0–5.5)	20–37 °C	nd	LL-A ₂ pm	iso- and anteiso-branched and saturated fatty acids	DPG, PE, PME, PIMs	MK-9(H ₆ , H ₈)	72.1 to 73.6
<i>Phaeacidiphilus</i> gen. nov.	+	3.0–6.5 (4.5)	28–37 °C	Gal, Glc, Man, Rib	LL- & meso-A ₂ pm	iso-C _{15:0} , anteiso-C _{15:0} , anteiso-C _{17:0} , iso-C _{17:0} , iso-C _{16:0}	DPG, PE, PI, PIMs	MK-9(H ₆ , H ₈)	73.4
<i>Mangrovactinospora</i> gen. nov.	–	5.0–8.0	Optimum pH 6.0–7.0	Gal, Glc, Man, Rib and Rha.	LL-A ₂ pm	anteiso-C _{15:0} , iso-C _{16:0} , iso-C _{15:0} and anteiso-C _{17:0}	DPG, PI, PE, OH-PE, PME and OH-PME	MK-9(H ₆ , H ₈)	73
<i>Peterkaempferia</i> gen. nov.	+	5–7 (5-6)	20 to 40 °C	Glc, Man (trace), Rib	LL-A ₂ pm;	anteiso-C _{15:0} , C _{16:0}	PE, PI, DPG, GPL, AGL, & unknown L	MK-9(H ₆ , H ₈)	72.5
<i>Streptoantibioticus</i> gen. nov.	+	7	20–40 °C	Gal, Rha	LL-A ₂ pm	Tiso- and anteiso-branched and saturated fatty acids	DPG, PE, PI, PIMs	MK-9(H ₆ , H ₈)	71.5–71.8
<i>Wenjunlia</i> gen. nov.	–	6 to 8	15 - 45 °C (25 - 34 °C)	Frc, Man, Rib, Glc	LL-A ₂ pm	anteiso-C _{15:0} , anteiso-C _{17:0} , iso-C _{16:0} , iso-C _{17:1} and/or anteiso-C _{17:1} B	DPG, PE, PME, PIMs	MK-9(H ₆ , H ₈ , H ₁₀)	72
Previously described genera									
<i>Allostreptomyces</i>	+	5.0–11.0 (7.0)	10–50 °C (28–30 °C)	Gal, Man	LL-A ₂ pm	anteiso-C _{15:0} , C _{16:0} , iso-C _{16:0} , iso-C _{15:0} and anteiso-C _{17:0} .	DPG, PIMs	MK-9(H ₆ , H ₈)	75.3
<i>Embleya</i>	–	6–11 (6-9)	10–28 °C (25–28 °C)	Arb	LL-A ₂ pm	C _{16:0} , iso-C _{16:0} , anteiso-C _{15:0} and iso-C _{15:1} , C _{16:1} cis 9	PE	MK-9(H ₄ , H ₆)	70.9 to 71.6
<i>Kitasatospora</i>	+	5.5–9.0	10–37 °C	Gal or Rib, Glc and Man	LL- & meso-A ₂ pm ^d	iso- and anteiso-branched and saturated fatty acids	DPG, PE, PI, PIMs	MK-9(H ₄ , H ₆ , H ₈) or MK-9(H ₆ , H ₈)	65-80
<i>Streptacidiphilus</i>	+	3.5–6.0 (4.5–5.5)	20–40 °C	Gal, Rha	LL-A ₂ pm	iso- and anteiso-branched and saturated fatty acids	DPG, PE, PI, PIMs	MK-9(H ₆ , H ₈)	71.5–71.8
<i>Streptomyces</i>	+	5.0–11.5 (6.5–8.0) ^b	28–37 °C	Gal	LL- & meso-A ₂ pm	iso- and anteiso-branched and saturated fatty acids	DPG, PE, PI, PIMs	MK-9(H ₆ , H ₈)	66–73
<i>Yinghuangia</i>	+	5–9 (6–7)	15–37 °C	Arb, Glc, Rha and Rib, or Rib, Man and Gal	LL-A ₂ pm	iso-C _{16:0} , anteiso-C _{15:0} , iso-C _{15:0} and C _{16:0}	PE, DPG and PI	MK-9(H ₆ , H ₈)	70-75

831 ^aData obtained from Kim *et al.* [69]; Shirling and Gottlieb [70]; Ōmura *et al.* [71]; Lonsdale [72]; Williams *et al.* [73]; Nakagaito *et al.* [74]; Antony-Babu and
 832 Goodfellow [75]; Nouioui *et al.* [36]; Komaki *et al.* [76]; Komaki and Tamura [77]; Roh *et al.* [78]; Ser *et al.* [54]; Huang *et al.* [12]; Ping *et al.* [79]; Nagai *et al.*
 833 [80]; ^bAlkalophilic strains, which grow between pH 5.0 and 11.0, have an optimum at pH 9 to 9.5 Mikami *et al.* [81] Antony-Babu and Goodfellow [75]; ^cCell
 834 wall sugars: Arb, arabinose; Frc, fructose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnase, Rib, ribose; ^dAerial and submerged spores contain LL-
 835 A₂pm (LL-diaminopimelic acid) and vegetative mycelia meso-A₂pm or DL-A₂pm (Meso-diaminopimelic acid); ^eDPG, diphosphatidyl glycerol; PE, phosphatidyl
 836 ethanolamine; PI, phosphatidyl inositol; PIMs, phosphatidyl inositol mannosides; OH-PE, hydroxy phosphatidyl ethanolamine; OH-PME, hydroxy phosphatidyl
 837 methyl ethanolamine; GPL, glycopospholipid; AGL, aminoglycophospholipid; ^fMK-9(H₂, H₄, H₆, H₈, H₁₀), di-, tetra-, hexa-, octa- and deca-hydrogenated
 838 menaquinones with nine isoprene units; nd, not determined. *Allostreptomyces* and *Yinghuangia* -genome data not available at the time of analysis.

839 **Fig. 1.** Midpoint rooted maximum likelihood phylogeny of members of *Streptomycetaceae*. The
840 tree was constructed using PhyloPhlAn 3.0. The scale bar indicates the normalized fraction of total
841 branch length as explained by Segata *et al.* [31]. Bolded taxa indicate the type species of each
842 genus based on current taxonomic assignments. Strain information and accession numbers can be
843 found in Table S1.

844 **Fig. 2.** AAI from pairwise whole-genome comparisons within the taxa of family
845 *Streptomycetaceae*. The heat map shows AAI values between selected type strains genomes, along
846 with the tree cladogram to show phylogenetic relationships. In the genus *Streptomyces*, only
847 selected members from each clade were used for analysis. Based on AAI comparisons and
848 monophyly observed in the genome-based phylogeny, inferred members of the novel genera are
849 marked in boxes named A, B, C, D, E and F indicating the novel genera *Actinacidiphila*,
850 *Mangrovactinospora*, *Peterkaempfera*, *Phaeacidiphilus*, *Streptantibioticus* and *Wenjunlia*
851 respectively. Information on *Streptomycetaceae* taxa and accession numbers can be found in Table
852 S1.

853 **Fig. 3.** POCP from pairwise whole-genome comparisons within the taxa of family
854 *Streptomycetaceae*. The heat map shows POCP values between selected type strains genomes,
855 along with the tree cladogram to show phylogenetic relationships. Based on POCP comparisons
856 and monophyly observed in the genome-based phylogeny, inferred members of the novel genera
857 are marked in boxes named A, B, C, D, E and F indicating the novel genera *Actinacidiphila*,
858 *Mangrovactinospora*, *Peterkaempfera*, *Phaeacidiphilus*, *Streptantibioticus* and *Wenjunlia*
859 respectively. Coherent grouping of all members of these genera was observed for, with the
860 exception of *Wenjunlia*. Information on *Streptomycetaceae* taxa and accession numbers can be
861 found in Table S1.