Five new members of the *Streptomyces violaceusniger* 16S rRNA gene clade: *Streptomyces castelarensis* sp. nov., comb. nov., *Streptomyces himastatinicus* sp. nov., *Streptomyces mordarskii* sp. nov., *Streptomyces rapamycinicus* sp. nov. and *Streptomyces ruanii* sp. nov.

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The taxonomic status of six strains received as *Streptomyces hygroscopicus* was established using a polyphasic approach. The organisms had phenotypic and morphological properties consistent with their classification in the genus *Streptomyces*. Almost complete 16S rRNA gene sequences of the strains were determined and aligned with corresponding sequences of representatives of the genus *Streptomyces* and phylogenetic trees were inferred using four tree-making algorithms. All of the strains fell within the *Streptomyces violaceusniger* 16S rRNA gene clade and four of them were considered to merit species status using a combination of genotypic and phenotypic data. These organisms were designated *Streptomyces himastatinicus* sp. nov. (type strain ATCC 53653^T=DSM 41914^T), *Streptomyces mordarskii* sp. nov. (type strain NRRL B-1346^T=DSM 40771^T), *Streptomyces rapamycinicus* sp. nov. (type strain NRRL 5491^T=ATCC 29253^T) and *Streptomyces ruanii* sp. nov. (type strain ISP 5276^T=DSM 40276^T). DNA–DNA relatedness and phenotypic data showed that the two remaining strains, '*Streptomyces hygroscopicus* subsp. *enhygrus*' NRRL 3664 and *Streptomyces hygroscopicus* subsp. *hygroscopicus* NRRL 3111 belong to the same species as the type strain of *Streptomyces rutgersensis* subsp. *castelarensis*. This taxon is designated *Streptomyces castelarensis* sp. nov., comb. nov. (type strain ATCC 15191^T=DSM 40830^T) following the proposal that *Streptomyces rutgersensis* subsp. *rutgersensis* is a heterotypic synonym of *Streptomyces albidoflavus*.

The application of genotypic and phenotypic methods has clarified the taxonomy of the genus *Streptomyces* (Manfio *et al.*, 1995; Anderson & Wellington, 2001), leading to the delineation of several multi-membered species-groups (Sembiring *et al.*, 2000; Duangmal *et al.*, 2005; Lanoot *et al.*, 2005; Liu *et al.*, 2005; Xu *et al.*, 2006). Members of one of these taxa, the *Streptomyces violaceusniger* 16S rRNA gene clade, form a grey aerial spore mass and a greyish-yellow substrate mycelium on oatmeal agar, produce aerial hyphae that differentiate into spiral chains of rugose ornamented spores (Sembiring *et al.*, 2000; Goodfellow *et*

al., 2007) and give a characteristic amplification product with taxon-specific primers (Kumar *et al.*, 2007).

The *S. violaceusniger* clade currently encompasses 24 species with validly published names that are mainly circumscribed using a combination of DNA–DNA relatedness and phenotypic data (Labeda & Lyons, 1991; Sembiring *et al.*, 2000; Goodfellow *et al.*, 2007). It is apparent from these studies that some strains received as *Streptomyces hygroscopicus* and *S. violaceusniger* have been misclassified as they belong to novel species classified within and outside the *S. violaceusniger* 16S rRNA gene clade. It is important to correctly classify members of this clade, not least because they are a rich source of antimicrobial metabolites (Tripathi *et al.*, 2004), biological control agents (Chamberlain & Crawford, 1999) and immunosuppressants (Vezina *et al.*, 1975; Kino *et al.*, 1987).

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *S. castelarensis* NRRL 3111, *S. rapamycinicus* NRRL 5491^T, *S. castelarensis* NRRL 3664, *S. mordarskii* NRRL B-1346^T, *S. himastatinicus* ATCC 53653^T and *S. ruanii* ISP 5276^T are EF408732–EF408737, respectively.

The aim of the present study was to establish the taxonomic status of strains received as *S. hygroscopicus* ATCC 53653 (Lam *et al.*, 1990), '*Streptomyces hygroscopicus* subsp. *enhygrus*' NRRL 3664 (Johnson & Dietz, 1971), *Streptomyces hygroscopicus* subsp. *hygroscopicus* ISP 5276 (Sveshnikova, 1957), *S. hygroscopicus* subsp. *hygroscopicus* NRRL B-1346 (Ettlinger *et al.*, 1958), *S. hygroscopicus* subsp. *hygroscopicus* NRRL 3111 (Zbinovsky *et al.*, 1968) and *S. hygroscopicus* subsp. *hygroscopicus* NRRL 5491 (Vezina *et al.*, 1975) using a polyphasic approach. It is evident from the literature that all of these organisms produce interesting bioactive compounds and have cultural and morphological properties in line with those shown by members of the *S. violaceusniger* 16S rRNA gene clade. These assignments were confirmed in the present investigation, which also showed that four of the organisms, *S. hygroscopicus* ATCC 53653, ISP 5276, NRRL B-1346 and NRRL 5491, merit recognition as representing novel species. The three remaining strains, '*S. hygroscopicus* subsp. *enhygrus*' NRRL 3664, *S. hygroscopicus* subsp. *hygroscopicus* NRRL 3111 and the type strain of *Streptomyces rutgersensis* subsp. *castelarensis* (Cercós, 1954) formed a fifth novel species.

The organisms were maintained on oatmeal agar (DSMZ, 1998) slopes and as mycelial fragments and spores in glycerol (20 %, v/v) at -20°C . Biomass for the molecular systematic studies was grown in shake flasks of tryptic soy broth at 160 r.p.m. at 28°C for 14 days, and then harvested by centrifugation. Genomic DNA extraction, PCR amplification and 16S rRNA gene sequencing were carried out using minor modifications (Kumar *et al.*, 2007) of an existing procedure (Goodfellow *et al.*, 2007). The resultant sequences were aligned with those of corresponding sequences of the type strains of *Streptomyces* species retrieved from the GenBank/EMBL/DBJ and RDP databases using the PHYDIT program (available at <http://plaza.snu.ac.kr/~jchun/phydit/>) and the alignments checked manually. Phylogenetic trees were inferred by using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from the PHYLIP 3.5c suite of programs (Felsenstein, 1993). Evolutionary distance matrices were prepared for the neighbour-joining and least-squares methods, as described by Jukes & Cantor (1969). Bootstrap analyses (Felsenstein, 1985) were performed with 1000 resampled neighbour-joining datasets by using the SEQBOOT and CONSENSE programs from the PHYLIP package (Felsenstein, 1993).

Almost complete 16S rRNA gene sequences were obtained for the six strains (1436–1445 nt). All of the organisms were recovered within the evolutionary radiation occupied by the *S. violaceusniger* 16S rRNA gene clade, the taxonomic integrity of which was supported by a 90 % bootstrap value in the neighbour-joining analysis (Fig. 1). It is also apparent from the figure that the six strains were

recovered in two broad phylogenetic groups each of which included a number of *Streptomyces* type strains.

The six strains were examined for colonial and morphological properties considered to be typical of members of the *S. violaceusniger* 16S rRNA gene clade, using previously described procedures (Shirling & Gottlieb, 1966; Sembiring *et al.*, 2000; Goodfellow *et al.*, 2007). To this end, they were screened for their ability to produce pigments on oatmeal and peptone-yeast extract-iron agars, and for spore chain morphology and spore surface ornamentation. They were also probed using the *S. violaceusniger* clade-specific oligonucleotide primers described by Kumar *et al.* (2007). All of the organisms formed a grey to blackish aerial spore mass on oatmeal agar, did not produce melanin pigments on peptone-yeast extract-iron agar, formed spiral chains of rugose ornamented spores, and gave a PCR amplification product characteristic of members of the *S. violaceusniger* clade. These data show that all of the strains are authentic members of the *S. violaceusniger* 16S rRNA gene clade (Goodfellow *et al.*, 2007; Kumar *et al.*, 2007).

The six tested strains and the type strains of *Streptomyces antimycoticus* and *S. rutgersensis* subsp. *castelarensis* were screened for diverse phenotypic properties known to be of value in streptomycete systematics (Atalan *et al.*, 2000; Xu *et al.*, 2006; Goodfellow *et al.*, 2007); the various tests were carried out using media and methods described by Williams *et al.* (1983). It is evident from Table 1 and from the species descriptions that the organisms had many phenotypic characteristics in common though members of the two broad phylogenetic groups can be separated by such features.

It can be seen from Fig. 1 that '*S. hygroscopicus* subsp. *enhygrus*' NRRL 3664 and *S. hygroscopicus* subsp. *hygroscopicus* NRRL B-1346 and NRRL 3111 form a 16S rRNA subclade together with *S. rutgersensis* subsp. *castelarensis* DSM 40830^T. The standing of this taxon, the *S. rutgersensis* subsp. *castelarensis* subclade, is supported by all of the tree-making algorithms and by a bootstrap value of 61 %. It is also evident that the type strains of *Streptomyces antimycoticus*, *Streptomyces geldanamycininus* and *S. melanosporofaciens* are closely related to members of this subclade. Indeed, the taxonomic integrity of this broader group was supported by all of the tree-making algorithms and by a bootstrap value of 88 %.

All of the organisms in the broader phylogenetic group shared 16S rRNA gene similarities within the range 99.0–99.8 %, values that correspond to between 3 and 11 nt differences at 1449 and 1446 locations, respectively. The highest 16S rRNA similarity found between the type strains belonging to this taxon was between *S. geldanamycininus*, *S. antimycoticus* and *S. rutgersensis* subsp. *castelarensis*, 99.5 % similarity in each case, a value equivalent to 7 nt differences at 1447 sites. Extensive DNA–DNA relatedness experiments indicated that members of the *S. violaceusniger* 16S rRNA gene clade that share similarities at or below 99.7 %, that is, with four or more nucleotide differences,

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost complete 16S rRNA gene sequences showing the relationships between the tested strains and between them and representatives of the *Streptomyces violaceusniger* 16S rRNA gene clade. Asterisks denote branches that were also recovered using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms. F and P indicate branches that were recovered using the least-squares and maximum-parsimony methods, respectively. Bar, 0.1 substitutions per site. Numbers at nodes indicate the levels of bootstrap support (%) based on the neighbour-joining analysis of 1000 resampled datasets. The strains tested are shown in bold. GenBank accession numbers are given in parentheses.

The highest 16S rRNA gene similarity between the organisms assigned to the broader phylogenetic group was between *S. hygroscopicus* subsp. *hygroscopicus* NRRL 3111 and *S. rutgersensis* subsp. *castelarensis* DSM 40830^T; these strains shared a similarity of 99.8 % (3 nt differences at 1449 locations). Similarly, '*S. hygroscopicus* subsp. *enhygrus*' NRRL 3664 was most closely related to *S. hygroscopicus* subsp. *hygroscopicus* NRRL 3111 (99.7 % similarity; 5 nt differences at 1448 sites), although the latter organism also shared high 16S rRNA gene similarities with the type strains of *S. antimycoticus* (99.7 %, 5 nt differences at 1449 locations), *S. geldanamycininus* (99.7 %, 4 nt differences at 1447 locations) and *S. melanosporofaciens* (99.5 %, 7 nt differences at 1448 sites). The '*S. hygroscopicus* subsp. *enhygrus*' strain showed relatively low

In contrast to '*S. hygroscopicus* subsp. *enhygrus*' NRRL 3664 and *S. hygroscopicus* subsp. *hygroscopicus* NRRL 3111, the remaining strain, *S. hygroscopicus* subsp. *hygroscopicus* NRRL B-1346, is not especially closely related to most of the other organisms in the broader phylogenetic group. This strain shared its highest 16S rRNA sequence similarities with '*S. hygroscopicus* subsp. *enhygrus*' NRRL 3664 (99.7%, 5 nt differences), *S. rutgersensis* subsp. *castelarensis* DSM 40830^T (99.3%, 10 nt differences), *S. hygroscopicus* subsp. *hygroscopicus* NRRL 3111 (99.5%, 11 nt differences), *S. geldanamycininus* NRRL 3602^T (99.2%, 11 nt differences) and *S. melanosporofaciens* (99.0%, 14 nt differences).

Table 1. Phenotypic properties that separate organisms assigned to two phyletic lines in the *S. violaceusniger* 16S rRNA clade

Strains: 1, *S. antimycoticus* NBRC 12839^T; 2, *S. geldanamycininus* NRRL 3602^T; 3, '*S. hygroscopicus* subsp. *enhygrus*' NRRL 3664; 4, *S. hygroscopicus* subsp. *hygroscopicus* NRRL B-1346; 5, *S. hygroscopicus* subsp. *hygroscopicus* NRRL 3111; 6, *S. melanosporofaciens* NRRL B-12234^T; 7, *S. rutgersensis* subsp. *castelarensis* DSM 40830^T; 8, *S. hygroscopicus* ATCC 53653; 9, *S. hygroscopicus* subsp. *hygroscopicus* ISP 5276; 10, *S. hygroscopicus* subsp. *hygroscopicus* NRRL 5491^T. +, Positive; – negative. All are susceptible to ampicillin (32 µg ml⁻¹), cefoxitin (32 µg ml⁻¹), cephaloridine (64 µg ml⁻¹), fusidic acid (8 µg ml⁻¹), novobiocin (4 µg ml⁻¹) and tetracycline hydrochloride (4 µg ml⁻¹). All are negative for allantoin hydrolysis; and do not grow on inulin, malonic acid, sodium acetate, sodium citrate or sodium pyruvate as sole carbon sources.

Characteristic	Group 1							Group 2		
	1	2*	3	4	5	6*	7	8	9	10
Enzyme activity										
Nitrate reduction	+	–	–	–	–	–	–	+	–	–
Arbutin hydrolysis	+	+	+	+	+	+	+	+	–	+
Degradation tests (% w/v)										
Casein (1)	+	+	+	+	+	+	+	+	–	+
Elastin (0.3)	+	–	–	–	–	–	–	–	+	–
Guanine (0.05)	–	–	+	–	+	–	+	–	+	+
Hypoxanthine (0.4)	+	+	+	+	+	+	+	–	+	+
Uric acid (0.5)	+	+	+	+	+	+	+	+	–	+
Growth on sole carbon source at 1 % (w/v)										
Adonitol	–	+	+	+	+	+	+	–	–	–
L-Arabinose	+	+	+	+	+	+	+	–	+	+
Arbutin	+	–	–	–	–	–	–	–	+	–
Dextrin	–	+	+	+	+	+	+	–	+	+
meso-Erythritol	+	+	+	+	+	+	+	–	–	+
L-Fucose	–	–	–	–	–	–	–	+	–	–
Inositol	–	+	+	+	+	+	+	–	+	+
α-D-Lactose	+	+	+	+	+	+	+	+	–	+
Melezitose	+	+	+	–	+	+	+	–	–	+
Salicin	+	–	–	+	–	–	–	–	+	+
Growth on sole nitrogen source										
α-Alanine	–	–	–	+	–	–	–	+	+	+
L-Alanine	–	–	–	–	–	–	–	+	+	+
L-Glycine	+	+	+	+	+	+	+	–	+	–
DL-Methionine	–	–	–	+	–	–	–	+	–	+
L-Phenylalanine	–	+	+	+	+	–	+	+	+	+
L-Serine	+	–	–	+	–	–	–	+	+	+
L-Tryptophan	–	–	–	–	–	–	–	–	+	–
L-Valine	–	+	+	+	+	–	+	+	+	+
Growth at:										
pH 5.0	–	–	–	–	–	–	–	–	+	–
pH 9.0	–	–	–	+	–	–	–	+	+	+
10 °C	–	–	–	+	–	–	–	+	+	+
Resistance to antibiotics (µg ml ⁻¹)										
Cefoxitin (64)	+	+	+	+	+	+	+	+	–	+
Cephaloridine hydrochloride (32)	+	+	+	+	+	–	+	+	+	+
Chlortetracycline hydrochloride (4)	–	–	–	+	–	–	–	+	+	–
Chlortetracycline hydrochloride (8)	–	–	–	+	–	–	–	+	+	–
Doxycycline hydrochloride (8)	–	–	–	+	–	–	–	+	+	+
Erythromycin (32)	–	–	+	+	+	–	+	+	–	–
Erythromycin (64)	–	–	–	–	–	–	–	+	–	–
Fusidic acid (16)	+	+	+	+	+	–	+	+	+	+
Lincomycin hydrochloride (32)	+	+	+	+	+	+	+	–	+	+
Lincomycin hydrochloride (64)	+	+	+	–	+	+	+	–	+	+
Rifampicin (32)	+	+	+	+	+	+	+	–	–	–
Rifampicin (64)	+	+	+	+	+	–	+	–	–	–

*Data taken from Goodfellow *et al.* (2007).

DNA–DNA relatedness experiments were carried out between selected pairs of strains (Table 2) by measuring the divergence between the thermal denaturation midpoint of homoduplex DNA and heteroduplex DNA (ΔT_m), as described by Gonzalez & Saiz-Jimenez (2005).

It can be seen from Table 2 that *S. hygroscopicus* subsp. *hygroscopicus* NRRL B-1346 represents a distinct taxon as the DNA–DNA relatedness analyses performed using thermal denaturation revealed differences in melting temperature with its closest phylogenetic neighbours that were well below the 70 % guideline recommended for the delineation of genomic species ($\Delta T_m > 5^\circ\text{C}$; Wayne *et al.*, 1987; Rosselló-Mora & Amann, 2001). This organism can also be distinguished from its closest phylogenetic neighbours using a combination of phenotypic properties, notably by its ability to grow at 10°C and pH 9.0 (Table 1). The excellent agreement between the genotypic and phenotypic data indicate that *S. hygroscopicus* subsp. *hygroscopicus* NRRL B-1346 merits recognition as a novel species in the *S. violaceusniger* 16S rRNA gene clade. The name proposed for this taxon is *Streptomyces mordarskii* sp. nov.

DNA–DNA relatedness experiments were also carried out to establish the finer taxonomic relationships between *S. rutgersensis* subsp. *castelarensis* DSM 40830^T, '*S. hygroscopicus* subsp. *enhygrus*' NRRL 3664 and *S. hygroscopicus* subsp. *hygroscopicus* NRRL 3111; these strains share 16S

rRNA gene similarities within the range 99.6–99.8 %, values that correspond to between 5 and 3 nt differences at 1447 and 1449 locations, respectively. It can be seen from Table 2 that these strains belong to a single genomic species as the respective ΔT_m values recorded in DNA–DNA relatedness studies fell within the range $3.4\text{--}4.5^\circ\text{C}$; that is, below the recommended guideline for the delineation of genomic species when measuring the divergence between the thermal denaturation midpoint of homo- and heteroduplex DNA, as described by Gonzalez & Saiz-Jimenez (2005). It is apparent from Table 2 that *S. hygroscopicus* subsp. *hygroscopicus* NRRL 3111 can be distinguished from other phylogenetically close neighbours as it shared ΔT_m values between 5.1 and 5.4°C with the type strains of *S. antimycoticus*, *S. geldanamycininus* and *S. melanosporofaciens*. Similarly, '*S. hygroscopicus* subsp. *enhygrus*' NRRL 3664 can be distinguished readily from *S. geldanamycininus* NRRL 3602^T and *S. melanosporofaciens* NRRL B-12234^T based on ΔT_m values (Table 2).

Confidence can be placed in the DNA–DNA relatedness data as it has been shown that comparable results are obtained when the same strains are examined using thermal denaturation and spectrophotometric procedures (Jurado *et al.*, 2005; Goodfellow *et al.*, 2007). The estimated DNA–DNA relatedness values obtained for *S. rutgersensis* subsp. *castelarensis* DSM 40830^T, '*S. hygroscopicus* subsp. *enhygrus*' NRRL 3664 and *S. hygroscopicus* subsp. *hygro-*

Table 2. DNA–DNA relatedness values found between '*S. hygroscopicus* subsp. *enhygrus*' NRRL 3664 and *S. hygroscopicus* subsp. *hygroscopicus* NRRL B-1346 and NRRL 3111 and their closest phylogenetic neighbours based on ΔT_m values

Strains	ΔT_m ($^\circ\text{C}$)	DNA–DNA relatedness (%)	Nucleotide base differences/total no. of 16S rRNA nucleotides
<i>S. antimycoticus</i> NBRC 12839 ^T vs <i>S. hygroscopicus</i> subsp. <i>hygroscopicus</i> NRRL 3111	5.4	64	5/1449
<i>S. antimycoticus</i> NBRC 12839 vs ' <i>S. hygroscopicus</i> subsp. <i>enhygrus</i> ' NRRL 3664	5.8	61	9/1447
' <i>S. hygroscopicus</i> subsp. <i>enhygrus</i> ' NRRL 3664 vs <i>S. geldanamycininus</i> NRRL 3602 ^T	5.1	65	8/1445
' <i>S. hygroscopicus</i> subsp. <i>enhygrus</i> ' NRRL 3664 vs <i>S. melanosporofaciens</i> NRRL B-12234 ^T	5.5	63	11/1446
<i>S. hygroscopicus</i> subsp. <i>hygroscopicus</i> NRRL B-1346 vs ' <i>S. hygroscopicus</i> subsp. <i>enhygrus</i> ' NRRL 3664	6.0	60	5/1448
<i>S. hygroscopicus</i> subsp. <i>hygroscopicus</i> NRRL B-1346 vs <i>S. hygroscopicus</i> subsp. <i>hygroscopicus</i> NRRL 3111	6.5	56	11/1449
<i>S. hygroscopicus</i> subsp. <i>hygroscopicus</i> NRRL B-1346 vs <i>S. melanosporofaciens</i> NRRL B-12234 ^T	7.0	55	14/1448
<i>S. hygroscopicus</i> subsp. <i>hygroscopicus</i> NRRL 3111 vs ' <i>S. hygroscopicus</i> subsp. <i>enhygrus</i> ' NRRL 3664	3.9	77	5/1448
<i>S. hygroscopicus</i> subsp. <i>hygroscopicus</i> NRRL 3111 vs <i>S. melanosporofaciens</i> NRRL B-12234 ^T	5.3	64	7/1448
<i>S. hygroscopicus</i> subsp. <i>hygroscopicus</i> NRRL 3111 vs <i>S. geldanamycininus</i> NRRL 3602 ^T	5.1	65	4/1447
<i>S. rutgersensis</i> subsp. <i>castelarensis</i> DSM 40830 ^T vs <i>S. hygroscopicus</i> subsp. <i>hygroscopicus</i> NRRL 3111	3.4	82	3/1449
<i>S. rutgersensis</i> subsp. <i>castelarensis</i> DSM 40830 ^T vs ' <i>S. hygroscopicus</i> subsp. <i>enhygrus</i> ' NRRL 3664	4.5	73	7/1447

scopius NRRL 3111 (Table 2) indicate that they belong to the same genomic species as all of the values are above the 70 % cut-off point recommended for the delineation of genomic species (Wayne *et al.*, 1987). Indeed, two of the estimated values are above or near the 80 % level, which Labeda and his colleagues considered might apply to species level relatedness in the genus *Streptomyces* (Labeda, 1993, 1998; Labeda & Lyons, 1992). The assignment of all three strains to a single species is strongly supported by the phenotypic data. It can be seen from Table 1 that the three organisms shared an identical phenotypic profile that distinguishes them from the type strains of *S. antimycoticus*, *S. geldanamycininus* and *S. melanosporofaciens*. The congruence found between the DNA–DNA relatedness and phenotypic data demonstrate that strains DSM 40830^T, NRRL 3111 and NRRL 3664 belong to a novel species, albeit one in search of a name.

S. rutgersensis subsp. *castelarensis* DSM 40830^T and *S. rutgersensis* subsp. *rutgersensis* DSM 40077^T have markedly different properties and should not be retained in the same species. The latter organism, for example, produces straight to flexuous chains of smooth ornamented spores (Shirling & Gottlieb, 1968) and the latter spiral chains of rugose ornamented spores as shown in the present investigation; the two strains have distinct 16S-ITS RFLP fingerprint patterns (Lanoot *et al.*, 2005). There is also an abundance of chemotaxonomic, molecular systematic and numerical taxonomic data which show that strain DSM 40077^T is a bona fide member of the species *S. albidoflavus* (Mordarski *et al.*, 1986; Williams *et al.*, 1989; Manchester *et al.*, 1990; Kämpfer *et al.*, 1991; Goodfellow *et al.*, 1992; Ferguson *et al.*, 1997). Therefore, it is proposed that *S. rutgersensis* subsp. *rutgersensis* (Waksman and Curtis 1916) Waksman and Henrici 1948 be recognized as a heterotypic synonym of *S. albidoflavus* (Rossi Doria 1891) Waksman and Henrici 1948.

The classification of *S. rutgersensis* subsp. *castelarensis* DSM 40830^T in the *S. violaceusniger* 16S rRNA gene clade underpins the results of a numerical taxonomic study in which this organism was assigned to a cluster that contained organisms known to produce spiral chains of rugose ornamented spores (Kämpfer *et al.*, 1991). It is evident from this and from an earlier study (Lanoot *et al.*, 2004) that strain DSM 40830^T forms a distinct centre of taxonomic variation in the genus *Streptomyces* that corresponds to a species. These findings together with the reclassification of *S. rutgersensis* subsp. *rutgersensis* as a synonym of *S. albidoflavus* mean that *S. rutgersensis* subsp. *castelarensis* Cercós 1954 has to be given species status as *Streptomyces castelarensis* (Cercós 1954) Kumar and Goodfellow according to Rule 50a of the *International Code of Nomenclature of Bacteria* (Lapage *et al.*, 1992). This taxon also encompasses the strains received as '*S. hygroscopicus* subsp. *enhygrus*' NRRL 3664 and *S. hygroscopicus* subsp. *hygroscopicus* NRRL 3111.

It can be seen from Fig. 1 that the strains received as *S. hygroscopicus* ATCC 53653 and ISP 5276 formed a subclade

together with the type strains of *Streptomyces demainii*, *S. hygroscopicus* and *Streptomyces sporocinereus*. The taxonomic status of this group, the *S. hygroscopicus* subclade, is supported by all of the tree-making algorithms and by a bootstrap value of 55 % in the neighbour-joining analysis. *S. hygroscopicus* ATCC 53653 and ISP 5276 share a 16S rRNA gene similarity of 98.5 %, a value that corresponds to 22 nt differences at the 1444 locations available for study. These strains are most closely related to *S. hygroscopicus* NRRL 2387^T, sharing 16S rRNA gene similarities with the latter of 98.4 % (23 nt differences at 1446 locations) and 98.8 % (17 nt differences at 1446 locations), respectively. DNA–DNA relatedness experiments were not carried out between these strains and their closest phylogenetic neighbours as type strains of species classified in the subclade share higher 16S rRNA gene similarities and have DNA–DNA relatedness values well below the 70 % cut-off point recommended for the delineation of genomic species (Wayne *et al.*, 1987). *S. demainii* NRRL B-1478^T and *S. hygroscopicus* NRRL 2387^T, for example, share a 16S rRNA gene similarity of 99.7 % (4 nt differences at 1449) and a DNA–DNA relatedness value of 38 % (Labeda & Lyons, 1991).

S. hygroscopicus subsp. *hygroscopicus* NRRL 5491 is loosely associated with members of the *S. hygroscopicus* 16S rRNA subclade and with the type strains of *Streptomyces albiflaviniger* and *S. violaceusniger*. It is most closely related to *S. hygroscopicus* NRRL 2387^T and *S. violaceusniger* NRRL B-1476^T, with a 16S rRNA gene similarity with each of these organisms of 98.9 % (15 nt differences at 1448 locations). The type strains of *S. hygroscopicus* and *S. violaceusniger* are more closely related to one another than to *S. hygroscopicus* subsp. *hygroscopicus* NRRL 5491 as they share a 16S rRNA gene similarity of 99.5 % (8 nt differences at 1449 sites). DNA–DNA relatedness studies were not carried out between the *S. hygroscopicus* subsp. *hygroscopicus* strain and its two closest phylogenetic neighbours, as the latter belong to distinct genomic species given a DNA–DNA relatedness value of 46 % (Labeda & Lyons, 1991).

It is apparent from the 16S rRNA gene sequence data that *S. hygroscopicus* ATCC 53653 and *S. hygroscopicus* subsp. *hygroscopicus* ISP 5276 and NRRL 5491 form distinct, but related, phyletic lines within the *S. violaceusniger* 16S rRNA gene tree (Fig. 1). These findings are underpinned by corresponding phenotypic data which show that the three organisms have many properties in common but can be separated readily using a combination of phenotypic properties (Table 1). It is evident, therefore, that the three organisms form new centres of taxonomic variation within the *S. violaceusniger* clade. Consequently, we propose the names *Streptomyces himastatinicus* sp. nov. for strain ATCC 53653, *Streptomyces rapamycinicus* sp. nov. for strain NRRL 5491 and *Streptomyces ruanii* sp. nov. for strain ISP 5276.

The present investigation provides further evidence that some members of the *S. violaceusniger* 16S rRNA gene clade

share very high 16S rRNA gene sequence similarities, but can be assigned as representing distinct species using a combination of DNA–DNA relatedness and phenotypic features. Descriptions of the novel species assigned to the *S. violaceusniger* 16S rRNA gene clade are given below.

Description of *Streptomyces castelarensis* (Cercós 1954) Kumar and Goodfellow sp. nov., comb. nov.

Streptomyces castelarensis (cas.tel.ar.en'sis. N.L. masc. adj. *castelarensis* from Castelar, Argentina, the source of the organism).

Basonym: *Streptomyces rutgersensis* subsp. *castelarensis* Cercós 1954.

The description is based on data taken from this and previous studies (Cercós, 1954; Kämpfer *et al.*, 1991). Rugose ornamented spores ($0.8 \times 1.6 \mu\text{m}$) are borne on spiral spore chains with two to six turns on inorganic salts-starch agar. Grows well on modified Bennett's, glycerol-asparagine, oatmeal and yeast extract-malt extract agars. On oatmeal agar the aerial spore mass colour is grey, becoming black and moist when mature, and the substrate mycelium greyish-yellow; diffusible pigments are not formed. Melanin pigments are not produced on either peptone-yeast extract-iron or tyrosine agars. *N*-Acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, cellobiose, D-fructose, D-galactose, D-glucose, D-glycerol, glycogen, maltose, D-mannitol, D-mannose, melibiose, raffinose, L-rhamnose, D-ribose, trehalose, D-xylitol and D-xylose are used as sole carbon sources, but not sucrose, L-sorbose or D-tagalose. Additional phenotypic properties are shown in Table 1. Produces the antibiotic camphomycin.

The type strain, ATCC 15191^T (=DSM 40830^T), was isolated from dust in Castelar, Argentina.

Description of *Streptomyces himastatinicus* sp. nov.

Streptomyces himastatinicus (hi.ma.sta.ti'ni.cus. N.L. neut. n. *himastatinum*; L. suffix *-a-um* related to; N.L. masc. adj. *himastatinicus* related to himastatin, referring to the ability to produce himastatin).

The description is based upon data taken from this and a previous study (Lam *et al.*, 1990). Rugose ornamented, oval to barrel-shaped spores ($0.5\text{--}0.7 \times 0.5\text{--}1.2 \mu\text{m}$) are borne in spiral chains with two to eight turns. Grows well on modified Bennett's, glycerol-asparagine, oatmeal and yeast extract-malt extract agars. On oatmeal agar the aerial spore mass colour is grey, becoming black and moist when mature, and the substrate mycelium greyish-yellow; diffusible pigments are not formed. Melanin pigments are not produced on either peptone-yeast extract-iron or tyrosine agars. Growth occurs between 10 and 37 °C, but not at 4 or 40 °C, and at pH values from 5 to 10, but not at pH 4.0 or 11.0. Aesculin is hydrolysed. Degrades starch and

Tween 40, but not cellulose, chitin, DNA, pectin, RNA, tributyrin, xanthine or xylan. D-Fructose, D-galactose, D-glucose, D-glycerol, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, D-ribose, soluble starch, sucrose, trehalose and D-xylose are used as sole sources of carbon for energy and growth, but not amygdalin, D-arabinose, cellobiose, dulcitol or D-sorbitol (all at 1 %, w/v). Butan-1,4-diol is used as a sole carbon source, but not methanol or propanol (all at 1 %, v/v). L-Arginine, L-asparagine, L-glutamic acid, L-histidine, L-isoleucine, L-ornithine, L-proline and L-threonine are used as sole nitrogen sources for energy and growth, but not aminobutyric acid, DL-aspartic acid, L-leucine, DL-norleucine or L-norvaline (all at 1 %, w/v). Grows in the presence of 8 % (w/v) NaCl, but not in the presence of 10 % (w/v) NaCl or 0.1 % (v/v) phenol. Sensitive to gentamicin sulphate, kanamycin sulphate, neomycin sulphate, streptomycin sulphate and tobramycin sulphate (each at $8 \mu\text{g ml}^{-1}$). Additional phenotypic properties are shown in Table 1. Whole-organism hydrolysates contain LL-diaminopimelic acid; major phospholipids are phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylinositol. Produces the antibiotic himastatin that shows antimicrobial activity against Gram-positive bacteria. The G + C content of the DNA of the type strain is 71.2 mol%.

The type strain, ATCC 53653^T (=DSM 41914^T), was isolated from soil collected from the State of Himachal Pradesh, India.

Description of *Streptomyces mordarskii* sp. nov.

Streptomyces mordarskii (mor.dar'ski.i. N.L. masc. gen. n. *mordarskii* of Mordarski, named in honour of Marian Mordarski for his many contributions to the biology of actinomycetes).

The description is based on data taken from this and a previous study (Ettlinger *et al.*, 1958). Rugose ornamented spores ($0.8 \times 1.8 \mu\text{m}$) are borne in spiral spore chains with two to five turns on inorganic salts-starch agar. On oatmeal agar the aerial spore mass colour is grey, becoming black and moist when mature, and the substrate mycelium is greyish-yellow; diffusible pigments are not formed. Melanin pigments are not produced on either peptone-yeast extract-iron or tyrosine agars. Growth occurs between 10 and 37 °C, but not at 4 or 40 °C, and from pH 6.0 to 10.0, but not at pH 5.0 or 11.0. Aesculin is hydrolysed. Degrades tyrosine, but not chitin, cellulose, DNA, pectin, RNA, tributyrin, xanthine or xylan. D-Arabitol, cellobiose, D-fructose, D-galactose, D-glucose, glycogen, maltose, D-mannose, raffinose, L-rhamnose, D-ribose, sucrose, trehalose and D-xylose are used as sole sources of carbon for energy and growth, but not aesculin, amygdalin, D-arabinose, L-arabitol, dulcitol, glucosamine, inulin, pectin or L-sorbose (all at 1 %, w/v). Butan-1,4-diol is used as a sole carbon source, but not methanol or propanol (all at 1 %, v/v). L-Glutamic acid, L-histidine, L-leucine, L-isoleucine, L-ornithine, L-proline and L-threonine are used

as sole nitrogen sources for energy and growth, but not L-aminobutyric acid, DL-aspartic acid, DL-norleucine, L-norvaline or L-ornithine (all at 1 %, w/v). Does not grow in the presence of 13 % (w/v) NaCl or 0.1 % (v/v) phenol. Sensitive to gentamicin sulphate, kanamycin sulphate, neomycin sulphate, streptomycin sulphate and tobramycin sulphate (each at 8 µg ml⁻¹). Additional phenotypic properties are shown in Table 1. The G+C content of the DNA of the type strain is 70.4 mol%.

The type strain, NRRL B-1346^T (=DSM 40771^T), was isolated from soil collected in Japan.

Description of *Streptomyces rapamycinicus* sp. nov.

Streptomyces rapamycinicus (ra.pa.my.ci'ni.cus. N.L. neut. n. *rapamycinum* rapamycin; L. suffix *-icus-a-um* related to; N.L. masc. adj. *rapamycinicus* related to rapamycin, referring to the ability to produce rapamycin).

The description is based on data taken from this and a previous study (Vezina *et al.*, 1975). Oval, rugose ornamented spores (0.8 × 1.6 µm) are borne in spirals of three or more turns; ten or more spores per spiral. Grows well on modified Bennett's, glucose-asparagine, oatmeal, sabouraud-glucose, sabouraud-maltose, tomato paste, oatmeal, tryptone-yeast extract and yeast extract-malt extract agars. On oatmeal agar the aerial spore mass colour is grey, becoming black and moist when mature, the substrate mycelium is greyish-yellow and a light-yellow diffusible pigment is formed. Melanin pigments are not produced on either peptone-yeast extract-iron or tyrosine agars. Growth occurs between 10 and 37 °C, but not at 4 or 40 °C, and from pH 6.0 to 10.0, but not at pH 5.0 or 11.0. Aesculin is hydrolysed, but hydrogen sulphide is not produced. Degrades adenine, gelatin, starch, Tweens 20, 40, 60 and 80 and tyrosine, but not cellulose, chitin, DNA, keratin, pectin, RNA, tributyrin, xanthine or xylan. D-Fructose, D-glucose, glycerol, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, D-ribose, starch, sucrose, trehalose and D-xylose are used as sole carbon sources for energy and growth, but not D-aesculin, amygdalin, arabinose, L-arabitol, dulcitol, glucosamine, inulin, pectin, D-sorbitol or L-sorbose (all at 1 %, w/v). Butan-1,4-diol is used as a sole carbon source, but not methanol or propanol (all at 1 %, v/v). L-Arginine, L-asparagine, L-glutamic acid, L-histidine, L-isoleucine, L-ornithine, L-proline and L-threonine are used as sole nitrogen sources for energy and growth, but not L-aminobutyric acid, DL-aspartic acid, L-leucine, DL-norleucine or L-norvaline (all at 1 %, w/v). Does not grow in the presence of 13 % (w/v) NaCl or 0.1 % (v/v) phenol. Sensitive to gentamicin sulphate, kanamycin sulphate, neomycin sulphate, streptomycin sulphate and tobramycin sulphate (each at 8 µg ml⁻¹). Antimicrobial activity is shown against *Candida albicans*, *Enterobacter aerogenes*, *Escherichia coli*, *Microsporium gypseum*, *Proteus mirabilis*, *Staphylococcus aureus* and *Trichophyton granulosum*.

Additional phenotypic properties are shown in Table 1. Produces the antibiotic rapamycin. The G+C content of the DNA of the type strain is 71 mol%.

The type strain, NRRL 5491^T (=ATCC 29253^T), was isolated from a soil sample collected from Easter Island.

Description of *Streptomyces ruanii* sp. nov.

Streptomyces ruanii (rua'ni.i. N.L. masc. gen. n. *ruanii* of Ruan, named in honour of Jisheng Ruan, a celebrated Chinese systematist).

The description is based on data taken from this and a previous study (Sveshnikova, 1957). Rugose ornamented spores (1.1 × 0.8 µm) are borne in spiral spore chains of three or more turns on inorganic salts-starch agar. On oatmeal agar the aerial spore mass colour is grey, becoming black and moist when mature, and the substrate mycelium is greyish-yellow; diffusible pigments are not formed. Melanin pigments are not produced on either peptone-yeast extract-iron or tyrosine agars. Growth occurs between 10 and 37 °C, but not at 4 or 40 °C, and from pH 6 to 10, but not at pH 5.0 or 11.0. Aesculin is hydrolysed. Degrades adenine, gelatin and Tweens 20, 40, 60 and 80, but not cellulose, chitin, DNA, keratin, pectin, RNA, starch, tributyrin, xanthine or xylan. D-Arabinose, D-arabitol, cellobiose, D-fructose, D-galactose, glycogen, maltose, D-mannose, L-rhamnose, D-ribose, sucrose, trehalose and D-xylose are used as sole sources of carbon for energy and growth, but not aesculin, amygdalin, L-arabitol, dulcitol, glucosamine, inulin, D-sorbitol or D-sorbose (all at 1 %, w/v). Butan-1,4-diol is used as a sole carbon source, but not methanol or propanol (all at 1 %, v/v). L-Asparagine, L-glutamic acid, L-histidine, L-isoleucine, L-ornithine and L-proline are used as sole nitrogen sources for energy and growth, but not L-aminobutyric acid, DL-aspartic acid, L-leucine, DL-norleucine or L-norvaline. Growth does not occur in the presence of 13 % (w/v) NaCl or 0.1 % (v/v) phenol. Sensitive to gentamicin sulphate, kanamycin sulphate, neomycin sulphate and tobramycin sulphate (each at 8 µg ml⁻¹). Antimicrobial activity is shown against *Bacillus cereus*, *Bacillus subtilis* and *Candida albicans*, but not against *Enterobacter aerogenes* or *Escherichia coli*. Additional phenotypic properties are shown in Table 1. The G+C content of the DNA of the type strain is 70.5 mol%.

The type strain, ISP 5276^T (=DSM 40276^T), was isolated from soil.

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