Supplementary materials

New insights into the relationship between the average nucleotide identity and the digital DNA-DNA hybridization values in the genus *Amycolatopsis* and *Amycolatopsis cynarae* sp. nov., a novel actinobacterium from the rhizosphere soil of *Cynara scolymus* and proposal of *Amycolatopsis niigatensis* as a synonym of *Amycolatopsis echigonensis*

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Fig.S1 The correlations between ANIb and dDDH from the 29 pairs of *Amycolatopsis* species

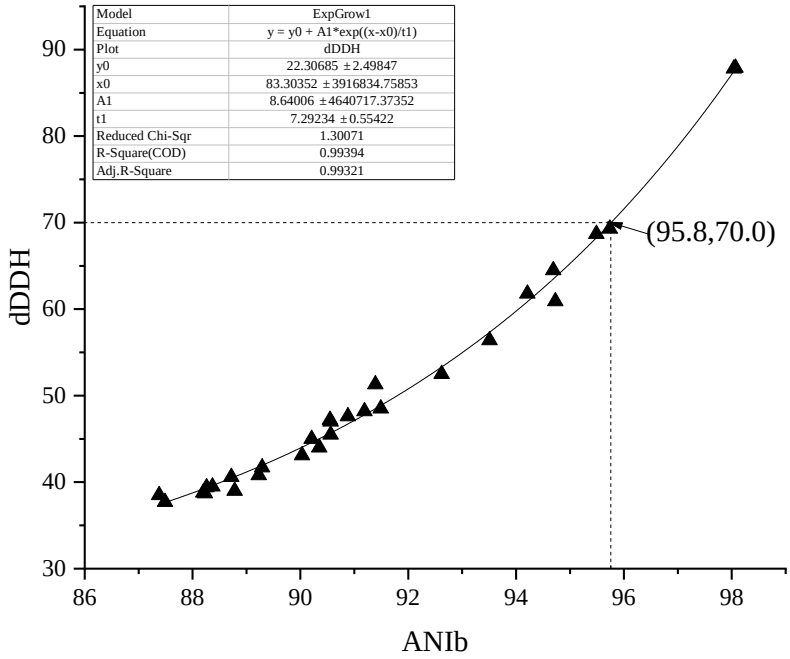

Fig.S2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between selected species of the genus *Amycolatopsis*. *Nocardia farcinica* NBRC 15532^{T} was used as an outgroup. Bootstrap percentages over 50 % derived from 1000 replications are showed at the nodes. Dots indicate branches also recovered in the neighbor-joining and maximum-parsimony trees. Bar, 0.010 substitutions per site.

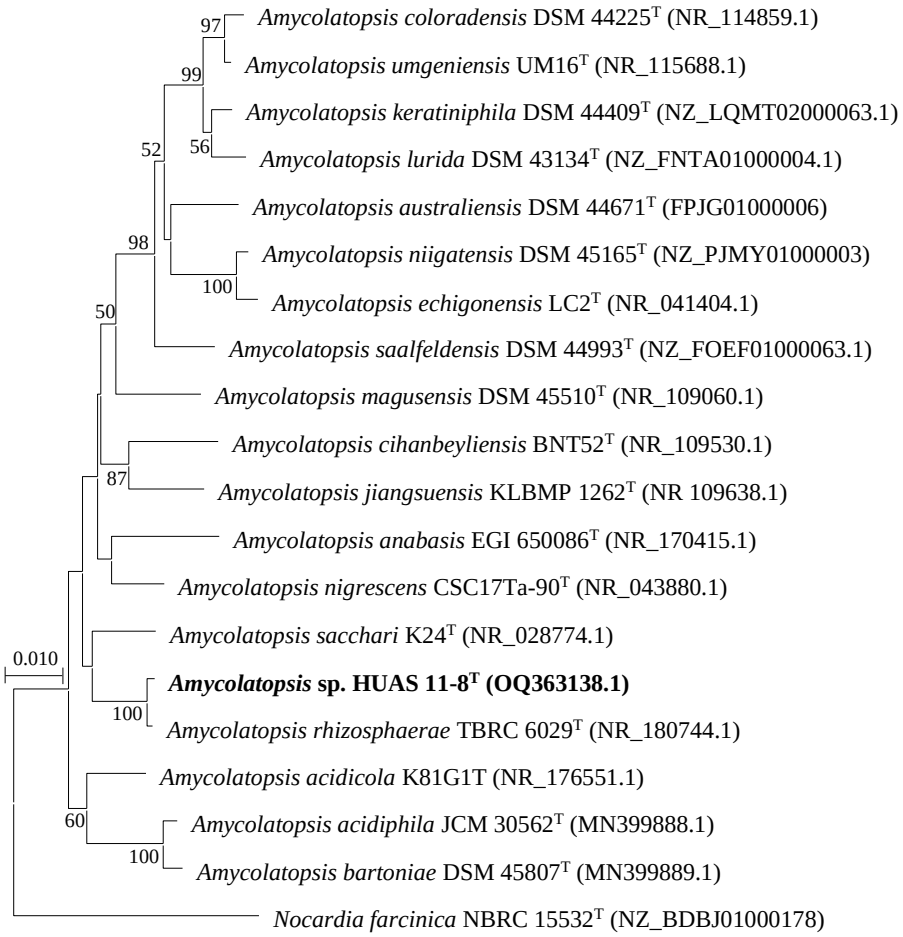

Fig.S3. Maximum-parsimony phylogenetic tree based on 16S rRNA gene sequences showing the relationship between selected species of the genus *Amycolatopsis*. *Nocardia farcinica* NBRC 15532^{T} was used as an outgroup. Bootstrap percentages over 50 % derived from 1000 replications are showed at the nodes. Dots indicate branches also recovered in the neighbor-joining and maximum-parsimony trees.

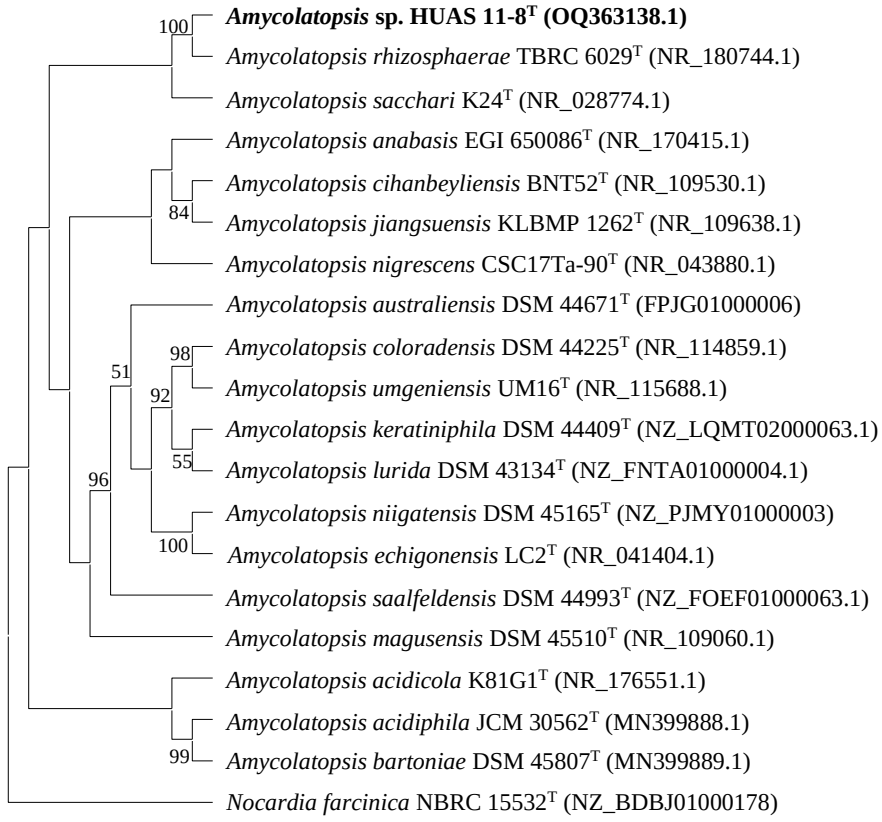

Fig.S4. Polar lipids composition of strain HUAS 11-8^{T}.

The plate dotted with sample was subjected to two-dimensional development, with the first solvent of chloroform-methanol-water (65:25:4, v/v/v) followed by the second solvent of chloroform-methanol-acetic acid-water (80:18:12:5, v/v/v/v).

Note: Molybdophosphoric acid, molybdenum blue reagent, anisaldehyde and ninhydrin were used to detect total lipids, phospholipids, phosphatidylinositol mannosides and aminolipids respectively. A, Molybdophosphoric acid (for total lipids); B, Molybdenum blue reagent (for phospholipids); C, Anisaldehyde (for phosphatidylinositol mannosides); D, Ninhydrin (for aminolipids). DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidyl ethanolamine; PC, Phosphatidylcholine; PI, phosphotidylinositol.

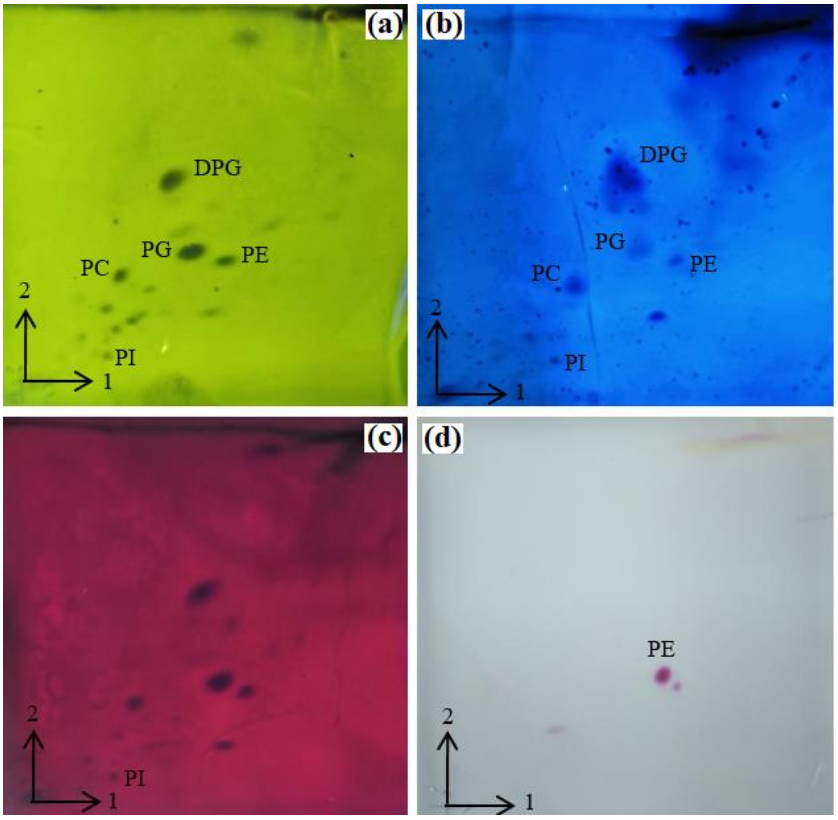

Table S1 Quality analysis and GenBank assembly of genomes of *Amycolatopsis* species in this work

No.	Specie	Strain	GenBank assembly	COM(%)	CON(%)	No.	Species	Strain	GenBank assembly	COM(%)	CON(%)
1.	*A. alba*	DSM 44262^T	GCA_000384215.1	98.97	0	14.	*A. orientalis*	DSM 40040^T	GCA_000478275.1	99.16	0.75
2.	*A. balhimycina*	FH 1894^T	GCA_000384295.1	98.98	0.17	15.	*A. panacis*	YIM PH21725^T	GCA_003600245.1	96.70	3.27
3.	*A. coloradensis*	DSM 44225^T	GCA_001953865.1	98.66	1.49	16.	*A. regifaucium*	DSM 45072^T	GCA_900113515.1	99.00	1.52
4.	*A. decaplanina*	DSM 44594^T	GCA_000342005.1	98.47	0.58	17.	*A. rifamycinica*	DSM 46095^T	GCA_000695625.1	98.98	1.15
5.	*A. dendrobii*	DR6-1^T	GCA_014145675.1	99.06	2.43	18.	*A. roodepoortensis*	DSM 46661^T	GCA_014873915.1	99.00	4.21
6.	*A. echigonensis*	JCM 21831^T	GCA_014174495.1	96.71	2.90	19.	*A. sulphurea*	DSM 46092^T	GCA_002564045.1	97.03	2.52
7.	*A. eurytherma*	DSM 44348^T	GCA_003752125.1	99.06	0.44	20.	*A. thailandensis*	JCM 16380^T	GCA_002234405.1	95.12	0.44
8.	*A. japonica*	DSM 44213^T	GCA_000732925.1	98.47	0.65	21.	*A. thermalba*	NRRL B-24845^T	GCA_003385215.1	98.57	1.52
9.	*A. kentuckyensis*	NRRL B-24129^T	GCA_002155975.1	96.19	0.86	22.	*A. thermoflava*	N1165^T	GCA_000473265.1	98.40	0
10.	*A. lurida*	DSM 43134^T	GCA_900105055.1	99.00	0.56	23.	*A. tolypomycina*	DSM 44544^T	GCA_900105945.1	98.65	1.1
11.	*A. methanolica*	239^T	GCA_000371885.1	98.66	0.33	24.	*A. tucumanensis*	ABO^T	GCA_021654135.1	99.06	1.83
12.	*A. niigatensis*	DSM 45165^T	GCA_002846615.1	98.57	3.46	25.	*A. vancoresmycina*	NRRL B-24208^T	GCA_000716785.1	98.84	0.83
13.	*A. nivea*	CFH S0261^T	GCA_004522235.1	99.06	2.10	26.	*A. vastitatis*	H5^T	GCA_002234595.1	98.59	1.16

Note: COM, Completeness; CON, Contamination.

Table S2 ANI and dDDH values of 29 pairs of *Amycolatopsis* species

No.	Species 1	Strain	Species 2	Strain	ANIm(%)	ANIb(%)	dDDH(%)
1.	*A. niigatensis*	DSM 45165^T	*A. echigonensis*	JCM 21831^T	98.74	98.07	87.9
2.	*A. thermoflava*	N1165^T	*A. eurytherma*	DSM 44348^T	98.74	98.04	87.8
3.	*A. eurytherma*	DSM 44348^T	*A. methanolica*	239^T	96.64	95.74	69.3
4.	*A. thermoflava*	N1165^T	*A. methanolica*	239^T	96.62	95.49	68.7
5.	*A. tucumanensis*	ABO^T	*A. thermoflava*	N1165^T	96.09	94.69	64.5
6.	*A. tucumanensis*	ABO^T	*A. methanolica*	239^T	95.54	94.21	61.8
7.	*A. sulphurea*	DSM 46092^T	*A. panacis*	YIM PH21725^T	95.41	94.73	60.9
8.	*A. roodepoortensis*	DSM 46661^T	*A. lurida*	DSM 43134^T	94.40	93.51	56.4
9.	*A. dendrobii*	DR6-1^T	*A. nivea*	CFH S0261^T	93.85	92.62	52.5
10.	*A. balhimycina*	FH 1894^T	*A. vastitatis*	H5^T	93.49	91.39	51.3
11.	*A. japonica*	DSM 44213^T	*A. decaplanina*	DSM 44594^T	92.81	91.49	48.5
12.	*A. alba*	DSM 44262^T	*A. thailandensis*	JCM 16380^T	92.79	91.19	48.2
13.	*A. niigatensis*	DSM 45165^T	*A. nivea*	CFH S0261^T	92.78	90.88	47.6
14.	*A. dendrobii*	DR6-1^T	*A. echigonensis*	JCM 21831^T	92.65	90.55	47.2
15.	*A. nivea*	CFH S0261^T	*A. echigonensis*	JCM 21831^T	92.62	90.56	47.0
16.	*A. thermoflava*	N1165^T	*A. thermalba*	NRRL B-24845^T	92.25	90.56	45.5
17.	*A. methanolica*	239^T	*A. thermalba*	NRRL B-24845^T	92.07	90.21	45.0
18.	*A. orientalis*	DSM 40040^T	*A. regifaucium*	DSM 45072^T	91.61	90.35	44.0
19.	*A. rifamycinica*	DSM 46095^T	*A. tolypomycina*	DSM 44544^T	91.70	90.03	43.1
20.	*A. alba*	DSM 44262^T	*A. roodepoortensis*	DSM 46661^T	91.15	89.29	41.7
21.	*A. kentuckyensis*	NRRL B-24129^T	*A. vancoresmycina*	NRRL B-24208^T	90.89	88.72	40.6
22.	*A. alba*	DSM 44262^T	*A. coloradensis*	DSM 44225^T	90.84	89.23	40.8
23.	*A. rifamycinica*	DSM 46095^T	*A. vancoresmycina*	NRRL B-24208^T	90.63	88.78	39.0
24.	*A. tolypomycina*	DSM 44544^T	*A. vancoresmycina*	NRRL B-24208^T	90.57	88.23	38.7
25.	*A. japonica*	DSM 44213^T	*A. coloradensis*	DSM 44225^T	90.42	88.26	39.4
26.	*A. thailandensis*	JCM 16380^T	*A. orientalis*	DSM 40040^T	90.41	88.37	39.5
27.	*A. kentuckyensis*	NRRL B-24129^T	*A. vastitatis*	H5^T	90.27	87.38	38.5
28.	*A. lurida*	DSM 43134^T	*A. orientalis*	DSM 40040^T	90.12	88.20	38.8
29.	*A. rifamycinica*	DSM 46095^T	*A. vastitatis*	H5^T	90.07	87.49	37.7

Table S3 Cultural characteristics of strain HUAS 11-8^{T} and *Amycolatopsis rhizosphaerae* JCM 32589^{T}.

Characteristics	1	2
Color of aerial mycelium on Reasoner' 2A	Green	Gray
Color of aerial mycelium on Reasoner' 2A	White	White
Diffusible pigment on Reasoner' 2A	None	None
Color of aerial mycelium on No. 1	White	White
Color of aerial mycelium on No. 1	White	White
Diffusible pigment on No. 1	None	None
Color of aerial mycelium on ISP 2	Gray	White
Color of substrate mycelium on ISP 2	Green	Yellow
Diffusible pigment on ISP 2	None	None
Color of aerial mycelium on ISP 3	White/Gray	None
Color of substrate mycelium on ISP 3	White	None
Diffusible pigment on ISP 3	None	None
Color of aerial mycelium on ISP 4	White	None
Color of substrate mycelium on ISP 4	White	None
Diffusible pigment on ISP 4	None	None
Color of aerial mycelium on ISP 5	Gray	White
Color of substrate mycelium on ISP 5	Gray	Yellow
Diffusible pigment on ISP 5	None	None
Color of aerial mycelium on ISP 6	Yellow	Light magenta
Color of substrate mycelium on ISP 6	Yellow	Light magenta
Diffusible pigment on ISP 6	None	None
Color of aerial mycelium on ISP 7	Gray	White
Color of substrate mycelium on ISP 7	Gray	White
Diffusible pigment on ISP 7	None	None

Note: 1, strain HUAS 11-8^{T}; 2, *Amycolatopsis rhizosphaerae* JCM 32589^{T}; No. 1, Gause's synthetic No. 1 medium. All data were from this study.

Table S4 The fatty acids composition of HUAS 11-8^{T} and *Amycolatopsis rhizosphaerae* JCM 32589^{T}.

Fatty acids	1	2	Fatty acids	1	2
$C_{10:0}$	tr	–	anteiso-$C_{16:0}$	0.6	tr
iso-$C_{10:0}$	tr	tr	iso-$C_{16:0}$	**30.5**	**37.8**
anteis*o*-$C_{11:0}$	tr	–	$C_{16:0}$	**10.8**	4.9
iso-$C_{11:0}$ 3 OH	–	tr	iso-$C_{16:1}$ G	4.4	5.8
iso-$C_{12:0}$	–	tr	iso-$C_{17:0}$	3.8	1.4
$C_{13:0}$	tr	–	anteiso-$C_{17:0}$	7.0	3.0
anteiso-$C_{14:0}$	tr	–	anteiso-$C_{17:1\ \omega9c}$	0.6	tr
$C_{14:0}$	1.0	tr	$C_{17:1\ \omega8c}$	0.6	5.0
$C_{14:1}$ ω5c	tr	tr	$C_{17:1\ \omega6c}$	**10.0**	**17.2**
iso-$C_{14:0}$	1.6	2.0	$C_{17:0}$	tr	1.6
iso-$C_{14:0}$ 3OH	tr	–	$C_{18:1}$ ω9c	4.0	1.0
$C_{15:0}$ 2OH	–	tr	$C_{18:0}$	1.1	0.9
iso-$C_{15:1}$ G	tr	tr	Summed Feature 3	9.4	**10.1**
iso-$C_{15:0}$	4.7	3.3	Summed Feature 5	1.4	0.9
anteiso-$C_{15:0}$	1.3	0.7	Summed Feature 9	0.8	tr
$C_{15:1}$ ω6c	tr	1.9			

Note: 1, strain HUAS 11-8^{T}; 2, *Amycolatopsis rhizosphaerae* JCM 32589^{T}; tr, trace amount (<0.5%); –, not detected; Summed Feature 3, $C_{16:1}$ ω7c/$C_{16:1}$ ω6c; Summed feature 5, $C_{18:0}$ ante/$C_{18:2}$ ω6,9c; Summed feature 9, iso-$C_{17:1}$ ω9c/$C_{16:0}$ 10-methyl. All data were from this study.

Table S5 16S rRNA gene sequence similarity, *gyrB*-based genetic distance, *recN*-based genetic distance between *Amycolatopsis echigonensis* JCM 21831^T and it relatives

Strains	*Amycolatopsis echigonensis* JCM 21831^T		
	16S rRNA gene sequence similarity	*gyrB*-based genetic distance (1297 bp)	*recN*-based genetic distance (1228 bp)
Amycolatopsis niigatensis DSM 45165^T	99.37%	0.011	0.014
Amycolatopsis halotolerans NRRL B-24428^T	99.10%	0.051	0.055
Amycolatopsis albidoflavus NRRL B-24149^T	98.96%	0.036	0.059
Amycolatopsis rubida NRRL B-24150^T	98.82%	0.049	0.052
Amycolatopsis circi S1.3^T	98.82%	0.039	0.053
Amycolatopsis nivea CFH S0261^T	98.81%	0.025	0.059
Amycolatopsis equina SE (8)3^T	98.73%	0.039	0.053
Amycolatopsis dendrobii DR6-1^T	98.72%	0.041	0.051
Amycolatopsis hippodromi S3.6^T	98.70%	0.039	0.055

Table S6 GenBank accession numbers of the *gyrB* and *recN* genes used in this work

Strains	GenBank accession number	
	gyrB	*recN*
Amycolatopsis echigonensis JCM 21831^T	EU822892.1	JF772782.1
Amycolatopsis niigatensis DSM 45165^T	NZ_PJMY01000003.1 (2944383-2946350)	NZ_PJMY01000003.1 (6770045-6771829)
Amycolatopsis halotolerans NRRL B-24428^T	EU822895.1	JF772784.1
Amycolatopsis albidoflavus NRRL B-24149^T	EU822886.1	JF772776.1
Amycolatopsis rubida NRRL B-24150^T	EU822911.1	JF772801.1
Amycolatopsis circi S1.3^T	HQ021205.1	JX465741.1
Amycolatopsis nivea CFH S0261^T	NZ_SDLT01000004.1 (295153-297120)	NZ_SDLT01000063.1 (5732-7516)
Amycolatopsis equina SE (8)3^T	HQ021207.1	JX465742.1
Amycolatopsis dendrobii DR6-1^T	NZ_JACGZW010000009.1 (10410-12377)	NZ_JACGZW010000029.1 (5559-7343)
Amycolatopsis hippodromi S3.6^T	HQ021206.1	JX465743.1