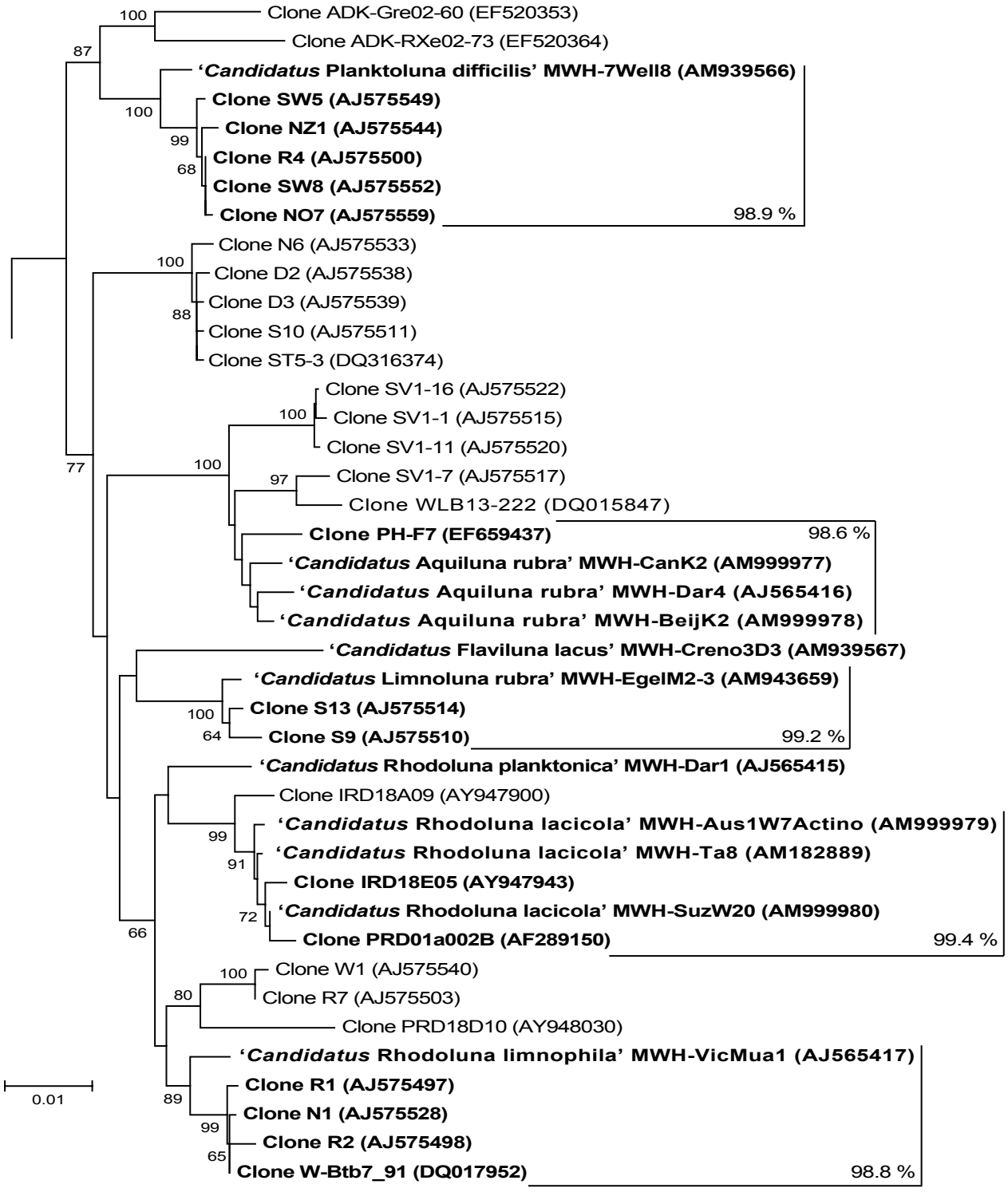
Supplementary Fig. S1. Growth of '*Candidatus* Limnoluna rubra' strain MWH-EgelM2-3 and an accompanying non-actinobacterial strain on NSY agar plates. The colonies of the candidate species are red-pigmented and those of the other strain are unpigmented. The plated liquid culture contained about 10^9 cells ml^{-1} and was dominated by the actinobacterial strain (>90 % of cells). The photo shows a section of a plate that received an inoculum of about 10^5 cells. The strain of the candidate species grows well when in direct contact with the non-pigmented colonies; however, the size of the actinobacterial colonies decreases with distance from the non-pigmented colonies and, beyond a crucial distance from these colonies, no actinobacterial growth occurs.



Supplementary Fig. S2. Neighbour-joining tree based on almost full-length 16S rRNA gene sequences (*E. coli* positions 47–1465). The tree reconstructs the phylogenetic relationships between the novel candidate species and environmental sequences of uncultured actinobacteria. The phylogenetic group presented in the tree represents almost the entire Luna-1 cluster (Hahn *et al.*, 2003) and the major part of the acII group (Warnecke *et al.*, 2004). Sequences that contain the diagnostic oligonucleotide sequences for discrimination of the candidate species from other taxa are shown in bold, and sequence similarities within the respective groups are presented (as percentage identity). Bootstrap values represent percentage support of nodes based on 1000 resamplings (only values ≥60% are shown). Bar, 1 % estimated sequence divergence. The sequence of *Brevibacterium linens* DSM 20425^T (GenBank accession no. X77451) served as an outgroup (not shown). References cited are given in the main paper.

Hahn, M. W. (2009). Description of seven candidate species affiliated with the phylum *Actinobacteria*, representing planktonic freshwater bacteria. *Int J Syst Evol Microbiol* **59**, 112–117.

Supplementary Table S1. Pairwise comparison of the sequence similarity of 16S rRNA genes of the candidate species

Values are percentages of sequence identity.

Strain	1	2	3	4	5	6	7
1. '*Candidatus* Aquiluna rubra' strain MWH-Dar4	(100)	95.5	96.9	96.2	96.0	96.2	96.9
2. '*Candidatus* Flaviluna lacus' strain MWH-Creno3D3		(100)	96.8	96.5	96.4	95.9	95.8
3. '*Candidatus* Limnoluna rubra' strain MWH-EgelM2-3			(100)	97.1	97.1	97.6	97.4
4. '*Candidatus* Rhodoluna limnophila' strain MWH-VicMua1				(100)	98.3	97.9	96.6
5. '*Candidatus* Rhodoluna planktonica' strain MWH-Dar1					(100)	97.8	96.8
6. '*Candidatus* Rhodoluna lacicola' strain MWH-Ta8						(100)	96.0
7. '*Candidatus* Planktoluna difficilis' strain MWH-7Well8							(100)

Supplementary Table S2. Geographical, limnological and climatic characteristics of habitats from which the strains representing the seven candidate species were isolated

Strain	Source of isolation	Geographical coordinates	Habitat characteristics	Climate
'*Candidatus* Planktoluna difficilis' MWH-7Well8	Lake Taihu, China	31° 26′ 5.78″ N 120° 19′ 14.36″ E	Large, shallow, eutrophic to hypertrophic lake	Subtropical
'*Candidatus* Aquiluna rubra' MWH-Dar4	Pond, Tanzania	6° 49′ 16.83″ S 39° 16′ 46.31″ E	Small, artificial pond on the university campus in Dar es Saalam	Tropical
'*Candidatus* Limnoluna rubra' MWH-EgelM2-3	Lake Egelsee, Austria	47° 57′ 43.70″ N 13° 7′ 28.56″ E	Shallow, eutrophic, slightly alkaline lake	Temperate
'*Candidatus* Flaviluna lacustris' MWH-Creno3D3	Lake Creno, Corsica, France	42° 12′ 17.32″ N 8° 56′ 44.73″ E	Oligotrophic, montane lake	Temperate
'*Candidatus* Rhodoluna lacicola' MWH-Ta8	Lake Taihu, China	31° 26′ 5.78″ N 120° 19′ 14.36″ E	Large, shallow, eutrophic to hypertrophic lake	Subtropical
'*Candidatus* Rhodoluna limnophila' MWH-VicMua1	Lake Victoria, Tanzania	2° 34′ 32.41″ S 32° 51′ 19.68″ E	Large, deep, tropical lake with circumneutral pH	Tropical
'*Candidatus* Rhodoluna planktonica' MWH-Dar1	Pond, Tanzania	6° 49′ 16.83″ S 39° 16′ 46.31″ E	Small, artificial pond on the university campus in Dar es Saalam	Tropical

Hahn, M. W. (2009). Description of seven candidate species affiliated with the phylum *Actinobacteria*, representing planktonic freshwater bacteria. *Int J Syst Evol Microbiol* **59**, 112–117.