Antonie van Leeuwenhoek (2020) 113:1823–1837
https://doi.org/10.1007/s10482-019-01378-1

ORIGINAL PAPER

Description of the novel planctomycetal genus *Bremerella*, containing *Bremerella volcania* sp. nov., isolated from an active volcanic site, and reclassification of *Blastopirellula cremea* as *Bremerella cremea* comb. nov.

Stephanie Rensink · Sandra Wiegand · Nicolai Kallscheuer · Patrick Rast · Stijn H. Peeters · Anja Heuer · Christian Boedeker · Mike S. M. Jetten · Manfred Rohde · Mareike Jogler · Christian Jogler

Received: 1 October 2019 / Accepted: 12 December 2019 / Published online: 1 January 2020

Abstract Planctomycetes are part of the PVC superphylum together with Verrucomicrobia, Chlamydiae and others. They are budding bacteria with very distinctive characteristics, such as a remarkable morphology and cell biology. Planctomycetes can be found in almost all habitats, and seem to have a preference for marine biotic and abiotic surfaces, on which they frequently occur in biofilm-forming communities. To extend the number of axenic cultures of planctomycetal strains, we isolated Pan97^T from a biofilm in a volcanic site close to the Italian island Panarea in the Thyrrhenian Sea. The physiology, genome and morphology of the novel strain were characterised revealing typical planctomycetal characteristics, such as, division by polar budding and presence of crateriform structures. The strain shows pear-shaped cells of 1.5 ± 0.3 µm × 0.8 ± 0.2 µm and forms white- to cream-coloured colonies on solid medium. Strain Pan97^T is mesophilic and neutrophilic, since growth was observed at a pH range of 5.5–9.5 with optimal growth at pH 7.0 and at a temperature range of 15–40 °C with a maximal growth rate at 36 °C. Pan97^T has a genome size of 6,496,182 bp with a G + C content of 56.2%. 5264 protein-coding genes were identified, of which 2141 genes (41%) encode hypothetical proteins. Based on the phylogenetic analysis, we suggest that Pan97^T (DSM 101992^T = LMG 29460^T) represents a novel species of a novel genus within the family *Planctomycetaceae*, for which we propose the name *Bremerella* gen. nov., with strain Pan97^T classified as *Bremerella volcania* sp. nov. Based on our analysis, we also propose the reclassification of *Blastopirellula cremea* Lee et al. 2013 as *Bremerella cremea* comb. nov., as this species is considered to be the type species of the novel genus *Bremerella*.

Keywords *Bremerella volcania* · Planctomycetes · Volcanic landscape · Hydrothermal vent · Panarea · *Blastopirellula marina* · *Blastopirellula cremea*

S. Rensink · S. Wiegand · N. Kallscheuer · S. H. Peeters · M. S. M. Jetten · M. Jogler · C. Jogler (✉)
Department of Microbiology, Radboud University, Nijmegen, The Netherlands
e-mail: christian@jogler.de

P. Rast · A. Heuer · C. Boedeker
Leibniz Institute DSMZ, Brunswick, Germany

M. Rohde
Central Facility for Microscopy, Helmholtz Centre for Infection Research, Brunswick, Germany

M. Jogler · C. Jogler
Department of Microbial Interactions, Institute of Microbiology, Friedrich Schiller University, Jena, Germany

Introduction

Planctomycetes were first discovered in 1924 and were initially classified as eukaryotes (Gimesi 1924). Subsequently, they were acknowledged to be bacteria (Hirsch 1972). Phylogenetically, they are classified as a phylum, and together with Verrucomicrobia and Chlamydiae, they are part of the so-called PVC superphylum (Wagner and Horn 2006). Planctomycetes were considered exceptional due to their compartmentalised cell plan (Lindsay et al. 1997), nucleus-like structure (Fuerst and Webb 1991), endocytosis (Lonhienne et al. 2010) and the lack of a peptidoglycan cell wall (König et al. 1984). However, Planctomycetes faced a paradigm shift resulting from new insights into their cell biology (Wiegand et al. 2018). Before their recognition as Gram-negative bacteria, they were seen as microorganisms beyond the bacterial cell plan (Devos et al. 2013; Fuerst and Sagulenko 2011) and were postulated to link bacteria and eukaryotes (Devos and Reynaud 2010). The development of new microscopic techniques and genetic tools led to new insights into the cell organisation and physiology of the phylum *Planctomycetes* (Jogler and Jogler 2013; Overmann et al. 2017; Rivas-Marin et al. 2016). Several distinctive characteristics were discovered. These include an uncommon mechanism of cell division associated with the absence of canonical divisome proteins including FtsZ (Jogler et al. 2012; Pilhofer et al. 2008; Wiegand et al. 2018, 2019). A complex cytoplasmic membrane and an unusual macromolecule uptake system were found, which potentially might both take part in the digestion of internalised polysaccharides (Boedeker et al. 2017). Finally, it was found that Planctomycetes and also the closely related Verrucomicrobia (Boedeker et al. 2017) do possess a peptidoglycan cell wall (Jeske et al. 2015; van Teeseling et al. 2015). The planctomycetal cell envelope architecture is now considered Gram-negative (Devos 2014).

Planctomycetal species were found to divide either by budding or binary fission and some species even seem to use both mechanisms (Wiegand et al. 2019). Members of the class *Phycisphaerae* and the family *Candidatus* Brocadiaceae divide by binary fission while *Planctomycetia* divide by budding, which can be arbitrary budding in *Gemmataceae* and *Isosphaeraceae* or polar budding in *Planctomycetaceae* in association with a lifestyle switch (Wiegand et al. 2019). The lifecycle switch features the attachment of a swimming cell to a surface or to other cells. Subsequently, the sessile mother cell starts to divide by polar budding, thereby developing a swimming flagellated daughter cell (Jogler et al. 2011; Wiegand et al. 2018).

Besides cell biology, Planctomycetes have great ecological relevance as they are involved in global nitrogen and carbon cycles (Wiegand et al. 2018). Members of *Candidatus* Brocadiaceae are known for their performance of the anammox (anaerobic ammonium oxidation) reaction (Strous et al. 1999), in which ammonium is converted to dinitrogen gas (Peeters and van Niftrik 2019). Therefore, Planctomycetes can be used for the industrial removal of ammonium from wastewater (Kartal et al. 2010; Strous et al. 1999). Typically, Planctomycetes can be found in diverse habitats, especially on aquatic surfaces (Wiegand et al. 2018). They even make up the majority of cells in some biofilm communities (Webster and Negri 2006), in particular on marine algal surfaces (Bengtsson et al. 2012; Bondoso et al. 2014, 2015, 2017; Vollmers et al. 2017). In general, they often live associated with aquatic phototrophs such as diatoms, cyanobacteria and kelp, or in association with fungi, sponges or lichens (Jeske et al. 2016; Lage and Bondoso 2014). For example, Planctomycetes can make up 70% of the microbial community on the macroalgae *Ecklonia radiata* of the Australian shore (Wiegand et al. 2018). Also, in biofilms on surfaces of the kelp *Laminaria hyperborea*, Planctomycetes account for more than 50% of the bacterial biofilm (Bengtsson and Ovreas 2010). Given such high values and the fact that Planctomycetes are slow growers compared to other natural competitors in their ecological niche (Frank et al. 2015; Wiegand et al. 2018), it was hypothesised that their survival in such communities is mediated by the production of bioactive small molecules (Graça et al. 2016; Jeske et al. 2013). Also, the natural resistance of Planctomycetes to several classes of antibiotics (Cayrou et al. 2010)—often a result of the lack of targets due to their unusual cell division mechanism—could be associated with the production of bioactive compounds (Godinho et al. 2019; Graça et al. 2016; Jeske et al. 2013, 2016). Planctomycetal characteristics, including large genomes, the presence of giant genes (Kohn et al. 2016, 2019a; Wiegand et al. 2019) and their complex lifecycles has some

similarities with other 'talented' small compound-producing bacteria, such as Actinobacteria, in particular members of the family *Streptomycetaceae* (Challis and Hopwood 2003).

To extend the collection of axenic cultures of Planctomycetes, we here sampled an active volcanic area in the Thyrrhenian Sea close to the Italian island Panarea (Fig. 1) and isolated the novel strain Pan97^T from a red biofilm in the so called Hot Lake (Gugliandolo et al. 2015). Characterisation of its physiology and morphology, and analysis of the genome, suggest that strain Pan97^T represents a novel species of a novel genus within the family *Planctomycetaceae*.

Materials and methods

Sampling and cultivation

Strain Pan97^T was isolated on the 9th of September 2013 from a red biofilm sampled in a hydrothermal vent close to the island Panarea in Italy (sampling site 38.5568 N, 15.1097 E) (Fig. 1). The samples were collected as previously described (Wiegand et al. 2019). Initial amplification and sequencing of the 16S rRNA gene was performed as described (Boedeker et al. 2017), which was intended to check that the strain is a Planctomycete. The strain was subsequently cultivated in M1 medium with HEPES as buffering agent and additionally supplemented with *N*-acetyl glucosamine (NAG) and artificial seawater (ASW). This medium, designated M1H NAG ASW, was prepared according to a protocol published earlier (Kallscheuer et al. 2019a).

Physiological analysis

Temperature and pH optima were determined by cultivating Pan97^T in M1H NAG ASW medium. To this end, the strain was cultivated at temperatures of 10, 15, 20, 22, 24, 27, 30, 33, 36 and 40 °C in a shaking incubator at 110 rpm with an initial pH of 8.0. The growth rates were determined from optical density measurements at 600 nm (OD_{600}) recorded with a UV–Vis spectrophotometer (Ultrospec II photometer LKB Biochrom). For determination of the pH optimum 100 mM HEPES was used as buffering agent for cultivations at pH 7.0, 7.5 and 8.0. For cultivations at pH 5.0 and 6.0 HEPES was replaced by 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) and at pH 9.0 and 10.0 100 mM *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES) was used as buffering agent. Cultivations for determination of the pH optimum were performed at 28 °C in a shaking incubator at 110 rpm. For determination of the salt tolerance of Pan97^T different amounts of NaCl were added to M1H NAG ASW in order to yield a final NaCl concentration ranging from 0 to 2 M.

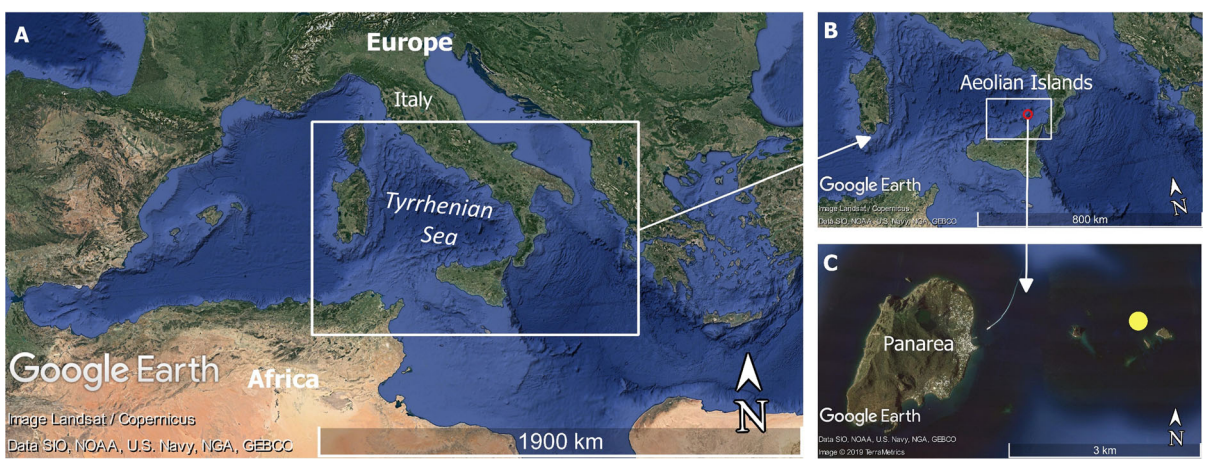

Fig. 1 Sampling location of the novel strain Pan97^T. The sampling spot is located in Italy, Europe (**a**) close to the island Panarea (**b**, red circle) which is part of the Aeolian Islands (**b**, white box). Pan97^T was isolated from a hydrothermal vent (**c**, yellow dot), located close to Panarea. Satellite images were obtained using Google Earth Pro. (Color figure online)

Microscopic techniques

Light microscopy and field emission scanning electron microscopy were performed as previously described (Kallscheuer et al. 2019a).

Genome information

Genome information of strain Pan97^T is available from GenBank under accession number CP036289 (Wiegand et al. 2019) and the 16S rRNA gene sequence is available from GenBank under accession number MK554518.

Phylogenetic analysis

The 16S rRNA gene-based phylogenetic analysis of strain Pan97^T was computed together with sequences of all described planctomycetal species (assessed in May 2019) and all isolates recently described (Boersma et al. 2019; Kallscheuer et al. 2019a, b, c, d; Kohn et al. 2019b; Peeters et al. 2019; Wiegand et al. 2019).

The 16S rRNA gene sequence alignment was made using SINA (Pruesse et al. 2012). The phylogenetic inference was calculated with RAxML (Stamatakis 2014) using the maximum likelihood method with 1000 bootstraps, nucleotide substitution model GTR, gamma distributed rate variation and estimation of proportion of invariable sites (GTRGAMMAI option) (Stamatakis 2014). In this phylogenetic analysis, three 16S rRNA genes of bacterial strains from the PVC superphylum (accession numbers AJ229235, CP010904.1 and NR_027571) were used as outgroup. The average nucleotide identity (ANI) was calculated using OrthoANI (Lee et al. 2016) and the average amino acid identity (AAI) was obtained using the aai.rb script of the enveomics collection (Rodriguez-R and Konstantinidis 2016). The percentage of conserved proteins (POCP) was calculated as described (Qin et al. 2014). The *rpoB* gene sequences were taken from publicly available genome annotations and sequence identities were determined as previously described (Bondoso et al. 2013). The alignment and matrix calculation was performed upon extracting only those parts of the sequence that would have been sequenced with the described primer set and the alignment and matrix calculation was done with Clustal Omega (Sievers et al. 2011).

Results and discussion

Sampling and cultivation

After sampling an active volcanic area close to the island Panarea in Italy, a novel Planctomycetes strain, designated Pan97^T, was isolated from a red biofilm from the so-called Hot Lake (Gugliandolo et al. 2015; Sieland et al. 2009), a submarine depression of the seafloor at a water depth of 19.1 m (Fig. 1). Panarea is one out of seven islands of the volcanic archipelago of the Aeolian Islands (Raab et al. 2017), which have a quaternary active volcanic structure and are characterised by a volcanic landform derived from repeated episodes of volcanic activity and volcano-tectonic collapse (Lucchi et al. 2017). Increased temperatures and the presence of different sources of carbon, nitrogen and sulphur render this area an interesting location for the isolation of novel planctomycetal strains. At the time of sampling, the sampling site was filled with water at pH 5.62 and 27.6 °C, compared to the surface water at pH 8.06 and 20.0 °C.

Phylogenetic analysis

The phylogenetic analysis of Pan97^T showed that this strain belongs to the *Pirellula* clade (Wiegand et al. 2019). Upon 16S rRNA gene sequence-based phylogenetic inference (Fig. 2), Pan97^T clustered with *Blastopirellula cremea* LHWP2^T (Lee et al. 2013), the second described species of the genus *Blastopirellula*. The two strains show a 16S rRNA gene identity of 99.0%. As this value is above the proposed species threshold of 98.7% (Stackebrandt and Ebers 2006), both strains may belong to the same species. However, it has been shown previously that Planctomycetes tend to have high 16S rRNA sequence identities despite belonging to different species (Bondoso et al. 2013; Kohn et al. 2019b) and higher thresholds can be applied to particular taxonomic groups (Meier-Kolthoff et al. 2013). As there is no genome available for *B. cremea* LHWP2^T, genome-based analysis of these strains was not possible. To come to an educated decision, we compared all genomes from species annotated as belonging to *B. cremea* in NCBI (Table 1). By 16S rRNA gene analysis only, all of the three genomes may also belong to the species *B. cremea*. However, when comparing genome-based markers, this picture changed distinctly. For *rpoB*

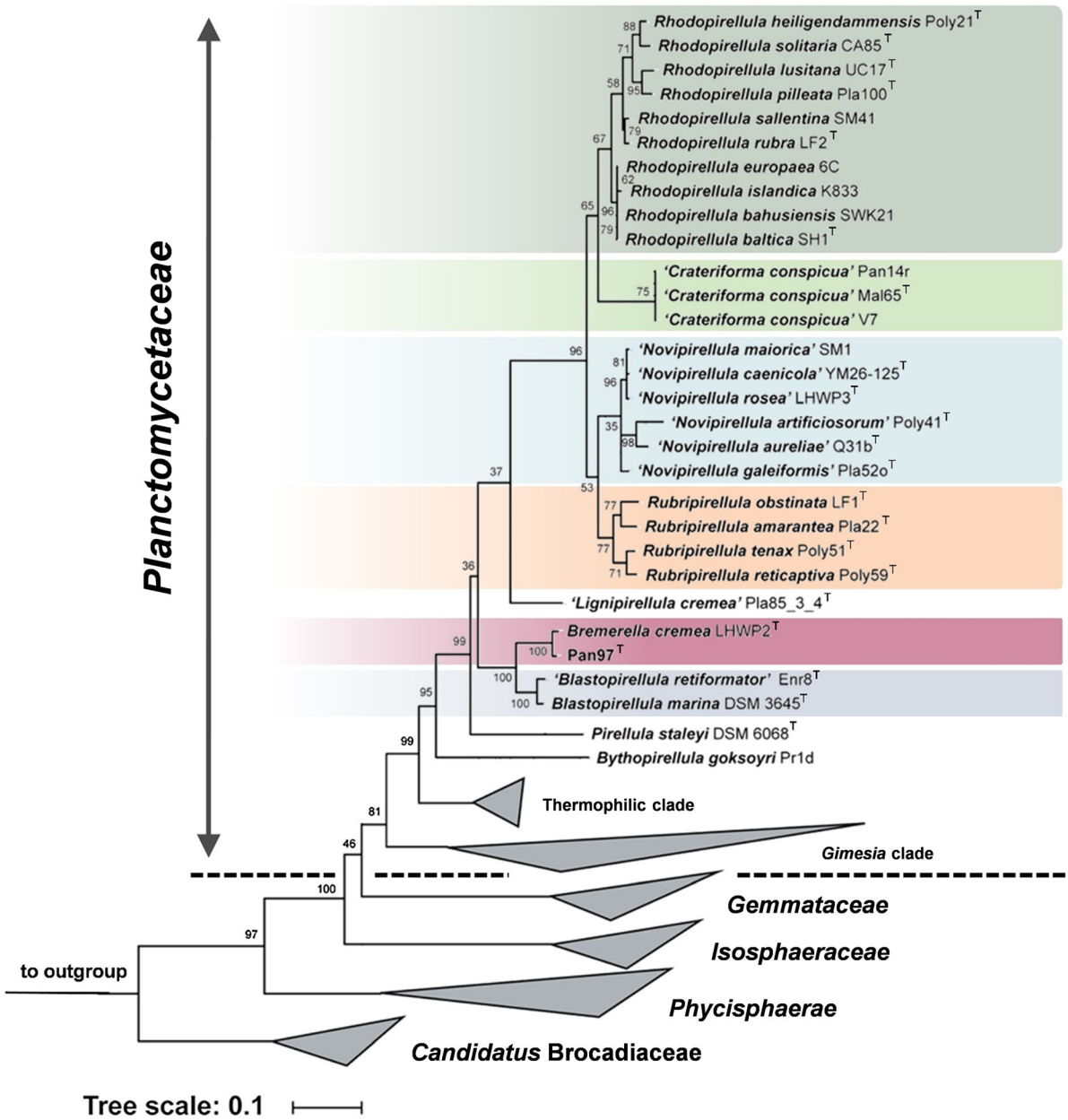

Fig. 2 Phylogenetic tree of the phylum *Planctomycetes* and of strain Pan97^T. Phylogeny was computed with 16S rRNA genes using a maximum likelihood approach. At the nodes the bootstrap values after 1000 re-samplings are given in %. The outgroup contains three 16S rRNA genes from the PVC superphylum (accession numbers AJ229235, CP010904.1 and NR_027571). The *Gimesia* clade includes species of the genera *Gimesia*, *Planctopirus*, *Fuerstiella*, *Schlesneria*, *Rubinisphaera* and *Planctomicrobium*, while the thermophilic clade includes species of the genera *Thermostilla*, *Thermogutta* and *Thermopirellula*

sequence identity, the species threshold is 95.5% (Bondoso et al. 2013) and for ANI a threshold of 95–96% is accepted (Kim et al. 2014). The ANI values as well as the *rpoB* sequence identities for the analysed strains are significantly below these thresholds (Table 1). We therefore assume that 16S rRNA gene identity comparisons in this clade do not follow the species threshold of 98.7% (Stackebrandt and Ebers

Table 1 Comparison of the genomes and 16S rRNA gene sequences of strain Pan97^T, *Blastopirellula marina* SH106^T and several *Blastopirellula cremea* strains

	Pan97^T	GCF_003335485	GCF_003335505	GCF_003335525	*Blastopirellula cremea*	*Blastopirellula marina*
16S rRNA gene						
Pan97^T	100.0	99.5	99.3	99.7	99.0	94.4
GCF_003335485	99.5	100.0	99.8	99.4	99.5	94.1
GCF_003335505	99.3	99.8	100.0	99.6	99.3	94.1
GCF_003335525	99.7	99.4	99.6	100.0	98.9	94.5
Blastopirellula cremea	99.0	99.5	99.3	98.9	100.0	93.9
Blastopirellula marina	94.4	94.1	94.1	94.5	93.9	100.0
ANI						
Pan97^T	100.0	73.9	74.0	83.7	n.d	69.7
GCF_003335485	73.9	100.0	78.0	73.9	n.d	69.1
GCF_003335505	74.0	78.0	100	73.9	n.d	69.2
GCF_003335525	83.7	73.9	73.9	100.0	n.d	69.6
Blastopirellula marina	69.7	69.1	69.2	69.6	n.d	100.0
rpoB						
Pan97^T	100.0	83.9	84.1	89.7	n.d	74.6
GCF_003335485	83.9	100.0	84.7	86.4	n.d	74.4
GCF_003335505	84.1	84.7	100.0	85.7	n.d	73.6
GCF_003335525	89.7	86.4	85.7	100.0	n.d	74.9
Blastopirellula marina	74.6	74.4	73.6	74.9	n.d	100.0
AAI						
Pan97^T	100.0	74.6	74.8	88.3	n.d	58.5
GCF_003335485	74.6	100.0	83.6	74.6	n.d	58.6
GCF_003335505	74.8	83.6	100.0	74.7	n.d	58.5
GCF_003335525	88.3	74.6	74.7	100.0	n.d	58.8
Blastopirellula marina	58.5	58.6	58.5	58.8	n.d	100.0
POCP						
Pan97^T	100.0	71.7	70.7	81.5	n.d	60.0
GCF_003335485	71.7	100	78.1	70.5	n.d	57.2
GCF_003335505	70.7	78.1	100	70.5	n.d	58.3
GCF_003335525	81.5	70.5	70.5	100.0	n.d	59.2
Blastopirellula marina	60.0	57.2	58.3	59.2	n.d	100.0

For *B. cremea*, the 16S rRNA of the type strain is used, next to genome sequences (given by RefSeq accession number) from three other isolates available from NCBI. The genomes are compared for average nucleotide identity (ANI), rpoB sequence identity, average amino acid identity (AAI) and percentage of conserved proteins (POCP)

n.d. not determined

2006) and conclude that Pan97^T does not belong to the species *B. cremea*.

When assessing the values for species differentiation it became evident that Pan97^T and *B. cremea* do not belong to the same genus as *Blastopirellula marina*. Their 16S rRNA gene identities are below the genus threshold of 94.5% (Yarza et al. 2014) and the *rpoB* sequence identities are below the corresponding genus threshold of 75.5–78.0% (Kallscheuer et al. 2019d) (Fig. 3 and Table 1). The same is true for AAI values, which were also below the genus threshold of 60–80% (Luo et al. 2014). Only the

POCP is slightly above the proposed genus threshold of 50% (Qin et al. 2014).

Taken together, we propose that the genus *Blastopirellula* genus of the family *Planctomycetaceae* is divided into *Blastopirellula* and the novel genus *Bremerella*. As *B. marina* is the type species of the genus *Blastopirellula* (Schlesner et al. 2004), we propose the reclassification of *B. cremea* (Lee et al. 2013) as *Bremerella cremea* comb. nov., the type species of the genus *Bremerella* gen. nov. We further propose that strain Pan97^T represents a novel species within this novel genus named *Bremerella volcania* sp. nov.

Morphological and physiological analyses

The morphology of strain Pan97^T was characterised from cells harvested during the exponential growth phase. The strain shows pear-shaped cells of 1.5 ± 0.3 µm × 0.8 ± 0.2 µm (Fig. 4a–c) and forms white- to cream-coloured colonies on solid medium indicating a lack of carotenoid biosynthesis. Pan97^T cells are arranged in rosettes and usually form aggregates (Fig. 4d). The cells divide by polar budding (Fig. 4a) with the daughter cells having the same shape as the mother cell. The surface of the cell shows overall crateriform structures and fibre-like structures occur at the division pole regions of the cells (Fig. 4e).

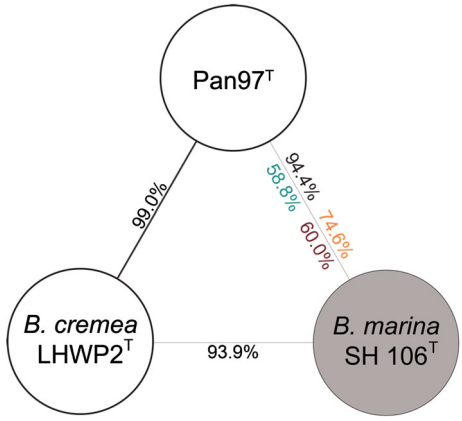

Fig. 3 Similarity values of the novel isolate Pan97^T with the two described *Blastopirellula* species *B. cremea* and *B. marina*. The black font gives the 16S rRNA gene identity, the orange font gives the *rpoB* sequence identity, the turquoise font the whole genome-based average nucleotide identity (ANI) and the red font the percentage of conserved proteins (POCP). The colour of the circles discriminates between strains that are concluded to form different genera

No holdfast structures or flagella were observed during microscopic analyses. The phenotypic features show similarities between Pan97^T and the close relatives *B. cremea* and *B. marina* in terms of cell size, aggregate formation and cell division mechanism (Table 2). Daughter cells of Pan97^T have a pear-like shape, whereas they have bean-like shapes in case of *B. cremea* and *B. marina*.

In the physiological analyses, strain Pan97^T was found to grow at a pH range of 5.5–9.5 with optimal growth at pH 7.0 (Fig. 5a) and at a temperature range of 15–40 °C with a maximal growth rate at 36 °C (Fig. 5b). Based on these values, strain Pan97^T is mesophilic and neutrophilic. In our experiments, a maximal growth rate of 0.079 h^{-1} was observed, which corresponds to a generation time of 9 h. The in vitro determined optimum growth conditions of Pan97^T do not fit to the environmental conditions found at the sampling location in the sampling year (pH 5.62 and 27.6 °C), indicating that this might not be the preferred location of the strain. However, the temperature optimum of 36 °C is high compared to the close relatives *B. cremea* and *B. marina*, both showing optimal growth at 30 °C, which might in some way reflect the higher temperatures at the sampling location in comparison to other locations from which we have isolated strains (e.g. the North Sea or the Baltic Sea; Wiegand et al. 2019). In cultivations with different NaCl concentrations, Pan97^T showed the highest growth rate of 0.035 h^{-1} at an NaCl concentration of 0.1 M, but was also capable of growth at the highest tested concentration of 2 M (with 13% of the growth rate observed at 0.1 M NaCl) (Fig. 5c).

Genome characteristics

The phenotypic and genomic characteristics of Pan97^T and its close relatives *B. cremea* LHWP2^T (Lee et al. 2013) and *B. marina* DSM 3645^T (Schlesner et al. 2004) are summarised in Table 2. Pan97^T has a genome size of 6,496,182 bp with a G + C content of 56.2%. 5264 protein-coding genes were identified, of which 2141 genes (41%) encode hypothetical proteins. The values correspond to 820 protein-coding genes per Mb and a coding density of 88.5%. Compared to *B. marina*, with a genome size of 6,663,851 bp and a G + C content of 57.4%, strain Pan97^T has a slightly smaller genome and G + C content, but a slightly larger coding density. Pan97^T

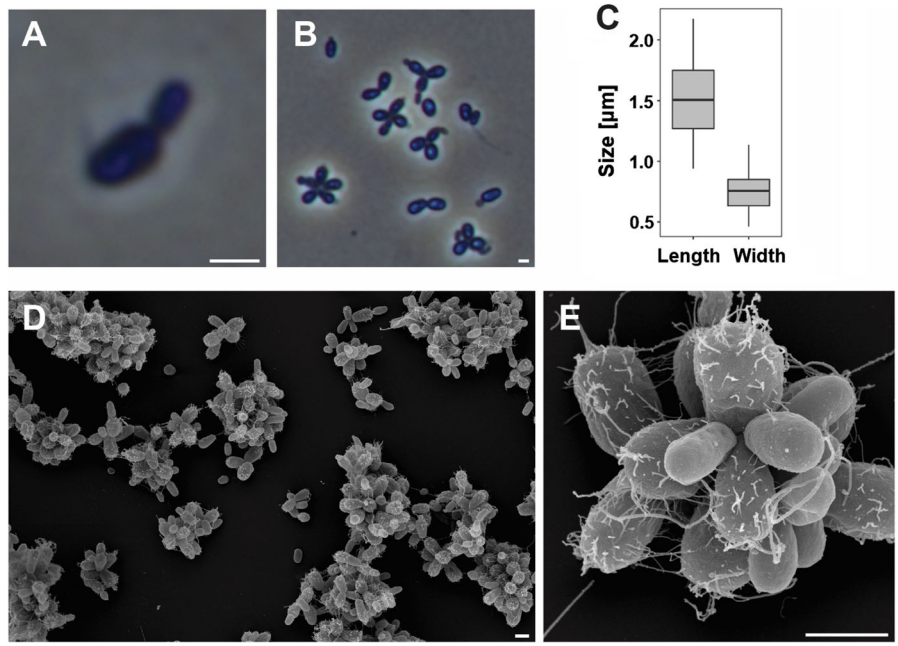

Fig. 4 Morphological characteristics of strain Pan97^T. Morphologic characteristics were analysed by phase contrast (**a**, **b**) and scanning electron microscopy (**d**, **e**). The scale bar is 1 µm. Pan97^T cells divide by polar budding (**a**) and grow in rosettes and usually form aggregates (**b**). Average cell size (**c**) was determined based on phase contrast images

harbours two copies of the 16S rRNA gene, while the gene is present in single copy in *B. marina* DSM 3645^T (Table 2).

Genome-based analysis of the central carbon metabolism

As the central carbon metabolism of Planctomycetes has not been investigated in detail, we performed a genome-based analysis in order to check for presence of key metabolic enzymes participating in the central carbon metabolism in Pan97^T. We included genes coding for enzymes involved in glycolytic pathways, gluconeogenesis, the tricarboxylic acid (TCA) cycle and anaplerotic reactions and compared the data obtained for Pan97^T to *B. marina* DSM 3645^T. Both strains harbour genes coding for enzymes of the Embden–Meyerhof–Parnas (EMP) pathway (the most common glycolytic pathway) as well as for the Entner–Doudoroff pathway (Table 3). From this data alone, we could not ultimately decide which pathway(s) both strains actually follow for sugar degradation. It should be noted that we were not able to identify a gene coding for phosphoglycerate mutase in *B. marina* DSM 3645^T, however, as all other genes belonging to the EMP pathway could be identified, we assume that this glycolytic route is functional in *B. marina*. Pan97^T and *B. marina* DSM 3645^T harbour a complete gene set required for a functional pentose phosphate pathway and TCA cycle (Table 2), which leads to the assumption that the central carbon metabolism of both strains is similar to most heterotrophic bacteria. For the gluconeogenesis, we could only identify a partial set of genes in both strains, with only two genes, *ppdK* (encoding pyruvate phosphate dikinase) and *pckA* (encoding phosphoenolpyruvate carboxykinase), present in both strains. It thus remains to be elucidated if the two strains are capable of de novo sugar biosynthesis. Additionally, both strains lack the glyoxylate shunt, which is typically required as the anaplerotic pathway during growth either with acetate or with compounds that are degraded to acetate or acetyl-CoA (e.g. fatty acids). Absence of the glyoxylate shunt suggests that both strains are either unable to use such compounds as sole carbon and energy source or that they follow other pathways with a related function.

Table 2 Phenotypic and genotypic features of strain Pan97^T compared to the two closely related strains '*Bremerella cremea*' LHWP2^T and *Blastopirellula marina* DSM 3645^T

Features	Pan97^T	*B. cremea* LHWP2^T [a]	*B. marina* DSM 3645^T [b]
Phenotypic features			
Size (µm)	1.5 × 0.8	0.6–1.5 × 0.6–1.4	1.0–2.0 × 0.7–1.5
Shape	Pear-shaped	Ovoid-shaped	Ovoid, ellipsoidal or pear-shaped
Aggregates	Yes	Yes	Yes
Flagella	n.o.	Yes	n.o.
Crateriform structures	Overall	Yes	Yes
Fimbriae	Polar matrix or fiber	n.o.	Reproductive pole
Capsule	n.o.	n.o.	n.o.
Bud shape	Pear-shaped	Bean-shaped	Bean-shaped
Budding pole	Polar	Polar	Polar
Stalk	n.o.	n.o.	n.o.
Holdfast structure	n.o.	n.o.	n.o.
Temperature range	15–40 °C	20–36 °C	< 38 °C
Temperature optimum	36 °C	30 °C	27–33 °C
pH range	5.5–9.5	6.0–8.0	n.a.
pH optimum	7.0	7.0	n.a.
Genotypic features			
Genome size [bp]	6,496,182	n.a.	6,663,851
Plasmids [bp]	no	n.a.	n.a.
G+C [%]	56.2 ± 0	n.a.	57.4
Completeness [%]	98.28	n.a.	96.55
Contamination [%]	3.45	n.a.	1.72
Protein-coding genes	5264	n.a.	5406
Hypothetical proteins	2141	n.a.	3023
Protein-coding genes/Mb	820	n.a.	811
Coding density [%]	88.5	n.a.	86.8
16S rRNA genes	2	n.a.	1
tRNA genes	81	n.a.	56

n.o. not observed, *n.a.* not available

[a]Lee et al. (2013), [b]Schlesner et al. (2004)

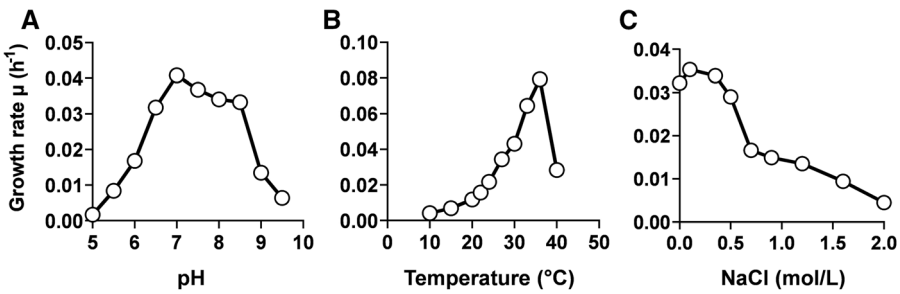

Fig. 5 Temperature and pH optimum and NaCl tolerance of strain Pan97^T. The figure shows the growth rates calculated from cultivations performed at different temperatures (**a**), different pH (**b**) and different NaCl concentrations (**c**). The growth rates were obtained from the slope of the plot of ln(OD_{600}) against the cultivation time for each tested condition

Table 3 Genome-based analysis of the central carbon metabolism of Pan97^T in comparison to *Blastopirellula marina* DSM 3645^T

Enzyme	EC number	Gene	Pan97^T	*B. marina* DSM 3645^T
Glycolysis				
Glucose-6-phosphate isomerase	5.3.1.9	*pgi*	Pan97_33820	GCF_000153105_52400
ATP-dependent 6-phosphofructokinase isozyme 1	2.7.1.11	*pfkA*	Pan97_18800	GCF_000153105_47750
Fructose-bisphosphate aldolase class 2	4.1.2.13	*fbaA*	Pan97_49530	GCF_000153105_01670
Triosephosphate isomerase	5.3.1.1	*tpiA*	Pan97_35530	GCF_000153105_41410
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*gapA*	Pan97_03050	GCF_000153105_02490
Phosphoglycerate kinase	2.7.2.3	*pgk*	Pan97_47370	GCF_000153105_10610
2,3-bisphosphoglycerate-independent phosphoglycerate mutase	5.4.2.12	*gpmI*	n	n
2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	5.4.2.11	*gpmA*	Pan97_15800	n
Enolase	4.2.1.11	*eno*	Pan97_19920	GCF_000153105_25320
Pyruvate kinase I	2.7.1.40	*pykF*	Pan97_15110	GCF_000153105_18700
Pyruvate dehydrogenase E1 component	1.2.4.1	*aceE*	Pan97_30420	GCF_000153105_45170
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	2.3.1.12	*aceF*	Pan97_30410	GCF_000153105_45160
Gluconeogenesis				
Phosphoenolpyruvate carboxylase	4.1.1.31	*ppc*	Pan97_04440	n
Phosphoenolpyruvate synthase	2.7.9.2	*ppsA*	Pan97_15930	n
Pyruvate, phosphate dikinase	2.7.9.1	*ppdK*	Pan97_43660	GCF_000153105_45620
Phosphoenolpyruvate carboxykinase (ATP)	4.1.1.49	*pckA*	Pan97_31190	GCF_000153105_41130
Fructose-1,6-bisphosphatase class 2	3.1.3.11	*glpX*	n	n
Fructose-1,6-bisphosphatase class 1	3.1.3.11	*fbp1*	n	GCF_000153105_40330
Pyrophosphate–fructose 6-phosphate 1-phosphotransferase	2.7.1.90	*pfp*	n	n
Pentose phosphate pathway				
Glucose-6-phosphate 1-dehydrogenase	1.1.1.49	*zwf*	Pan97_21990	GCF_000153105_27210
6-phosphogluconolactonase	3.1.1.31	*pgl*	Pan97_52900	GCF_000153105_20950
6-phosphogluconate dehydrogenase, decarboxylating	1.1.1.44	*gndA*	Pan97_21970	GCF_000153105_27230
Transketolase 2	2.2.1.1	*tktB*	Pan97_44840	GCF_000153105_04910
Transaldolase B	2.2.1.2	*tal*	Pan97_52010	GCF_000153105_51220
Entner–Doudoroff pathway				
KHG/KDPG aldolase	4.1.3.16	*kdgA*	Pan97_13340	GCF_000153105_22230
Phosphogluconate dehydratase	4.2.1.12	*edd*	Pan97_15010	GCF_000153105_14920
Tricarcoxylic acid cycle				
Citrate synthase	2.3.3.16	*gltA*	Pan97_42980	GCF_000153105_47880
Aconitate hydratase A	4.2.1.3	*acnA*	Pan97_24010	GCF_000153105_26150
Isocitrate dehydrogenase [NADP]	1.1.1.42	*icd*	Pan97_08720	GCF_000153105_14510
2-oxoglutarate dehydrogenase E1 component	1.2.4.2	*sucA*	Pan97_27730	GCF_000153105_37900
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	2.3.1.61	*sucB*	Pan97_27720	GCF_000153105_37910
Succinate–CoA ligase [ADP-forming] subunit alpha	6.2.1.5	*sucD*	Pan97_51530	GCF_000153105_05740
Succinate–CoA ligase [ADP-forming] subunit beta	6.2.1.5	*sucC*	Pan97_51540	GCF_000153105_05730
Succinate dehydrogenase flavoprotein subunit	1.3.5.1	*sdhA*	Pan97_46000	GCF_000153105_00080
Succinate dehydrogenase iron-sulfur subunit	1.3.5.1	*sdhB*	Pan97_45990	GCF_000153105_00070
Succinate dehydrogenase cytochrome b556 subunit	1.3.5.1	*sdhC*	Pan97_46010	GCF_000153105_00090

Table 3 continued

Enzyme	EC number	Gene	Pan97^T	*B. marina* DSM 3645^T
Fumarate hydratase class II	4.2.1.2	*fumC*	Pan97_45900	GCF_000153105_50000
Malate dehydrogenase	1.1.1.37	*mdh*	Pan97_14160	GCF_000153105_14490
Glyoxylate shunt				
Isocitrate lyase	4.1.3.1	*aceA*	n	n
Malate synthase G	2.3.3.9	*glcB*	n	n

Genes involved in biosynthesis of flagella

As we were not able to confirm presence of flagella in Pan97^T during microscopic analyses (Fig. 4), we searched for genes coding for enzymes involved in the biosynthesis of flagella in the genomes of Pan97^T and *B. marina* DSM 3645^T. Indeed, the same set of genes required for assembly of flagella is present in both species (Table 4), suggesting no major differences in mechanism of motility of the two related species. The analysis also showed that proteins with regulatory activity, e.g. transcriptional regulators (FlhC, FlhD, FliT) and (anti-)sigma factors (FlgM, FliA) are probably absent in both strains (Table 4). This indicates that regulation of flagella biosynthesis might involve other regulatory proteins distinct from those of model organisms in which flagella biosynthesis was investigated before.

Putative gene clusters involved in secondary metabolite production

Species of the family *Planctomycetaceae* typically have complex lifestyles and are often found in higher abundance in biofilms on nutrient-rich biotic surfaces in marine environments. Such a lifestyle is believed to require an additional level of regulation/interaction by production of secondary metabolites, which may allow its producer to cope with abiotic (e.g. UV light) or biotic stresses (competing bacteria, etc.). To obtain a first insight into the secondary metabolism of strain Pan97^T we performed an AntiSMASH analysis based on the genome sequence (Blin et al. 2019). Five clusters coding for enzymes known to produce secondary metabolites were identified. One cluster is associated with terpene production, two with polyketide biosynthesis (one putative type I polyketide synthase and one putative mixed type I polyketide synthase-non-ribosomal peptide synthetase). In addition, one putative cluster each was found to code for putative bacteriocin- and resorcinol biosynthetic proteins. The number of biosynthetic clusters is in the middle range, as 3–13 putative biosynthetic clusters were found for the planctomycetal genomes analysed so far (Wiegand et al. 2019), thereby suggesting some potential of Pan97^T as a source of novel bioactive compounds.

Description of *Bremerella* gen. nov.

Bremerella (Bre.me.rel'la. N.L. dim. fem. n. *Bremerella*; in honour of the German microbiologist Erhard Bremer).

Species belonging to this genus have pear- to ovoid shaped cells, form aggregates and divide by polar budding. The type species is *Bremerella cremea* comb. nov. (basonym: *Blastopirellula cremea*).

Description of *Bremerella cremea* comb. nov.

Bremerella cremea (cre.me'a. N.L. fem. adj. *cremea* cream–white).

Basonym: *Blastopirellula cremea* Lee et al. 2013.

Characteristics are as described previously (Lee et al. 2013). The type strain is LHWP2^T (KACC 15559^T = JCM 17758^T).

Description of *Bremerella volcania* sp. nov.

Bremerella volcania (vol.ca'ni.a. L. fem. adj. *volcania* corresponding to the volcano area in which the type strain was found).

Cells are pear-shaped (1.5 ± 0.3 µm × 0.8 ± 0.2 µm) and form white- to cream-coloured colonies on solid medium. Cells are arranged in rosettes and usually form aggregates. Division is performed by

Table 4 Genome-based analysis of genes required for assembly of flagella

Gene	Protein annotation	Pan97^T	*B. marina* DSM 3645^T
flgA	Flagellar basal body P-ring formation protein	Pan97_46170	GCF_000153105_48570
flgB	Flagellar basal-body rod protein	Pan97_14220	GCF_000153105_14600
flgC	Flagellar basal-body rod protein	Pan97_14230	GCF_000153105_14610
flgD	Flagellar biosynthesis, initiation of hook assembly	Pan97_14320	GCF_000153105_14700
flgE	Flagellar hook protein	Pan97_14330	GCF_000153105_14710
flgF	Flagellar basal-body rod protein	Pan97_46150	GCF_000153105_48550
flgG	Flagellar basal-body rod protein	Pan97_46160	GCF_000153105_48560
flgH	Flagellar L-ring protein	Pan97_46180	GCF_000153105_48580
flgI	Flagellar P-ring protein	Pan97_46190	GCF_000153105_48590
flgK	Flagellar hook-filament junction protein 1	Pan97_46220	GCF_000153105_48620
flgL	Flagellar hook-filament junction protein 2	Pan97_46230	GCF_000153105_48630
flgM	Anti-sigma factor for FliA (sigma(28))	n	n
flgN	Flagellar biosynthesis protein	Pan97_46210	GCF_000153105_48610
flhA	Flagellar biosynthesis protein	Pan97_14460	GCF_000153105_11490
flhB	Flagellar biosynthesis protein	Pan97_14430	GCF_000153105_14810
flhC	Flagellar transcriptional activator	n	n
flhD	Flagellar transcriptional activator	n	n
fliA	RNA polymerase sigma 28 (sigma F) factor	n	n
fliC	Flagellar filament structural protein (flagellin)	Pan97_46260	GCF_000153105_48660
fliD	Flagellar filament capping protein	Pan97_46270	GCF_000153105_48670
fliE	Flagellar basal-body protein	Pan97_14240	GCF_000153105_14620
fliH	Flagellar biosynthesis protein	Pan97_14270	GCF_000153105_14650
fliI	Flagellum-specific ATP synthase	Pan97_14280	GCF_000153105_14660
fliJ	Flagellar biosynthesis protein	Pan97_14290	GCF_000153105_14670
fliK	Flagellar hook-length control protein	Pan97_14310	GCF_000153105_14690
fliO	Flagellar biosynthesis protein	Pan97_14390	GCF_000153105_14770
fliP	Flagellar biosynthesis protein	Pan97_14400	GCF_000153105_14780
fliQ	Flagellar biosynthesis protein	Pan97_14410	GCF_000153105_14790
fliR	Flagellar biosynthesis protein	Pan97_14420	GCF_000153105_14800
fliS	Flagellar biosynthesis protein	Pan97_46280	GCF_000153105_48680
fliT	Flagellar biosynthesis protein	n	n
motA	Motility protein A	Pan97_14350	GCF_000153105_14730
motB	Motility protein B	Pan97_14360	GCF_000153105_14740

polar budding with the daughter cell having the same shape as the mother cell. Cells of the type strain grow at a temperature range of 15–40 °C and a pH range of 5.5–9.5. Genome information of the type strain is available from GenBank under accession number CP036289 and the 16S rRNA gene sequence is available from GenBank accession number MK554518. The type strain is Pan97^T (DSM 101992^T = LMG 29460^T) isolated from a red biofilm obtained from a hydrothermal vent close to the island Panarea in Italy in September 2013.

Acknowledgements Part of this research was funded by the Deutsche Forschungsgemeinschaft Grants KA 4967/1-1 and JO 893/4-1, Grant ALWOP.308 of the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO), SIAM (Soehngen Institute for Anaerobic Microbiology) Grant No. 024002002 and the Radboud Excellence fellowship. We thank Ina Schleicher for skillful technical assistance. Brian Tindall and Regine Fähnrich from the DSMZ as well as the BCCM/LMG

Bacteria collection we thank for support during strain deposition. We thank the Scientific Diving Center of the Bergakademie Freiberg, Germany, Thomas Pohl, Peter Hornburger and all participants of the 2013 Panarea Expedition for sampling support.

Author contributions SR wrote the manuscript, analysed the data and prepared the figures, SW and MJ performed the genomic and phylogenetic analysis, AH and PR isolated the strains and performed the cultivation and strain deposition, SHP and CB performed the light microscopic analysis, MSMJ and NK contributed to text preparation and revised the manuscript, MR performed the electron microscopic analysis, CJ took the samples and supervised the study. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical statement This article does not contain any studies with animals performed by any of the authors.

References

Bengtsson MM, Ovreas L (2010) Planctomycetes dominate biofilms on surfaces of the kelp *Laminaria hyperborea*. BMC Microbiol 10:261

Bengtsson MM, Sjotun K, Lanzen A, Ovreas L (2012) Bacterial diversity in relation to secondary production and succession on surfaces of the kelp *Laminaria hyperborea*. ISME J 6:2188–2198

Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T (2019) AntiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res 47:W81–W87

Boedeker C, Schuler M, Reintjes G, Jeske O, van Teeseling MC, Jogler M, Rast P, Borchert D, Devos DP, Kucklick M, Schaffer M, Kolter R, van Niftrik L, Engelmann S, Amann R, Rohde M, Engelhardt H, Jogler C (2017) Determining the bacterial cell biology of planctomycetes. Nat Commun 8:14853

Boersma A, Kallscheuer N, Wiegand S, Rast R, Peeters S, Mesman R, Heuer A, Boedeker C, Jetten M, Rohde M, Jogler M, Jogler C (2019) *Alienimonas californiensis* gen. nov. sp. nov., a novel Planctomycete isolated from the kelp forest in Monterey Bay. Antonie Van Leeuwenhoek. https://doi.org/10.1007/s10482-019-01367-4

Bondoso J, Harder J, Lage OM (2013) *rpoB* gene as a novel molecular marker to infer phylogeny in Planctomycetales. Antonie Van Leeuwenhoek 104:477–488

Bondoso J, Balague V, Gasol JM, Lage OM (2014) Community composition of the Planctomycetes associated with different macroalgae. FEMS Microbiol Ecol 88:445–456

Bondoso J, Albuquerque L, Nobre MF, Lobo-da-Cunha A, da Costa MS, Lage OM (2015) *Roseimaritima ulvae* gen. nov., sp. nov. and *Rubripirellula obstinata* gen. nov., sp. nov. two novel planctomycetes isolated from the epiphytic community of macroalgae. Syst Appl Microbiol 38:8–15

Bondoso J, Godoy-Vitorino F, Balague V, Gasol JM, Harder J, Lage OM (2017) Epiphytic Planctomycetes communities associated with three main groups of macroalgae. FEMS Microbiol Ecol 93:fiw255

Cayrou C, Raoult D, Drancourt M (2010) Broad-spectrum antibiotic resistance of Planctomycetes organisms determined by Etest. J Antimicrob Chemother 65:2119–2122

Challis GL, Hopwood DA (2003) Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces species*. Proc Natl Acad Sci USA 100:14555–14561

Devos DP (2014) Re-interpretation of the evidence for the PVC cell plan supports a Gram-negative origin. Antonie Van Leeuwenhoek 105:271–274

Devos DP, Reynaud EG (2010) Evolution. Intermediate steps. Science 330:1187–1188

Devos DP, Jogler C, Fuerst JA (2013) The 1st EMBO workshop on PVC bacteria—Planctomycetes–Verrucomicrobia–Chlamydiae superphylum: exceptions to the bacterial definition? Antonie Van Leeuwenhoek 104:443–449

Frank O, Michael V, Pauker O, Boedeker C, Jogler C, Rohde M, Petersen J (2015) Plasmid curing and the loss of grip–the 65-kb replicon of *Phaeobacter inhibens* DSM 17395 is required for biofilm formation, motility and the colonization of marine algae. Syst Appl Microbiol 38:120–127

Fuerst JA, Sagulenko E (2011) Beyond the bacterium: planctomycetes challenge our concepts of microbial structure and function. Nat Rev Microbiol 9:403–413

Fuerst JA, Webb RI (1991) Membrane-bounded nucleoid in the eubacterium *Gemmata obscuriglobus*. Proc Natl Acad Sci USA 88:8184–8188

Gimesi N (1924) Hydrobiologiai Tanulmanyok (Hydrobiologische Studien). I: *Planctomyces bekefii* gim. nov. gen. et sp. Kiadja a Magyar Ciszterci Rend Budapest, pp 1–8

Godinho O, Calisto R, Ovreas L, Quinteira S, Lage OM (2019) Antibiotic susceptibility of marine Planctomycetes. Antonie Van Leeuwenhoek 112:1273–1280

Graça AP, Calisto R, Lage OM (2016) Planctomycetes as novel source of bioactive molecules. Front Microbiol 7:1241

Gugliandolo C, Lentini V, Bunk B, Overmann J, Italiano F, Maugeri TL (2015) Changes in prokaryotic community composition accompanying a pronounced temperature shift of a shallow marine thermal brine pool (Panarea Island, Italy). Extremophiles 19:547–559

Hirsch P (1972) Two identical genera of budding and stalked bacteria: Planctomyces Gimesi 1924 and Blastocaulis Henrici and Johnson 1935. Int J Syst Evol Microbiol 22:107–111

Jeske O, Jogler M, Petersen J, Sikorski J, Jogler C (2013) From genome mining to phenotypic microarrays: Planctomycetes as source for novel bioactive molecules. Antonie Van Leeuwenhoek 104:551–567

Jeske O, Schuler M, Schumann P, Schneider A, Boedeker C, Jogler M, Bollschweiler D, Rohde M, Mayer C, Engelhardt H, Spring S, Jogler C (2015) Planctomycetes do possess a peptidoglycan cell wall. Nat Commun 6:7116

Jeske O, Surup F, Ketteniss M, Rast P, Forster B, Jogler M, Wink J, Jogler C (2016) Developing techniques for the

utilization of planctomycetes as producers of bioactive molecules. Front Microbiol 7:1242

Jogler M, Jogler C (2013) Toward the development of genetic tools for Planctomycetes. In: Fuerst JA (ed) Planctomycetes: cell structure, origins and biology. Humana Press, Totowa, pp 141–164

Jogler C, Glockner FO, Kolter R (2011) Characterization of Planctomyces limnophilus and development of genetic tools for its manipulation establish it as a model species for the phylum Planctomycetes. Appl Environ Microbiol 77:5826–5829

Jogler C, Waldmann J, Huang X, Jogler M, Glöckner FO, Mascher T, Kolter R (2012) Identification of proteins likely to be involved in morphogenesis, cell division, and signal transduction in Planctomycetes by comparative genomics. J Bacteriol 194:6419–6430

Kallscheuer N, Jogler M, Wiegand S, Peeters S, Heuer A, Boedeker C, Jetten M, Rohde M, Jogler C (2019a) *Rubinisphaera italica* sp. nov. isolated from a hydrothermal area in the Tyrrhenian Sea close to the volcanic island Panarea. Antonie Van Leeuwenhoek. https://doi.org/10.1007/s10482-019-01329-w

Kallscheuer N, Jogler M, Wiegand S, Peeters S, Heuer A, Boedeker C, Jetten M, Rohde M, Jogler C (2019b) Three novel *Rubripirellula* species isolated from artificial plastic surfaces submerged in the German part of the Baltic Sea and the estuary of the river Warnow. Antonie Van Leeuwenhoek. https://doi.org/10.1007/s10482-019-01368-3

Kallscheuer N, Wiegand S, Jogler M, Boedeker C, Peeters S, Rast P, Heuer A, Jetten M, Rohde MCJ (2019c) *Rhodopirellula heiligendammensis* sp. nov., *Rhodopirellula pilleata* sp. nov., and *Rhodopirellula solitaria* sp. nov. isolated from natural or artificial marine surfaces in Northern Germany and California, USA. Antonie Van Leeuwenhoek. https://doi.org/10.1007/s10482-019-01366-5

Kallscheuer N, Wiegand S, Peeters SH, Jogler M, Boedeker C, Heuer A, Rast P, Jetten MSM, Rohde M, Jogler C (2019d) Description of three bacterial strains belonging to the new genus *Novipirellula* gen. nov., reclassificiation of *Rhodopirellula rosea* and *Rhodopirellula caenicola* and readjustment of the genus threshold of the phylogenetic marker *rpoB* for *Planctomycetaceae*. Antonie van Leeuwenhoek. https://doi.org/10.1007/s10482-019-01374-5

Kartal B, Kuenen JG, van Loosdrecht MC (2010) Sewage treatment with anammox. Science 328:702–703

Kim M, Oh HS, Park SC, Chun J (2014) Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol 64:346–351

Kohn T, Heuer A, Jogler M, Vollmers J, Boedeker C, Bunk B, Rast P, Borchert D, Glockner I, Freese HM, Klenk HP, Overmann J, Kaster AK, Rohde M, Wiegand S, Jogler C (2016) *Fuerstia marisgermanicae* gen. nov., sp. nov., an Unusual Member of the Phylum Planctomycetes from the German Wadden Sea. Front Microbiol 7:2079

Kohn T, Heuer A, Jogler M, Vollmers J, Boedeker C, Bunk B, Rast P, Borchert D, Glockner I, Freese HM, Klenk HP, Overmann J, Kaster AK, Rohde M, Wiegand S, Jogler C (2019a) Corrigendum: *Fuerstia marisgermanicae* gen. nov., sp. nov., an unusual member of the phylum Planctomycetes from the German Wadden Sea. Front Microbiol 10:1029

Kohn T, Wiegand S, Boedeker C, Rast P, Heuer A, Schüler M, Rohde C, Müller R-W, Brümmer F, Rohde M, Engelhardt H, Jogler M, Jogler C (2019b) *Planctopirus ephydatiae*, a novel planctomycetal species isolated from the freshwater sponge *Ephydatia fluviatilis*. Syst Appl Microbiol. https://doi.org/10.1016/j.syapm.2019.126022

König E, Schlesner H, Hirsch P (1984) Cell wall studies on budding bacteria of the *Planctomyces/Pasteuria* group and on a *Prosthecomicrobium* sp. Arch Microbiol 138:200–205

Lage OM, Bondoso J (2014) Planctomycetes and macroalgae, a striking association. Front Microbiol 5:267

Lee HW, Roh SW, Shin NR, Lee J, Whon TW, Jung MJ, Yun JH, Kim MS, Hyun DW, Kim D, Bae JW (2013) *Blastopirellula cremea* sp. nov., isolated from a dead ark clam. Int J Syst Evol Microbiol 63:2314–2319

Lee I, Ouk Kim Y, Park SC, Chun J (2016) OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66:1100–1103

Lindsay MR, Webb RI, Fuerst JA (1997) Pirellulosomes: a new type of membrane-bounded cell compartment in planctomycete bacteria of the genus *Pirellula*. Microbiol UK 143:739–748

Lonhienne TG, Sagulenko E, Webb RI, Lee KC, Franke J, Devos DP, Nouwens A, Carroll BJ, Fuerst JA (2010) Endocytosis-like protein uptake in the bacterium *Gemmata obscuriglobus*. Proc Natl Acad Sci USA 107:12883–12888

Lucchi F, Romagnoli C, Tranne CA (2017) Volcanic landforms and landscapes of the Aeolian Islands (Southern Tyrrhenian Sea, Sicily): implications for hazard evaluation. In: Soldati M, Marchetti M (eds) Landscapes and landforms of Italy. Springer, Cham, pp 443–453

Luo C, Rodriguez RL, Konstantinidis KT (2014) MyTaxa: an advanced taxonomic classifier for genomic and metagenomic sequences. Nucleic Acids Res 42:e73

Meier-Kolthoff JP, Göker M, Spröer C, Klenk HP (2013) When should a DDH experiment be mandatory in microbial taxonomy? Arch Microbiol 195:413–418

Overmann J, Abt B, Sikorski J (2017) Present and future of culturing bacteria. Annu Rev Microbiol 71:711–730

Peeters SH, van Niftrik L (2019) Trending topics and open questions in anaerobic ammonium oxidation. Curr Opin Chem Biol 49:45–52

Peeters S, Wiegand S, Kallscheuer N, Jogler M, Heuer A, Jetten M, Rast P, Boedeker C, Rohde M, Jogler C (2019) Three marine strains constitute the novel genus and species *Crateriforma conspicua* gen. nov. sp. nov. in the phylum Planctomycetes. Antonie van Leeuwenhoek. Accepted manuscript (ANTO-D-19-00328)

Pilhofer M, Rappl K, Eckl C, Bauer AP, Ludwig W, Schleifer KH, Petroni G (2008) Characterization and evolution of cell division and cell wall synthesis genes in the bacterial phyla Verrucomicrobia, Lentisphaerae, Chlamydiae, and Planctomycetes and phylogenetic comparison with rRNA genes. J Bacteriol 190:3192–3202

Pruesse E, Peplies J, Glöckner FO (2012) SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28:1823–1829

Qin Q-L, Xie B-B, Zhang X-Y, Chen X-L, Zhou B-C, Zhou J, Oren A, Zhang Y-Z (2014) A proposed genus boundary for the prokaryotes based on genomic insights. J Bacteriol 196:2210–2215

Raab G, Halpern D, Scarciglia F, Raimondi S, Norton K, Pettke T, Hermann J, de Castro Portes R, Aguilar Sanchez AM, Egli M (2017) Linking tephrochronology and soil characteristics in the Sila and Nebrodi mountains, Italy. CATENA 158:266–285

Rivas-Marin E, Canosa I, Santero E, Devos DP (2016) Development of genetic tools for the manipulation of the Planctomycetes. Front Microbiol 7:914

Rodriguez-R LM, Konstantinidis KT (2016) The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. PeerJ Preprints 4:e1900v1

Schlesner H, Rensmann C, Tindall BJ, Gade D, Rabus R, Pfeiffer S, Hirsch P (2004) Taxonomic heterogeneity within the Planctomycetales as derived by DNA-DNA hybridization, description of *Rhodopirellula baltica* gen. nov., sp. nov., transfer of *Pirellula marina* to the genus *Blastopirellula* gen. nov. as *Blastopirellula marina* comb. nov. and emended description of the genus *Pirellula*. Int J Syst Evol Microbiol 54:1567–1580

Sieland R, Steinbrückner D, Hamel M, Merkel B, Schipek M (2009) Geochemical investigations and gas quantification of submarine fluid discharges in the hydrothermal system of Panarea (Aeolian Islands, Italy). In: 1st International workshop research in shallow marine and fresh water systems, pp 87

Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7:539

Stackebrandt E, Ebers J (2006) Taxonomic parameter revisited: tarnished gold standards. Microbiol Today 33:152–155

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313

Strous M, Fuerst JA, Kramer EH, Logemann S, Muyzer G, van de Pas-Schoonen KT, Webb R, Kuenen JG, Jetten MS (1999) Missing lithotroph identified as new planctomycete. Nature 400:446–449

van Teeseling MC, Mesman RJ, Kuru E, Espaillat A, Cava F, Brun YV, VanNieuwenhze MS, Kartal B, van Niftrik L (2015) Anammox Planctomycetes have a peptidoglycan cell wall. Nat Commun 6:6878

Vollmers J, Frentrup M, Rast P, Jogler C, Kaster AK (2017) Untangling genomes of novel planctomycetal and verrucomicrobial species from Monterey Bay kelp forest metagenomes by refined binning. Front Microbiol 8:472

Wagner M, Horn M (2006) The Planctomycetes, Verrucomicrobia, Chlamydiae and sister phyla comprise a superphylum with biotechnological and medical relevance. Curr Opion Biotechnol 17:241–249

Webster NS, Negri AP (2006) Site-specific variation in Antarctic marine biofilms established on artificial surfaces. Environ Microbiol 8:1177–1190

Wiegand S, Jogler M, Jogler C (2018) On the maverick Planctomycetes. FEMS Microbiol Rev 42:739–760

Wiegand S, Jogler M, Boedeker C, Pinto D, Vollmers J, Rivas-Marín E, Kohn T, Peeters SH, Heuer A, Rast P, Oberbeckmann S, Bunk B, Jeske O, Meyerdierks A, Storesund JE, Kallscheuer N, Lücker S, Lage OM, Pohl T, Merkel BJ, Hornburger P, Müller R-W, Brümmer F, Labrenz M, Spormann AM, Op den Camp H, Overmann J, Amann R, Jetten MSM, Mascher T, Medema MH, Devos DP, Kaster A-K, Øvreås L, Rohde M, Galperin MY, Jogler C (2019) Cultivation and functional characterization of 79 Planctomycetes uncovers their unique biology. Nat Microbiol. https://doi.org/10.1038/s41564-019-0588-1

Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W, Schleifer KH, Whitman WB, Euzeby J, Amann R, Rossello-Mora R (2014) Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. Nat Rev Microbiol 12:635–645