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Emended description of the genus *Polynucleobacter* and the species *P. necessarius* and proposal of two subspecies, *P. necessarius* subspecies *necessarius* subsp. nov. and *P. necessarius* subsp. *asymbioticus* subsp. nov

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Heckmann and Schmidt described the genus *Polynucleobacter* for bacterial endosymbionts of freshwater ciliates within the genus *Euplotes*, and the species *Polynucleobacter necessarius* for obligate endosymbionts living in the cytoplasm of *E. aediculatus*. Pure cultures of the type strain could never be established due to the obligate nature of the symbiotic relationship between the endosymbionts and their hosts. Therefore, *P. necessarius* is one of a few validly described bacterial species lacking a deposited pure culture. Meanwhile, it was demonstrated that the endosymbiotic *P. necessarius* type strain is closely related to obligately free-living strains. Similarity values of the 16S rRNA sequence of the type strain and free-living isolates in the range of 99.1 to 99.4% indicate that these organisms belong to the same species. Here, we emend the description of *P. necessarius* by characterization of free-living strains. The species *P. necessarius* is characterized by low G + C contents of the DNA (44–46 mol%), small genome sizes (1.5–2.5 Mbp), and a lack of motility. Due to distinct differences in lifestyle and genome size of *P. necessarius* strains, we propose to establish two subspecies, *P. necessarius* subsp. *necessarius* subsp. nov. [with a nameless type strain contained in a deposited *E. aediculatus* culture (ATCC 30859)], and *P. necessarius* subsp. *asymbioticus* subsp. nov. [with the type strain QWL-P1DMWA-1^T (=DSM 18221^T = YYYYYY^T)], for the obligate endosymbionts of *E. aediculatus* and *E. harpa* and obligately free-living strains, respectively.

The genus *Polynucleobacter* and the species *P. necessarius* were proposed by K. Heckmann and H. J. Schmidt in 1987 for bacterial endosymbionts of freshwater ciliates affiliated with the genus *Euplotes*. The presence of bacterial endosymbionts in *Euplotes* spp. was reported for the first time by Fauré-Fremiet in 1952. He already described the presence of several nucleoid-like structures in cells of endosymbionts, which could be observed after sufficient staining by light microscopy. This trait, later characterized in detail by ultrastructural investigations (Heckmann, 1975), is described by the genus name. Furthermore, Fauré-Fremiet already suggested that the endosymbionts are essential for the host cells (Fauré-

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The GenBank/EMBL/DDJB accession numbers for the 16S rRNA gene sequences of strains QWL-P1DMWA-1^T, MWH-JaK3, MWH-MoK4, and MWH-HuW1 are AJ879783, AJ550657, AJ550654, and AJ550666, respectively.

Fremiet, 1952). The bidirectional obligate nature of the symbiotic relationship between the host cells and the intracellular bacterial symbionts was later demonstrated by sophisticated experiments (Heckmann, 1975; Fujishima & Heckmann, 1984; Heckmann *et al.*, 1983). The obligately symbiotic lifestyle of the endosymbionts, indicated by the epithet of the species *P. necessarius*, seems to be responsible for the failure of all experiments aiming on cultivation of these strains (Heckmann, 1975; Heckmann & Schmidt, 1987; Vannini *et al.*, 2007). These failures resulted in an incomplete phenotypic characterization of the taxon, as well as in the lack of deposition of a pure culture of the *P. necessarius* type strain proposed by Heckmann and Schmidt. Instead, the type strain is included in a culture of its host *E. aediculatus* deposited at the American Type Culture Collection. In 1996, the 16S rRNA gene of the *P. necessarius* type strain was sequenced (Springer *et al.*, 1996), which revealed that the endosymbionts are related to strains currently classified as *Ralstonia* and *Cupriavidus* species. *Polynucleobacter*-like endosymbionts characterized by multiple nucleoid-like structures were reported for eight freshwater *Euplotes* spp. (Foissner, 1978; Heckmann & Schmidt, 1987), and the brackish-water species *E. harpa* (Vannini *et al.*, 2005), however, verification of the affiliation with the genus *Polynucleobacter* by comparative analysis of 16S rRNA genes was only performed for the endosymbionts of three *E. harpa* strains and a second *E. aediculatus* strain (Vannini *et al.*, 2005; Vannini *et al.*, 2007).

From 1996 on, many investigations employing cultivation-independent techniques for revealing the diversity of freshwater bacterioplankton retrieved 16S rRNA sequences closely related to the sequence of the endosymbiotic *P. necessarius* type strain (e.g., Bahr *et al.*, 1996; Hiorns *et al.*, 1997; Crump *et al.*, 1999). Thus, *P. necessarius*-like bacteria were detected in the water column of lakes and rivers, which is an environment usually not inhabited by the benthos-dwelling *Euplotes* spp. hosting *Polynucleobacter* endosymbionts, therefore, it was uncertain if these environmental sequences represent endosymbionts of ciliates. Recently, the first strains matching environmental sequences have been brought into pure cultures in the laboratory (Hahn, 2003). Phylogenetic analyses with 16S rRNA gene sequences of the endosymbiont, the cultivated strains, and environmental sequences revealed one monophyletic cluster (operationally designated as ‘Polynucleobacter cluster’) subdivided in four narrow subclusters (designated as PnecA, PnecB, PnecC, and PnecD, respectively), each characterized by internal minimal 16S rRNA similarity values >98.2% (Hahn, 2003; Wu & Hahn, 2006a). All available sequences of endosymbionts but also several sequences of free-living strains clustered within subcluster PnecC (Hahn, 2003; Vannini *et al.* 2005), which is characterized by a minimal 16S rRNA similarity value of 98.5%, and thus resembles a species-like taxon (Stackebrandt & Ebers, 2006). Development and application of a FISH probe specific for the entire subcluster PnecC demonstrated the presence of free-living bacteria affiliated with this subcluster in the water column of freshwater systems (Hahn *et al.*, 2005; Wu & Hahn, 2006a), thus, this species-like subcluster contains both free-living and endosymbiotic strains. Recently, it was demonstrated that the endosymbiotic *P. necessarius* and the cultivated strains possess the closest phylogenetic relationship ever revealed between obligate endosymbionts and obligately free-living bacteria (Vannini *et al.*, 2007).

Free-living strains affiliated with the ‘Polynucleobacter cluster’ possess a wide distribution in freshwater habitats. Environmental sequences and cultivated strains were obtained from an ecologically broad variety of habitats located in all climatic zones (Hahn, 2003) and characterized by various chemical conditions. Bacteria affiliated with subcluster PnecC were even detected in lakes located at altitudes of 5000 meters above sea level (Wu *et al.*, 2006). Furthermore, investigations employing FISH probes specific for the phylogenetically characterized subclusters revealed relative abundances in the range of <1% to 60% of total bacterial numbers in the water column of various freshwater habitats (Hahn *et al.*, 2005; Wu

& Hahn, 2006a; Wu & Hahn, 2006b), thus, this group of bacteria comprises an important part of the freshwater bacterioplankton.

Here, we present an emended description of the genus *Polynucleobacter* and its sole species *P. necessarius*, which considers both endosymbiotic and free-living strains. This description is based on the phenotypic and chemotaxonomic characterization of four free-living strains affiliated with subcluster PnecC.

The investigated strains were isolated from freshwater habitats (Supplemental Material Table S1) by using the filtration-acclimatization method (Hahn *et al.*, 2004) or the dilution-acclimatization method as reported previously (Hahn, 2003; Hahn *et al.*, 2005). Strains closely related to the strains presented here, could also be isolated from NSY agar plates directly inoculated with freshwater samples (Hahn, unpublished data). Strains were routinely grown on NSY medium (Hahn *et al.*, 2004) with strength of 3 g L⁻¹ (Supplemental Material Fig. S1). All strains also grew on R2A, peptone and Luria-Bertani agar (Hahn, 2003). Growth at different temperatures (5–38 °C), and growth under anoxic conditions in an anaerobic chamber were examined on NSY agar. NaCl tolerance was determined using NSY agar supplemented with different NaCl concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 1.0, 1.25, 1.5, 1.75, and 2.0%, w/v). The temperature range supporting growth was tested on NSY agar plates exposed to different temperatures (5, 10, 15, 20, 30, 33, 34, 35, and 36 °C). Three of the four investigated strains could grow on a mineral medium (Hahn *et al.*, 2004) supplemented with vitamin B12 and acetate as sole carbon and energy source, however, in some experiments the inoculated cultures did not grow on this minimal medium but repetition of the experiment clearly resulted in growth. By contrast, experiments with three *Cupriavidus* spp. and the *Burkholderia gladioli* type strain always resulted in strong and reliable growth. Due to the unreliability of growth and due to the establishment of only relatively low cell concentrations, tests on assimilation of various organic compounds were neither performed with media containing only a sole substrate source, nor by utilization of commercially available using test kits. Instead, experiments were performed by comparison of OD established in liquid one-tenth-strength NSY medium (0.3 g L⁻¹) with and without 0.5 g L⁻¹ test substance (pH 7.2). OD differences of <10%, of 10–50%, and of >50% of the OD established on the medium without test substance were scored as no assimilation, weak assimilation and assimilation, respectively. Sequencing and phylogenetic analyses of 16S rRNA genes was mainly preformed as described previously (Hahn, 2003; Hahn *et al.*, 2005). Neighbour-Joining trees were calculated by using the software MEGA4 (Tamura *et al.*, 2007) and Maximum Likelihood trees were constructed by using the software RAxML version 7.0.4 (Stamatakis *et al.*, 2008). Both, the G+C content of DNA, and the composition of cellular fatty acids were determined as described by Tóth *et al.* (2008). Genome sizes were examined by pulsed field gel electrophoresis (PFGE) as described previously (Vannini *et al.*, 2007), and for two strains genome sizes were determined in a genome-sequencing project.

The phenotypic characterization of the investigated free-living strains was complicated by their relatively weak growth on complex media (Supplemental Material Fig. S2), and by their weak and unreliable growth performance on media with only a single carbon source. The results of the phenotypic and chemotaxonomic characterization of the investigated free-living strains is presented in Tables 1 and 2.

The phylogenetic analyses of 16S rRNA genes of free-living strains and endosymbiotic *Polynucleobacter* strains revealed the affiliation with the family *Burkholderiaceae* (Fig. 1). The cluster formed by the *Polynucleobacter* taxa is closest related to the *Ralstonia* spp./*Cupriavidus* spp. lineage (94% maximal sequence similarity). The minimal 16S rRNA sequence similarity of the four free-living strains and the originally proposed endosymbiotic

type strain of *Polynucleobacter necessarius* is 99.1% (1500bp sequence stretches analysed), and the minimal sequence similarity among the four free-living strains is 99.6%. A very close phylogenetic relationship between the obligate endosymbionts of *E. aediculatus* and *E. harpa*, and the four obligately free-living strains was also demonstrated by the analysis of 16S-23S ITS sequences (Vannini *et al.*, 2007). Furthermore, the endosymbionts and the free-living strains share low and almost identical G+C contents of their DNA, small genome sizes, and the lack of motility. All these characteristics are absent or exceptional among other *Burkholderiaceae* (Table 3). Almost all observed differences between free-living and endosymbiotic strains (Table 4) are likely a result of the evolutionary adaptation of endosymbionts to an obligate dependence on their host cells (Heckmann, 1975; Vannini *et al.*, 2007). A lack of dependence on self-synthesis of essential compounds due to delivery of such compounds by the host could have made possible the deletion of involved genes, which could have resulted in a decrease of genome size. This could have also resulted in more complex nutritional demands of the obligate endosymbionts causing the lack of cultivability. An increase in size is usually a disadvantage for free-living planktonic bacteria with small cell sizes, because of a resulting increase of predation by bacterivorous protists (Hahn & Höfle, 2001), however, the obligate endosymbionts are completely protected by their hosts against direct predation.

Based on the close phylogenetic relationship between the free-living and the endosymbiotic strains, we propose to consider these organisms differing so strongly in lifestyle as members of the species *Polynucleobacter necessarius*. Unfortunately, this proposal cannot be verified by DNA-DNA reassociation experiments due to the lack of sufficient amounts of pure DNA extracted from endosymbiotic strains. Strains affiliated with *P. necessarius* can be clearly differentiated from other valid taxa within the family *Burkholderiaceae* by means of FISH with the *P. necessarius*-specific probe PnecC-16S-445 (Hahn *et al.*, 2005), by the G+C content of their DNA, and by the lack of motility (Table 3). Members of *Polynucleobacter necessarius* may be differentiated from most *Ralstonia* and *Cupriavidus* species by the lack of 2-hydroxylated fatty acids others than 2-OH-C_{12:0} (Table 2). The mentioned FISH probe also enables the differentiation of *P. necessarius* from so far undescribed taxa putatively affiliated with the genus *Polynucleobacter*.

Based on distinct differences in lifestyle and genome size, we propose to place obligately endosymbiotic, and obligately free-living *P. necessarius* separately in two new subspecies.

Emended description of the genus *Polynucleobacter* (Heckmann and Schmidt, 1987)

Polynucleobacter [Pol.y.nuc'leo.bac.ter. Gr. adj. *polys* numerous; L. masc. n. *nucleus* nut, kernel; masc. *bacter* the equivalent of Gr. neut. n. *bactrum* a rod; N.L. masc. *Polynucleobacter* rod with many nucleoids].

The monotypic genus *Polynucleobacter* belongs to the family *Burkholderiaceae*, and harbours endosymbiotic strains of several *Euplotes* species and free-living strains dwelling in the water column of freshwater lakes, ponds and streams. According to the description by Heckmann and Schmidt (1987), endosymbionts of the freshwater species *E. aediculatus*, *E. eurytomus*, *E. plumipes*, *E. daidaleos*, *E. octocarinatus*, *E. patella*, and *E. woodruffi*, which are penicillin-sensitive and possess multiple nuclei-like structures belong to this genus. Furthermore, endosymbionts of *Euplotes moebiusi* f. *quadricirratu*s (Foissner, 1978) possess the morphological characteristics described for *Polynucleobacter* spp. However, for most of these endosymbionts the examination of their phylogenetic affiliation with the genus *Polynucleobacter* is lacking. Vannini and colleagues (2005) demonstrated by phylogenetic

and ultrastructural investigations that endosymbionts of the brackish-water species *E. harpa* also belong to this genus. Type species is *P. necessarius*.

Emended description of *Polynucleobacter necessarius* (Heckmann and Schmidt, 1987)

Polynucleobacter necessarius (nec.es.sar'i.us. L. adj. *necessarius* indispensable, necessary). The species contains obligate endosymbionts living in ciliates of the species *E. aediculatus* and *E. harpa*, as well as obligately free-living strains inhabiting freshwater systems. The species possesses the following characteristics. The DNA G+C content is 44–46 mol%, and genome sizes range from 1.5 to 2.5 Mbp. All strains are non-motile. Members of the species can be distinguished from other taxa by the presence of the oligonucleotide sequence 5'-GAG CCG GTG TTT CTT CCC-3' (*E. coli* position 445–463) within the 16S rRNA gene. The presence of this sequence can be determined by fluorescent in situ hybridization of whole cells by using the fluorescently labelled oligonucleotide probe PnecC-16S-445 (Hahn *et al.*, 2005).

Description of subspecies *Polynucleobacter necessarius* subsp. *necessarius* subsp. nov

Polynucleobacter necessarius subspecies *necessarius* (nec.es.sar'i.us. L. adj. *necessarius* indispensable, necessary).

The subspecies possesses the characteristics previously described by Heckmann and Schmidt (1987) for the species *P. necessarius*. This subspecies contains *P. necessarius* strains obligately living as endosymbionts in cells of the ciliates *E. aediculatus* and *E. harpa*. This includes the *P. necessarius* type strain contained in the *E. aediculatus* E24 culture (= ATCC 30859, = clone 15 (Heckmann and Schmidt, 1987)) and the endosymbiotic strain STIR1 contained in the *E. aediculatus* STIR1 culture (Petroni *et al.*, 2002; Vannini *et al.*, 2007), as well as three strains detected in three cultures of the brackish water ciliate *E. harpa* (Vannini *et al.*, 2005, Vannini *et al.*, 2007). Strains usually have elongated cells with cell lengths > 2.5 µm. Genome size is ranging from 1.5 to 1.8 Mbp. All attempts to cultivate the obligate endosymbionts outside of their host cells failed so far, therefore the subspecies is lacking a type strain cultivated as pure culture.

Description of subspecies *Polynucleobacter necessarius* subsp. *asymbioticus* subsp. nov

Polynucleobacter necessarius subspecies *asymbioticus* (a.sym.bio'ti.cus. Gr. pref *a* not; M.L. *symbioticus*, -a, -um living together; M.L. masc. adj. *asymbioticus* not symbiotic).

This subspecies exclusively contains free-living *P. necessarius* strains. Strains have cell morphologies of straight or curved rods, 0.5–3.0 µm in length and 0.3–0.5 µm in width. Strains forming short curved rods grow in NSY medium with relatively even cell lengths, but strains with larger cells usually show uneven lengths distributions, and even a few elongated cells of up to 15 µm were observed in some of the cultures, however, the majority of cells possess cell lengths < 2 µm. Chemoorganotroph, aerobic, at least some of the strains facultatively anaerobic. Can be cultivated on NSY, R2A, Luria-Bertani, and peptone medium. Colonies grown on NSY agar are unpigmented, circular and convex with smooth surface. Growth occurs at 5–30 °C, some strains also grow at 34 and 35 °C. Growth occurs with 0–0.3 % (w/v) NaCl; some strains also grow at 0.4 and 0.5 % (w/v) NaCl. Growth does not occur at 0.6 % (w/v) NaCl or higher. Assimilates acetate, pyruvate, malate, succinate,

fumarate, D-galacturonic acid and L-cysteine. None of the strains assimilates glycolate, oxalate, L-serine, and citrate. Major cellular fatty acids are C_{16:1w7c}, C_{16:0}, C_{18:1 w7c} and summed feature 2 (including 3-OH-C_{12:0}). Sole 2-hydroxylated compound is 2-OH-C_{12:0}. Genome size is ranging from 2.1 to 2.5 Mbp.

The type strain is QLW-P1DMWA-1^T (= DSM 18221^T = yyy), isolated from a small acidic freshwater pond located in the Austrian Alps at an altitude of 1300 meters above sea level (Hahn *et al.*, 2005).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

FISH	Fluorescent in situ hybridization
PFGE	pulsed field gel electrophoresis
Mbp	mega base pairs

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Fig. 1. Neighbour-joining (NJ) tree based on almost complete 16S rRNA gene sequences, showing the phylogenetic position of the four investigated free-living strains within the family *Burkholderiaceae*. Sequences of *Thermotrix* spp. and *Lautropia mirabilis* could not be considered due to the lack of sequences of sufficient lengths. The *Bordetella* and *Comamonas* sequences were used as outgroup not affiliated with the family *Burkholderiaceae*. Bootstrap values obtained from the NJ tree (first value) and the Maximum Likelihood (ML) tree constructions (second value) are presented (only values above 60 % are shown). Trees constructed with the two algorithms mainly differed in the position of *Paucimonas lemoignei*, which formed in the ML tree a monophyletic group (bootstrap support 95%) together with *Polynucleobacter*, *Ralstonia*, and *Cupriavidus*. Furthermore, *B. pertussis* and *C. terrigena* did not form a monophyletic group in the ML tree. The kind of lifestyle, and where applicable, the host species is indicated for all *Polynucleobacter* taxa. Strain *P. necessarius* 'E24' is contained in the *E. aediculatus* culture E24 (ATCC 30859). Bar, 0.02 substitutions per nucleotide position.

Table 1

Traits characterizing the four investigated free-living *P. necessarius* strains. All strains have the following characteristics in common. DAPI stained cells show only rarely nucleoid-like structures; none-motile; catalase and oxidase positive; assimilation of acetic acid, pyruvic acid, succinic acid; weak assimilation of D-galacturonic acid; no assimilation of glycolic acid, L-serine, oxalic acid, and citric acid. –, Negative; +, positive; w, weakly positive; n.d., not determined.

	QLW-P1DMWA-1 ^T	MWH-JaK3	MWH-MoK4	MWH-HuW1
Cell morphology	short rods	short rods	curved rods	short curved rods
Cell length (μm)	0.7-1.2	0.5-1.5	0.9-2.9	0.6-1.4
Cell width (μm)	0.4-0.5	0.3-0.5	0.4-0.5	0.4-0.5
Temperature range of growth (°C)	5 - 34(w)	5 - 35	5 - 30	5 - 35
NaCl tolerance (%NaCl, w/v)	0 - 0.5(w)	0 - 0.3(w)	0 - 0.3	0 - 0.5
Anaerobic growth	+	+	–	–
Growth in mineral medium with acetic acid and B12	w	w	w	–
Assimilation of:				
Urea	+	+	–	–
Thiosulfate	+	+	–	–
Formic acid	w	–	–	–
Glyoxylic acid	w	–	–	–
Propionic acid	+	+	–	+
Malonic acid	w	w	–	+
Oxaloacetic acid	–	+	+	+
Malic acid	+	+	w	+
Fumaric acid	+	+	w	+
Levulinic acid	w	–	–	–
D-Mannose	w	–	–	–
D-Glucose	w	–	w	–
D-Galactose	w	–	–	–
D-Lyxose	w	w	w	–
D-Fructose	w	w	w	–
L-Fucose	w	w	–	–
D-Sorbitole	w	–	–	–
L-Glutamate	+	–	–	+
L-Aspartate	+	–	–	–
L-Cysteine	+	+	+	w
L-Alanine	w	–	–	–
L-Asparagine	w	–	–	–
Betaine	w	–	–	–
Genome size (Mbp)	2.15 ^{\$}	2.2 ^{\$\$}	n.d.	2.5 ^{\$\$}
DNA G+C content (mol%)	44.8 ^{\$}	45.5	n.d.	n.d

^{\$} Genome sequencing (unpublished)

^{\$\$} PFGE (Vannini et al., 2007)

Table 2

Major fatty acid composition of *Polynucleobacter necessarius* ssp. *asymbioticus*. Values are percentages of the summed fatty acids named in the Peak library file of the MIDI system (contents >0.2%). Strains grown on R2A plates for 3-4 days at 28 °C.

	QLW-P1DMWA-1 ^T DSM 18221 ^T	JaK3	HuK1
C _{12:0}	3.4	3.7	5.5
C _{14:0}	0.9	1.2	0.3
C _{15:0}	0.3	-	0.3
C _{16:0}	22.2	15.5	29.6
C _{17:0}	-	-	0.5
C _{18:0}	1.2	0.5	2.4
C _{20:0}	1.1	-	-
C _{14:1} w5c	-	0.6	0.2
C _{15:1} w6c	-	-	0.6
C _{16:1} w5c	0.9	0.4	-
C _{16:1} w7c	41.3	35.6	45.0
C _{18:1} w9c	-	-	0.4
C _{18:1} w7c	12.9	20.4	1.1
11Methyl-C _{18:1} w7c	3.1	8.1	1.1
C _{12:0} -2OH	2.5	2.2	1.3
Feat. 1 (including C _{12:0} ALDE?)	0.4	0.5	1.0
Feat. 2 including C _{14:0} -3OH	9.6	9.2	9.9
Feat. 7 including C _{19:1} w6c	0.4	2.0	0.3

Table 3
 Characteristics that differentiate *P. necessarius* (including both subspecies) from other *Burkholderiaceae*.

	<i>P. necessarius</i>	<i>Cupriavidus</i> spp.	<i>Ralstonia</i> spp.	<i>Burkholderia</i> spp.	<i>Lautropia mirabilis</i>	<i>Limnobacter</i> spp.	<i>Pandoraea</i> spp.	<i>Pa. lemoignei</i>	<i>Thermothrix</i> spp.
DNA G+C content (mol%)	44 - 46	63 - 67	63 - 67	62 - 68	65	55	61 - 65	59	40
Genome size (Mbp)	1.5 - 2.5	6.5 - 7.4	5.3 - 5.9	5.2 - 9.8	ND	3.4 ^{\$}	ND	ND	ND
Motility	-	+	+	+	+	+	+	+	+
Thermophilic	-	-	-	-	-	-	-	-	+
Obligately chemolithotrophic	-	-	-	-	-	-	-	-	+

ND, no data available; *Pa.*., *Paucimonas*

^{\$} *Limnobacter*-sp. MED105

Table 4

Characteristics that differentiate *P. necessarius* subsp. *necessarius* and *P. necessarius* subsp. *asymbioticus*.

	<i>P. necessarius</i> subsp. <i>necessarius</i>	<i>P. necessarius</i> subsp. <i>asymbioticus</i>
Lifestyle	obligate endosymbionts in <i>Euplotes</i> spp.	obligately free-living, planktonic
Genome size (Mbp)	< 1.8	> 2.1
Multiple nucleoids ^{\$}	always visible	rarely visible
Cell length ^{\$\$} (μm)	> 2.5	< 2.0
Culturability	no pure cultures	pure cultures

^{\$} Cells stained by DAPI; detection by epifluorescence microscopy

^{\$\$} Referring to the majority of cells present in pure cultures or in a ciliate host. Elongated cells > 2μm may appear in cultures of free-living strains, however, they commonly represent only a minority of the population. By contrast, in endosymbiotic populations, the majority of cells is always >3 μm.