

Supplementary Information

Ugly ducklings – The dark side of plastic materials in contact with potable water

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Table S1: Exemplary online blog entries on biofouling inside bath toys

Issue - What is the slime?	Link
Rub-a-dub-dub, what's in the tub?	https://www.babble.com/baby/whats-in-the-tub/
What's the black stuff in your squeeze toys?	http://blogs.babycenter.com/momstories/whats-the-black-stuff-in-your-squeeze-toys/
Friday Find: NBC's Today Show segment: Do bath toys carry germs?	http://www.betravekeepgoing.com/2010/03/friday-find-nbcs-today-show-segment-do.html
Yuck. Yuck. Yuck! (A.K.A. Why my kids no longer have rubber duckies!)	http://www.imperfecthomemaking.com/2012/11/yuck-yuck-yuck-aka-why-my-kids-no.html
Issue – How to prevent or remove it?	Link
How to clean bath toys & prevents mould	http://www.maids.com/blog/how-to-clean-and-prevent-mold-in-bath-toys/
Glue gun the rubber ducky	http://lajollamom.com/glue-gun-the-rubber-ducky/
How to remove mould from bath toys	http://www.howtocleanstuff.net/how-to-remove-mold-from-bath-toys/
Ask Martha: How can I clean my children's bath toys?	http://www.marthastewart.com/1125723/cleaning-childrens-bath-toys
What's the best way to clean bath toys?	http://www.realsimple.com/magazine-more/inside-magazine/ask-real-simple/best-way-clean-bath-toys



Figure S1: Images of all examined bath toys, both sound and cut open. A-L: bath toys from real households.

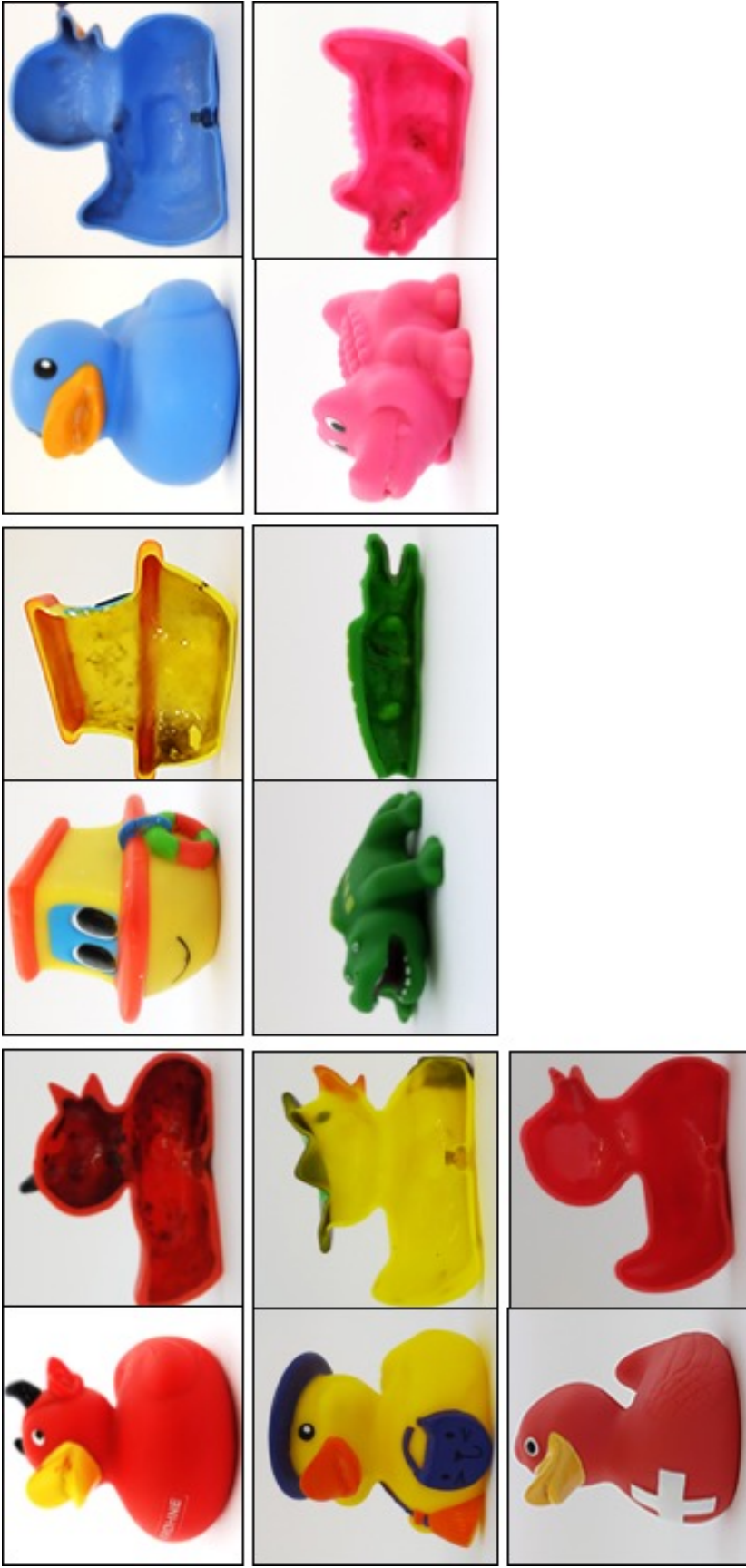


Figure S1: Images of all examined bath toys, both sound and cut open. M-S: bath toys from real households.

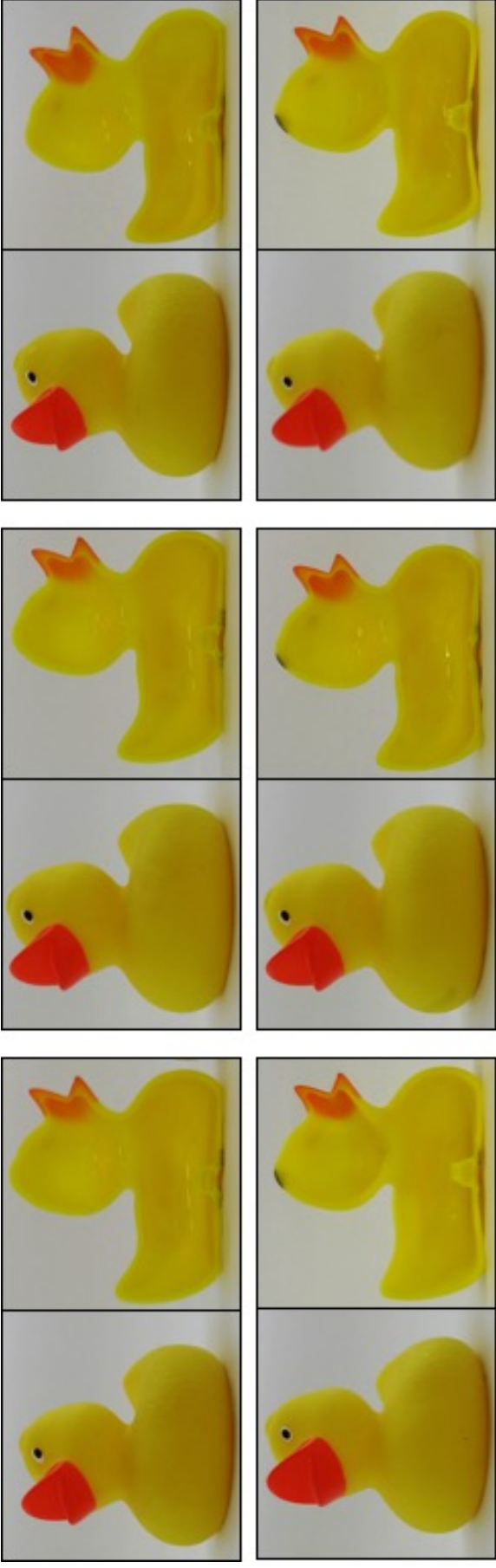


Figure S1: Images T-V: control bath toys processed with water prior to bathing (clean water controls). W-Y: control bath toys used with water after bathing (dirty water controls).

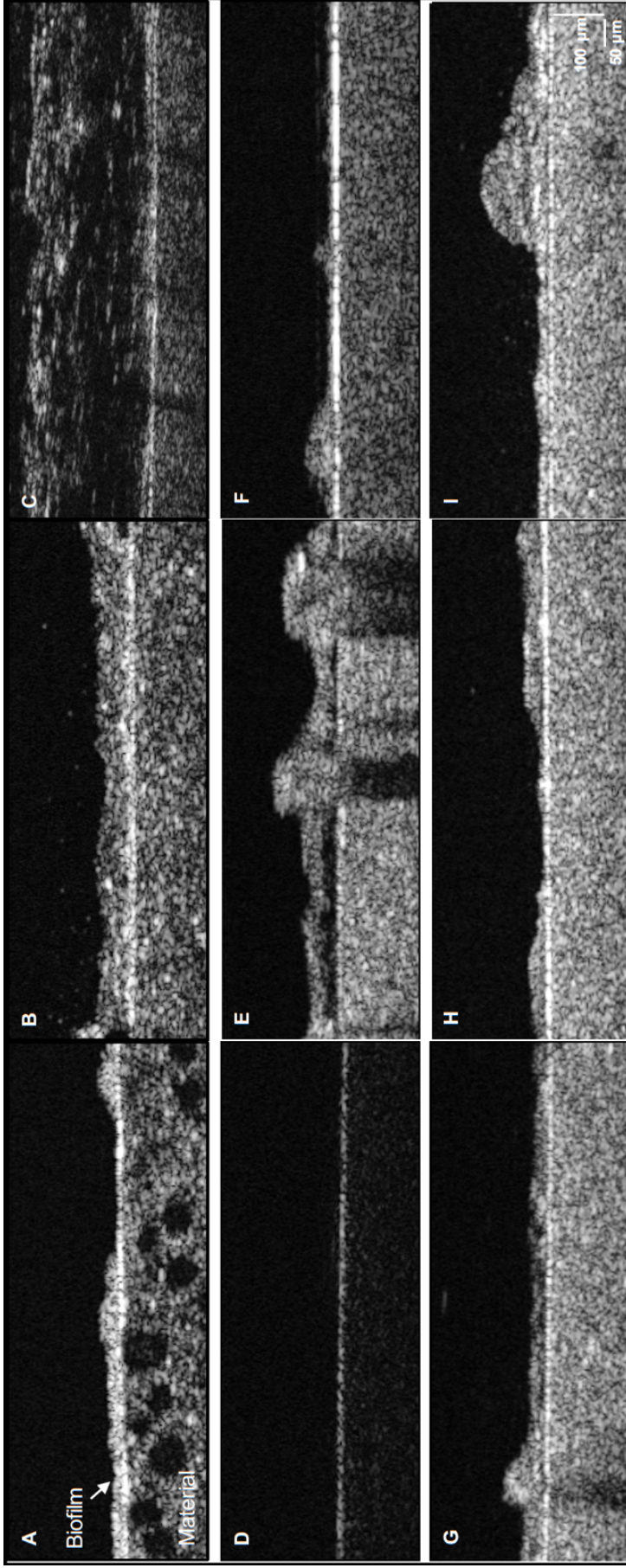


Figure S2: Optical coherence tomography (OCT) was used to image biofilm shape and thickness of selected bath toys. For real bath toys three exemplary biofilms are shown (A-C). Biofilms of clean water controls, which were used with water prior to bathing, are shown in D-F, while biofilms of dirty water controls, processed with water after bathing, are shown in images G-I.

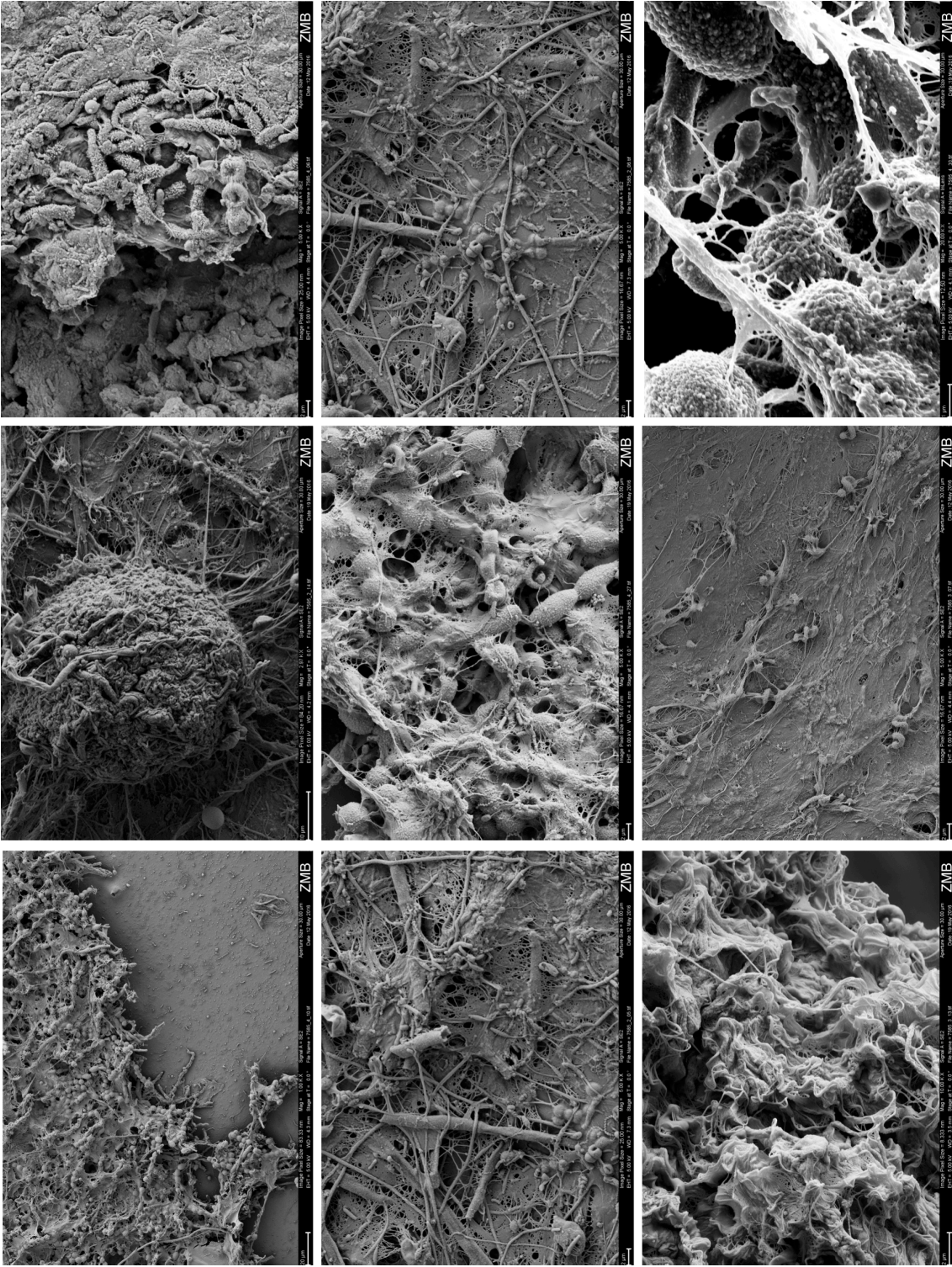


Figure S3: Additional images for biofilm composition by scanning electron microscopy. SEM was used to visualize the microbial community composition in some real bath toy biofilms. Images were taken by the Center for Microscopy and Image Analysis, University Zurich.

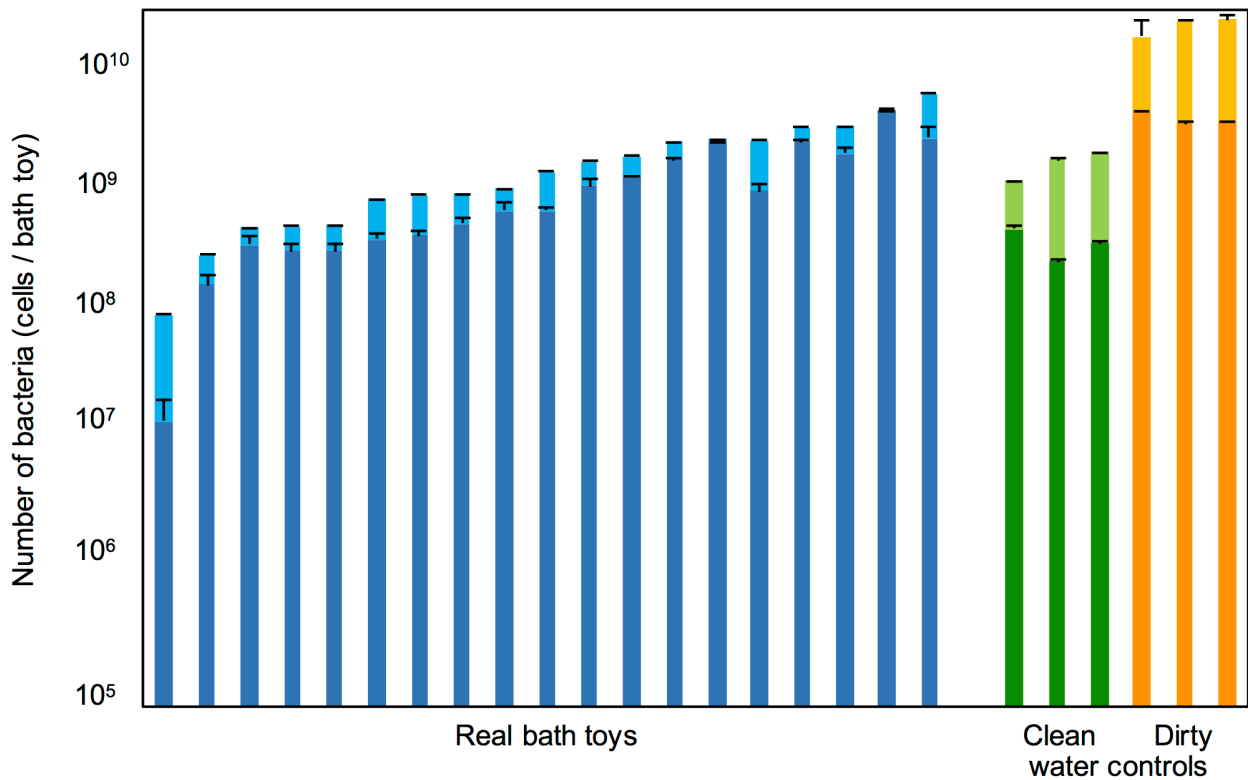


Figure S4: Number of bacteria in bath toy biofilms. Flow cytometry was used to differentiate between the total number of cells (SYBR[®] Green I staining) and the amount of intact cells (SYBR[®] Green I and Propidium Iodide staining) in biofilms grown in bath toys from real households or in control bath toys processed with either water prior (clean water controls) or after bathing (dirty water controls). The darker colours represent the number of intact bacteria in each bath toy, the lighter colours the damaged amount of the total number of cells. Error bars represent standard deviations of triplicate measurements.

Table S2: Classification of operational taxonomic units (OTUs) shared by biofilms originating from all bath toys (), all real bath toys (), all control bath toys (), all clean water controls (), or all dirty water controls (). NA indicates that no further classification could be made for that. "OTU-ID name" gives identity of clustered OTUs for comparison between categories.

	Phylum	Class	Order	Family	Genus	OTU-ID name
1	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	1
2	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	2
3	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	3
4	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	9
5	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	15
6	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium	16
7	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	NA	20
8	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	NA	75
1	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	1
2	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	2
3	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	3
4	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	4
5	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	9
6	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	15
7	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium	16
8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	NA	20
9	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	NA	45
10	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	57
11	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	NA	75
12	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	289
13	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	25548
1	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	1
2	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	2
3	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	3
4	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA	8
5	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	9
6	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Enhydrobacter	13
7	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	15

8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium	16
9	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	NA	20
10	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	33
11	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	37
12	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	41
13	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Hylemonella	55
14	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	60
15	Actinobacteria	Actinobacteria	Actinomycetales	Brevibacteriaceae	Brevibacterium	62
16	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	NA	75
17	Planctomycetes	Phycisphaerae	Phycisphaerales	NA	NA	84
18	TM7	TM7-3	NA	NA	NA	85
19	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	111
20	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	Methylopila	119
21	Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Chryseobacterium	126
22	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	150
23	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	171
24	Actinobacteria	Actinobacteria	Actinomycetales	Williamsiaceae	Williamsia	191
25	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	254
26	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	317
27	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	338
28	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NA	376
29	Armatimonadetes	[Fimbriimonadia]	[Fimbriimonadales]	[Fimbriimonadaceae]	Fimbriimonas	403
30	Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Chryseobacterium	448
31	Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	Ochrobactrum	718
32	WPS-2	NA	NA	NA	NA	1003
33	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium	2702
34	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	9064
35	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Xanthobacter	9623
36	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	NA	15174
37	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	24425
38	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	24749
39	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	NA	25533
40	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	25657
41	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	27237
42	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	27622
43	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	33484
44	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	34670
45	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	36313

46	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	36581
47	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	39507
1	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	1
2	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	2
3	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	3
4	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	4
5	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Comamonas	5
6	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA	8
7	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	9
8	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Enhydrobacter	13
9	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	15
10	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium	16
11	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	NA	20
12	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenyllobacterium	33
13	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	37
14	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	41
15	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Hylemonella	55
16	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	57
17	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	60
18	Actinobacteria	Actinobacteria	Actinomycetales	Brevibacteriaceae	Brevibacterium	62
19	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	68
20	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	NA	75
21	Planctomycetes	Phycisphaerae	Phycisphaerales	NA	NA	84
22	TM7	TM7-3	NA	NA	NA	85
23	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	111
24	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	Methylopila	119
25	Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Chryseobacterium	126
26	WPS-2	NA	NA	NA	NA	146
27	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	150
28	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	171
29	Actinobacteria	Actinobacteria	Actinomycetales	Williamsiaceae	Williamsia	191
30	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	Planctomyces	226
31	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	NA	233
32	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	254
33	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	289
34	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	292
35	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	317
36	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	338

		ria	es			
37	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NA	376
38	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Spirosoma	381
39	Armatimonadetes	[Fimbriimonadia]	[Fimbriimonadales]	[Fimbriimonadaceae]	Fimbriimonas	403
40	Firmicutes	Bacilli	Bacillales	Planococcaceae	Lysinibacillus	447
41	Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Chryseobacterium	448
42	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Azospirillum	533
43	Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	Ochrobactrum	718
44	WPS-2	NA	NA	NA	NA	1003
45	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Citricoccus	1261
46	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium	2702
47	Proteobacteria	Deltaproteobacteria	Myxococcales	0319-6G20	NA	3053
48	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	9064
49	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Xanthobacter	9623
50	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	11155
51	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	14526
52	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	NA	15174
53	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	19848
54	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	21114
55	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	NA	21892
56	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	24425
57	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	24749
58	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	NA	25305
59	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	NA	25533
60	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	25657
61	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	27237
62	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	27622
63	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	28140
64	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	33484
65	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	34670
66	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	35651
67	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	36313
68	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	36581
69	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium	39228
70	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	39507
71	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	41388
72	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	44242

1	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	1
2	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	2
3	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	3
4	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA	8
5	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	9
6	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Enhydrobacter	13
7	TM7	TM7-3	EW055	NA	NA	14
8	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	15
9	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium	16
10	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	NA	20
11	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenyllobacterium	33
12	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas	34
13	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	37
14	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	38
15	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	41
16	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Hylemonella	55
17	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	60
18	Actinobacteria	Actinobacteria	Actinomycetales	Brevibacteriaceae	Brevibacterium	62
19	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	NA	75
20	Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Chryseobacterium	80
21	Planctomycetes	Phycisphaerae	Phycisphaerales	NA	NA	84
22	TM7	TM7-3	NA	NA	NA	85
23	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	111
24	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	NA	117
25	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	Methylopila	119
26	Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Chryseobacterium	126
27	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	150
28	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	171
29	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Prosthecoacter	180
30	Actinobacteria	Actinobacteria	Actinomycetales	Williamsiaceae	Williamsia	191
31	Proteobacteria	Alphaproteobacteria	BD7-3	NA	NA	198
32	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter	200
33	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	254
34	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	258
35	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Dokdonella	287
36	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	317
37	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	318
38	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	338

39	Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae	Aquicella	367
40	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NA	376
41	Armatimonadetes	[Fimbriimonadia]	[Fimbriimonadales]	[Fimbriimonadaceae]	Fimbriimonas	403
42	Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Chryseobacterium	448
43	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	NA	559
44	Proteobacteria	Alphaproteobacteria	Rhizobiales	Brucellaceae	Ochrobactrum	718
45	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	820
46	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	910
47	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	NA	959
48	WPS-2	NA	NA	NA	NA	1003
49	Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]	Chryseobacterium	1090
50	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	1186
51	Planctomycetes	Planctomycetia	Gemmatales	Isosphaeraceae	NA	1648
52	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium	2702
53	Actinobacteria	Actinobacteria	Actinomycetales	Gordoniaceae	NA	4496
54	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Delftia	4964
55	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	6141
56	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	9064
57	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Xanthobacter	9623
58	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	13280
59	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	NA	13697
60	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	NA	15174
61	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	16770
62	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	18398
63	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	18530
64	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	20814
65	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Roseateles	21094
66	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Roseateles	21116
67	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Roseateles	22107
68	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Roseateles	22334
69	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	24425
70	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	24749
71	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	25154
72	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	25286
73	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	NA	25380
74	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	25387
75	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	NA	25533
76	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	25548
77	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	25552

78	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	25649
79	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	25650
80	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	25657
81	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	27237
82	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	27622
83	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Roseateles	27910
84	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	27981
85	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	28085
86	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	28098
87	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	28102
88	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	28130
89	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	32272
90	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	32696
91	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	33213
92	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	33240
93	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	33281
94	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	33484
95	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	33496
96	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	33868
97	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Mycoplana	33931
98	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	34122
99	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	34125
100	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	34670
101	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	36313
102	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	36569
103	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	36581
104	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	36622
105	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	38225
106	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	39507
107	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA	43316

Table S3: ‘Household-specific core communities’ in real bath toys originated from five different Swiss households, with bath toys provided ranging between 1 – 6 bath toys per household. The OTUs in each core community are (1) present in all samples from the household, and (2) not present in other households; while shared OTUs are (1) present in all samples from the household, but (2) not necessarily exclusively.

	n(bath toys) / household	Sum of reads	n(household specific core OTUs)	% of core-reads / total number of reads within household	n(shared OTUs) / household
Household_I	1	30'133	33	0.1	-
Household_II	2	60'266	1'289	2.9	1'445
Household_III	3	90'399	741	2.1	124
Household_IV	5	150'665	1'059	1.1	41
Household_V	6	180'798	3'904	4.2	29

Table S4: Number of operational taxonomic units (OTUs) per individual bath toy and Shannon-Wiener index (H') as a measure of diversity.

Bath toy	Number of observed OTUs	H'
Real bath toy 01	2'826	0.52
Real bath toy 02	239	0.31
Real bath toy 03	597	0.43
Real bath toy 04	3'199	0.62
Real bath toy 05	3'483	0.52
Real bath toy 06	2'458	0.47
Real bath toy 07	281	0.35
Real bath toy 08	192	0.27
Real bath toy 09	6'196	0.71
Real bath toy 10	3'562	0.57
Real bath toy 11	429	0.40
Real bath toy 12	204	0.26
Real bath toy 13	329	0.39
Real bath toy 14	311	0.33
Real bath toy 15	No data available	
Real bath toy 16	335	0.33
Real bath toy 17	527	0.35
Real bath toy 18	No data available	
Real bath toy 19	435	0.32
Clean water control	214	0.26
Clean water control	188	0.23
Clean water control	265	0.17
Dirty water control	269	0.32
Dirty water control	268	0.25
Dirty water control	252	0.25



Figure S5: Classification of abundant OTUs in real bath toy biofilms. The ten most abundant OTUs for each single bath toy (i.e., OTUs with the most numbers of reads with all samples set to an even depth of 30'133 reads), originating from random households, were separated on different classification levels (phylum, class, order) to highlight differences in community compositions.

Table S5: Comparison of most abundant operational taxonomic units (OTUs) in control bath toy biofilms. Most abundant OTUs in clean and dirty water control biofilms are specified on different classification levels. Bold highlighted OTUs were dominant in both clean and dirty water controls.

		Phylum	Class	Order	Family	Genus
Clean water controls		Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter
		Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA
		Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA
		Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA
		Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
		Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	NA
		Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA
		Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
		Planctomycetes	Phycisphaerae	Phycisphaerales	NA	NA
		Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA
	Rest	/	/	/	/	
		Phylum	Class	Order	Family	Genus
Dirty water controls		Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter
		Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA
		Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA
		Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium
		Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium
		Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteraceae	Mycobacterium
		Bacteroidetes	Flavobacteriia	Flavobacteriales	[[Weeksellaceae]	Chryseobacterium
		TM7	TM/-3	NA	NA	NA
		Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA
		Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA
	Rest	/	/	/	/	

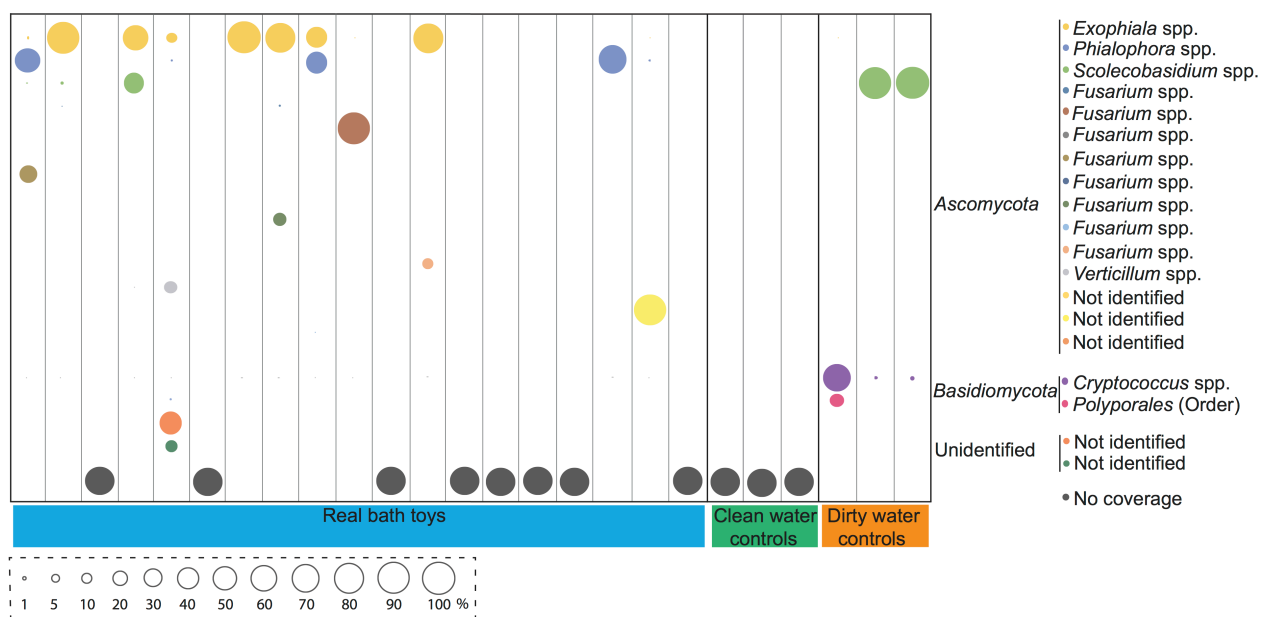


Figure S6: Dominant operational taxonomic units (OTUs) in fungal communities of bath toy biofilms. Colors represent different OTUs on genus level, with the majority belonging to the phylum Ascomycota and the rest either to Basidiomycota or being unidentified. Samples indicated with black circles did not show any results for fungi representatives. In general, the size of the circles indicates the absolute abundance of each OTU, with bigger volumes indicating higher percentages.

Table S6: Conventional plating results testing biofilms from the inner surface of bath toys for several indicator bacteria or groups. Samples were taken from either real bath toys (A) which were used randomly in different households or from control bath toys (B) which were used either with clean water prior to bathing (clean water controls) or with used water after bathing (dirty water controls). Compact Dry Plates were used for testing the presence of *Pseudomonas aeruginosa*, *Listeria* spp., *Enterococci* spp., Coliforms, and *Escherichia coli*. Special agar plates (*Legionella* BMP α Selective Medium) were used for the detection of *Legionella pneumophila*. Red, empty boxes indicate negative cultivation results. Green boxes indicate positive results including numbers of colony forming units (CFU). The number of CFUs was recalculated to their total presence per bath toy, with a detection limit of 3.3×10^2 CFU/bath toy.

A	Real bath toys																	
<i>P. aeruginosa</i>		3x10 ²		2x10 ⁴		14x10 ⁵		4x10 ⁵		5x10 ⁵	1x10 ⁵	4x10 ⁴		3x10 ³	4x10 ⁵	8x10 ⁵	3x10 ³	
<i>Listeria</i> spp.		3x10 ³				1x10 ⁵	2x10 ⁴								4x10 ²	4x10 ⁵	5x10 ³	
<i>Enterococci</i> spp.		1x10 ³					3x10 ²				3x10 ²						1x10 ⁵	
Coliforms																		
<i>E. coli</i>																		
<i>L. pneumophila</i>		7x10 ⁴	7x10 ⁴					4x10 ⁴						1x10 ⁵		2x10 ⁶	7x10 ³	
B	Clean water controls						Dirty water controls											
<i>P. aeruginosa</i>	2x10 ⁴	1x10 ⁴	1x10 ³	3x10 ⁴	8x10 ⁴	3x10 ⁴												
<i>Listeria</i> spp.	8x10 ³	1x10 ³	1x10 ³	4x10 ⁴	6x10 ⁴	1x10 ⁵												
<i>Enterococci</i> spp.																		
Coliforms	3x10 ³	2x10 ⁴																
<i>E. coli</i>				4x10 ⁴	1x10 ⁵	1x10 ⁵												
<i>L. pneumophila</i>	1x10 ⁴		7x10 ³	2x10 ³	2x10 ³	2x10 ³												

Table S7: Carbon migration potential (MP) and biomass formation potential (BFP) of the control bath toys' plastic material. Carbon migration was measured after 1 (M1), 3 (M3), and 7 (M7) days of incubation (with water renewal on day 2, 4, 5, and 6 without measurement) and values for total organic carbon (TOC), total cell counts/cm² (TCC), as well as the proportion of assimilable organic carbon (AOC) to total organic carbon were summarized afterwards ($\sum_{M1/M3/M7}$). Standard deviations are given for experimental triplicates (*Stdev*). For the BFP, planktonic (pBFP) and biofilm (bBFP) growth after 14 d of incubation was measured.

Carbon migration	TOC [$\mu\text{g}/\text{cm}^2$]	TOC [$\mu\text{g}/\text{cm}^2/\text{day}$]	TCC [cells/cm^2]	AOC/TOC [%]
$\sum_{M1/M3/M7}$	11.8	-	8.7×10^7	73.5
<i>Stdev</i>	0.8	-	1.1×10^6	-
M1	-	5.59		
<i>Stdev</i>	-	0.07		
M3	-	3.59		
<i>Stdev</i>	-	0.62		
M7	-	2.59		
<i>Stdev</i>	-	0.12		
Biofilm formation	Planktonic cells/cm²	Biofilm cells/cm²	\sumBFP [cells/cm^2]	pBFP/bBFP [%]
t_{14}	3.0×10^8	3.6×10^8	6.6×10^8	82.5
<i>Stdev</i>	5.1×10^7	5.6×10^7	1.1×10^8	-

Characteristics of tap water in the control experiment prior to and after bathing

Method

Water samples were taken before and after bathing in the control experiment. Total cell numbers were analyzed (FCM, SG staining; see above) and the concentrations of dissolved organic carbon (DOC), total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP) were measured. Chemical measurements were performed in a TOC-L_{CSH} (SHIMADZU GmbH, Switzerland) where carbon dioxide (CO₂) was measured with an infrared (IR) detector after an initial catalytically burning at 720 °C. For differentiating between total and dissolved organic carbon, DOC samples were filtered (0.45 µm) before separate measurement. For the T-N measurement, NO_x was measured with a chemiluminescence detector.

Results

Table S8: Biological and chemical parameters for water before and after bathing. Total cell numbers per milliliter as well as concentrations for dissolved organic carbon (mg DOC/L), total organic carbon (mg TOC/L), total nitrogen (mg TN/L), and total phosphate (µg TP/L) were measured for clean water prior to bathing and dirty water after bathing including dirt and soap. Standard deviations (Stdev) are given for the measurement of three individual samples.

	DOC [mg/L]	TOC [mg/L]	TN [mg/L]	TP [µg/L]	TCC / mL
Before bathing	1.0	1.2	2.6	13.7	2.1 x 10 ⁵
<i>Stdev</i>	0.1	0.1	0.3	6.0	1.1 x 10 ⁵
After bathing	11.3	15.7	4.9	21.2	4.1 x 10 ⁵
<i>Stdev</i>	1.6	1.2	1.0	6.0	2.5 x 10 ⁵

Table S9: Settings for Amplicon PCR and Index PCR.

Amplicon PCR	Temperature	Duration	Cycles
	95°C	5:00 min	29 x
	95°C	0:20 min	
	51°C	0:15 min	
	72°C	0:30 min	
	4°C	hold	
Index PCR	Temperature	Duration	Cycles
	95°C	3:00 min	8 x
	95°C	0:30 min	
	51°C	0:35 min	
	72°C	0:35 min	
	4°C	hold	