Supplementary Material

Table S1. Number of *P. n. asymbioticus* strains isolated from habitats of different pH categories and number and percentage of them covered by the 13 employed RLBH probes. #, number.

| pH  | # of strains | % of strains | # of strains in category targeted by probes | % of strains in category targeted by probes |
|-----|--------------|--------------|---------------------------------------------|--------------------------------------------|
| < 4 | 2            | 1            | 1                                           | 50                                         |
| 4 - 5 | 27       | 18           | 21                                          | 78                                         |
| 5 - 6 | 50       | 33           | 29                                          | 58                                         |
| 6 - 7 | 28       | 18           | 17                                          | 61                                         |
| 7 - 8 | 24       | 16           | 5                                           | 21                                         |
| > 8   | 14       | 9            | 9                                           | 64                                         |
| unknown pH | 7 | 5 | 4 | 57 |


Table S2. List of investigated habitats and detection of *Polynucleobacter necessarius* ssp. *asymbioticus* groups by RLBH probes. Detailed environmental data of these habitats were presented previously (Jezberová et al., 2010). Black rectangles indicate detection by a probe in at least one sample of the respective habitat, and red diamonds indicate isolation of at least one strain from the respective habitat. Note that strains were not always isolated from those water samples investigated by RLBH. PnecC (%), relative abundance of *P. n. asymbioticus* as % of total bacterial numbers (data from Jezberová et al., 2010).

| Habitat                              | F1 | F2 | F4 | F5 | F10 | F11 | F13n | F14 | F15 | F15-1 | F16 | F17 | pH | PnecC (%) |
|--------------------------------------|----|----|----|----|-----|-----|------|-----|-----|--------|-----|-----|----|-----------|
| 1 Loiberbacher pond 1                |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 2 Loiberbacher pond 2                |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 3 Loiberbacher pond 3                |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 4 Loiberbacher pond 4                |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 5 Hintersee                           |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 6 Fuschlsee                           |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 7 Füllingsee                          |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 8 Ebensee                             |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 9 Irsee                               |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 10 Mondsee (Scharlfing)              |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 11 Pond Seepromenade                 |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 12 Egelsee (Scharlfing)              |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 13 Krottensee                        |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 14 Wolfgangsee (Winkel)              |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 15 Eisla                              |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 16 Schwarzensee                      |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 17 Mönichsee                         |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 18 Mittersee                         |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 19 Süssensee                         |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 20 Haleswiessee                      |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 21 Attersee (Unterach)               |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 22 Egelsee (near Attersee)           |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 23 Unterer Langbathsee               |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 24 Oberer Langbathsee                |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 25 Taterlauensee                     |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 26 Traunsee (Altmünster)             |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 27 Offensee                          |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 28 Nussensee                         |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 29 Altauseer See                     |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 30 Grundtsee                         |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 31 Töupilzsee                        |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 32 Sommersbergsee                   |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 33 Hauslattensee (Steeg)             |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 34 Vorderer Gosausesee               |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 35 Hinterer Gosausesee               |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 36 Gosau 3 (fishpond)                |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 37 Vorderer Lahngangsee              |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 38 Wildensee                         |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 39 Pond Sportland                    |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 40 Pond Rossmeiss                    |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 41 Elmsee                            |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 42 Lacke near Elmsee                 |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 43 Post1                              |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 44 Post2                              |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 45 Post3                              |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 46 Post4                              |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 47 Illinger 1                         |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 48 Illinger 2                         |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 49 Egelsee I near Mattsee            |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 50 Egelsee II near Mattsee            |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 51 Wengen Moor 1                     |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 52 Wengen Moor 2                     |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 53 Puddle near Wengen Moor           |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 54 Wallensee                          |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 55 Pond 5 (garden pond)              |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 56 Schwarze Lacke                    |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 57 Doppel-Lacke                      |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
| 58 Gerzinsel                         |    |    |    |    |     |     |      |     |     |        |     |     |    |           |
|   |   |   |   |   |
|---|---|---|---|---|
| 59 | Unterer Teich | 65 | Kl. Inselteich | 67 | Sesslenteich |
| 60 | Holzteich | 66 | Grosser Inselteich | 68 | Stegenteich |
| 61 | Rechteckteich | 69 | I. Holzteich | 69 | Trög 1 |
| 62 | Grosser Teich | 70 | Trög 2 | 70 | Trög 2 |
| 63 | Birnenteich | 71 | Trög 3 | 71 | Trög 3 |
| 64 | Pisateich | 72 | Trög 4 | 72 | Trög 4 |
| 65 | Kleiner Inselteich | 73 | Trög 5 | 73 | Trög 5 |
| 66 | Grosser Inselteich | 74 | Trög 6 | 74 | Trög 6 |
| 67 | Trög 1 | 75 | Trög 7 | 75 | Trög 7 |
| 68 | Trög 2 | 76 | Trög 8 | 76 | Trög 8 |
| 69 | Trög 1 | 77 | Letten 1 | 77 | Letten 1 |
| 70 | Trög 2 | 78 | Letten 2 | 78 | Letten 2 |
| 71 | Trög 3 | 79 | Letten 3 | 79 | Letten 3 |
| 72 | Trög 4 | 80 | Letten 4 | 80 | Letten 4 |
| 73 | Trög 5 | 81 | Reiter Alpe | 81 | Reiter Alpe |
| 74 | Trög 6 | 82 | Schwägalpe | 82 | Schwägalpe |
| 75 | Trög 7 | 83 | Schwägalpe 2 | 83 | Schwägalpe 2 |
| 76 | Trög 8 | 84 | Adlerlacke | 84 | Adlerlacke |
| 77 | Letten 1 | 85 | SchFe1 | 85 | SchFe1 |
| 78 | Letten 2 | 86 | Hor1 | 86 | Hor1 |
| 79 | Letten 3 | 87 | Hor2 | 87 | Hor2 |
| 80 | Letten 4 | 88 | Hor3 | 88 | Hor3 |
| 81 | Reiter Alpe | 89 | Hor4 | 89 | Hor4 |
| 82 | Schwägalpe | 90 | Salz1 | 90 | Salz1 |
| 83 | Schwägalpe 2 | 91 | Salz2 | 91 | Salz2 |
| 84 | Adlerlacke | 92 | Salz4 | 92 | Salz4 |
| 85 | SchFe1 | 93 | Grasteich | 93 | Grasteich |
| 86 | Hor1 | 94 | Triangle Lacke | 94 | Triangle Lacke |
| 87 | Hor2 | 95 | Oko Lacke | 95 | Oko Lacke |
| 88 | Hor3 | 96 | Kleiner Brauner Lacke | 96 | Kleiner Brauner Lacke |
| 89 | Hor4 | 97 | Kreuzungs Lacke | 97 | Kreuzungs Lacke |
| 90 | Salz1 | 98 | Portier Horn Teich | 98 | Portier Horn Teich |
| 91 | Salz2 | 99 | U-Lacke | 99 | U-Lacke |
| 92 | Salz4 | 100 | Hidden Lacke | 100 | Hidden Lacke |
| 93 | Grasteich | 101 | Joint Lacke | 101 | Joint Lacke |
| 94 | Triangle Lacke | 102 | Jitka’s Lacke | 102 | Jitka’s Lacke |
| 95 | Oko Lacke | 103 | Funkla Lacke | 103 | Funkla Lacke |
| 96 | Kleiner Brauner Lacke | 104 | Seilbahn Lacke 1 | 104 | Seilbahn Lacke 1 |
| 97 | Kreuzungs Lacke | 105 | Seilbahn Lacke 2 | 105 | Seilbahn Lacke 2 |
| 98 | Portier Horn Teich | 106 | Oberer Klaffersee | 106 | Oberer Klaffersee |
| 99 | U-Lacke | 107 | Unterer Klaffersee | 107 | Unterer Klaffersee |
| 100 | Hidden Lacke | 108 | Breitenbergssee | 108 | Breitenbergssee |
| 101 | Joint Lacke | 109 | Rauhenbergssee | 109 | Rauhenbergssee |
| 102 | Jitka’s Lacke | 110 | Gerlos 1 | 110 | Gerlos 1 |
| 103 | Funkla Lacke | 111 | Gerlos 2 | 111 | Gerlos 2 |
| 104 | Seilbahn Lacke 1 | 112 | Gerlos 3 | 112 | Gerlos 3 |
| 105 | Seilbahn Lacke 2 | 113 | Gerlos 4 | 113 | Gerlos 4 |
| 106 | Oberer Klaffersee | 114 | Gerlos 5 | 114 | Gerlos 5 |
| 107 | Unterer Klaffersee | 115 | Gerlos 6 | 115 | Gerlos 6 |
| 108 | Breitenbergssee | 116 | Chalupská Slat | 116 | Chalupská Slat |
| 109 | Rauhenbergssee | 117 | Piedrno | 117 | Piedrno |
| 110 | Gerlos 1 | 118 | Čertovo | 118 | Čertovo |
| 111 | Gerlos 2 | 119 | Černé | 119 | Černé |
| 112 | Gerlos 3 | 120 | Pražské | 120 | Pražské |
| 113 | Gerlos 4 | 121 | Laka | 121 | Laka |

*PnecC bacteria (P. n. ssp. asymbioticus) could be detected by FISH but could not be reliably quantified due to low cell numbers.
Fig. S1. Comparison of phylogenetic trees based on complete 16S-23S ITS sequences (B) and partial glnA sequences (C). Two markers are located at almost opposite positions in the genome of *P. n. ssp. asymbioticus* strain QLW-P1DMWA-1 (A) (accession number NC_009379).
**Fig. S2.** Depth distribution of *P. n. asymbioticus* groups in meromictic Lake Krottensee (May 2007) as revealed by RLBH. Left graph, oxygen concentrations and total abundance of *P. n. asymbioticus* determined by a species-specific FISH probe (Jezberová et al., 2010). Note the non-linear scaling of the axis indicating the water depths. Right graph, detection of *P. n. asymbioticus* groups. All probes but probe F17 were applied to the samples. Besides the three presented probes, only application of probe F15-1 resulted in detections but the signals were very faint. Black – strong signal, dark grey – average signal, grey – weak signal, white – no signal. Note that sampling of the depths profile was performed on a different date than sampling of surface waters, which resulted in the detections presented in Table S1 and Fig. 1B.
**Fig. S3.** Comparison of pH ranges of investigated groups as indicated by RLBH (red) and pH range of habitats from which isolates were obtained (blue). Polyphyletic and paraphyletic groups are indicated by an asterisk.