

Commensal scale-worms of the genus *Branchipolynoe* (Polychaeta: Polynoidae) at deep-sea hydrothermal vents and cold seeps

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The polychaete family Polynoidae (scale worms) is very diverse, and particularly well represented worldwide at deep-sea hydrothermal vents, cold seeps, and at sites of decomposing organic matter such as whale carcasses, sunken wood or even experimentally-enriched substrates. To date, more than 40 species (placed in 7 different subfamilies) are described that are only known from such chemosynthetically-driven ecosystems, and many more undoubtedly still await discovery or formal description. Most species are free-living in a wide range of habitats: from high-temperature hydrothermal "chimney" walls to peripheral habitats with little or no influence from the reduced fluids.

In contrast with those free-living species, polynoids of the genus *Branchipolynoe* Pettibone, 1984 (placed in the monogeneric subfamily Branchipolynoinae) occur exclusively in the mantle cavity of *Bathymodiolus*-like mytilid bivalves. As opposed to most other polynoids, *Branchipolynoe* species have well-developed gills in a dorsal position that most likely represent a favourable adaptation to oxygen-depleted environments. The true nature of this "symbiotic" association is still unclear, but a few facts are known that suggest the worms are opportunistic commensals specially adapted to their hosts: Pettibone (1984) pointed out some morphological features seemingly connected with commensalism, and the protection such a life habit must provide against the numerous predators present at vents and seeps; Desbruyères et al. (1985) found bits of mussel gills and pseudofaeces in the worms' gut contents; finally, infestation rates vary from up to 6 individuals per host to none, and mussels are known

to occur without the worm, but not the contrary (pers. obs.). No report exists yet on a possible negative effect of the worm on its host.

Three species of *Branchipolynoe* have so far been described (see Fig. 1 for their range): *B. symmytilida* Pettibone, 1984 was originally discovered in the hydrothermal-vent mussel *Bathymodiolus thermophilus* at the Galapagos Rift vents. Later, the geographic range of this pair of species was extended to include the northern and southern parts of the fast-spreading East Pacific Rise (EPR), from 13°N, 11°N, 9°50'N and 17-18°S (pers. obs.). The second hydrothermal species to be described was *B. pettiboneae* Miura & Hashimoto, 1991, from species of *Bathymodiolus* living in venting areas of the Okinawa Trough and the Kaikata Seamount, south of Japan. *Branchipolynoe pettiboneae* was later reported from several other vent areas in the western Pacific: the Mariana, Manus, Lau, and North Fiji back-arc basins. This species is known to occur in at least three distinct mytilid species: *Bathymodiolus brevior*, *B. platifrons*, and *B. japonicus* (Miura, 1997).

Finally, as early as the discovery of chemosynthetic communities associated with cold seepage areas in the Gulf of Mexico, another species of commensal branchiate scale-worm, *Branchipolynoe seepensis* Pettibone, 1986 was discovered associated with the mytilids of the West Florida Escarpment hypersaline seep sites ("Seep Mytilid Va"). Other mussels, "Seep Mytilid III" and "Seep Mytilid IV", from shallower cold seeps on the Louisiana Continental Slope, also harbor commensal polynoids that have not yet been formally identified as *B. seepensis* (Gustafson et al., submitted).

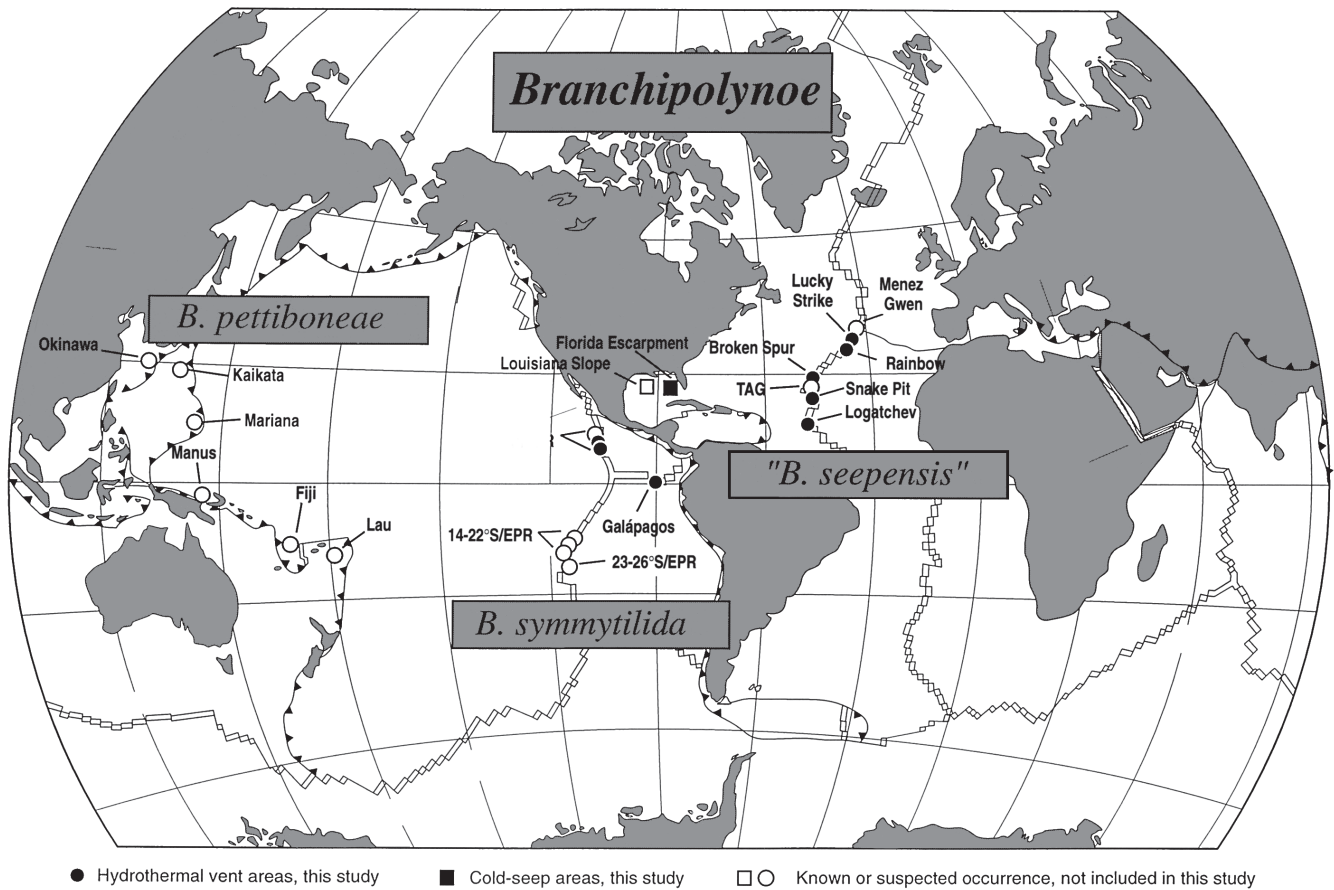


Figure 1. Distribution map of the commensal polynoid genus *Branchipolynoe*.

With the discovery of hydrothermal mytilid bivalves (see the review by Van Dover, 1995) at most of the known vent areas on the Mid-Atlantic Ridge (MAR), commensal polynoids were also found associated with two species of mussels: *Bathymodiolus puteoserpentis* on the southern MAR and another *Bathymodiolus* presently being described on the northern MAR (von Cosel et al., submitted; Jollivet et al., this volume). The commensal worms were assigned to *Branchipolynoe seepensis* based on morphological criteria: originally reported from Snake Pit (Segonzac, 1992), then Lucky Strike (Desbruyères et al., 1994), *Branchipolynoe* is now also known from Broken Spur, Logatchev and the newly discovered Rainbow area (P. Chevaldonné, pers. obs.) and was found in very small numbers at the shallow Menez Gwen area (S. Hourdez, pers. comm.).

Specialized species, however, never or rarely occur at both vents and seeps, and there has been a growing suspicion that MAR commensal polynoids and Gulf of Mexico *B. seepensis* are in fact cryptic species. Also, the question of the taxonomic identity of commensals found on a ridge system at vent sites separated by hundreds of kilometers or more has been raised. The reproductive

pattern in this genus is indeed not believed to favour the wide dispersal of offspring. Females only produce a few very large eggs suggesting direct development or lecithotrophy (Jollivet et al., this volume). It has also been demonstrated that the host mussels, species with a much higher dispersal potential, have diverged in at least two different species on the MAR (von Cosel et al., submitted; Jollivet et al., this volume) and in many others at the Gulf of Mexico cold seeps (Gustafson et al., submitted). Could the commensal worms also have genetically diverged along the ridges?

The analysis of DNA sequences can provide answers to such questions. Specimens of *Branchipolynoe* have therefore been collected from several locations on the Galapagos Rift, the East Pacific Rise, the Mid-Atlantic Ridge and the Louisiana Slope in the Gulf of Mexico. DNA was extracted from frozen specimens stored at -80°C using standard procedures (CTAB, phenol : chloroform extraction, ethanol precipitation), and 560-710 bp fragments of the mitochondrial large subunit (16S) ribosomal RNA gene (rDNA), and the mitochondrial cytochrome oxidase subunit I (COI) gene, respectively, were PCR-amplified

using previously existing “universal” primers. Purified PCR products were sequenced automatically, and nucleotide sequences were aligned to perform phylogenetic comparisons, using the free-living polynoid *Lepidonotopodium fimbriatum* Pettibone, 1983 as an outgroup. Genetic distances were calculated according to the Kimura 2-parameter model (K2P). A total of 10 operational taxonomic units (OTUs) was included in this study (see Fig. 1) and 2-5 individuals per OTU were sequenced (total of 27 individuals). Gaps (insertions and deletions) in the 16S rDNA sequences were removed prior to distance calculations.

A finer, more detailed phylogenetic analysis is presented elsewhere (Chevaldonné et al., in prep.), but genetic distances are presented here in Table 1. Pairwise K2P distances have been averaged within 4 distinct groups: (1) true *Branchiopolynoe seepensis* from Gulf of Mexico cold seeps; (2) “*B. seepensis*” from MAR hydrothermal vents; (3) *B. symmytilida*; and (4) *Lepidonotopodium fimbriatum*.

Table 1. Genetic distances, calculated according to the Kimura 2-parameter model (K2P) for the COI (upper part) and the 16S rRNA (lower part) genes. A total of 10 operational taxonomic units (OTUs) was included in this study and 2-5 individuals per OTU were sequenced (total of 27 individuals). Pairwise K2P distances have been averaged within 4 distinct groups: (1) true *B. seepensis* from Gulf of Mexico cold seeps; (2) “*B. seepensis*” from MAR hydrothermal vents; (3) *B. symmytilida*; and (4) *L. fimbriatum*.

COI	1	2	3	4
1 <i>B. seepensis</i> GoM	0.0048			
2 “ <i>B. seepensis</i> ” MAR	0.0536	0.0019		
3 <i>B. symmytilida</i>	0.1960	0.1719	0.0086	
4 <i>L. fimbriatum</i>	0.2738	0.2682	0.3004	0.0000

16S	1	2	3	4
1 <i>B. seepensis</i> GoM	0.0020			
2 “ <i>B. seepensis</i> ” MAR	0.0144	0.0007		
3 <i>B. symmytilida</i>	0.0786	0.0774	0.0038	
4 <i>L. fimbriatum</i>	0.1750	0.1808	0.1861	0.0000

There is a clear divergence between the Pacific and Atlantic species of *Branchiopolynoe* included in this study. The nucleotide divergence is 17-20% for the COI gene and ca. 8% for the 16S rDNA, which is evidence for an ancient isolation of the two groups. Within the Atlantic, the vent and the seep commensals appear to have diverged as well. The nucleotide divergence is higher than 5% for the COI and 1.4% for the 16S rDNA. Such values are not negligible and strongly suggest isolation of the two groups. Within each group, the distance values clearly indicate infra-specific relationships, but there seem to be a greater genetic variability in *B. symmytilida* than for the Atlantic species.

The first significant information derived from this preliminary phylogenetic analysis of the genus *Branchiopolynoe* is that *B. seepensis* from the Gulf of Mexico and the MAR commensal appear to have recently diverged, and must be considered as two isolated phylogenetic species. It is not possible to determine at this stage, whether this divergence results from the geographic distance involved, or from differences in the habitats (seeps vs. vents).

Second, MAR *Branchiopolynoe* are monospecific, at least from Lucky Strike to Logatchev, with little or no variability in the studied DNA sequences. As a consequence, this species is commensal with at least two different mytilid hosts, and it is remarkable for its depth range. Also, gene flow is most probably maintained over the great distances involved, and over the large transform faults that offset the MAR, despite a suggested non-planktotrophic development pattern (Jollivet et al., this volume).

Finally, *Branchiopolynoe symmytilida* occurs at both the Galapagos vents and along the northern EPR, with similar constraints (distance and development) as the MAR species. The observed nucleotide sequence variability is much greater than for the MAR species, although apparently not correlated with a geographic pattern. This might be the result of a younger age in the MAR species. More detailed results and discussion are provided in Chevaldonné et al. (in prep.).

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