Microbial communities associated with the degradation of oak wood in the Blanes submarine canyon and its adjacent open slope (NW Mediterranean)

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Abstract

Submarine canyons can trap and concentrate organic falls, like terrestrial debris, including wood. Sunken wood creates a unique ecosystem in the deep sea, which base, i.e. the microbial communities directly degrading this wood, remains poorly studied. Our aim was thus to examine the wood degrading microbial community by comparing oak samples experimentally deployed in experimental mooring arrays in the Blanes Canyon (BC) and its adjacent open slope (north western Mediterranean Sea). We analyzed the microbial community by parallel tag pyrosequencing of the16S rRNA genes from wood samples recovered from different depths after 9 and 12 months of deployment. In this first study of the phylogenetic description of wood associated microbial community by high throughput molecular techniques, we found that the microbial diversity was higher in samples from BC compared to the open slope. The structure of the communities were, however, not significantly different from each other, although we observed an apparent clustering according to time of immersion. Furthermore, an in depth taxonomic analysis revealed that *Alphaproteobacteria* was the dominant microbial taxa, with the *Roseobacter* clade seeming to have a specialized role in the degradation of oak in BC and its adjacent slope.

1. Introduction

Vegetal debris such as wood or plant remains are widely distributed on the seafloor and may represent an important source of organic carbon, especially in the deep sea (Wolff, 1979). The presence of wood allows the development of new ecological niches, the colonization by specialized communities (Gaudron et al., 2010; Romano et al., 2011) and may support high species richness and develop into biological hotspots (Bernardino et al., 2010; De Leo et al., 2010)

Despite their importance for the biodiversity of deep-sea fauna, relatively little is known about the degradation processes of wood falls. Wood consists primarily of cellulose, hemicellulose and lignin, cellulose being the most easily degraded by bacteria. Indeed, with a few exceptions, bacteria and fungi are the primary cellulose hydrolyzing organisms (King et al., 2010) and for a review, see Watanabe and Tokuda (2001). Generally, cellulolytic degrading bacteria are well described, especially in environments like the rumen and termite gut (Colberg, 1988; Ljungdahl and Eriksson, 1985; Lynd et al., 2002). In marine environments, both cellulose degrading (Distel et al., 2002; Distel and Roberts, 1997; Waterbury et al., 1983) and sulfide-oxidizing (Duperron et al., 2008; Lorion et al., 2009) symbiotic bacteria of wood boring organisms have been described.

There are some early descriptions of free-living (non-symbiotic) cellulose degrading bacteria (Austin et al., 1979; Cundell and Mitchell, 1977; Gareth Jones et al., 1976; Kohlmeyer, 1978), and the first studies on wood associated bacteria focused on describing their physical action on wood, i.e. tunneling vs. eroding (Jurgens et al., 2003; Mouzouras et al., 1988). However, this was before the era of molecular biology and the

16S rRNA gene taxonomy of these microorganisms remains poorly known. The first characterization of free living wood degrading bacteria using molecular biology methods was performed by Landy et al. (2008) who used clone libraries and DGGE to describe the Bacteria inhabiting archeological waterlogged wood, finding mostly *Bacteriodetes* and *Pseudomonas*. Furthermore, Palacios et al. (2009) described the microbiota associated to sunken wood using molecular fingerprinting methods and found different bacterial communities in wood with different signs of decay. Further examination showed that short time immersed wood harbored groups mainly implicated in the first steps of cellulose degradation, while anaerobic fermenting Bacteria, as well as sulfate reducing Bacteria (SRB) and methanogenic Archaea, were more abundant in long-time immersed wood (Fagervold et al., 2012).

Submarine canyons with their head close to the shore, like the ones located along the northwestern Mediterranean shelf, can channel organic substrates like wood directly from the continental shelf to the deep-sea. This unique property results in a significant increase of benthic productivity in canyons (De Leo et al., 2010). Here we wanted to test the hypothesis that the wood degrading microbial communities in submarine canyons are different from those usually found on woods from the continental margin and deep-sea floor. We compared oak wood deployed in the Blanes Canyon (BC) with wood deployed on its adjacent open slope, and looked at the possible role of depth and time of immersion on the microbial community. Bacterial communities were described by pyrosequencing targeting the 16S rRNA gene used as a phylogenetic marker. The approach allows a precise description of the wood associated microbial community and helped us hypothesize on their possible functions in wood degradation.

2. Materials and methods

2.1. Wood experimental immersion

Triplicate cubes (8 cm long) of oak wood were deployed in the Blanes Canyon (BC) along the canyon axis at three depths (900, 1200 and 1500 m) and at the western slope at 1200, 1500 and 1800 m depths. Samples were suspended 20 m above the seafloor. The samples from 1200 m depth were collected in November 2009 after 9 months of immersion and samples from 900, 1500 and 1800 m depth after 12 months of immersion, see Table 1 and Fig. 1 for details. After collection, wood chips to be used for microbial analysis were cut using sterilized tools, flash frozen in liquid nitrogen and placed at -20 °C directly on board. The rest of the wood cube was immersed in 4% buffered formaldehyde-seawater solution.

2.2. DNA extraction and PCR amplification

DNA was extracted according to Palacios et al. (2009), with some modifications. Wood chips (about 2g) were homogenized (powdered) using 25 ml grinding jars (Retsch, Inc. MM 400 Stainless steel) with a 20 mm diameter stainless steel ball using a RETSCH Mixer Mill (Retsch, Inc. MM301). The grinding jars with the wood and the stainless steel ball were dipped in liquid nitrogen before each bead beating. One cycle of bead beating for 1 min at 30 Hz followed by two cycles for 2 min at 30 Hz were performed.

Genomic DNA from approximately 100 mg of wood powder from each cube was extracted using the Mobio PowerPlant kit (Ozyme, Saint-Quentin-en-Yvelines, France) following the manufacturer's protocol, including an extra clean-up step. DNA from each of the triplicate oak cubes were pooled for further analysis.

Pooled DNA from triplicate wood cubes was subjected to PCR. Universal bacterial primers 27Fmod (5'-AGRGTTTGATCMTGGCTCAG-3') (Vergin et al., 1998) and a modified version of primer 519R (5'-GTVTTACCGCGGCTGCTG-3'), modified from 519R, 5'-GWATTACCGCGGCKGCTG (Teske et al., 1994), were used for the PCR reaction. The 27F primer sequences were modified by the 5' end addition of the Roche 454 A-adaptor sequence (5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG-3') and a 10-nucleotide identifying barcode (multiplex identifier, MID). For each sample, PCR was performed in triplicate and pooled to minimize PCR bias. Each reaction consisted of 1X High Fidelity PCR Buffer (Invitrogen), 0.4 mM of deoxynucleotide triphosphates (dNTPs, Eurogentec), 3 mM of Magnesium sulfate (MgSO4) (Invitrogen), 0.1 μM of each primer, 1.0 unit of Platinum® *Taq* High Fidelity (Invitrogen), 3 μl DNA template and water to bring the volume to 25 μl. The PCR included an initial denaturation step at 94°C for 1 min, followed by 26-30 cycles of denaturation at 95°C for 45 s, annealing at 54 °C for 45 s and elongation at 68 °C for 1 min; followed by a final 10 min elongation step at 68 °C. The number of cycles was determined for each sample after cycle optimization. PCR products were verified by gel electrophoresis (1 % agarose) with SmartLadder DNA as a size standard (Eurogentec). Bands were excised under UV light, and PCR product were extracted from the agarose gel and purified with the Ultrafree DA centrifugal filter kit. PCR products were quantified using a Picogreen

assay kit (Invitrogen). To obtain a similar number of reads from each sample, amplicons were mixed in equal concentration of 5 ng/ul. Emulsion PCR and the sequencing process were carried out by the Roche 454 pyrosequencing with a Genome Sequencer FLX Titanium chemistry at the Genotoul platform of INRA, Toulouse (France).

2.3. DNA sequence analysis

Sequences were processed and analyzed using the Qiime software (Caporaso et al., 2010a). Briefly, samples were denoised using the Ampliconnoise program and checked for chimeras using Perseus (Quince et al., 2011). The resulting 23893 clean sequences clustered into 1532 Operational Taxonomic Units (OTUs) at a 97% sequence identity level using the Uclust algorithm (Edgar, 2010). A representative sequence from each OTU was classified using the RDP classifier (Wang et al., 2007) and a training set extracted from the Silva108 database. Alpha and Beta diversity metrics were calculated using Qiime. Since we retrieved different amounts of sequences from each sample (Table 2), and since the alpha and beta diversity indexes are dependent of the sequencing depth, all samples were randomly resampled to the same size (2210 sequences). A two-sample t-test was performed using the Chao1 diversity metric to infer any statistical differences in alpha diversity between categories. To investigate whether the wood associated microbial community composition from the submarine canyon was different from the one at the open slope, unweighted and weighted unifrac metric (Lozupone and Knight, 2005; Lozupone et al., 2007) was used. These metrics takes into the account the phylogenetic distance between the OTUs and for weighted unifrac, the method also takes

into account abundance data. To test if the microbial community composition in any of the samples were significantly different, a P test (parsimony test) (Martin, 2002) was performed using the Bonferroni correction for sampling size. Phylogenetic trees used for Unifrac analysis was constructed using FastTree (Price et al., 2009) with sequences that had been aligned using PyNAST (Caporaso et al., 2010b) and filtered using the lanemask. Distance data was clustered using UPGMA (Unweighted Pair Group Method with Arithmetic mean, or average linkage) and jackknifed (10 iterations) to measure the robustness of the tree topology. The ARB software package (Ludwig et al., 2004) was used to perform phylogenetic analyses on Bacterial sequences. Pairwise distances between sequences were generated using the ARB neighbor-joining (NJ) algorithm with Jukes-Cantor corrected distance values. iTOL (Letunic and Bork, 2007) was used to display the phylogenetic tree together with OTUs abundance data. Sequences have been submitted to MG-RAST (http://metagenomics.anl.gov/linkin.cgi?metagenome=4485098.3) for public availability.

2.4. Analysis of wood boring bivalves

In the laboratory, formaldehyde preserved wood cubes were sectioned by hand and carefully dissected to separate and count all wood-boring bivalves. In order to insure that recently settled individuals were not overlooked, a magnifier was used throughout the extraction process. Density was expressed as number of individuals per dm^{-3}. A two-sample t-test was performed using the total density of wood-borers bivalves to infer any statistical differences among pair of samples.

3. Results

3.1. Bacterial diversity and wood borer density of oak samples

Overall, bacterial communities had a significantly higher diversity in BC than in open slope (two sample t-test, $p = 0.00498$), and see Table 2, however this was mostly driven by the increased diversity of BC oak at 900 m depth (OBC900W). Indeed, both alpha diversity indexes and rarefaction plots showed that OBC900W was the most diverse, followed by another canyon sample, OBC1200W, while the least diverse samples were both from the open slope (from 1500 and 1800 m) (Fig. 2A and Table 2). The other samples were more similar to each other in terms of alpha diversity. Furthermore, the different samples had different evenness, shown by the different rank-abundance plots (Fig. 2B) after sub-sampling to an even level of 2210 sequences per sample. The two samples that had been submerged for 9 months, OBC1200W and OOS1200W, were dominated by a few OTUs containing a large number of sequences. OBC1200W contained one OTU with over 500 sequences and this same OTU was present in OOS1200W with over 350 sequences. The other samples had a more even community distribution.

We collected a total of 465 individuals of wood borers belonging to the genus *Xylophaga* Turton, 1822, from the oak cubes. The density in BC was significantly higher than in the open slope (150 ± 20 *vs* 8 ± 2 ind dm^{-3}, t-test, $p<0.01$). In BC the oak cubes collected at 900 and 1200 m depth were more densely colonized than samples at 1500 m

depth. In contrast, there were no significant differences in the density among the deployments in the open slope samples (Suppl. Fig. 1).

3.2. Composition of the wood degrading microbial community.

One of our goals with this study was to determine if the wood location (BC vs. OS) was a driving factor for the microbial community composition. The comparison showed that samples were separated in two main clusters (Fig. 2). OOS1200W and OBC1200W, both being immersed for 9 months, clustered together strongly (Jackknife value >75%) and away from all other samples with both the weighted (Fig. 3) and unweighted (data not shown) Unifrac metrics. Thus, our data suggest that immersion time may be a stronger structuring factor for community composition than location. Within the second cluster, containing only samples that had been immersed for 12 months, BC samples (OBC900W and OBC1500W) grouped together (Jackknife >75%) separated from the open slope samples. Thus, for a similar immersion time, BC communities appeared more similar to each other than to slope communities. There was no apparent clustering of the communities according to depth. Furthermore, none of the communities were significantly different according to the P-test (Martin, 2002).

3.3. Major bacterial taxa in oak samples

Alphaproteobacteria was the major class of Bacteria in the oak samples, representing from around 35% to over 50% of the sequences recovered (Fig. 4). Within

the *Alphaproteobacteria*, the genus *Loktanella* was present in high relative amounts (Fig. 4). The most abundant OTU overall (Blanes 1043, Fig. 5) was 98% identical to *Loktanella* sp. K4B-4 (FJ889559) isolated from Arctic seawater. *Loktanella* sequences were most predominant in OBC1200W and OOS1200W (Fig. 4 and 5), constituting as much as one quarter of the sequences in OBC1200W. Three other abundant OTUs, Blanes 809, 896 and 717 also belonged to the *Alphaproteobacteria* (Fig. 5). The nearest BLAST hit sequence for all these OTUs came from the gut of a wood feeding sea urchin (*Asterechinus elegans*) found in deep wood falls in West-Pacific (Becker et al., 2009).

Bacteriodetes (mostly *Flavobacteria*) and *Gammaproteobacteria* were major groups recovered from the wood samples (Fig. 4). Two OTUs belonging to the *Flavobacteria*, Blanes 786 and 657, were very numerous containing over 400 sequences total and both were predominant in the OOS1800W sample (Fig. 5). Blanes 657 belonged to the genus *Zoobellia*, with the closest BLAST (94% identity) hit to *Zoobellia galactanivorans* strain DsiJT (Barbeyron et al., 2001). OTU Blanes 786 was 98% identical to a bacterial clone found inside the gut of a wood boring gastropod, *Pectinodonta* sp. (Zbinden et al., 2010). Within the *Gammaproteobacteria*, we obtained a large number of sequences from the genera *Pseudospirillum*. This genus, originally included within the *Oceanospirillum* (Satomi et al., 2002), consists of aerobic heterotrophic Bacteria ubiquitous in the marine environment. OTU Blanes 870 belongs to this genus and this OTU was most predominant in OOS1200W, but was present in all samples (Fig. 5). Also within the *Gammaproteobacteria*, OTU Blanes 250 (Fig. 5) was present in all samples in about the same amounts. This OTU belongs to the genus *Psychromonas*, of generally fermentative anaerobic, but aerotolerant, Bacteria

(Mountfort et al., 1998). Bacteria from this genus have been isolated from sediments around a whale fall (Miyazaki et al., 2008) and sequences from this genus were also found in sunken woods samples that had been immersed for 20 and 30 months (Fagervold et al., 2012).

We also found many *Planctomycetes* sequences belonging to the "Pir4 lineage" (Fig. 4), ranging from around 5 to 7.5 % of the total amount of sequences in different samples. This lineage does not contain any cultured representatives, so the exact function is uncertain. One of the most abundant OTUs, Blanes 105 also belonged to the *Planctomycetes* phylum. This OTU was 97% identical to a sequence from an epibacterial community found on marine macroalgae (Lachnit et al., 2011).

4. Discussion

Bacterial communities inhabiting oak cubes from Blanes Canyon were significantly more diverse compared to those from its outer slope. However, this increased diversity was mostly driven by one sample, OBC900W, that was markedly more diverse than the others. This increased diversity may be related to earlier findings showing that submarine canyons are caracterized by enhanced productivity, and can thus be considered "hotspots" of benthic biomass (De Leo et al., 2010; Vetter, 1994). Indeed, the higher densities of *Xylophaga* found on the BC Oak samples vs. the open slope (Suppl. Fig. 1) could be a cause for the increased microbial diversity we observed. One might hypothesize that the burrows that the *Xylophaga* excavate creates a greater number of microniches for microorganisms to colonize.

Abiotic features of BC versus its adjacent open slope might also influence microbial diversity. Distinctive hydrographic and hydrodynamic conditions (Canals et al., 2006; de Stigter et al., 2007; Durrieu de Madron, 1994) makes submarine canyons unique and dynamic environments and preferential conduits for the downward tranport of particulate material. Earlier studies in Blanes canyon have shown that the maximum particle flux recorded along the Blanes canyon axis during one year was three orders of magnitude higher than that the one recorded at the open slope, confirming the great capability of canyon as organic matter traps (Zúñiga et al., 2009; Romano et al, submitted$_b$, this issue). Indeed, the current inside the canyons were higher than on its outer slope during the period the woods were deployed (López Fernández et al, submitted, this issue). Higher particle fluxes might enhance the transport of bacteria that may colonize large organic falls such as wood. Furthermore, punctual strong currents leads to more sediment resuspension and bacteria from the sediment might thus settle on the wood. Moreover, since wood accumulates naturally in the canyon, woods that are already degrading could represent a bacterial seed bank for the new incoming woods. However, since PBC900W was also the shallowest of the samples, we cannot rule out the possibility that this increased diversity is also depth related.

The microbial community composition in any of the samples were, however, not statistically different from each other according to the P-test. There are obvious differences like the dominance of certain OTUs within the *Alphaproteobacteria*, but very few of the OTUs are specific to only one sample or only one category (i.e canyon, slope, 1200 m, etc). This implies that the wood itself, oak, might be the determining factor for the microbial community or that the conditions surrounding all the samples are not

sufficiently different to be driving factor for microbial community structure.

The predominance of *Alphaproteobacteria* in our oak samples is in contrast with a previous study by Fagervold et al. (2012) that showed *Deltaproteobactia* and/or *Bacteriodetes* to be the dominant phyla in sunken woods, depending on their immersion time. Indeed, this previous study showed that oak samples from the Mediterranean Sea from 57 meters depth (OakMed) were dominated by *Deltaproteobacteria*, with *Alphaproteobacteria* being a very minor part of the microbial community. OakMed also contained methanogens and sulfate reducing bacteria (SRB), suggesting that the wood was anaerobic (Fagervold et al., 2012). This difference in bacterial phyla distribution might be because the wood samples in the current study were deeper and not in contact with sediment, as in the previous study. Furthermore, the oak sample from the former study showed advanced signs of degradation. Conversely, in the present study, we only detect very small amounts of SRB, around 2% of total bacterial sequences (data not shown). However, the presence of the Pir4 lineage, originally found in anoxic soils and possibly anaerobic (Derakshani et al., 2001), suggest that at least some parts of the wood matrix had become anaerobic. This is corroborated by a recent study demonstrating that wood submerged in saltwater aquaria become suboxic within two days of immersion (Yucel et al., 2012). Most probable, the microbial communities in wood undergo succession with time and stage of decay. This is also suggested in this experiment by the clustering of the two 9 month samples away from the rest of the samples.

The dominance of *Alphaproteobacteria* in the oak samples might also be due to the characteristics of oak wood. Oak generally contains a lot of tannins and phenolic substances that can inhibit fungal growth (Scheefer and Cowling, 1966). These

substances presumably have an effect on the growth of bacteria as well. One might hypothesize that the most predominant OTUs being specialized toward the breakdown of these products or at least, be tolerant to these otherwise toxic compounds. Indeed, most of the *Alphaproteobacteria* we found, belonged to the *Roseobacter* clade, a clade that is shown to be nutritionally diverse (Buchan et al., 2005). Interestingly, one OTU, Blanes 1043, dominates two of the samples, OBC1200W and OOS1200W, both from 1200 m depth and both samples had been immersed for 9 months. OTU Blanes 1043 is related to *Loktanella* sp, an *Alphaproteobacteria* belonging to the *Rhodobacter* group (*Roseobacter* clade), and Bacteria within this genus are generally heterotrophic, moderately halotolerant and strictly aerobic. The species in this genus are b-galactosidase-positive (Van Trappen et al., 2004), meaning they can break down sugars. The specific role of Bacteria within this OTU in the oak wood remains speculative but the extraordinary high abundance of OTU Blanes 1043 suggest that these Bacteria have a specialized role in the degradation of oak in BC and its open slope.

We found several OTUs for which the closest relatives had been found in wood digesting sea urchin guts and associated with wood boring bivalves. These might have been associated with the wood boring bivalves found on the oak wood, but this needs to be confirmed. Furthermore, although we do not have proof, the fact that we find close relatives of these OTUs in other environments where wood degradation occurs, suggests that these OTUs might be important in the wood degradation pathway. For example, OTU Blanes 657 belonged to the genus *Zoobellia* and species in this genus cannot generally degrade cellulose but several marine polysaccharides (Barbeyron et al., 2001). This genus was also found in pine samples from the Pacific Ocean that had been

implanted for 20 months at 1100 m depth (Fagervold et al., 2012). However, only by obtaining pure cultures and directly test for cellulose digestion, can we be certain that these microorganisms can degrade cellulose, and not other wood components and/or secondary metabolites.

5. Conclusions

The microbial diversity was significantly higher in oak cubes from the Blanes Canyon compared to those from its outer slope. However, whether this is linked to the physico-chemical properties of water masses inside Blanes Canyon vs. the open slope or the increase abundance of *Xylophaga* on the woods inside BC, needs to be further investigated. Although the microbial community structure was not statistically different, there seemed to be a clustering according to time of immersion. Interestingly, we found that oak samples were dominated by *Alphaproteobacteria* within the *Roseobacter* clade and several of the most predominant OTUs were associated with wood boring macrofauna.

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Abbreviations:

PCR: Polymerase Chain Reaction

OTU: Operational Taxonomic Unit

Figure captions.

Fig. 1. Map showing the position of the experimental moorings in relation to the Blanes canyon (BC) and its outer slope (OS).

Fig. 2. Alpha diversity metrics of the microbial community from oak samples. Rarefaction curves (A) and rank abundance plots (B) of bacterial communities from oak immerged at different depths in the Blanes Canyon (OBC) and its outer slope (OOS).

Fig. 3. UPMGA clustering of bacterial communities from oak samples constructed using the weighted Unifrac metric. Grey nodes have jackknife support of 75-100% and dotted nodes have jackknife support of 50-75%.

Fig. 4. Percentage of the major bacteria Phyla retrieved from the oak samples. Minor phyla = phyla that constituted less than 1% of the sequences in any of the samples (*Acidobacteria*, *Actinobacteria*, *Deferribacteres*, *Verrumicrobia*). Unclassified = could not be assigned to any taxa with a probability of more than 0.8. *= *Loktanella*, Pir4lineage and *Pseudosprillum* belong to the phyla *Alphaproteobacteria*, *Planctomycetes* and *Gammaproteobacteria*, respectively.

Fig. 5. Phylogenetic tree of the 10 major OTUs found in Oak samples. The size of the circles is proportional to the number of sequences contained in the OTU.

Tables

Table 1. Details on the locations and sampling dates of the wood immersion experiment.

Trap	Seafloor depth (m)	Longitude	Latitude	Drop date	Sampling date	Duration (months)
BC 900	894	2º 54 19.14"	41º 34' 12.72"	Nov 2008	Nov 2009	12
BC 1200	1195	2º 50' 49.26"	41º 31' 15.06"	Feb 2009	Nov 2009	9
BC 1500	1468	2º 52' 58"	41º27' 28.80"	Nov 2008	Nov 2009	12
OS 1200	1184	2º 48' 54.6"	41º 13' 8.99"	Feb 2009	Nov 2009	9
OS 1500	1497	2º 53' 48"	41º 09' 0.59"	Nov 2008	Nov 2009	12
OS 1800	1806	2º 58' 9.0"	41º 04' 52.19"	Nov 2008	Nov 2009	12

Table 2. Number of sequence obtained for each sample and alpha diversity indexes. H'= Shannon diversity index. Subsampled data were calculated for 2210 sequences randomly resampled.

		All			Subsampled		
	Sequences	OTUs	H'	Chao1	OTUs	H'	Chao1
OBC900W	5916	807	5.39	1316	505	5.26	1013
OBC1200W	7388	562	4.41	815	339	4.28	551
OBC1500W	2577	341	4.79	476	318	4.76	457
OOS1200W	3046	390	4.50	579	341	4.46	502
OOS1500W	2210	274	4.53	425	274	4.53	425
OOS1800W	2756	324	4.49	570	290	4.48	506

Figure 1

Click here to download high resolution image

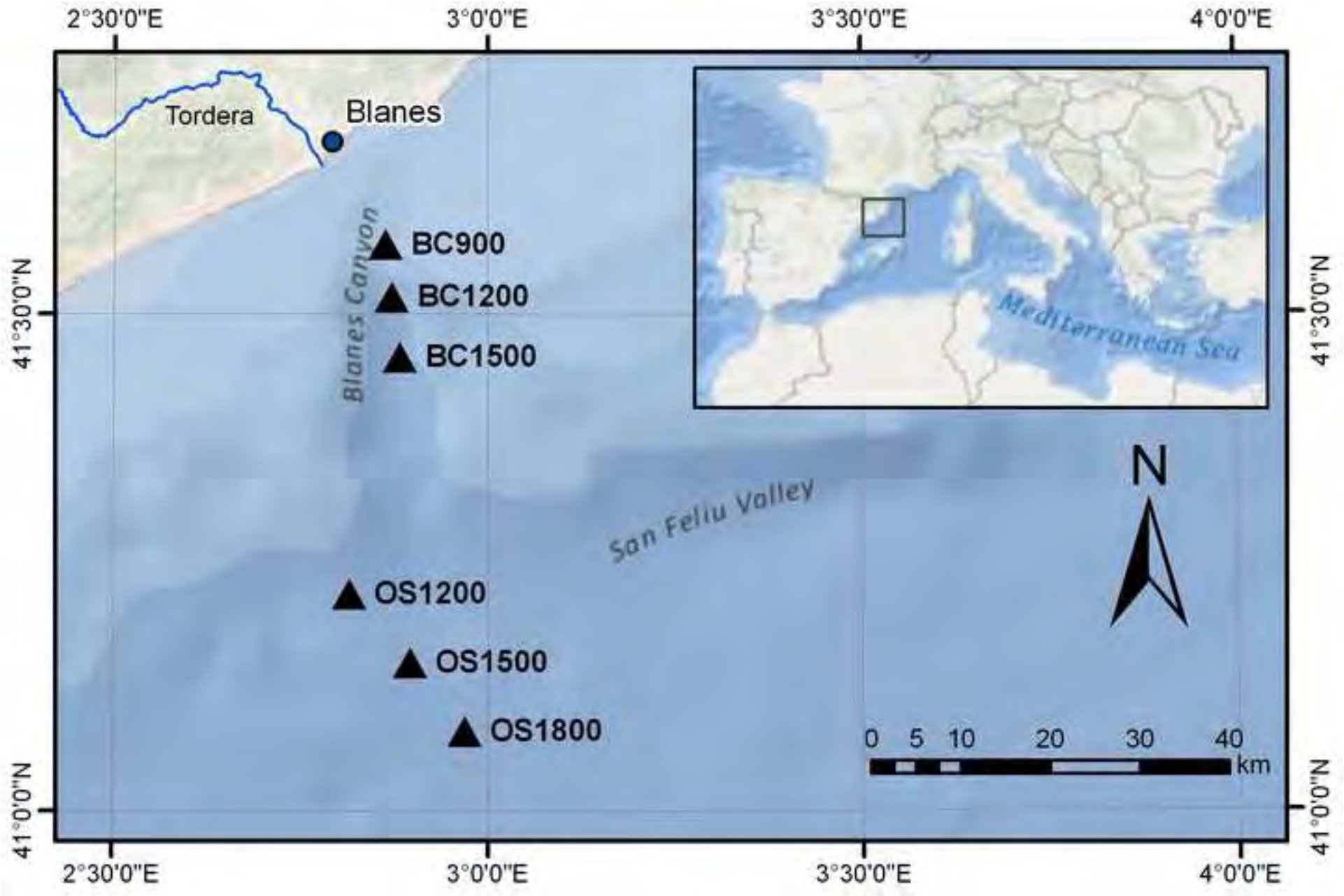
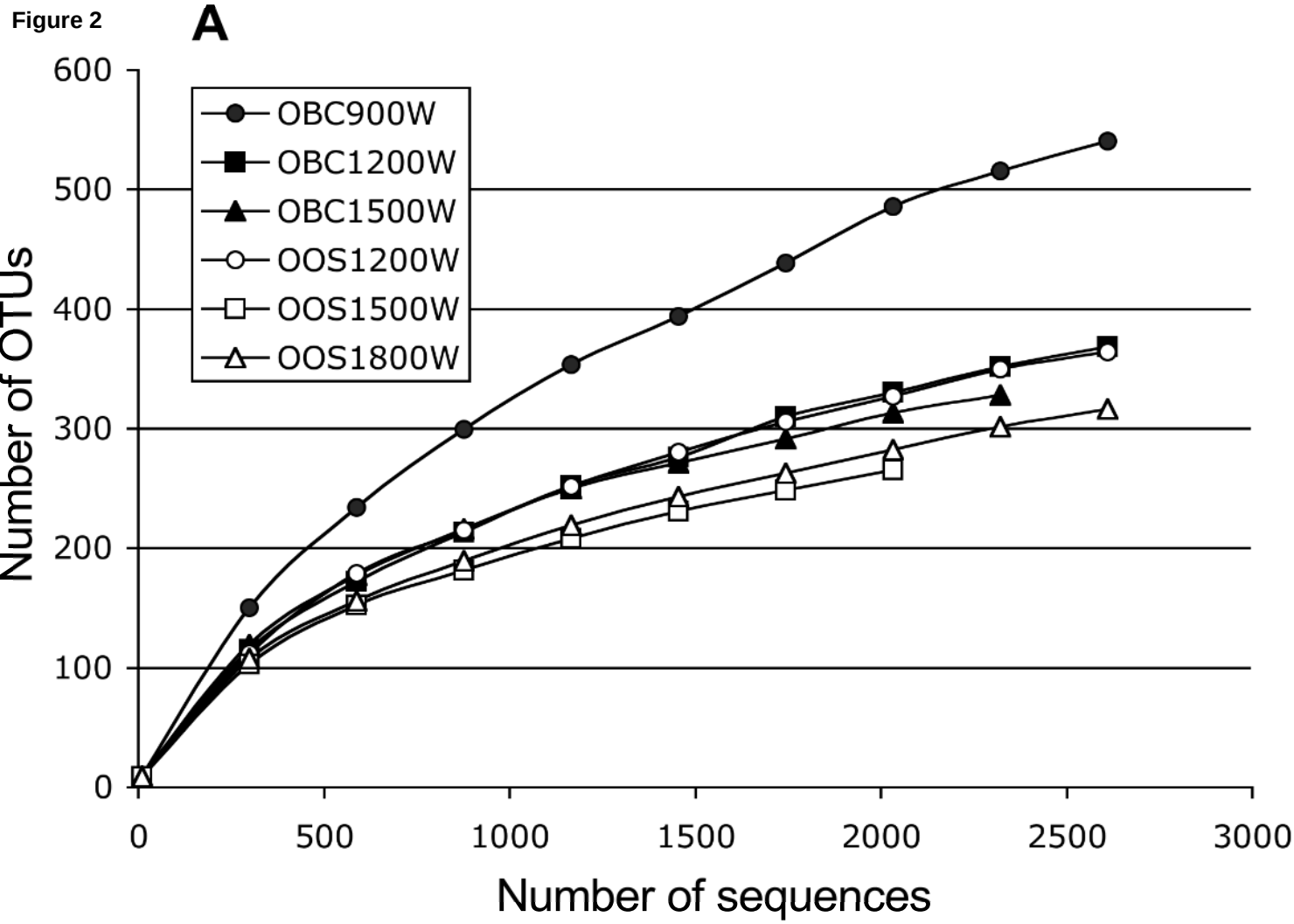


Figure 2


A
OBC900W
OBC1200W
OBC1500W
OOS1200W
OOS1500W
OOS1800W
Number of OTUs
0
100
200
300
400
500
600
0
500
1000
1500
2000
2500
3000
Number of sequences

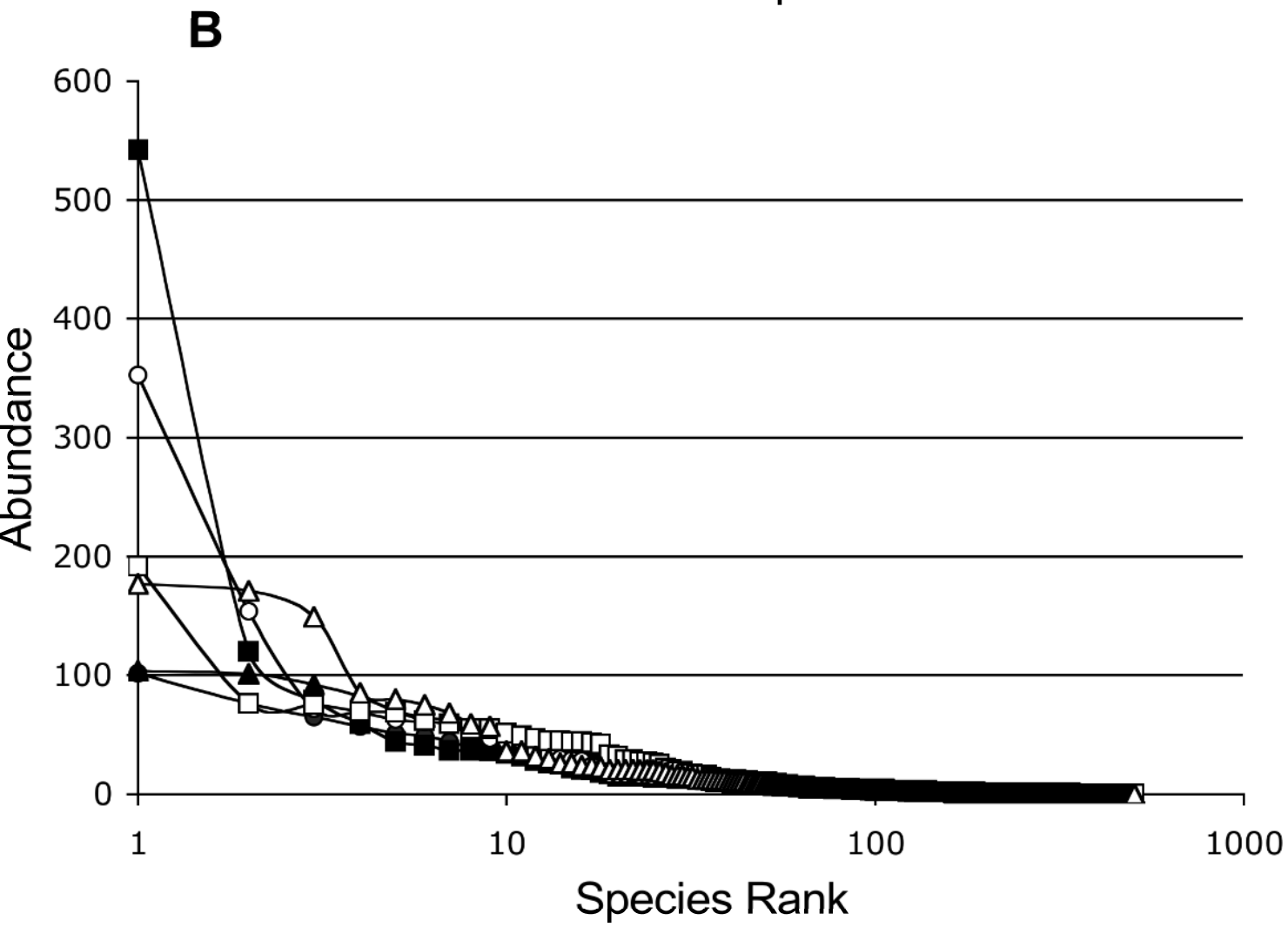

B
Abundance
0
100
200
300
400
500
600
1
10
100
1000
Species Rank

Figure 3

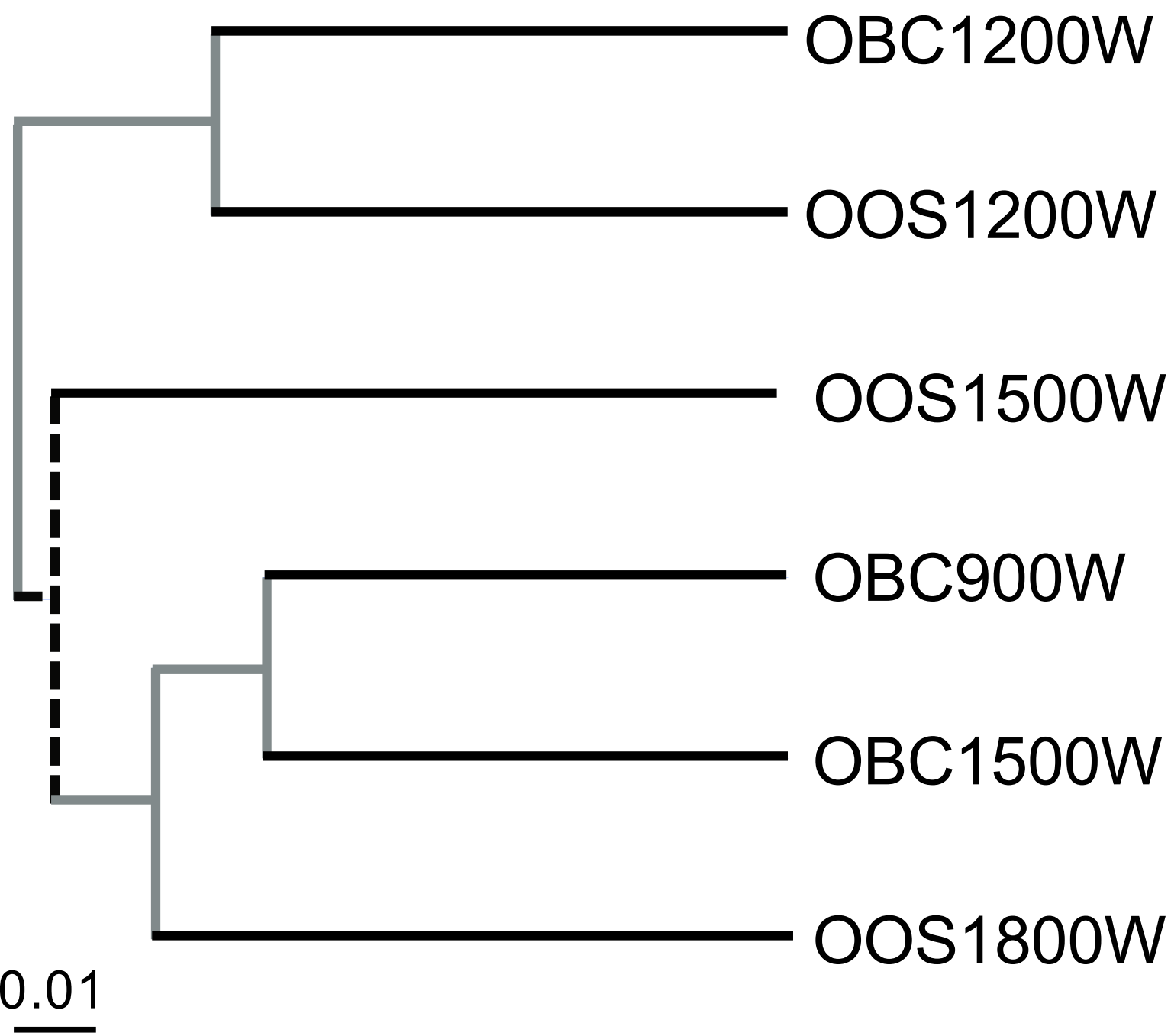

OBC1200W
OOS1200W
OOS1500W
OBC900W
OBC1500W
OOS1800W
0.01

Figure 4

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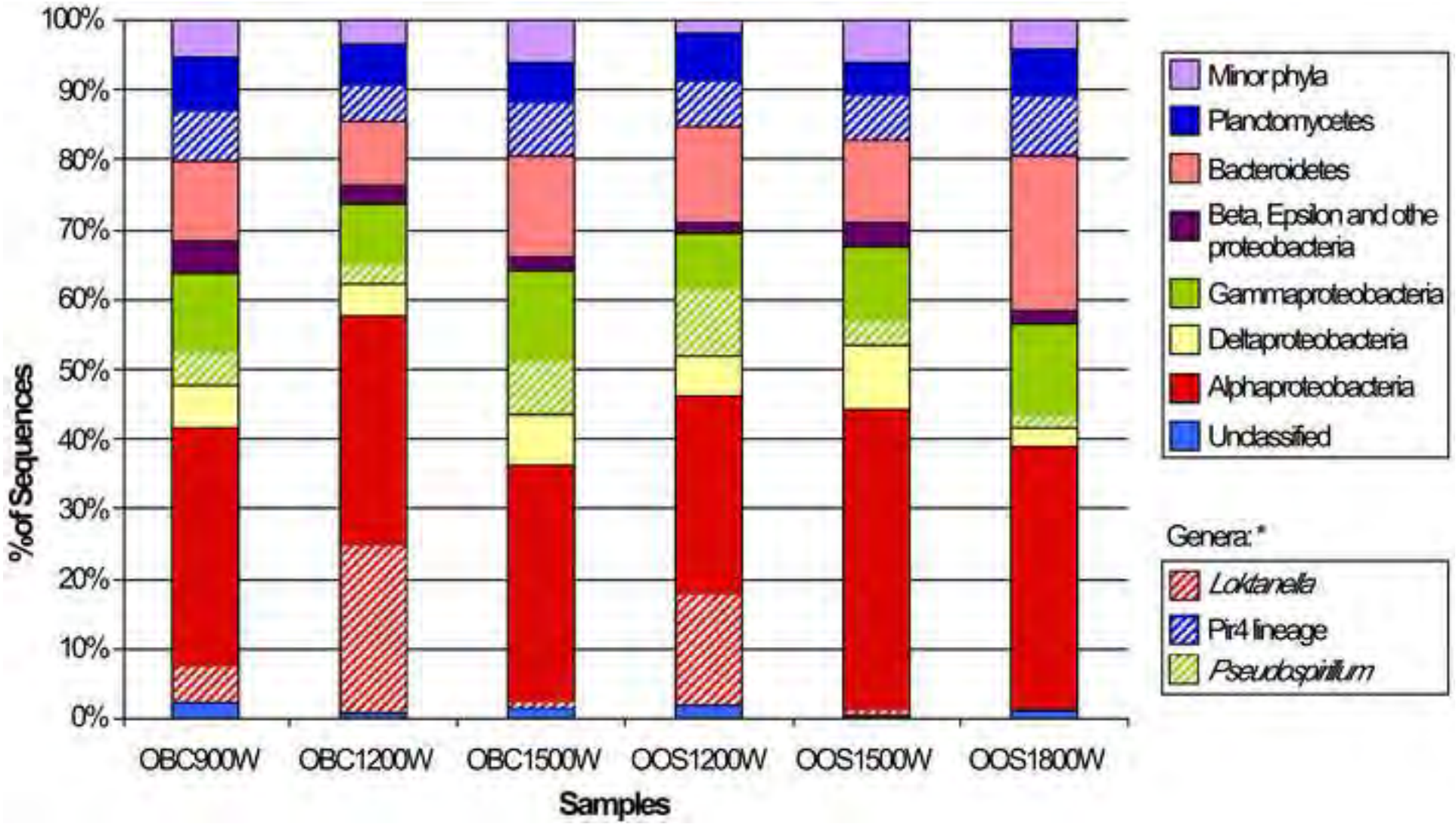

Figure 5

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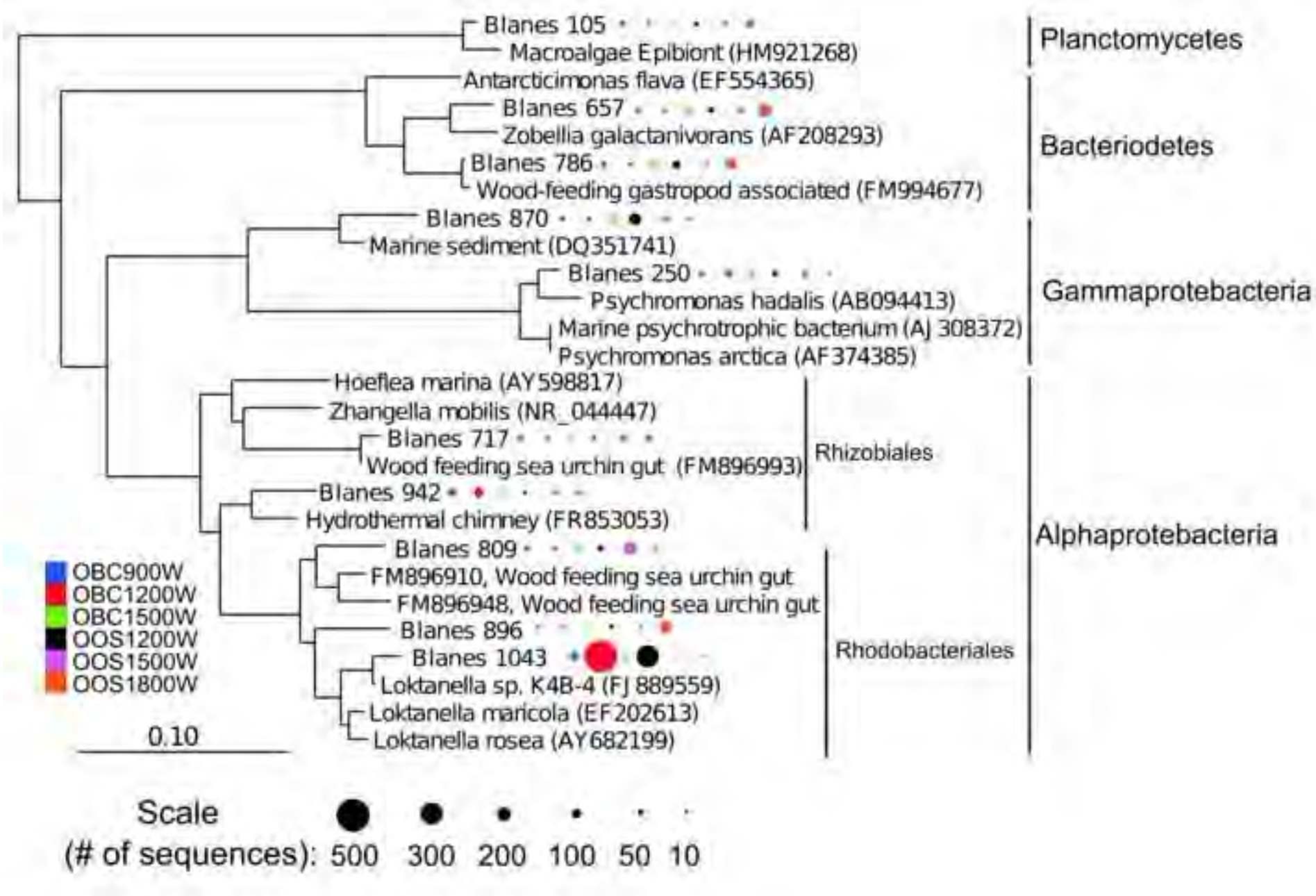

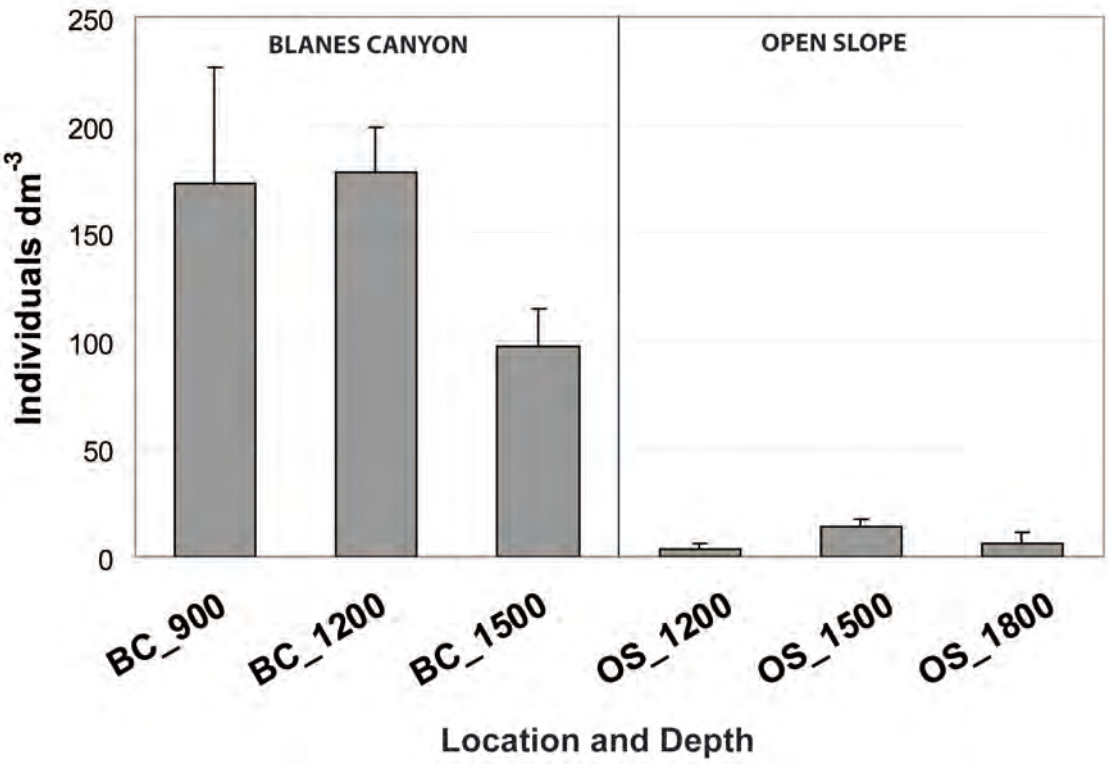

Supplemental Fig. 1. Density of *Xylophaga* spp. in the oak samples deployed at different depths inside the Blanes Canyon (BC) and in the adjacent Open Slope (OS).