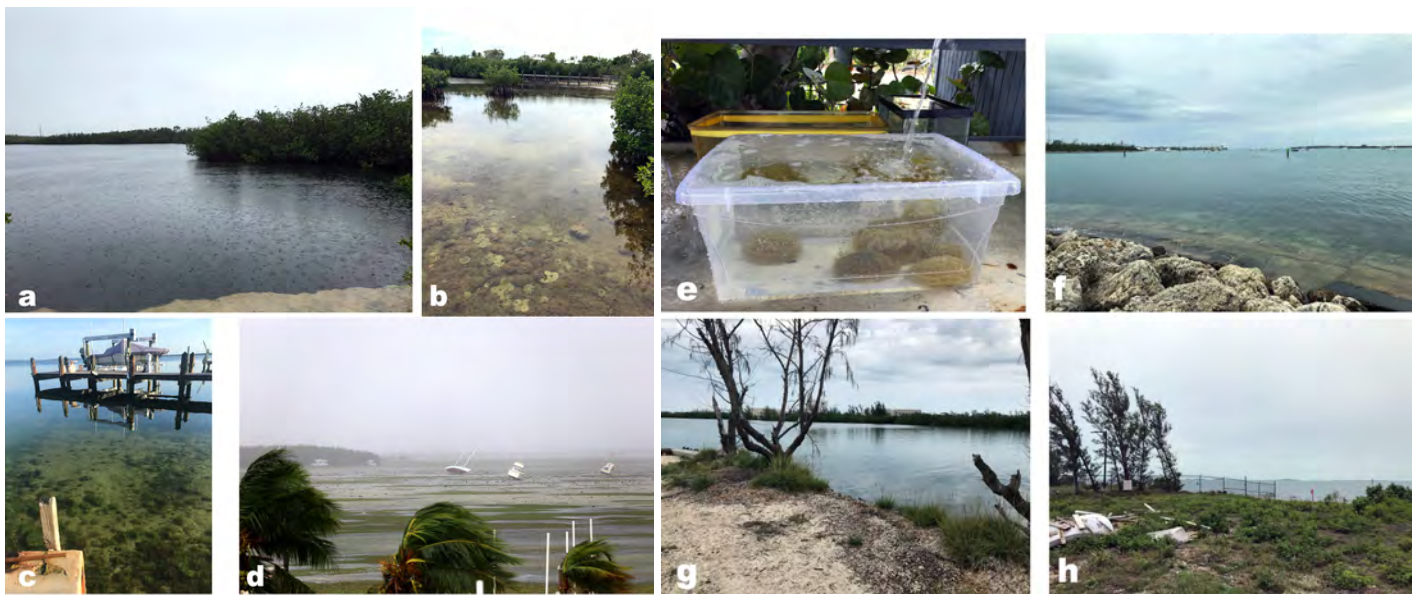
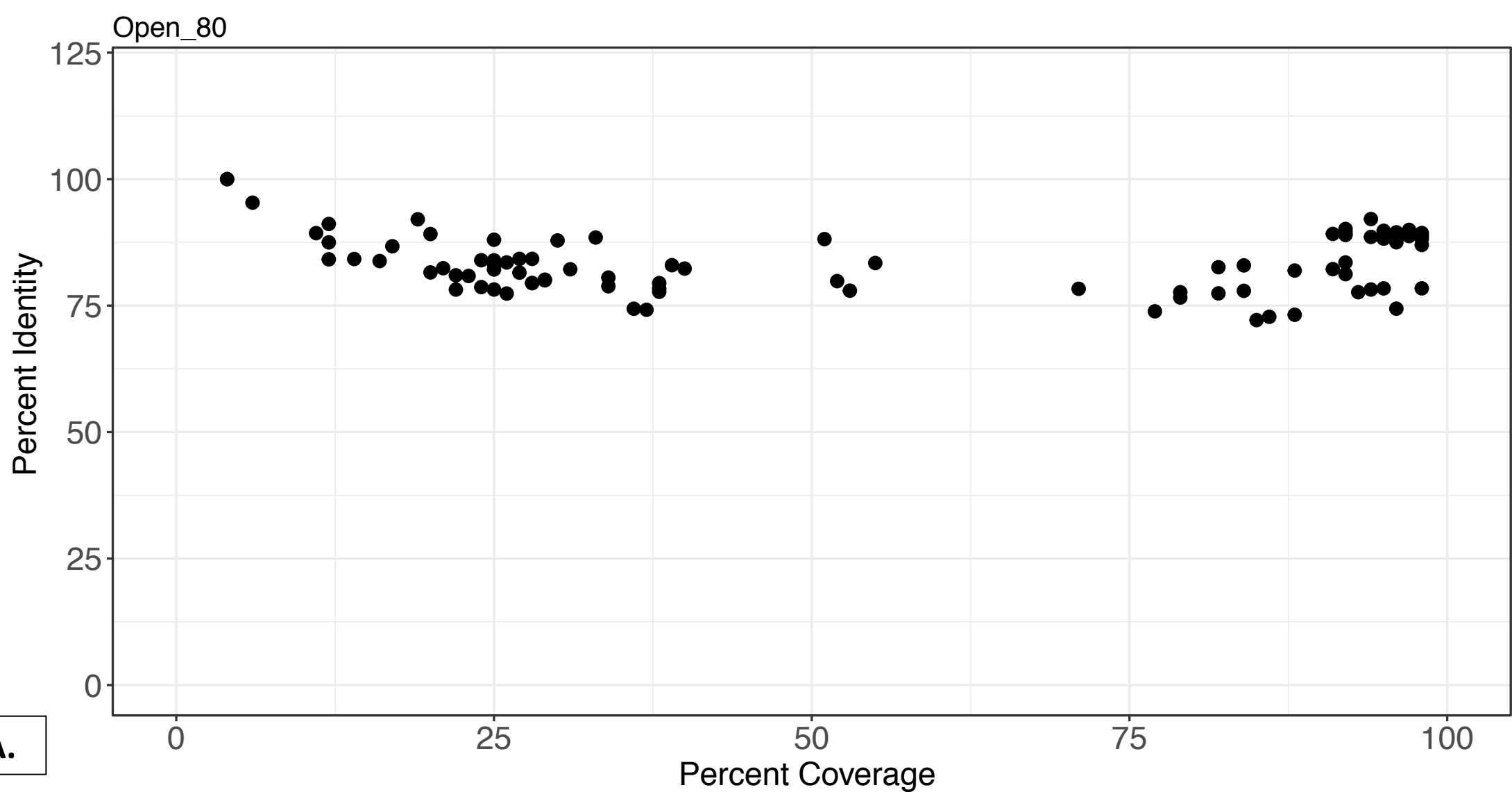
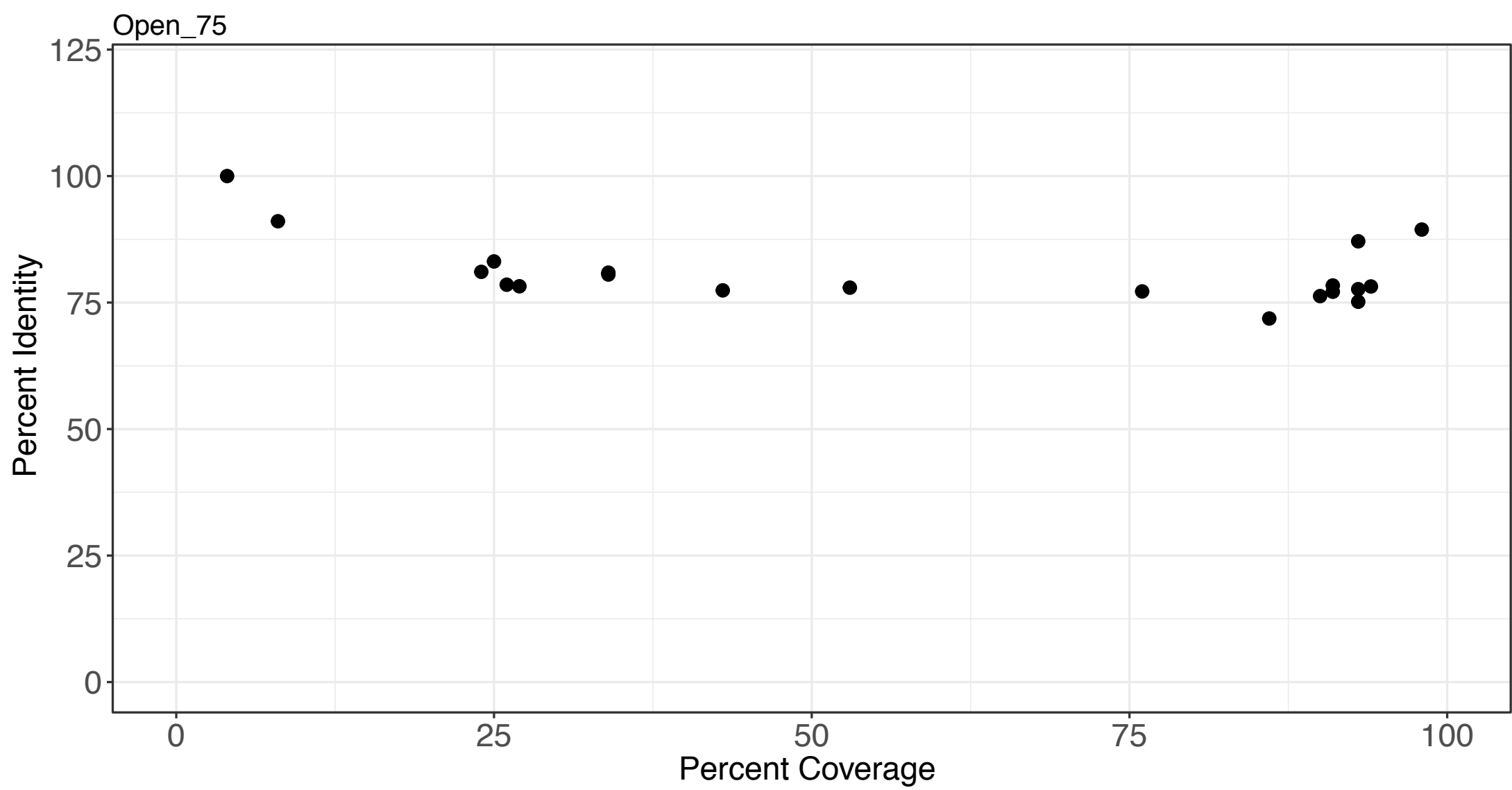
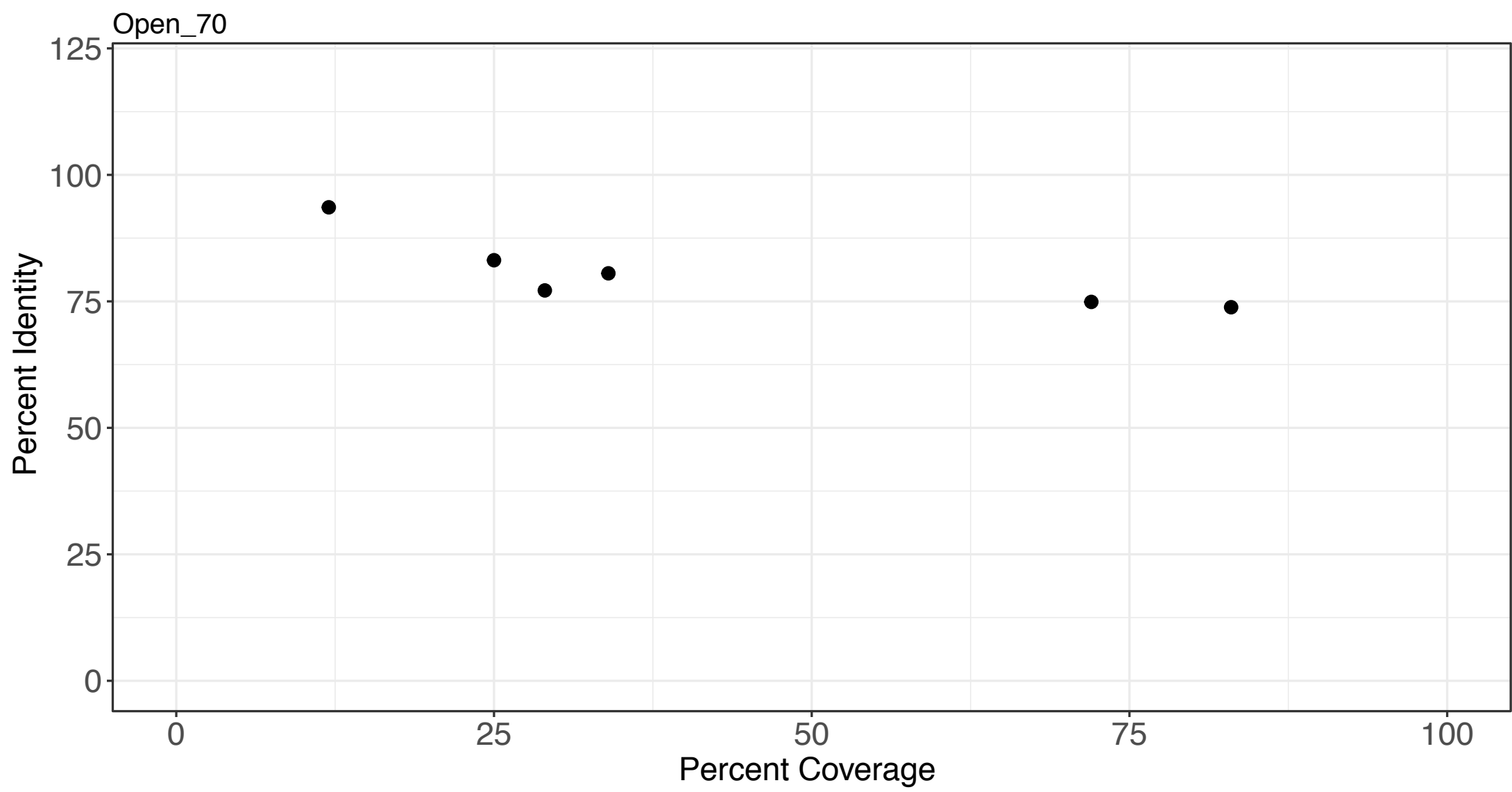


Supplementary Figure1. Typical life cycle of the upside-down Jellyfish (Cassiopea). (a) Motile planula (microscopic larval stage). (b) Four sessile polyps, attached to substrate of *Artemia* nauplii cysts. Yellow arrow denotes location in apical region of central-most polyp where groove forms during strobilation (start of metamorphosis) of apical portion of polyp. Blue arrow denotes motile planuloid following liberation as a lateral bud on a polyp. (c) Strobila (metamorphosing polyp), with yellow arrow denoting groove where strobilation (medusa metamorphosis) occurs. (d) Two free-swimming juvenile medusae, 1-2 days following strobilation and liberation from strobila apex. (Images in a-d are lab-reared *Cassiopea*). Scale bars, a=50 μm ; b-d=0.5 mm.

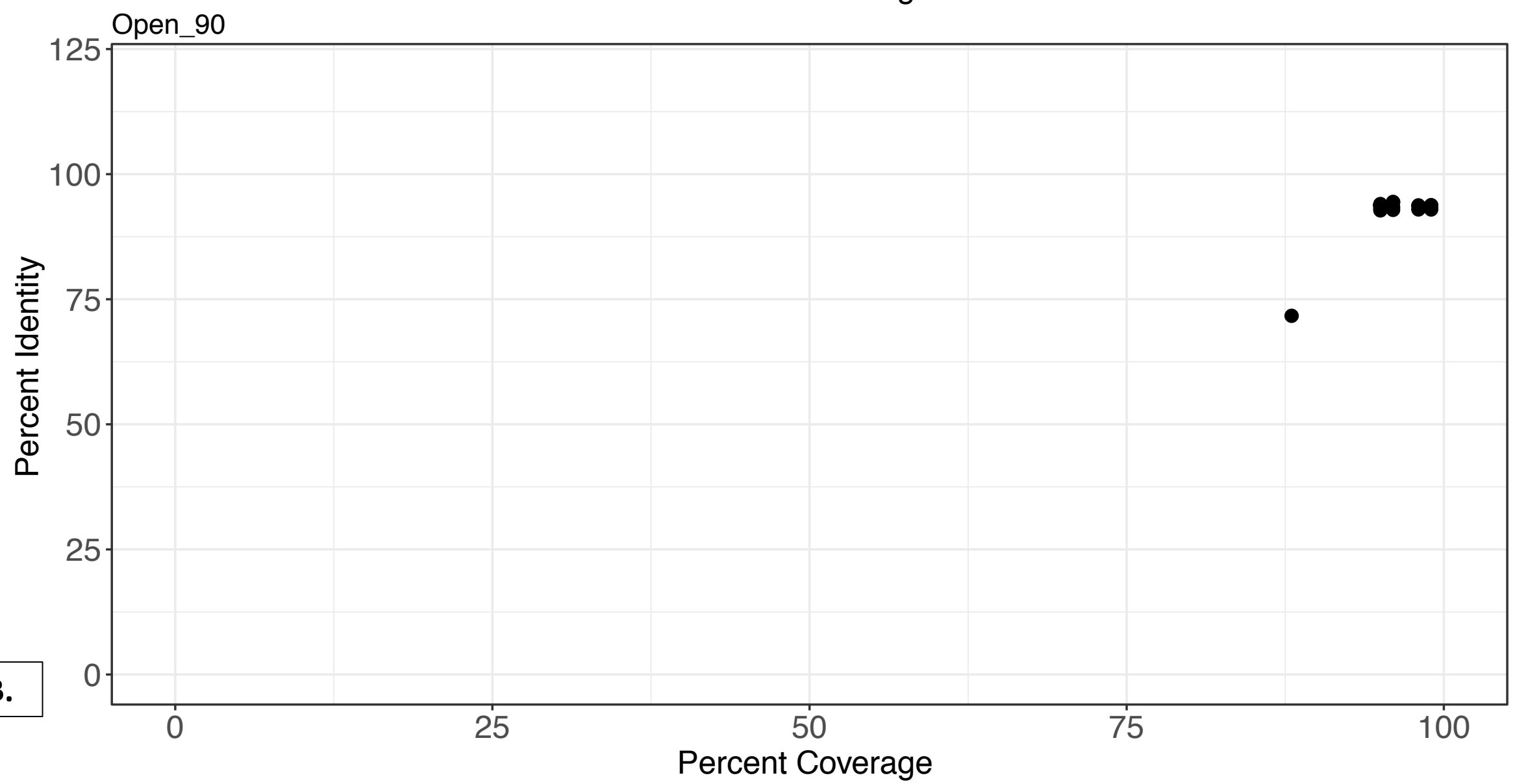
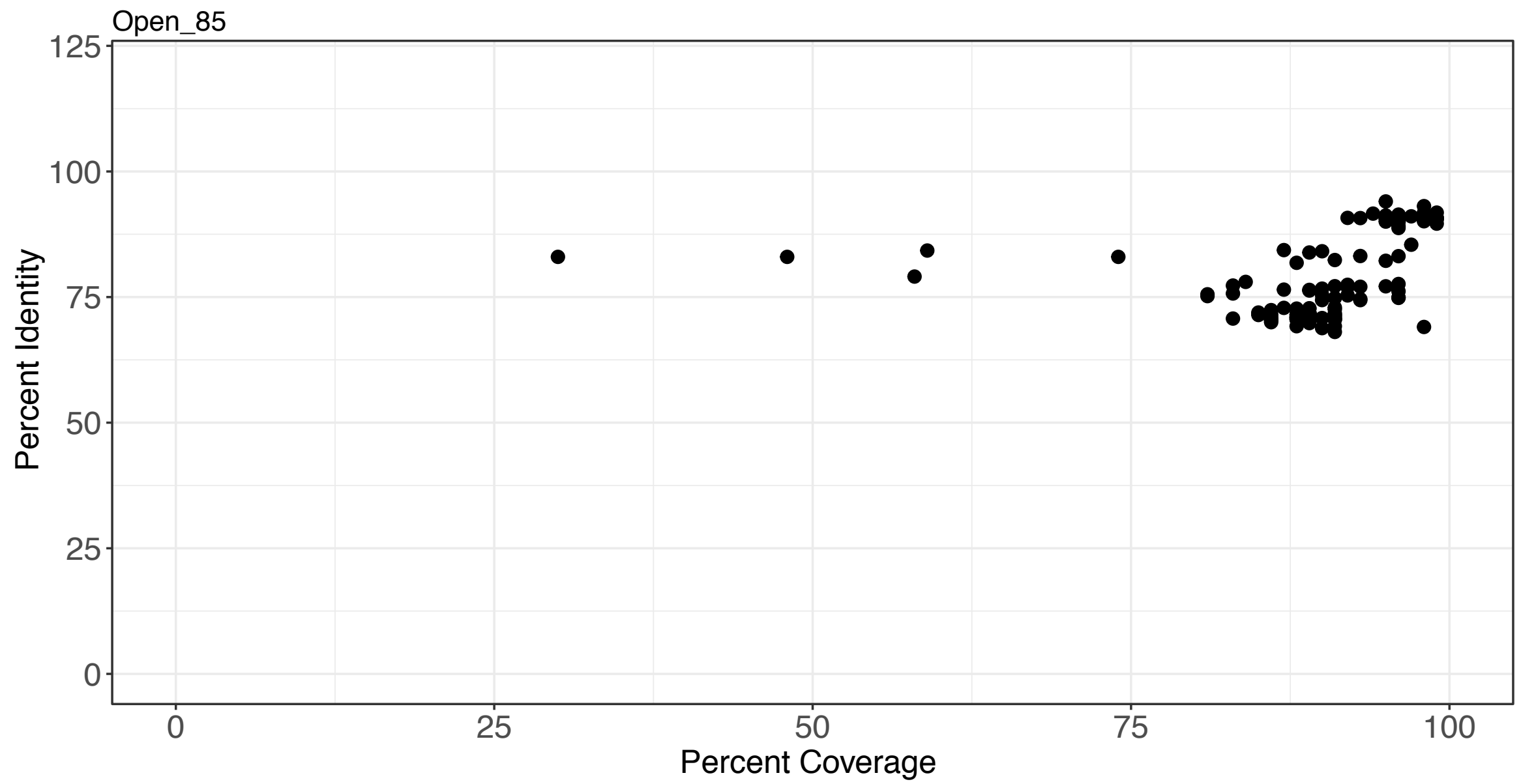


Supplementary Figure 2. Seven eDNA collection sites in the Florida Keys. (a–d) Upper Keys (Key Largo and Marathon Key). Soft substrate, varying degrees of dead mangrove leaf coverage, low visibility, weather conditions – rainy due to low pressure storm surge, sampling via plankton net from kayak. (a) Quarry (BC01). Sheltered; (b) Rock Harbor (BC02). Sheltered; (c) Buttonwood Sound (BC03). Pelagic; (d) Buttonwood Sound, September 10, 2017 showing the water in the Sound completely emptied out following direct hit by Hurricane Irma the previous day; (e) Aquarium (BC04). Positive control. Six *C. frondosa* medusae collected from Rock Harbor (BC02), held in plastic container into which natural seawater flows from Buttonwood Sound (BC03). Water sample collected from Aquarium with 1 L Nalgene jar. (f–h) Lower Keys (Fleming Key). Rocky, sandy substrate with sea grass, abundance of *Phymanthus crucifer* sea anemones on substrate, weather conditions slightly overcast, resulted in clear seawater, sampling via plankton net while snorkeling. No medusae witnessed. **Pelagic.** (f) Finger Pier (BC05); (g) SFUWOS FAA (BC06); (h) SFUWOS Drop (BC07). For geographic location details refer to map in Figure 6. Not shown here: Enclosure (BC08), Negative control.



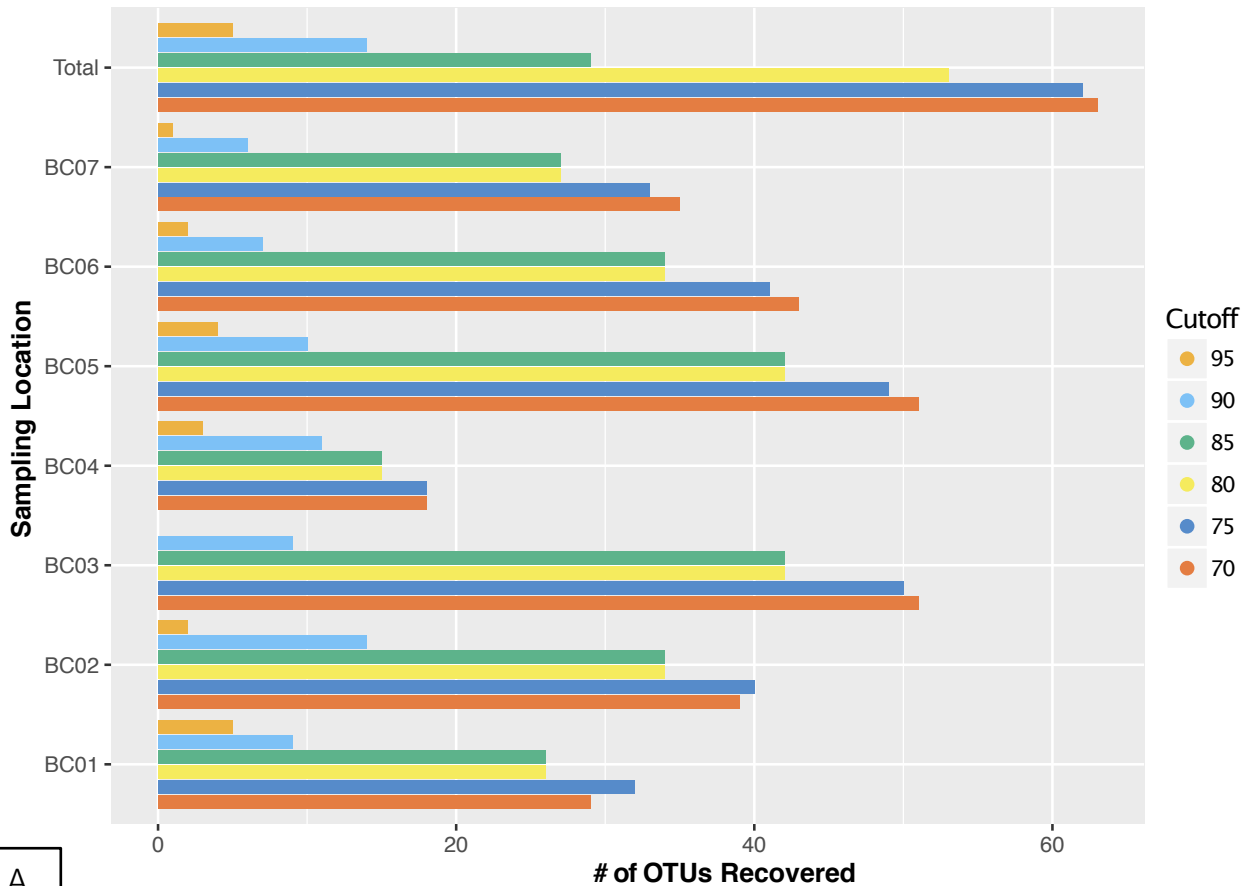
A.

Supplementary Figure 3a. Qiime2 de novo operational taxonomic units (OTUs) predicted using open vsearch revealed that in most instances over 50% of the representative sequences exhibited poor sequence alignment coverage (< 60%) to known Medusozoa sequences, despite high sequence identity.



B.

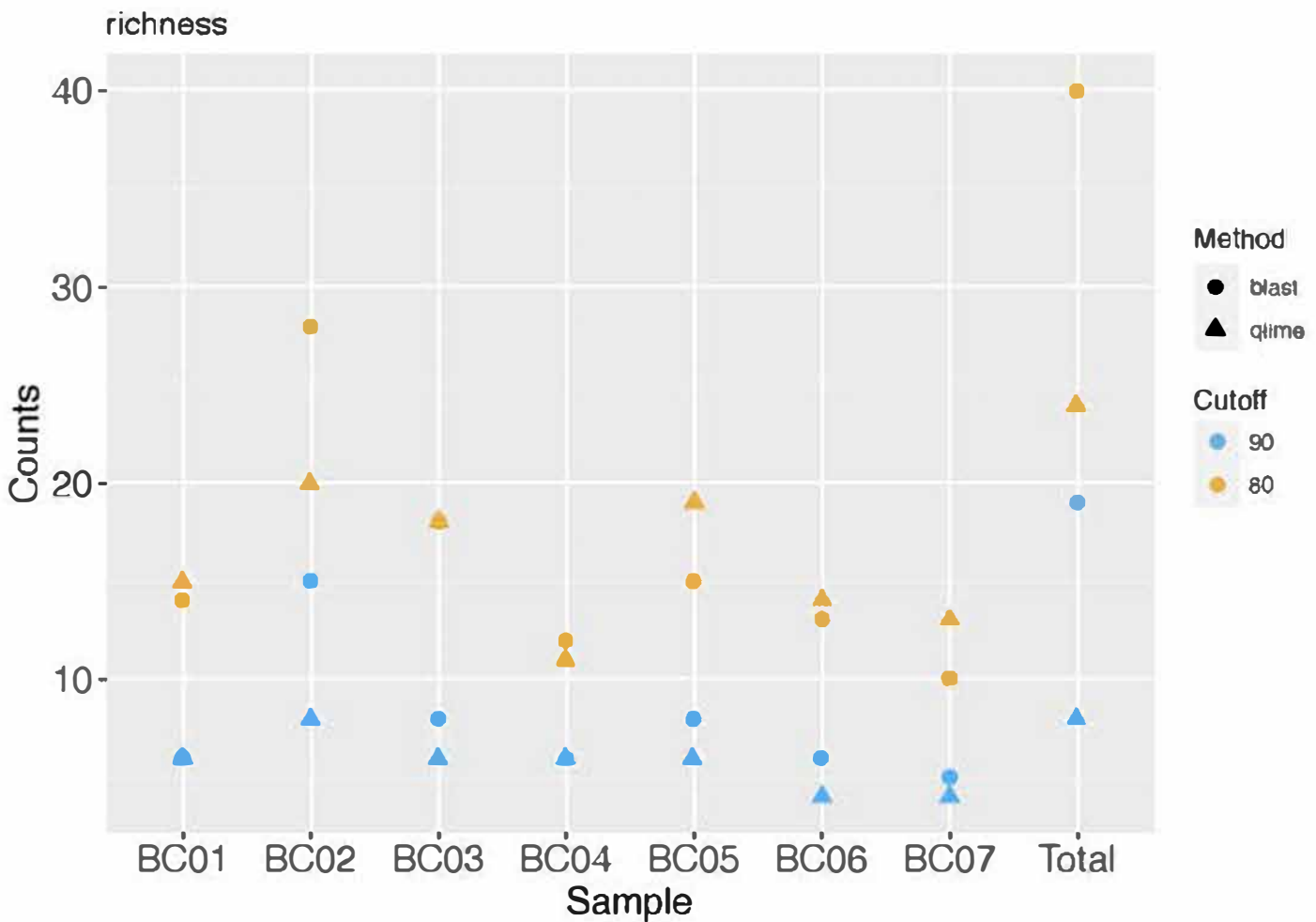
Supplementary Figure 3b. Qiime2 de novo operational taxonomic units (OTUs) predicted using open vsearch. Increasing the clustering cutoff value in open vsearch incrementally for thresholds higher than 75% led to a decrease in the number of predicted de novo OTUs, but did not improve coverage.



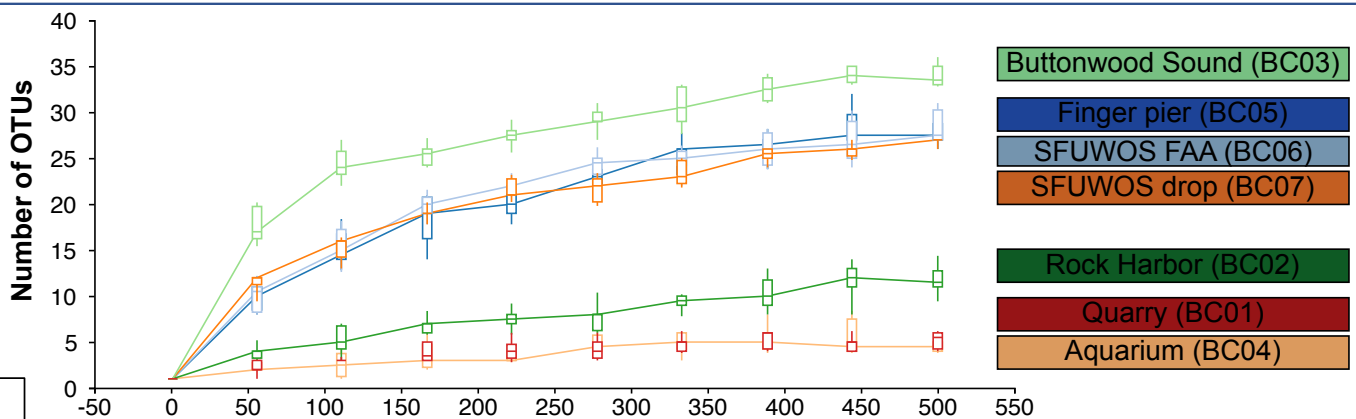
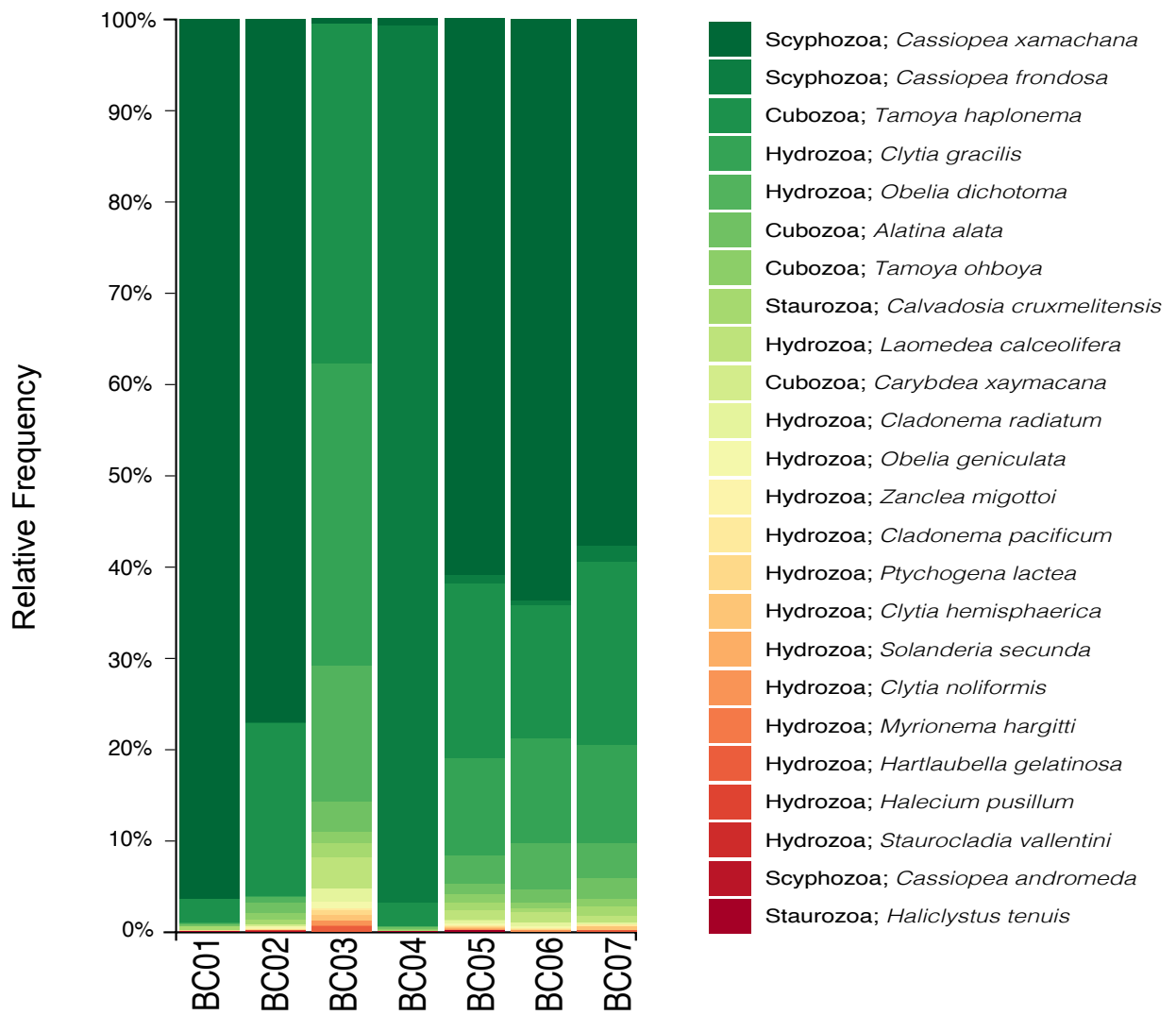
B.

	BC01	BC02	BC03	BC04	BC05	BC06	BC07	Total
# of Sequences	8484	27629	2480	17958	1643	1005	506	59705
closed_70	29	39	51	18	51	43	35	63
closed_75	32	40	50	18	49	41	33	62
closed_80	26	34	42	15	42	34	27	53
closed_85	13	24	21	15	20	18	14	29
closed_90	9	14	9	11	10	7	6	14
closed_95	5	2	0	3	4	2	1	5
Mean OTUs +/- per sample	19.5	29	31.5	15	31	26	20.5	

Supplementary Figure 4. A. Qiime2 operational taxonomic units (OTUs) predicted using closed vsearch (16S rRNA gene). In order to capture a suitable number of OTUs that might reflect the true biodiversity at sample collection sites, we conducted closed vsearch trial runs with different percent identity values (95%, 90%, 85%, 80%, 75%, 70%). B. Overall reduction in reads renaming after each incremental increase in percent identity.



Supplementary Figure 5. Comparison of species-richness of eDNA (mitochondrial 16S rDNA) samples based on Qiime2 and BLASTn classification methods, using 80% and 90% identity cutoffs. Taxonomic identity was determined by homology search against a custom MIDORI database (metazoan subset of the Gen Bank sequence database (NCBI)). BLASTN was performed with an 80% coverage value. Shapes correspond to homology search methods (Qiime2 or BLASTN), while color indicates % similar cutoff for each method {80% or 90%}. GenBank accession numbers provided in Table 2.



Supplementary Figure 6. Species-level biodiversity of jellyfish taxa (Medusozoa) in Florida Keys sampling site based on medusozoan 16S rRNA gene detection with MinION (ONT) metabarcoding results analyzed with Qiime2 software package. A. Barplot (modified from plot visualized in view.qiime2.org) of Qiime2 80% clustering, closed vsearch analysis shows relative frequency of sequences representing jellyfish species (level 7 OTUs), by sample site. B. Alpha-diversity rarefaction plot of the number of estimated OTUs (operational taxonomic units) as a function of sampling depth (normalized to sample BC04, which had the fewest number of reads after filtering, at 506). Distinct separation shown in biodiversity estimates between collections sites, with pelagic sites – Buttonwood Sound (Key Largo) and all Fleming Key sites – exhibiting higher estimates of jellyfish biodiversity, despite less visually documented biomass, while sheltered sites – Rock Harbor and Quarry (protected bays) and Aquarium (positive control) – exhibit distinct established populations of a few dominant taxa.



Supplementary Figure 7a. Heatmap reflecting background biodiversity in Florida Keys sampling site based on Metazoa COI eDNA detected with MinION (ONT) metabarcoding results analyzed with Qiime2 software package. Lighter shades indicated higher frequency (read count), while black indicates absence of sequences corresponding to a taxon. Homology search primarily returned matches to non-medusozoan insects (y-axis), indicating a dearth of COI sequences available for Florida Keys marine invertebrates in the Midori database (subset of GenBank COI data: <http://reference-midori.info/download.php#>). Medusozoan taxa shown in blue font. Sample locations are ordered (x axis) from least (BC04 – Aquarium) to highest (BC05 - Caribbean) biodiversity. Note: No MinION COI metabarcoding data were generated for BC03, as the sample failed to amplified in the first PCR.

>MRConsensus_of_17_i90c80_AY319467_Cassiopea_froncosa_COI Alignment of 17 sequences

```
GTCAACAAATCATAAAGATATTGGAACACTCTATTTAATATTTGGTATATTTGCTGGTGTATTAGGCGCA
GGTTTCAGTATGATAATTAGATTAGAACCTTCTGGGCCAGGATCTATGTTAGAGATGACCACTTATATAA
CGTAACTGTAACAGCCCATGGATTAATAATGATTTTCTTTTTTGTATGCCCGTCTTATTAGGGGCCCTTG
GTAATTGATTCATACCCATACGTAGGAGCACCTGACATGGCATTTCACGCCTTAACAATATAAGCTTT
TGATTATTACCTCCAGCAATTTTACTATTACTAGGATCTTCCTTAGTAGAACAAGGAGTAGGAACAGGTT
GGACAATATATCCACCACTCAGCTCCATTCAAGTGCATTCCGGAGGTTTCAGTAGATATGGTTATATTTAG
CTTACATTTAGCGGGTGTTCATCTATACTAGCTTCAATAAATTTTATAACTACAATTCCTTAATATGAGG
GCCCCCTGGAATGACTATGGATAAAAATGCCTTTTATATGTTTGATCAGTACTAGTTACAGCTGTACTATTA
TATTATCATTACCTGTTTTTGGCCGAGCAATCACAATGTTATTAAGTATAGAACTTTAATACCTCATT
TTTGATCCTGCAGGGGGGA
```

Note that this sequence does not translate due to the inference of five deletions within the sequence, based on an alignment to the genbank entry AY319467. To assess the inferred deletions, we substituted “N” for the inferred deletions, as in:

>MRConsensus_of_17_i90c80_AY319467_Cassiopea_froncosa_COI Alignment of 17 sequences, RC, Gap Corrected

```
GTCAACAAATCATAAAGATATTGGAACACTCTATTTAATATTTGGTATATTTGCTGGTGTATTAGGCGCA
GGTTTCAGTATGATAATTAGATTAGAACCTTCTGGGCCAGGATCTATGTTANAGATGACCACTTATATA
ACGTAACGTAACAGCCCATGGATTAATAATGATTTTCTNTTTTTGTTATGCCCGTCTTATTAGGGGCCCTT
TGTAATTGATTCATANCCCTATACGTAGGAGCACCTGACATGGCATTTCACGCCTTAACAATATAAGC
TTTTGATTATTACCTCCAGCAATTTTACTATTACTAGGATCTTCCTTAGTAGAACAAGGAGTAGGAACAG
GTTGGACAATATATCCACCACTCAGCTCCATTCAAGTGCATTCCGGAGGTTTCAGTAGATATGGTTATATT
TAGCTTACATTTAGCGGGTGTTCATCTATACTAGCTTCAATAAATTTTATAACTACAATTCCTTAATATG
AGGGCCCCCTGGAATGACTATGGATAAAAATGCCTTTTATATGTTTGATCAGTACTAGTTACAGCTGTACTAT
TAATATTATCATTACCTGTTTTTGGCCGAGCAATCACAATGTTATTAAGTATAGAACTTTAATACCTC
ANTTTTTGATCCTGCAGGGGGNGGA
```

And compared the amino acid sequence of our consensus to that of AY319467. We observe that each of ambiguous base calls, N, yield an ambiguous amino acid, X, as shown below. Therefore, we chose to forego GenBank accession.

✓ Query_10001	1	STNHNKDIGTLYLIFGIFAGVVLGAGFSMIIIRLELSGPGSMLXDDHLYNVTVTANGLIMIFXVMPVLLGAFGNWFIKLYVG	80
✓ Query_10002	1	-----GVLGAGFSMIIIRLELSGPGSMLGDDHLYNVTVTANGLIMIFFVMPVLLGGFGNWFIFLYVG	62
✓ Query_10001	81	APDMAFPRLNNSIFWLLPPAILLLLGSSLVEQGVTGWTIYPPSSIQVBSGGSVDMVIFSLHLAGVSSILASINFITTI	160
✓ Query_10002	63	APDMAFPRLNNSIFWLLPPAILLLLGSSLVEQGVTGWTIYPPSSIQVBSGGSVDMVIFSLHLAGVSSILASINFITTI	142
✓ Query_10001	161	LNMRAPGMTMDKMPYVWSVLVAVLLILSLPVFAGAITMLLTDRNFNTSXFDPAAGXG----	218
✓ Query_10002	143	LNMRAPGMTMDKMPYVWSVLVAVLLILSLPVFAGAITMLLTDRNFNTSFFDPAGGGDPIL	204

Supplementary Figure 7b. COI consensus of sequence reads that correspond to COI of *Cassiopea frondosa* (AY319467), with greater than 80% coverage and 90% identity.