Abstract

The cryptic nature of capitellid worms (Class Polychaeta), commonly studied as bioindicators for pollution, has led fellow student, Sebastian Bergen, to investigate their developmental biology in detail for his honors thesis. His research was spurred by Dr. George von Dassow's discovery that some capitellid larvae practice macrophagous carnivory in the plankton, a little known, but possibly widespread, larval strategy. The trouble was associating these observations with a particular species of *Capitella*. Reports of capitellid species for the Charleston, OR area include "Capitella spp.," in reference to the cryptic species complex *C. capitata*, an umbrella for some ~13 species. Developmental features and Laval mode are unknown for many species of *Capitella*, but differences in these have allowed researchers to distinguish between some species in the C. *capitata* complex. Aiding Bergen's efforts to identify the unknown larvae, we attempted to amplify and sequence two mitochondrial genes (16S, COI) from each individual in the hopes of finding a "parent" sequence within the GenBank database using BLAST. We also sought to confirm the identity of a local adult with lecithotrophic development. Of the 17 samples collected by Bergen between Spring and Summer 2021, only three were successfully sequenced. The adult worms (+larva) turn out to belong to *Capitella teleta*. It is clear that the GenBank database for *C. teleta* is well developed. BLAST sequences originated internationally (S. Korea, China, Portugal, Greenland, and the U.S.) indicating the possible cosmopolitan biology, making *C. teleta* a more accessible model to study the general biology of the *Capitella* genus. While we were unable to identify the carnivorous capitellid larvae, we can confirm the presence of *C. teleta* in Charleston, OR.

Introduction

The genus *Capitella* refers to a group of marine worms commonly found all over the world, most commonly in benthic studies (Pernet et al., 2015). WoRMS describes about 200 species belonging to 43 genera for the family Capitellidae (WoRMS, 2016). Two commonly studied groups, of which Bergen focuses on in his unpublished summary are *C. capitata* and *C. telata. C. capitata* comprises ~13 different species with nearly identical adult morphology (Grassle & Grassle, 1976, Blake et al. 2009, Silva et al. 2017). They differ from each other in allele combination and development. To understand the evolutionary history of larval strategies among the species we first must understand their form and function.

Adults of *Capitella* are very difficult to find and most of the specimens used in this study were captured in the larval stage and raised on the Oregon Institute of Marine Biology campus. The interest in capturing adults was to induce mating and observe possible behavioral differences between larvae that were born in the lab and those captured in the estuarine environment (Bergen direct communication, 2021). Additionally, identification of the adults were needed to attribute to species-specific observations.

The morphology and habit quirks for the captured specimens were observed for their feeding behavior, the study of their bodily and organ development, size increase, segmentation, and metamorphosis. Larvae were observed throughout their developmental process. All of these characteristics are morphologically based by Sebastian Bergen for the initial part of his unpublished summary of the worms. The effort of this study is to provide genetic analysis of the provided worms, so Bergen can relate his observations with a particular species of *Capitella*. The genetic analysis performed will not only further the understanding of the diversity of *Capitella* but allow behavioral and morphological quirks to be assigned. We predict this genetic analysis

will more frequently hit *C. telata* compared to the *C. capitata,* which lacks adequate genetic sequencing based on the preliminary observations made by Bergen.

Materials and Methods

Sample Information and Origin

Samples used in this study were sourced in the spring by Bergen from the heavily polluted Charleston Boat Basin (Bergen, 2021). If adults were found they were bred in the lab, and their subsequent larvae were observed. If adults were not found identification was reliant on genetic analysis. Sample titled Settlement B was obtained in the summer, where the goal was to determine settlement cues by placing one matured larva in a vial with mud collected from the boat basin. Also, collected during the summer was the morphologically identified *Capitella capitata* however, it was observed that the larvae were lecithotrophic, not planktotrophic. It was decided that although this nutritional mode differed from the other collected samples genetic analysis would still be relevant and informative.

A total of 17 *Capitella* samples are provided by S. Bergen that had been collected over the Summer of 2021. Larval samples were collected by a plankton tow in the Charleston Boat Basin, OR and adult samples were collected in the sediment of the Portside Mudflats, Charleston, OR (Bergen, 2021). Background information, photos, and sample information were all supplemented (**Figures 7-10**). S. Bergen estimated 20-30 larvae specimens were collected from plankton tows in the Spring of 2021, composing samples Cap-(A-N). Samples Settlement B, Adult 1-2, and their brooded larvae WB 1-2, were morphologically identified as *Capitella capitata* by S. Bergen, collected in the Summer of 2021. Settlement B and Adult 1-2 were collected from the Portside Mudflats. Samples WB 1-2 were matured larvae that metamorphosed in the lab. All samples were then cryopreserved (frozen in -80°C) in a minuscule amount of

seawater in 1.5 ml microcentrifuge tubes. All of the preceding processes were conducted by Bergen, S.

Genomic DNA Extraction and PCR Analysis

DNA extraction of all 17 *Capitella* samples was accomplished using the InstaGene Matrix (Bio-rad) following the manufacturer's procedure. This procedure was favored for its easy application to small and delicate animals; while also maintaining efficiency and minimizing sample transfer between tubes.

We attempted to amplify two gene regions, COI and 16S, using universal primers (Palumbi et al., 1991) for 16S, and LCO1490/HCO2198 (Folmer et al. 1994) [C1] for COI. When amplification with universal primers failed, we adjusted the annealing temperature and/or tried using degenerate COI primers jgLCO1490/jgHCO2198 (Geller et al. 2013). The final concentration of the master mix reagents were: 1X of 5X Green Buffer, 200 μM of dNTP mix 10mM, 1 U/Rx of Go Taq Polymerase (5 U/μl), 500 nM of forward and reverse universal primers (10μM) in the respective reagents, and Nuclease-free water (was added but final concentration was not given). All coming to a total volume of 20 μl for each 16S and COI master mix.

We used the following thermocycler program: initial denaturation at 95°C for 2 min, 35 cycles of cycle denaturation 40 sec at 95°C, primer annealing for 40 sec at 45°C (COI) or 50°C (16S), primer extension at 72°C for 1 minute, followed by a final extension to polish ends at 72°C for 2 min. To adjust for troubleshooting, lower annealing temperatures of 42°C (COI) and 48°C (16S). For COI samples using degenerate primers, the temperature was maintained at 45°C.

PCR products were verified by gel electrophoresis, (1% agarose gel) and image using a UV gel imager.

Purification was conducted using the materials and procedure from the Wizard SV gel and clean-up kit by Promega. Samples approved for sequencing were sent to Sequetech in Mountain View, CA to be sequenced in both directions; sequencing was of the Sanger variety. DNA sequence analyses were conducted using Geneious Prime Version 2022.01. Sequences with a HQ% lower than 50 were omitted from the analysis. Those that had an HQ% greater than 50 were trimmed and aligned with their complementary strand (MAFFT plug-in). Disagreements between the two sequences and low-quality ends were manually resolved or trimmed off. Afterwards, consensus sequences are generated. COI consensus sequences were converted into amino acids and checked for the presence of stop codons. We BLASTed all consensus sequences against the GenBank database, downloaded the top 10 matches, and aligned them with our sequences to produce neighbor-joining trees. All alignments were inspected for the presence of gaps and trailing ends. Analytic parameters assessed were the number and size of clades and the placement of our query sequences within the distance trees. Supplemental "distance" view tables (distance matrices) complement our distance trees by determining the number of putative species in relation to our query sequences based on identity percentage. All clade determinations were made using a 4% cutoff for COI and 2% was used for 16S (scale bars on phylogenetic trees have been readjusted to fit the cutoff standards).

Results and Discussion

PCR

Of the 17 samples, only three were successfully sequenced, yielding four good quality sequences: Adult 1 (COI and 16S), Adult 2 (16S), and WB1 (COI). These three samples were identified to the species level as *Capitata teleta*, a species with a surprisingly large global

distribution, based on the origins of the sequences from our BLAST result. The origins of the top BLAST sequences originated from the following countries: S. Korea, China, Portugal, Greenland, and the U.S.

The low PCR/sequencing success in this study (COI -2/17. 16S- 2/17; total 11.8%) could be explained by incompatibility of the primers we used with the unknown larvae DNA. Universal primers worked for *Capitella teleta* (Adulta 1, 2, WB1), but alternate primers will likely be needed to amplify either gene from the unknown larvae.

Previous barcoding of Capitella species (Tomioka et al. 2016, Silva et al. 2017) suggest Capitella- specific COI primers Capitella_COI_F/Capitella_COI_R, or degenerate COI primers, dgLOC1490/dgHCO2198 (Meyer 2003), may improve our success.

PCR Troubleshooting

For the 16S samples, troubleshooting with a lower annealing temperature resulted in no DNA amplification, possibly due to procedural conspecifics between other methods and InstaGene, as mentioned above. This result strongly suggests that 16SARL/16SBRH universal 16S primers are not compatible with our samples. Troubleshooting COI samples with faint bands using a decreased annealing temperature (42°C) only resulted in amplification of Settle B.TS1 and CA TS1. Since the original sample, Settle B, was composed of an unknown Capitella larva that was cultured and isolated in sediment by Bergen, the likelihood of predisposed impurity is high. As a result, the amplification of a moderately bright band may be induced by the contaminant and not the sample itself. Since all other samples in the plate (with the exception of CA TS1) were amplified. Although amplification occurred, we are unable to accept that lower the annealing temperature was effective; the quality of the amplification may suggest that the primer is not fully compatible with unknown *Capitella* larva. For COI samples with very faint

bands, PCR troubleshooting was conducted using jgLCO1490/jgHCO2198 degenerate primers and maintaining the default annealing temperature (45°C). Samples CI TS2 and CJ TS2 amplified with very faint bands, indicating possible species-primer incompatibility. Decreased annealing temperatures should have allowed the primers to bind nonspecifically to the CI TS2 and CJ TS2 DNA template, resulting in successful amplification of a single bright band (which occurred in CM TS2). The resulting amplification for CI TS2 and CJ TS2 may suggest that the DNA is incompatible with the universal primers at regular annealing temperatures. However, using degenerate primers at the regular annealing temperature has allowed the primers to successfully bind and amplify. If we had the time for an additional rerun, we could attempt to lower the annealing temperature by another 2°C (41°C) while maintaining the jgLCO1490/jgHCO2198 degenerate primers in our Master Mix. If successful amplification still fails to occur, it would be reliable to conclude that the primer is incompatible with the DNA. Further temperature decrease will not be suitable, since annealing temperatures that are too low will result in the formation of undesired non-specific duplexes which may reduce amplification efficiency (Integrated DNA Technologies).

All samples failed to produce adequate (bright) primer dimers, CA .TS1 and Settle B.TS1 produced moderately bright primer dimers. Only Settle B.TS1 amplified with a moderately bright band. All purified samples were sent for sequencing. TS1 refers to lower annealing temperatures (2°C for COI and 3°C for 16S). TS2 refers to jgLCO1490/jgHCO2189 degenerate primers.

BLAST Results

Only samples Settle B, WB1, and Adult 1 were of acceptable HQ% and were moved through the BLAST process. The following discussion and (**Table 1)** will reflect these three

samples for COI. The BLAST results for WB1 and Adult 1 did not match with the hypothesized species-level identification, morphologically identified as *C. capitata* by S. Bergen. Instead both samples had a species-level match with C. *telata*.

Settle B, unaligned sequences had a high-quality HQ% and reasonable sequence length that allowed confidence in a further investigation of the samples' species-level identification (**Table 1**). The consensus sequence for Settle B, 91.7% with a length of ~724 came back from BLAST with a top match to *Entomoneis sp.* The query coverage was on the lower side at 70.86% telling us there was an acceptable amount of sequence overlap with the database but not a perfect match. Identity, 91.8% reveals some base pair mismatch, and the E value of zero tells us we have a homologous hit to our query sequence. This confirms that Settle B was contaminated, since it was a completely different organism from the unknown *Capitella* observed by S. Bergen.

Sample WB1, COI unaligned sequences had a good and expected length with an accepted HQ% (**Table 1**). The consensus sequence for WB 1 had a quality of 100% telling us the sequences lined up accordingly with no mismatch of base pairs which is confirmed by the 100% Identity. The top BLAST hit identified *C.telata,* as the species level ID. The high query coverage, 96.49 %, and E value of zero support the identification. This top hit is the expected genus hypothesized at the beginning based on morphology by S. Bergen. The species-level identification was not given, but sequencing suggests that WB1 is *C. telata*. This BLAST hit does not match our prediction of a Capitella genus species, because it was a sequence from a contaminant.

Sample Adult 1, COI had a very high HQ% with an expected sequence length for the primers used (**Table 1**). The consensus sequence quality 100% and a query of 96.49% identified *Capitella telata,* as the species level identification this is supported by the 100% identity and E

value of zero concluding homology with the query. This top hit is the expected genus level hypothesized at the beginning based on morphology.

For the 16S specimens Adult 1 and Adult 2 consensus sequences had a species-level match of 100% identity with *Oxydromus sp.* The second top Blast hit was for *Capitella telata*, which makes more sense based on initial morphological ID. The top BLAST hit does not match our predicted morphological hypothesis of the genus *Capitella.* Further analysis of the BLAST results is speculated further with phylogenetic data.

Table 1: Below are the Geneious and BLAST results for COI samples.

Sequence name	Sample name & best guess at ID based on morphology	HQ%	Sequence length	Consensus sequence quality %; and length	The closest match in BLAST (with phylum)	Query coverage	Identity %	E value	Same species as closest match in BLAST?
Settle B HCO2198	Settle B *Capitella sp.*	73.6% noisy/ good	692	91.7%;724	*Entomoneis sp.*	70.86%	91.8%	0	No
Settle B LCO1490	Settle B *Capitella sp.*	84.6% good	693	91.7%;724	*Entomoneis sp.*	70.86%	91.8%	0	No
WB 1 HCO2198	WB 1 *Capitella sp.*	91.6% good	695	100%;655	*Capitella teleta*	96.49%	100%	0	Yes
WB 1 LCO1490	WB 1 *Capitella sp.*	83.4% good	680	100%;655	*Capitella teleta*	96.49%	100%	0	Yes
Adult 1 HCO2198	Adult 1 *Capitella sp.*	92.2% good	679	100%;655	*Capitella teleta*	96.49%	100%	0	Yes
Adult 1 LCO1490	Adult 1 *Capitella sp.*	79.6% noisy/ good	680	100%;655	*Capitella teleta*	96.49%	100%	0	Yes
CA LCO1490	CA *Capitella sp.*	1.9% noisy/ bad	728	N/A	N/A	N/A	N/A	N/A	N/A
CA HCO2198	CA *Capitella sp.*	11.4% short/ bad	161	N/A	N/A	N/A	N/A	N/A	N/A
CI LCO1490	CI *Capitella sp.*	0.1% noisy/	702	N/A	N/A	N/A	N/A	N/A	N/A

		bad							
CI HCO2198	CI *Capitella sp.*	0.4% short/ bad	549	N/A	N/A	N/A	N/A	N/A	N/A
CJ LCO1490	CJ *Capitella sp.*	2.1% noisy/ bad	698	N/A	N/A	N/A	N/A	N/A	N/A
CJ HCO2198	CJ *Capitella sp.*	0.3% noisy/ bad	658	N/A	N/A	N/A	N/A	N/A	N/A
CM LCO1490	CM *Capitella sp.*	0.9% noisy/ bad	636	N/A	N/A	N/A	N/A	N/A	N/A
CM HCO2198	CM *Capitella sp.*	35.9% short/ bad	337	N/A	N/A	N/A	N/A	N/A	N/A
CG LCO1490	CG *Capitella sp.*	13.6% noisy/ bad	682	N/A	N/A	N/A	N/A	N/A	N/A
CG HCO2198	CG *Capitella sp.*	61.7% noisy/ bad	684	N/A	N/A	N/A	N/A	N/A	N/A

Table 2: Below is the Geneious and BLAST results for 16S samples.

Sequence name	Sample name & best guess at ID based on morphology	HQ%	Sequence length	Consensus sequence quality %; and length	The closest match in BLAST (with phylum)	Query coverage	Identity %	E value	Same species as closest match in BLAST?
Adult 1 16SARL	Adult 1 *Capitella sp.*	97.2% short/ good	390	100%;483	*Oxydromus sp.*	93.79%	100%	0	Yes
Adult 1 16SBRH	Adult 1 *Capitella sp.*	96.4% Short /good	394	100%;483	*Oxydromus sp.*	93.79%	100%	0	Yes
Adult 2 16SARL	Adult 2 *Capitella sp.*	94.5% short/ good	399	100%;483	*Oxydromus sp.*	93.79%	100%	0	Yes
Adult 2 16SBRH	Adult 2 *Capitella sp.*	95.8% short/	402	100%;483	*Oxydromus sp.*	93.79%	100%	0	Yes

		good							

Phylogenetic Analysis

COI

Nucleotide alignments of Settle B.TS1, WB 1, and Adult 1 and resulting BLAST matches were converted into a phylogenetic tree (**Figure 1**). Eight major clades were observed: *Maritalea myrionectae strain* (single sequence), Uncultured Jiaozhou Bay zooplankton (single sequence), *Bradyrhizobium lablabi* and *B. ottawaense* strains (2 sequences), *Entomoneis sp.* (2 sequences), Settle B.TS1 (single sequence), *Cylindrotheca closterium* (single sequence), *Pythium cederbergense* (3 sequences), *C. telata* (12 sequences). Using a 2% cutoff for clade divergence, interpretation of the tree indicates that Settle B.TS1 was nearly grouped with a diatom, *Entomoneis sp.* (sister clade), sharing a 90.9 identity percentage with both sequences (**Table 2**). The BLAST results for Settle B.TS1 also supports the trends above, withe 91.2% pairwise identity with two *Entomoneis sp.* sequences. This is likely due to the collection method of the Settle B sample, cross contamination with microorganisms (diatoms, dinoflagellates, cyanobacteria, etc.) in the estuarine sediment is almost certain. Explaining the match and close divergence between Settle B.TS1 and *Entomoneis sp.* indicated in the phylogenetic tree, distance matrix, and BLAST results.

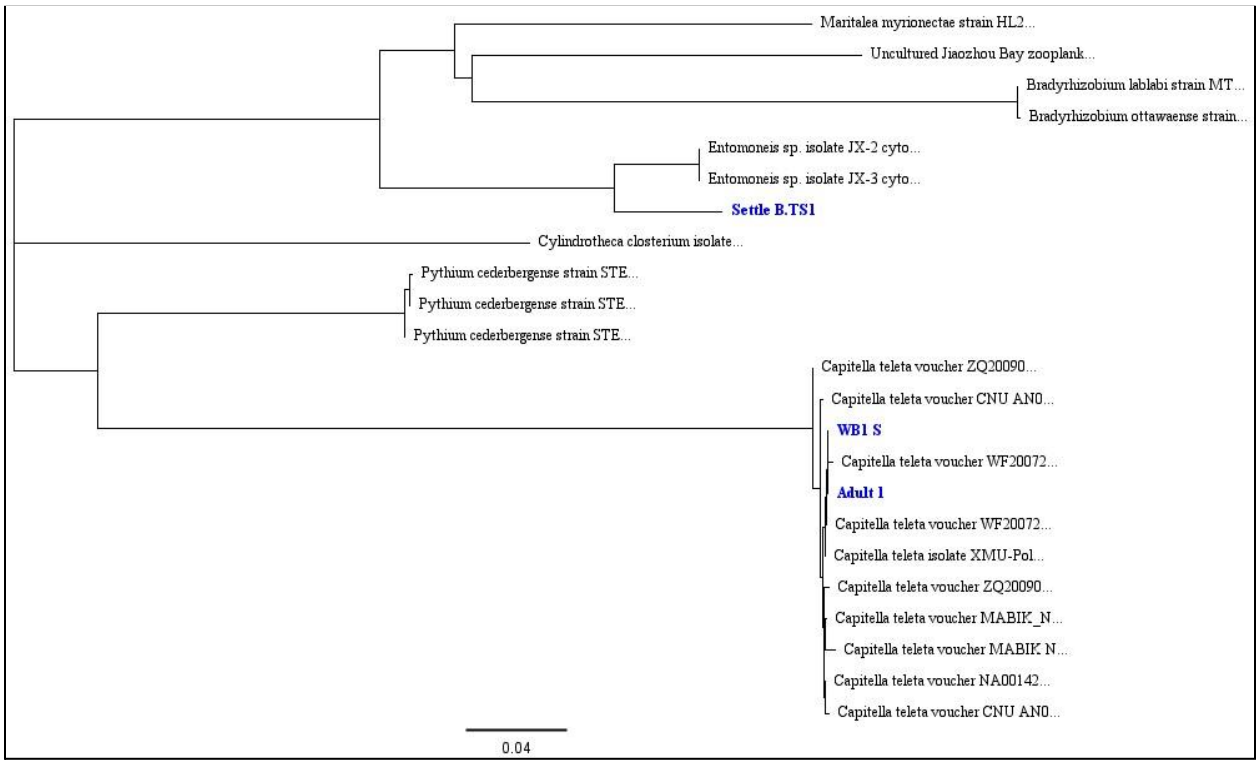
Additionally, the phylogenetic tree designated both WB 1 and Adult 1 in the 12-sequence *C. telata* clade. From the distance matrix both query sequences shared an average identity percentage of 99.7 with other *C. telata* sequences. Signifying that WB 1 and Adult 1 share species-level identification with each other as well as all other sequences in the *C. telata* clade. The top BLAST matches for WB 1 and Adult 1 for both sequences were *C. telata* isolate and *C. telata* voucher WF200726.24. All 19 sequences from our BLAST result satisfied the 4%

divergence cutoff, and are recognized as putative species (originating from the countries previously mentioned). Although it appears that *Pythium cederbergense* as the sister clade, it is only due to the format of the generated tree. Because all the top matches to WB1 and Adult1 are within the *C. telata*, a more definitive sister clade could be determined if more BLAST matches (i.e. 30) were downloaded and a tree was made with those additional sequences.

It is critical to address the unreliability of **Figure 1** for the interpretation for putative species and sister clades. The inclusion of the Settle B.TS1 nucleotide alignment and its BLAST matches introduced unnecessary and confounding obstacles. Sequences in clades *Maritalea myrionectae strain*, Uncultured Jiaozhou Bay zooplankton, *Bradyrhizobium lablabi* and *B. ottawaense* strains, are all waterborne bacteria. Additionally, the diatoms *Cylindrotheca* and *Entomoneis sp.*, and the parasitic water mold, *Pythium cederbergense*, were also resulting from the Settle B sample (Settle B.TS1). Conversely, WB 1 and Adult 1 were correctly grouped into a single clade that matched with various sequences of *C.telata*, indicating the sampling method that derived these two sequences avoided contamination. Therefore, Bergen should reconsider collecting samples that are in contact with sedimental contaminants (he could retain his settlement growth method but should rinse the organism in DI water to remove any sediment remnants such as microbes, from his samples).

Figure 1. The phylogenetic distance tree synthesized from nucleotide alignment of Settle B.TS1, WB 1(S), and Adult 1. The tree shows seven major clades. Six of which are the match results of the Nucleotide Alignment of Settle B.TS1: *Maritalea myrionectae strain* (single sequence), Uncultured Jiaozhou Bay zooplankton (single sequence), *Bradyrhizobium lablabi* and *B. ottawaense* strains (2 sequences), *Entomoneis sp.* (2 sequences), Settle B.TS1 (single

sequence), and are entirely different organisms (resulting from contamination of Settle B sample). Sequences WB 1(S) and Adult 1 all belong into a single major clade composed of *Capitella teleta*.



16S

Based on a 2% sequence divergence cutoff, the nucleotide alignment of 16S sequences, Adult 1 and 2, are converted into a phylogenetic distance tree of three major clades: *C. capitata* (single sequence), *Capitella sp.* (single sequence), *C. telata* (8 sequences). Our query sequences, Adults 1 and 2 were grouped in the 8-sequence *C. telata* clade. Adults 1 and 2 share a species-level match between each other, as well as six other sequences, all of which belong to *C. telata*. Of the sequences, *C. capitata* and the *Capitella sp.* fail to fall within the 2% divergence cutoff. The sister clade could not be determined, since all three clades come from the same node.

So the relationships between the three species are not clear due to the presence of polytomy. Furthermore, within the *C. telata* clade was also possible evidence of mislabelling. The *Oxydromus sp.* sequence was mislabelled with *C. telata* by GenBank even though they are different organisms(**Figure 2**). Furthermore, Adults 1 and 2 share the closest identity percentage (100%) with this *Oxydromus sp.* sequence. It is unclear whether this result actually indicates Adults 1 and 2 belong to *Oxydromus sp.*, since it was misplaced by GenBank. Conclusively, the results of phylogenetic analysis contradicts Bergen's preceding identification of Adults 1 and 2, as *C. capitata*. Although this contradiction will be further discussed in support of Bergen's identification.

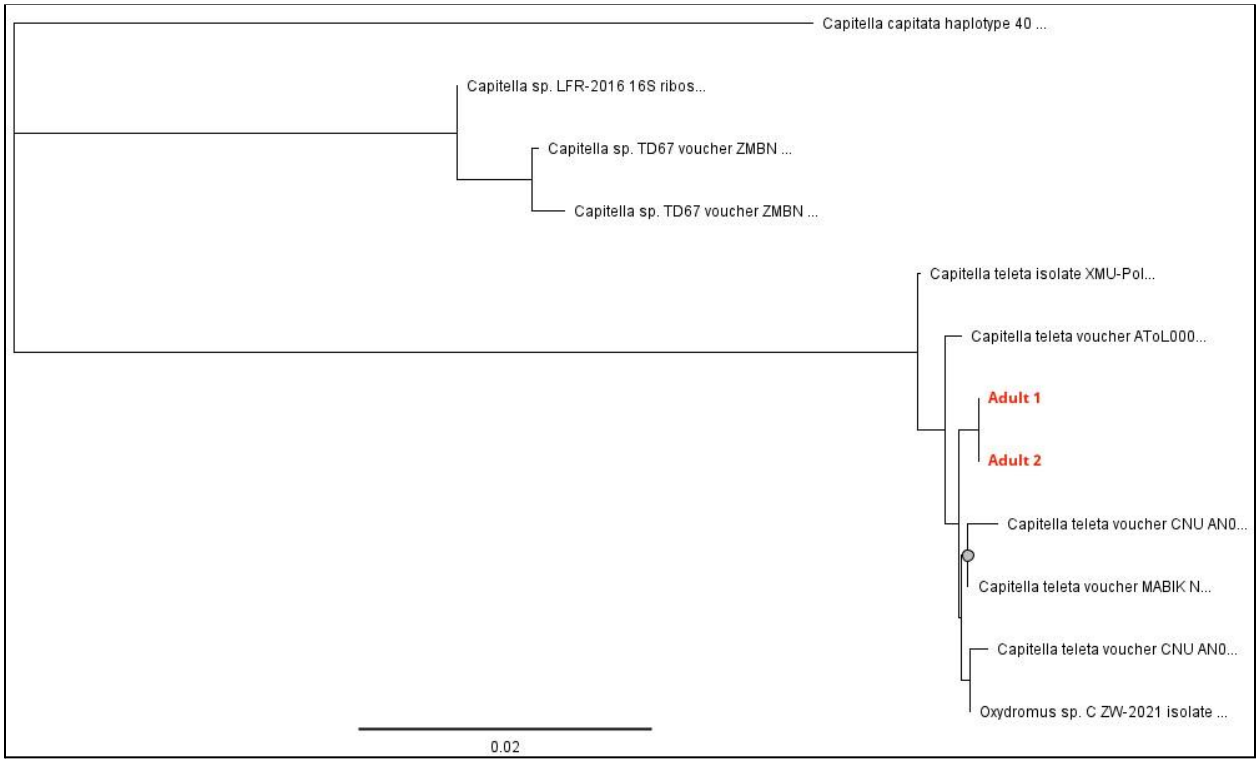


Figure 2. The phylogenetic tree synthesized from nucleotide alignment of Adults 1 and 2 (sequences used can be found in our Geneious file). The tree shows three clades, *Capitella sp.*, *C. capitata*, and *C. telata*. Adults 1 and 2 were grouped in the 8-sequence *C. telata* clade; a different species, *Oxydromus sp.*, was also grouped within this clade.

Conclusion

COI sample Settle B reveals an unpredicted BLAST hit which is unlikely due to contamination in the lab or human error. We can make this assumption because all positive and negative controls ran accordingly throughout the DNA analysis of all samples. Speculation as to why this hit was received could be due to gut content or bacteria present in the soil collected with the sample in its environment. COI sample WB1 can be confidently identified as *C. teleta,* mainly due to the high query and E value along with the 100% identity of sequences (**Table 1**); Sample Adult 1 can also be confidently identified as *C. telata,* supported by the 100% identity and E value of zero which concludes homology with the query and strengthens the data reliability.

The samples ran with 16S primers reveals a second top BLASThit for both Adult 1 and Adult 2 for *C. telata* (100% query and 99.8% identity match). The top BLAST match, *Oxydromus sp.*, is a result of mislabelling by GenBank. Taxonomically, *Oxydromus sp.* and *Capitella* are not closely related. Both belong to different subclasses, *Capitella* to Sedentaria, and *Oxydromus* to Errantia. This is indicated by **Figure 2** where we see the *Oxydromus* sequence is misgrouped with the *C. telata* clade. With both the BLAST information and evidence from the phylogenetic tree we conclude Adult 1 and 2 belong to *C. teleta* which aligns with morphologically based hypothesis. Further analysis of the WoRMS database shows the *Oxydromus* genus as included in the same Class as Capitella, Polychaeta. This may further indicate how in the GenBank database the sequence labeled as *Oxydromus* may be mislabeled.

Additionally, DNA and phylogenetic analysis contradict Bergen's initial identification of 16S: Adult 1 and 2 samples. It is justifiable to conclusively reject Bergen's identification based

on our results from Geneious Prime. Pernet, Harris, and Schroeder (2015) identified a clear gap in the research of various Capitellidae. The limited knowledge on capitellid such as *C. capitata* heavily constrains Bergen's effort in identifying and studying its planktotrophic larvae. Although C. telata is well studied and easier to capture, it limits Bergen's research, since capturing the larval and adult form *C. capitata* is extremely difficult due to their rarity (Bergen direct communication, 2021).

The inherent morphological indistinguishability in marine worms intrinsically contributes to their lack of a solid taxonomic foundation and understanding (Pernet et al., 2015). This notion is further supported by our phylogenetic and DNA analysis, by which both samples (Adult 1-2) had a 100% identity percentage with *Oxydromous sp*., under our assumption that the sequence was mislabelled (or misidentified). Furthermore, Bergen has emphasized the cryptic nature of capitellid worms as the rationale behind his research. Additionally, the "unknown" larvae samples (CA-N and Settle B) were studied most intensively and were the primary objective for genetic identification, due to the carnivorous behavior observed from the larvae. Although sequencing could not be conducted due to poor HQ%, Bergen should consider reconducting genetic analysis with alternative 16S and COI primers, which may produce sequences that would qualify for DNA and phylogenetic analysis. Capitellidae worms have indistinguishable and slight morphological differences between species, which could be an underlying possibility to a low-quality genetic database (Grassle, 1976). Therefore, Bergen should not be discouraged by the lack of successful identifications, and should work towards filling in the gaps of capitellid research through the identification of the carnivorous larvae studied in his research.

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