Final Report

Project Title: Marine Invasive Species Technical Support – Quantitative Survey of Nonindigenous Species (NIS) in Prince William Sound

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Introduction

To date, relatively few nonindigenous species (NIS) have been detected in coastal marine waters of Alaska compared to other regions of North America (Ruiz et al. 2000, 2011a). For Prince William Sound (PWS) in particular, this apparent low level of NIS is somewhat surprising, given the large influx of ballast water biota (and potential influx of hull fouling biota, which has not yet been assessed,) associated with oil tankers arriving to PWS (McGee et al. 2006). Most of these tankers arrive from ports in the western U.S., including California (Long Beach and San Francisco Bay / Estuary) and Washington (Puget Sound), where hundreds of NIS are now are documented, providing regular transfers of biota from these source ports (Hines & Ruiz 2000). Further, available evidence suggests many of these NIS that can tolerate environmental conditions in Alaska and are capable of establishing populations in PWS (Zabin et al. 2009, deRivera et al. 2011).

One possible explanation (hypothesis) for the low invasion rate in PWS is a lag time in detection (Ruiz et al. 2006, Ruiz & Hewitt 2009). Although ballast water discharge is high in PWS compared to many ports, this is a relatively recent development that began in the late 1970s, when the Alyeska terminal opened for business. There is often a lag time in detecting new invasions, that results from both the time it takes for populations to grow (in number of individuals and in area occupied) and also the level of search effort (Crooks and Soule 1999, Costello and Solow 2003). In the case of PWS, the time has been short, and the search effort has been low.

In addition, we note that ballast water treatment appears more limited for tankers that arrive to PWS, compared to other parts of the country (National Ballast Information Clearinghouse 2012). This low treatment is attributed to operational constraints of these vessels, and it suggests that that transfers of coastal organisms to PWS by tankers remains relatively high, creating opportunity for invasions to occur. Further, the rate of new invasions to the south has continued to increase, especially for California ports from which tankers arrive to Valdez (Cohen and Carlton 1995, 1998, Fofonoff et al. 2003). Thus, the pool (total species richness) of potential NIS arriving to PWS on tankers is likely to have increased through time.

In this project, we conducted a biotic survey of selected sites in PWS to test whether for evidence of new invasions occurring in PWS. We focused our surveys on biofouling communities, or those organisms that occur on hard substrate. We selected this community because: (a) most invasions to western North America occur on hard substrate (Ruiz et al. 2009), (b) we see taxa these "marching" northward (Ruiz et al. 2011a), including three species of tunicates detected only within the past decade (for the first time) in southeast Alaska, and (c) we have historical data from previous surveys of this community that occurred 10 years ago in PWS, providing a baseline for comparison of species occurrences. In addition, we used molecular genetic methods to provide further resolution and an independent assessment of morphological identifications.

Goals & Objectives

The overall objectives of this component were to (a) assess whether new (previously undetected) invasions have occurred in PWS and (b) to establish a baseline to assess changes in marine communities over time as a result of invasions and other potential forcing functions (e.g., climate change or other environmental changes).

The specific objectives of this component are to:

- Characterize species present in the sessile invertebrate community at multiple sites in PWS, using field-based surveys.
- Classify detected species as native, non-native, or cryptogenic.
- Use molecular genetic analyses to ground-truth consistency of identifications based on morphological analyses and test for cryptic species;
- Compare results for the number of NIS detected to previous surveys of PWS.

Approach

We used standardized, quantitative surveys to collect benthic marine invertebrates present in the hard substrate biofouling communities of PWS. The samples were analyzed using both morphological and genetic methods, to test for concordance in methods. We used these data, along with historical information on taxonomy and biogeography or the detected species, to classify organisms as non-native, native, or cryptogenic to PWS.

Methods

The biofouling communities in PWS were sampled using methods developed and applied in previous surveys throughout North America (Ruiz et al. 2006, unpublished). We deployed PVC settling plates (14x14 cm) as passive collectors, which were deployed in coastal waters for three months, allowing for colonization by marine invertebrates. Plates were deployed during summer, when recruitment is highest for temperate latitudes in the northern hemisphere.

Plates were deployed at each of six sites in PWS during May 2011, and they were retrieved in August 2011, after three months residence time in the water. The sites included: Valdez small boat harbor, Valdez ferry dock, Alyeska terminal, Solomon Gulch hatchery, Tatitlek, and Ellomar in Virgin Bay. The location and depth (from surface to bottom) is shown for each site in Table 1; note that depth varies between deployment and retrieval, reflecting large tidal amplitude and timing of measurements. At each site, we deployed 20 settling plates that are suspended 1m above low tide line (MLLW). In addition, we deployed an additional 20 plates in deeper water (~4m below MLLW) at the Aleyska terminal sites, to sample the community in higher salinity waters (below the low salinity surface conditions that are often present at this site).

Table 1. Site name, latitude, longitude, and water depth.

Site Name	LAT	LONG	Bottom depth, deployment (m)	Bottom depth, retrieval (m)
Tatitlek Ferry Dock	60.85923	-146.676	21.1	11
Ellamar Virgin Bay	60.89424	-146.704	6.6	6
Valdez Hatchery	61.08897	146.2976	30	16.25
Alyeska Terminal	61.0897	146.3684	9.2	11.9
Valdez boat harbor	61.12672	146.3437	6.5	4.5
Valdez Ferry Dock	61.12409	146.3638	10	

We used a stratified random design to determine the deployment location for each settling plate and deployed a cumulative total of 140 plates in this fashion across sites. Temperature, salinity, and dissolved oxygen were also measured for vertical profiles upon deployment and retrieval events.

Upon retrieval in August 2011, all plates were examined using a dissecting microscope while animals were still alive. We randomly selected 10 plates from each site for detailed analyses as follows: (a) for each plate, we collected voucher species of each morpho-species (i.e., unique morphotype) from every plate for morphological identification and (b) for each site, we collected up to 5 specimens of each morpho-species for genetic analyses. For colonial organisms (e.g., bryozoans, hydroids, and some tunicates), when sufficiently large, the morphological and genetic vouchers were collected as paired samples from the same colony, to allow direct comparison. The remaining 10 plates were scanned for any additional new taxa, or for additional DNA vouchers (as needed to achieve n=5 specimens), and these were vouchered as appropriate for morphological versus molecular analyses.

Following field analyses, most of the plates were kept and preserved, being shipped back to Smithsonian Environmental Research Center (SERC) for possible additional reference or future analyses. The voucher specimens were shipped to SERC (for morphological) or Moss Landing Marine Laboratory (for genetic analyses). All data for the voucher collections and morphological analyses were entered into database at SERC. Initial collections data were sent to Moss Landing Marine Laboratory (MLML), for local use, and subsequent molecular genetic data were collected, managed, and analyzed by MLML.

At the time of collection, each individual morphological and molecular voucher specimen was labeled with a unique identification number and preserved for subsequent analyses. Tentative morphological identification (Field Identification) and description was recorded for each voucher, including the specific plate, location, and time of collection. All morphological specimens were re-examined in the laboratory by SERC, using individual specialists for each taxonomic group, to produce a Final Identification to species or the lowest taxonomic unit possible.

1. Genetic Analyses. For genetic vouchers, tissues were removed from ethanol, rinsed in dH_20, and immersed in screw-cap flat bottom vials containing tissue lysis buffer (Qiagen ATL), 20 ul of Qiagen proteinase-K solution, and ~0.1 cc of ceramic beads. These vials were then vigorously shaken for 1 minute using a Biospec Bead-beater or tube vortexer fit with a tube-holding head. Homogenized tissue in lysis buffer was then incubated overnight with vertical orbital rotation in a hybridization oven set to 55^oC.

For the majority of samples, we used Qiagen DNeasy Tissue and Blood DNA extraction kits. These kits use a proteinase-K solution for tissue lysis and capture of DNA onto silica gel in spin columns. The captured DNA is washed in Qiagen buffer PE (a high salt Tris and ethanol solution) to remove impurities,

and eluted in Qiagen buffer EB (a low salt Tris solution). Extracted DNA was collected in 1.5 ml centrifuge tubes, from which subsamples were arrayed into 96 well plates. DNA tubes were boxed and archived, while further work used the 96 well DNA plates. All genomic DNA samples have been retained in frozen or lyophilized form at MLML.

COI PCR used Promega Green GoTaq master mix containing 1.5 mM $MgCl_2$. This was supplemented with additional 1.5 mM MgCl2, for a final concentration of 3 mM, and 0.2 mg/ml bovine serum albumen. PCR was primarily done in 96 well plate format. Melting temperature was 94^{o} C, annealing temperature was 48^{o} C, and extension temperature 72^{o} C, and incubation times were 1 minute for each step. 28S amplifications used Promega Green GoTaq master mix with 1.5 mM $MgCl_2$, 0.2 mg/ml BSA and 2% DMSO. The PCR program was the same as above.

PCR products were checked on 1% agarose gels and photographed. Positive PCR products were culled from PCR plates and loaded onto a fresh 96 well plate for DNA sequencing. PCR plates were then put back into the thermocycler for another 5 to 10 cycles to increase DNA yields from reactions showing initially faint amplification. The PCR products were again examined on agarose gels, and useful products moved to the sequencing plate. Failed PCR reactions or smeary PCR products were discarded. By this process, 96 well plates for sequencing were filled and sent for sequencing.

We made at least two attempts to obtain useful products from initially failed reactions. For recalcitrant templates, we attempted a nested PCR approach wherein a first round PCR products was used as a template with internal primers. Many of the specimens that were annotated as “tiny” did not produce amplifiable DNA. Good PCR products were sent to Elim Biopharmaceuticals in Hayward, CA for sequencing in both forward and reverse orientations

Sequences were downloaded from Elim Biopharmaceutical . We used the software package Geneious (Biomatters, Wellington, NZ) for most sequence editing and manipulation. Forward and reverse sequences for each reaction were aligned, and a consensus made. Thus, the majority of the PCR products were sequenced twice allowing for greater confidence. Where the forward and reverse sequences disagreed, the higher quality read was accepted; this was a relatively rare event except at the extreme 5’ ends of reads or 3’ end past ~700 bp where signal strength is often poor (a normal feature of Sanger sequencing). Sequences were then archived in folders according to initial field identifications.

For final analysis, we pooled and binned all sequences using the assembly feature of Geneious. We set thresholds of 95% and 97% similarity for COI and 28S, respectively, to create groups representing provisional genetic operational taxonomic units (OTU). Sequences were aligned using the MUSCLE program called from within Geneious, and then refined using the built-in Geneious alignment routine. We used the maximum likelihood program PhyML in Geneious to construct phylogenetic trees.

When sequences within an OTU matched Genbank (COI) records at >95%, or 28S at 98%, and the identification in the Genbank record was not in conflict with morphological sorters, we accepted that consensus identification. When an OTU did not match any records in Genbank, we compared sequences to those in a MLML database for species in San Francisco Bay. This database contains sequences from vouchers that were identified by Smithsonian personnel or contractors. When a sequence did not have a strong match to the Genbank or MLML-San Francisco databases, we could neither confirm nor refute a morphological identification. We suggested a broader taxonomic category supported by BLAST searches of Genbank. For example, one gastropod sequence did not match any known record, but appeared to be related to the genera *Littorina* or *Lacuna*. We therefore suggest that the specimen belongs to the gastropod family Littorinidae.

Results & Discussion

1. Environmental Measures. Environmental measurements collected at deployment and retrieval are shown in Table 2 for each site. As expected, there was considerable variation in salinity and temperature profiles among sites.

Table 2. Salinity, Temperature, and Dissolved Oxygen Profiles. Shown are measures collected for each site on (A) deployment and (B) Retrieval.

A. Deployment

Measure	Depth (m)	Alyeska Terminal 5/18/2011	Ellamar Virgin Bay 5/17/2011	Tatitlek Ferry Dock 5/17/2011	Valdez Ferry Dock 5/19/2011	Valdez Hatchery 5/17/2011	Valdez boat harbor 5/18/2011
DO	0	12.44	12.64	11.98	10.82	11.63	10.41
DO	1	12.65	12.46	12.22	10.54	11.98	10.93
DO	2	12.37	12.09	12.33	10.05	11.53	10.78
DO	5	12.22	12.3	13.7	11.04	11.87	11.56
DO	10			13.31	9.96	12.08	
Sal	0	26.8	29.7	29.2	18.3	22.8	19
Sal	1	27.5	29.9	29.5	22.7	24.8	24.2
Sal	2	28.2	29.9	29.6	25.6	27.3	27.1
Sal	5	28.8	30	30.1	28.4	29	29
Sal	10			30.5	29.6	30	
Temp	0	8.8	10	10.3	9	9.7	10.9
Temp	1	8.2	9.9	10.1	9.1	9.4	9.5
Temp	2	8.1	9.6	9.6	9.1	9.4	8.7
Temp	5	7.8	9	7.8	8.5	8.6	8.1
Temp	10			6.4	7.6	6.9	

B. Retrieval

Measure	Depth (m)	Alyeska Terminal 8/15/2011	Ellamar Virgin Bay 8/14/2011	Tatitlek Ferry Dock 8/14/2011	Valdez Ferry Dock 8/13/2011	Valdez Hatchery 8/10/2011	Valdez boat harbor 8/11/2011
DO	0	9.3	9.1	9.5	11.41	9.45	10.65
DO	1	9.4	9.2	9.1	9.2	7.52	10.41
DO	2	9.4	8.9	8.9	8.9	8.88	9.15
DO	4.3						7.59
DO	5	9.1	8.3	8.8	8.78	8.64	
DO	10	10.1		8.9	8.9	8.94	
Sal	0	14.6	21	22.3	8.5	1.6	3.3
Sal	1	18.1	21.5	22.5	16.7	2.1	4.6
Sal	2	18.3	24.6	22.6	17.9	20.2	17.6
Sal	4.3						23.2
Sal	5	25.4	25.8	25.9	20.9	24.5	
Sal	10	27.7		28.6	25.4	24.9	
Temp	0	12.3	14.1	14.5	10	8.6	8.7
Temp	1	13.2	14.1	14.6	12.3	8.2	9.1
Temp	2	13.2	14.2	14.6	12.7	12	11.4
Temp	4.3						12.8
Temp	5	12.9	13.9	14.2	13	13.4	
Temp	10	11.4		12.6	13.1	13.1	

2. Morphological Analyses. In total, we analyzed 423 vouchers to identify to the lowest possible taxonomic unit, based on morphological characteristics. The number of vouchers obtained varied greatly among sites (Table 3), reflecting the number of species present on plates. As expected, several of sites nearest Valdez had low overall species diversity (richness), and this most likely reflects the low and seasonally variable salinity of surface waters. The exception was the Alyeska Terminal site, because we had both surface (1m) and deeper (4m) samples, with the later providing the greatest species richness.

Table 3. Number of Morphological Vouchers Analyzed by Site.

Site	Vouchers
Alyeska Terminal	163
Ellamar Virgin Bay	71
Tatitlek Ferry Dock	102
Valdez Ferry Dock	63
Valdez Hatchery	7
Valdez boat harbor	17
Grand Total	423

Table 4 shows 32 different taxa that were identified across sites based solely on morphological analyses. Of these, two of these species are known to be non-native to Alaska, including the bryozoan *Schizoporella japonica* and the barnacle *Amphibalanus improvisus*. The non-native bryozoans had previously been documented in Alaskan waters. To our knowledge, however, the occurrence of *A. improvisus* is the first record for Alaska. This barnacle is native to the Atlantic Ocean and is known to be established on the Pacific Coast of North America, occurring as far north as British Columbia (49^{o} N; NEMESIS 2012). The genetic data confirms that the genotype of the Alaska specimen is consistent with that of other specimens from California (see section 3 below).

Table 4. Taxa of Sessile Invertebrates Identified by Morphological Analyses.
Asterisk indicates non-native species. We also note that the bivalve *Mytilus* sp. was assumed to be *M. trossulus* (confirmed by genetic analyses, below).

BIVALVIA
Hiatella arctica
Mytilus sp. (trossulus)
Pododesmus macrochisma

BRYOZOA
Bugula pacifica
Callopora craticula
Celleporella hyalina
cf. Fenestrulina sp.
cf. Pacificincola sp.
Cribrilina annulata
Cribrilina corbicula
Crisiella sp.
Cryptosula zavjalovensis
Filicrisia sp.
Lichenopora verrucaria
Membranipora villosa
Parasmittina cf. trispinosa
Schizoporella japonica*
Tubulipora sp.

CIRRIPEDIA
Amphibalanus improvisus*
Balanus balanus
Balanus crenatus
Semibalanus balanoides
Semibalanus cariosus

HYDROZOA
Clytia gracilis
Clytia hemisphaerica
Gonothyraea clarki
Obelia dichotoma
Sarsia sp.

TUNICATA
cf. Pyuridae
Corella inflata
Distaplia alaskensis
Distaplia occidentalis

We only found one specimen of *A. improvisus* in our analyses, at the Alyeska Terminal site. As a result, it is currently not evident whether the species has become established in PWS. In contrast, *S. japonica* occurred at two sites, Ellamar Virgin Dock and Tatitlek Ferry Dock, and 21 specimens were confirmed by

morphological analyses between these sites. The distribution of these and other occurrence records is shown in Appendix A.

We note that our analyses were restricted here to the sessile invertebrates collected during the surveys. While we retained mobile organisms (e.g., amphipods, non tube-building polychaetes) from the plate samples, these have not been included in the analyses here.

3. Genetic Analyses. In total, 656 voucher samples were collected for genetic analyses. Of these, 651 contained visible tissue, from which DNA was extracted; many samples were very small (due to the small size of many organisms), and tissue was not located in 5 of the voucher vials. Using these samples, a total of 901 PCR attempts were made for Cytochrome c oxidase subunit I (COI), yielding 277 sequences. Another 1277 PCR attempts were made for the large subunit ribosomal RNA gene (LSU, or synonymously, 28S rRNA), yielding 481 sequences.

Table 5 shows a comparison of COI data to the initial Field Identifications, for genotypes that occurred more than once (i.e., were shared by multiple specimens). Each Column indicates a different COI genotype, and the vertical lines distinguish those for which sequences differed at 5% or more, which are considered likely to represent distinct species.

In general, there was good concordance between morphological and genetic data for many taxa, when using the initial Field Identifications, which represent a very coarse first estimate. In several cases, the Field Identifications included multiple morphotaxa, which the genetic analyses grouped together. This is not unexpected, because (a) the taxonomic expertise among the field biologists was variable and (b) our approach strives explicitly to maximize morphological variation (i.e., oversplit), even we this may be intra-specific variation, for more detailed analysis during Final Identification. In some cases, the Final Identifications removed these additional morphotaxa. For example, in the Final Identifications below, Bryozoans 4 and 7 were considered the same species (*Membranipora villosa*) morphologically (see Table 4 and Appendix A).

For many specimens, the genetic data provided greater confidence and resolution than has been possible in previous surveys. For example, the genetic analyses were able to classify 27 of the hydroids in Table 5, recognizing two distinct genotypes, whereas the morphological analyses could only identify 7 of these to the species-level, because of small size or the absence of key characters (see Appendix A). In addition, the genetic data indicated three distinct gentoypes for Bryozoans 4 and 7 in Table 5, even though these were not discerned in the Final Identification based on morphological analyses. Thus, it is evident that the genetic methods can help overcome past challenges of identification for small, immature individuals.

The genetic data also confirmed the presence of *A. improvisus* and *S. japonica* genotypes found at other locations. For example, Figure 6 shows a tree for barnacle COI genotyopes present in Alaska and California, indicating that the *A. improvisus* specimen from PWS surveys (shown in blue) is virtually identical to that found in California. However, this tree also underscores the need for caution in assigning species names, since Genbank assigns this genotype to the congener *A. eburneus* (which we believe to be erroneous and are now genotyping additional *A. improvisus* (absent from GenBank) that were collected from the native Atlantic range). Genbank records of "*A. eburneus*" shown in red appear to be misidentified.

Overall, we encountered at least three types of issues related to taxonomic name for organisms, based on existing genetic data resources:

1. First, a mismatch was apparent between the taxonomic name provided by Genbank and the morphological identification. This was seen for *A. improvisus* (as above). In addition, our morphological analysis identified the hydroids in Table 5 as *Obelia dichotoma* whereas Genbank gave the same organism the name *Obelia longissima*. Moreover, the 28S rRNA sequences for voucher samples in our analyses were not a match those for either *O. dichotoma* or *O. longissima* sequences present in Genbank (see Figure 7), requiring further research to confirm the actual species binomial and its biogeographic origins.

2. Second, the genetic analyses identified wider variation than expected for individual species that were detected by morphological analyses. For example, the specimens identified morphologically as *Membranipora villosa* included many different gentoypes, which together spanned a wide genetic distance without clear separation (Figure 8), exceeding the 5% difference used typically in barcoding studies to designate distinct species. This may be a single species with considerable intraspecific genetic structure or multiple species with relative low divergence.

3. Third, we also encountered many species for which there are no genotypes available (in Genbank), to test for concordance with current taxonomic names. For example, in the bryozoans, there is considerable genetic differentiation among taxa sampled in PWS (see Figure 9), but there were no existing names for the respective sequences available in Genbank at the present time.

Conclusions

Our study revealed at least on new NIS (*A. improvisus*) was detected in PWS, representing a first record for the entire state of Alaska. This species may have arrived on the hulls of vessels (as adults) or in the ballast water of vessels (as larvae). We note that previous analyses by deRivera et al. 2011 (funded by Prince William Sound Regional Citizens' Advisory Council) predicted that this species is capable of colonizing Alaska over an extensive area. While we detected one specimen in the current survey, further occurrence records and evidence of reproduction is necessary to draw inferences about whether a self-sustaining population may be present in PWS. If the species becomes established, the potential impacts are not well understood at the present time (Ruiz et al. 2011b).

The genetic data allowed us to test for consistency in the morphological identifications and also to examine the level of sequence differentiation, which has not previously been available for PWS surveys of this biota. While this provides a new, powerful tool to examine species composition, further analysis is required to resolve the proper species name in many cases (as outlined above). We are now pursuing the work to resolve some of these issues, focusing particular attention on the barnacle *A. improvisus*, the bryozoans, and the hydroid *Obelia* (in this order).

We expect to use these results in multiple venues. First, for those NIS that are confirmed and resolved, we will make geo-referenced collections records available through NEMESIS (the National Exotic Marine and Estuarine Species Information System, an online and searchable database on marine NIS). Second,

we anticipate publishing new occurrence records and also taxonomic resolution for selected groups, as further analyses are completed. As outlined above, we will give initial attention to the barnacle *A. improvisus*, seeking to resolve the taxonomic issue that now exists. In addition, it would be useful to test whether the barnacle *A. improvisus* is now established in PWS, and we are pursuing opportunities for additional benthic community collections to test for the presence and persistence of this species. Third, we expect to compare the results of this survey to a genetic analysis of zooplankton assemblages that is schedule to begin shortly, allowing us to test (a) the efficacy of using plankton assemblages to detect the benthic species present (as a monitoring and detection tool) and (b) whether we detect additional *A. improvisus* present in the plankton community in PWS.

Table 5. Comparison of Morpho-species from initial Field Identifications to COI genotypes. See text for details. Shaded bins 17 and 21 have been combined based on phylogenetic analysis.

AKNIS COI Bins vs. Field IDs																										
Morpho-sp	**1**	**2**	**23**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**18**	**11**	**12**	**14**	**13**	**15**	**16**	**17**	**21**	**25**	**19**	**20**	**22**	**24**	**26**	*TOTAL*
Biv2	22																										*22*
Biv1	10																										*10*
Biv4	3																										*3*
Hyd2		11	2																								*13*
Hyd5		11																									*11*
Hyd4		3																									*3*
Cir2				16	3																						*19*
Cir1				8	8																						*16*
Cir6					6																						*6*
Cir5					1																						*1*
Ant2						12																					*12*
Ant1						3																					*3*
Nud5							13																				*13*
Nud6								12																			*12*
Biv3									7																		*7*
Biv5									2																		*2*
Bry6										8																	*8*
Bry2											6																*6*
Bry8											1	3															*4*
noID											1																*1*
Gas1													5														*5*
Gas2													2														*2*
Gas0													1														*1*
Nud7														7	1												*8*
Nud1														1													*1*
Nud9															6												*6*
Nud4																7											*7*
Tun4																	6										*6*
Bry1																		4									*4*
Bry7																			3	3	2						*8*
Bry4																			1								*1*
Bry9																						3					*3*
Nud8																							3				*3*
Bry11																								2			*2*
Cap1																									2		*2*
Scy1																										2	*2*
BIN TOTAL	*35*	*25*	*2*	*24*	*18*	*15*	*13*	*12*	*9*	*8*	*8*	*3*	*8*	*8*	*7*	*7*	*6*	*4*	*4*	*3*	*2*	*3*	*3*	*2*	*2*	*2*	*233*

Figure 6. Genetic Tree based on COI for Barnalces in Alaska (PWS) and California.

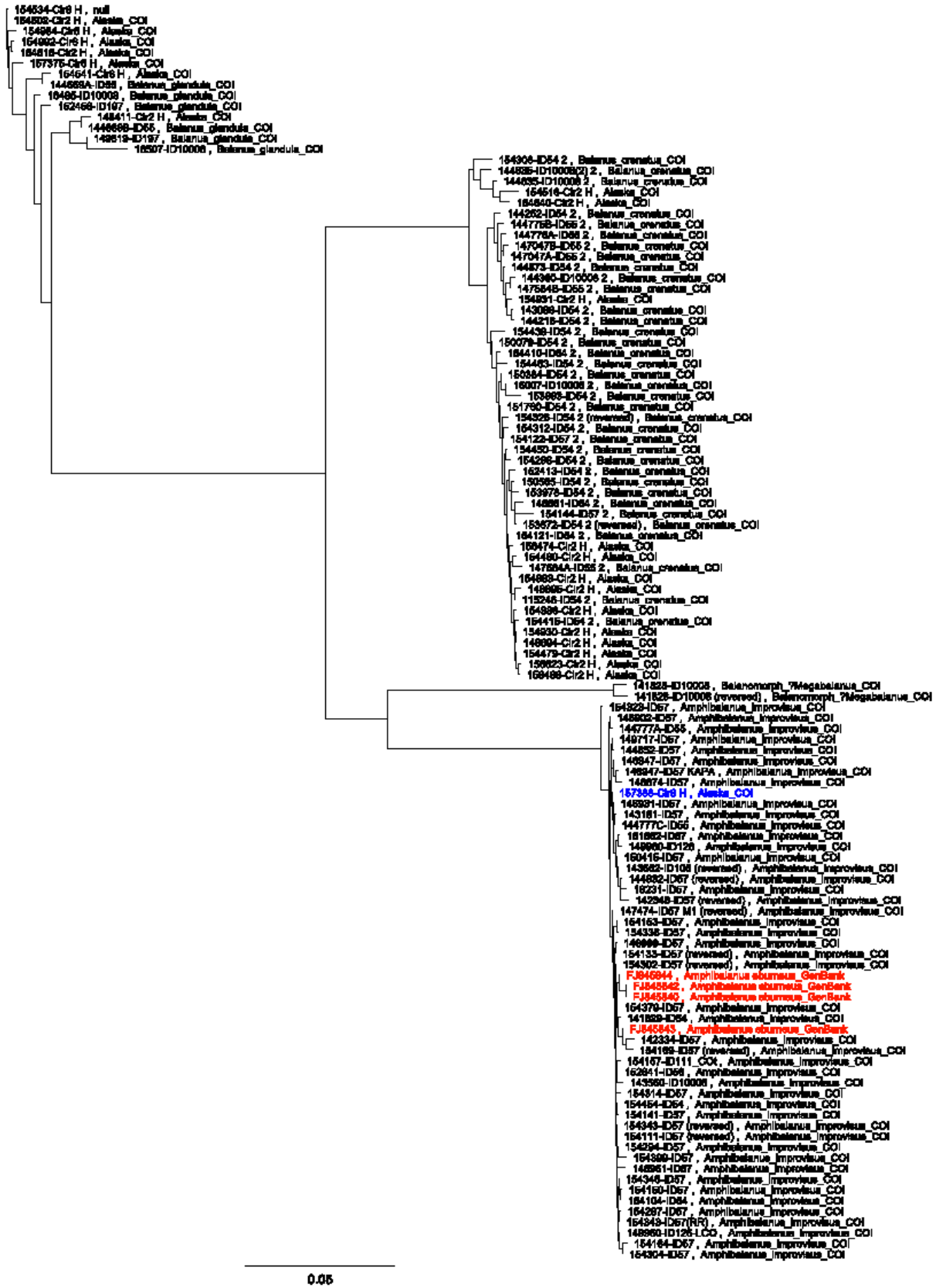


Figure 7. Genetic Tree based on 28S rRNA for *Obelia* spp. in PWS (shown as numbered samples).

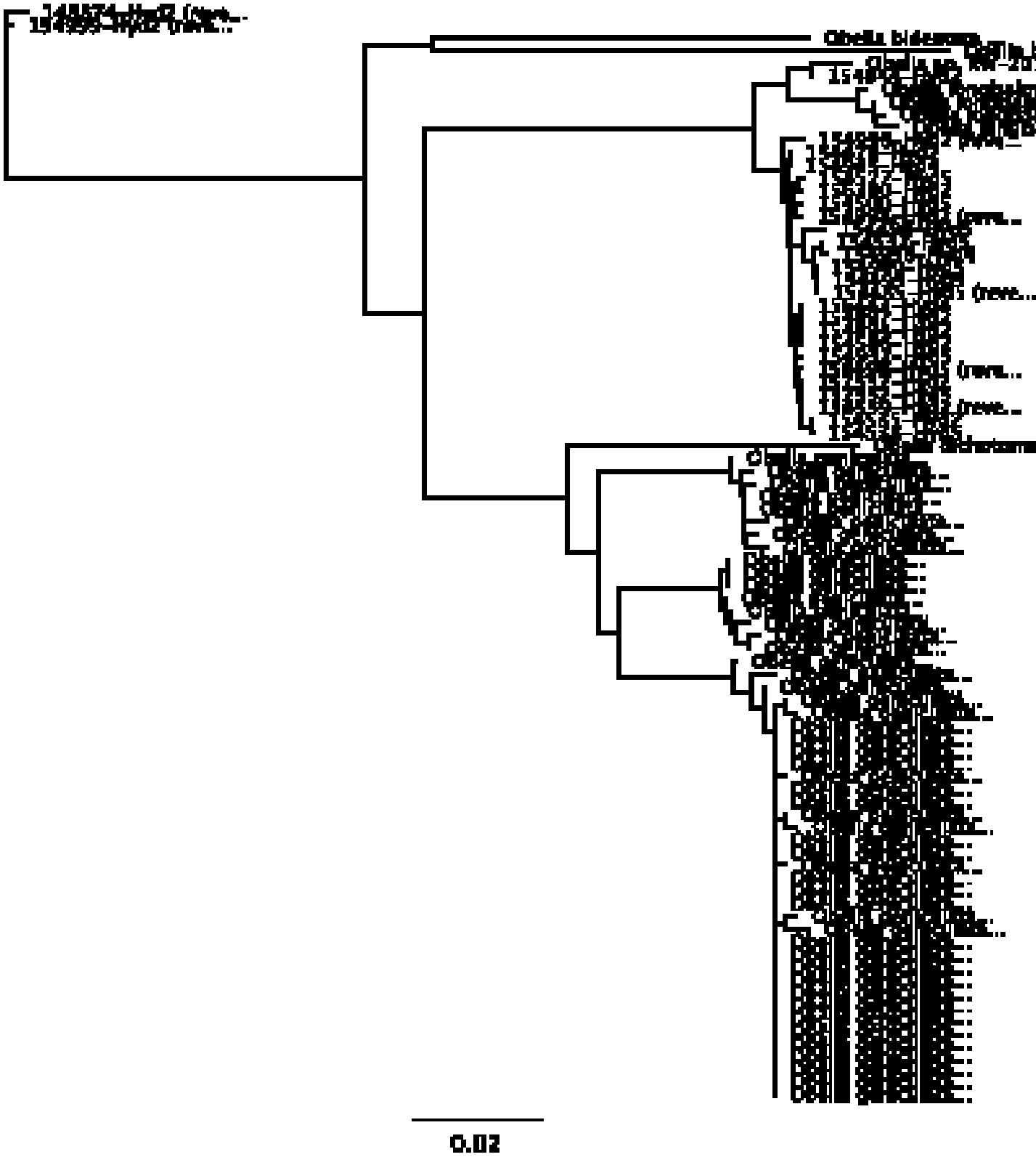


Figure 8. Genetic Tree based on COI for Species Identified Morphologically as *Membranipora villosa* in PWS.

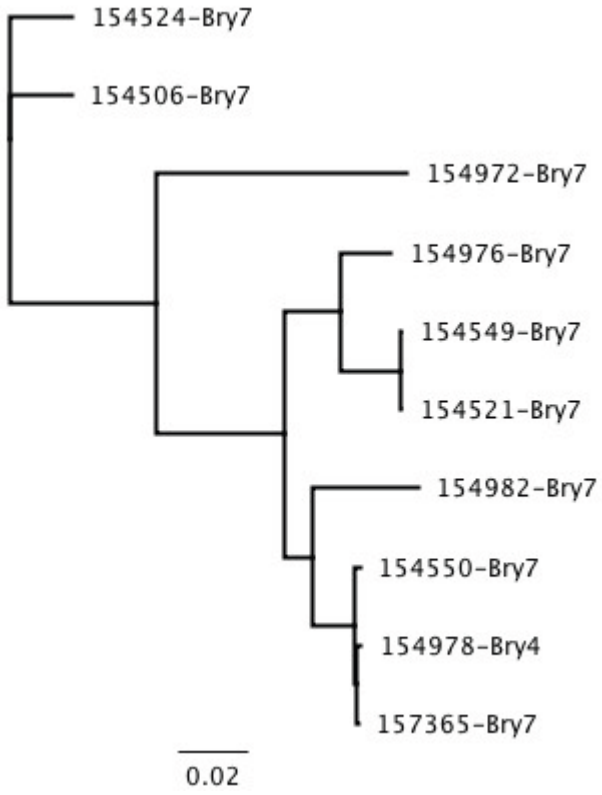
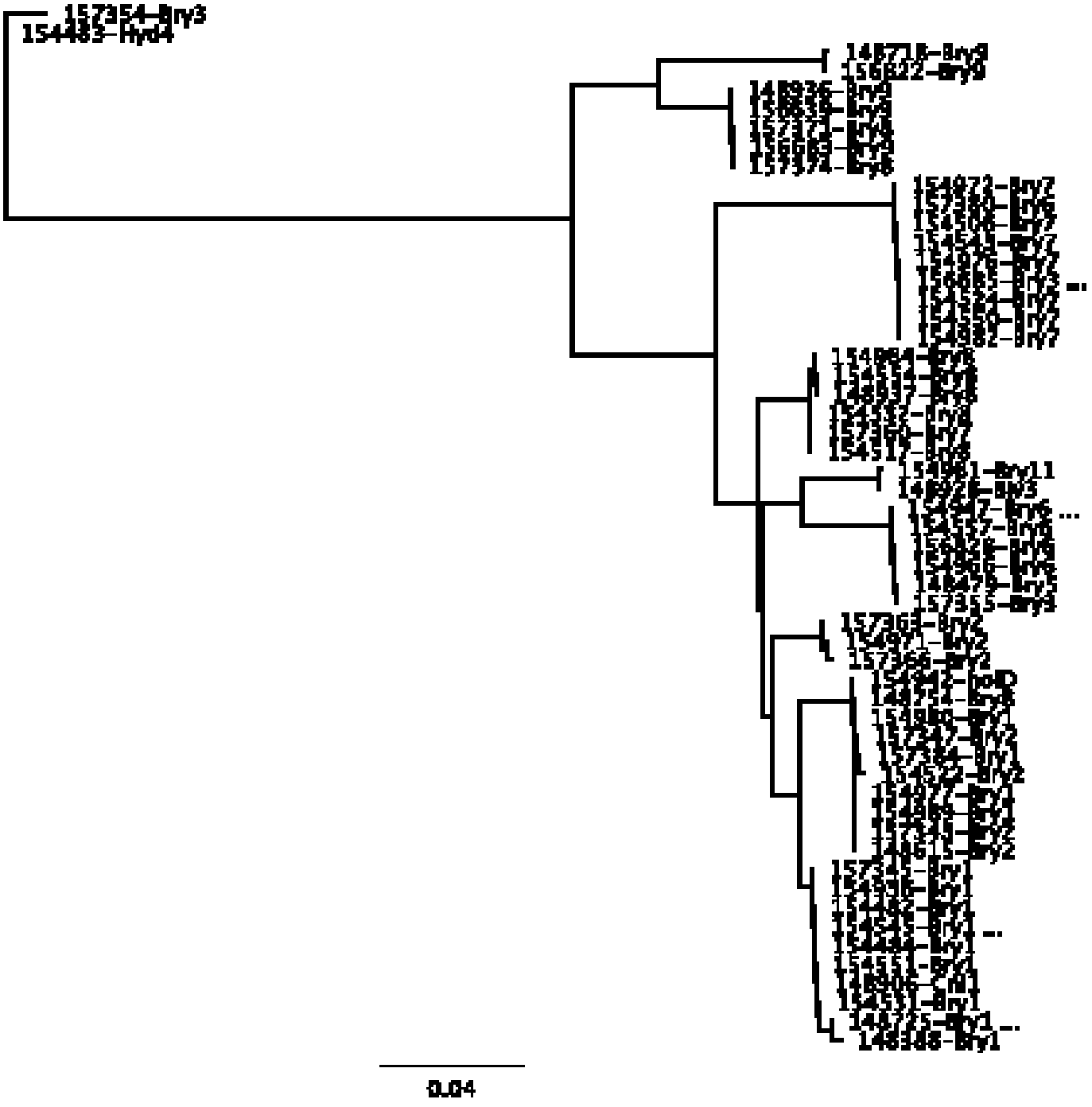


Figure 9. Genetic Tree based on 28S rRNA for bryozoans in PWS.



Appendix A. Taxa identified by morphological analyses by site. Shown is the number of voucher specimens with associated identifications per site.

Taxon	Alyeska Terminal	Ellamar Virgin Bay	Tatitlek Ferry Dock	Valdez Ferry Dock	Valdez Hatchery	Valdez boat harbor	Grand Total
Bivalvia							
Hiatella arctica	18	1	19	3			**41**
Hiatella sp.	5	4	3	5			**17**
Mytilidae	4		2	2	7	10	**25**
Mytilus sp.	1						**1**
Mytilus sp. (trossulus)	14		11	7			**32**
Pododesmus macrochisma		1					**1**
Bivalvia Total	**42**	**6**	**35**	**17**	**7**	**10**	**117**
Bryozoa							
Bugula cf. pacifica		1					**1**
Bugula pacifica	17						**17**
Callopora cf. craticula		1					**1**
Callopora craticula	5	9	6				**20**
Celleporella hyalina				1			**1**
cf. Callopora craticula	2	1					**3**
cf. Crisiella sp.		4					**4**
cf. Fenestrulina sp.		1					**1**
cf. Lichenopora verrucaria	1						**1**
cf. Pacificincola sp.	1						**1**
Cribrilina annulata		1					**1**
Cribrilina corbicula			10				**10**
Crisiella sp.		4		1			**5**
Crisiidae		1					**1**
Cryptosula zavjalovensis		1					**1**
Filicrisia sp.		1					**1**
Lichenopora verrucaria	1						**1**
Membranipora villosa	14	1	11				**26**
Parasmittina cf. trispinosa	1	2					**3**
Schizoporella japonica		4	17				**21**
Tubulipora sp.	4	4					**8**
Bryozoa Total	**46**	**36**	**44**	**2**			**128**

Appendix A (continued).

Taxon	Alyeska Terminal	Ellamar Virgin Bay	Tatitlek Ferry Dock	Valdez Ferry Dock	Valdez Hatchery	Valdez boat harbor	Grand Total
Cirripedia							
Amphibalanus improvisus	1						**1**
Balanidae	2	2	1			4	**9**
Balanus balanus			1				**1**
Balanus crenatus	18	5	7	1			**31**
Balanus sp.	1	1	2			2	**6**
Semibalanus balanoides	4		2				**6**
Semibalanus cariosus			1				**1**
Semibalanus sp.	1						**1**
Cirripedia Total	**27**	**8**	**14**	**1**		**6**	**56**
Hydrozoa							
Campanulariidae	14		5	12			**31**
Clytia gracilis	12			2			**14**
Clytia hemisphaerica	5			3			**8**
Clytia sp.	4	2	2	5			**13**
Gonothyraea clarki	5			12			**17**
Obelia dichotoma	3			4			**7**
Obelia sp.	5			4		1	**10**
Sarsia sp.				1			**1**
Hydrozoa Total	**48**	**2**	**7**	**43**		**1**	**101**
Tunicata							
cf. Pyuridae		4					**4**
Corella inflata		6					**6**
Distaplia alaskensis		1					**1**
Distaplia cf. occidentalis		2					**2**
Distaplia occidentalis		3					**3**
Distaplia sp.		1					**1**
Tunicata Total		**17**					**17**
Grand Total	**163**	**69**	**100**	**63**	**7**	**17**	**419**

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