UNIVERSITY OF CALIFORNIA SAN DIEGO

Biodiversity of *Diopatra* (Onuphidae, Annelida) and their tube-associated epifauna in the San Diego subtidal zone

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Marine Biology

by

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Committee in charge:

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Professor Paul Dayton
Professor James Leichter

2024

The Thesis of Isaiah Freedman is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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VITA

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ABSTRACT OF THE THESIS

Biodiversity of *Diopatra* (Onuphidae, Annelida) and their tube-associated epifauna in the San Diego subtidal zone

by

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Master of Science in Marine Biology

University of California San Diego, 2024

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This thesis investigates the biodiversity and ecological roles of the genus *Diopatra* in the San Diego subtidal zone, focusing on the epifaunal communities found on their tube-caps in both kelp forest and sandy bottom habitats. Through molecular and morphological analyses, two distinct species of *Diopatra* were identified: *Diopatra californica*, a resurrected species that was previously synonymized with *Diopatra splendidissima*, and *Diopatra ornata*, with both species demonstrating clear habitat preferences. A comprehensive survey of the epifaunal communities on *Diopatra* tube-caps revealed at least 103 associated species, with significant differences in community composition based on habitat type – both arthropods and molluscs were significantly more abundant in the kelp forest compared to sandy bottoms. The study argues for the resurrection of *Diopatra californica* as a distinct species due to insufficient support for its synonymization with *Diopatra splendidissima*, stemming from subjective historical taxonomic practices based on cosmopolitanism. By highlighting the role of Diopatra as ecosystem engineers and the need for further research on their conservation status amidst threats like climate change and fishing practices, this work underscores the complexity of marine biodiversity and the intertwined fates of species within their ecological niches.

INTRODUCTION

One of the southern California subtidal zone's claims to fame is its lush and diverse kelp forests. Along the coast of San Diego, dense stands of *Macrocystis pyrifera* (giant kelp) can reach heights of thirty meters, while an understory of *Eisenia arborea, Pterygophora californica,* and *Laminaria farlowii* blankets the rocky seabed. These forests contain diverse fish and invertebrate communities, many of which are important to commercial and recreational fisheries like rockfish, California spiny lobster, and sea urchins (Parnell et al., 2020). Other large invertebrates including turban snails, limpets, sponges, and anemones are found in abundance. While the substrate is primarily rocky, small depressions in the topography can fill with sand, allowing for animals typically found in soft-bottomed habitats to penetrate these kelp-dominated habitats (Foster & Schiel, 1985).

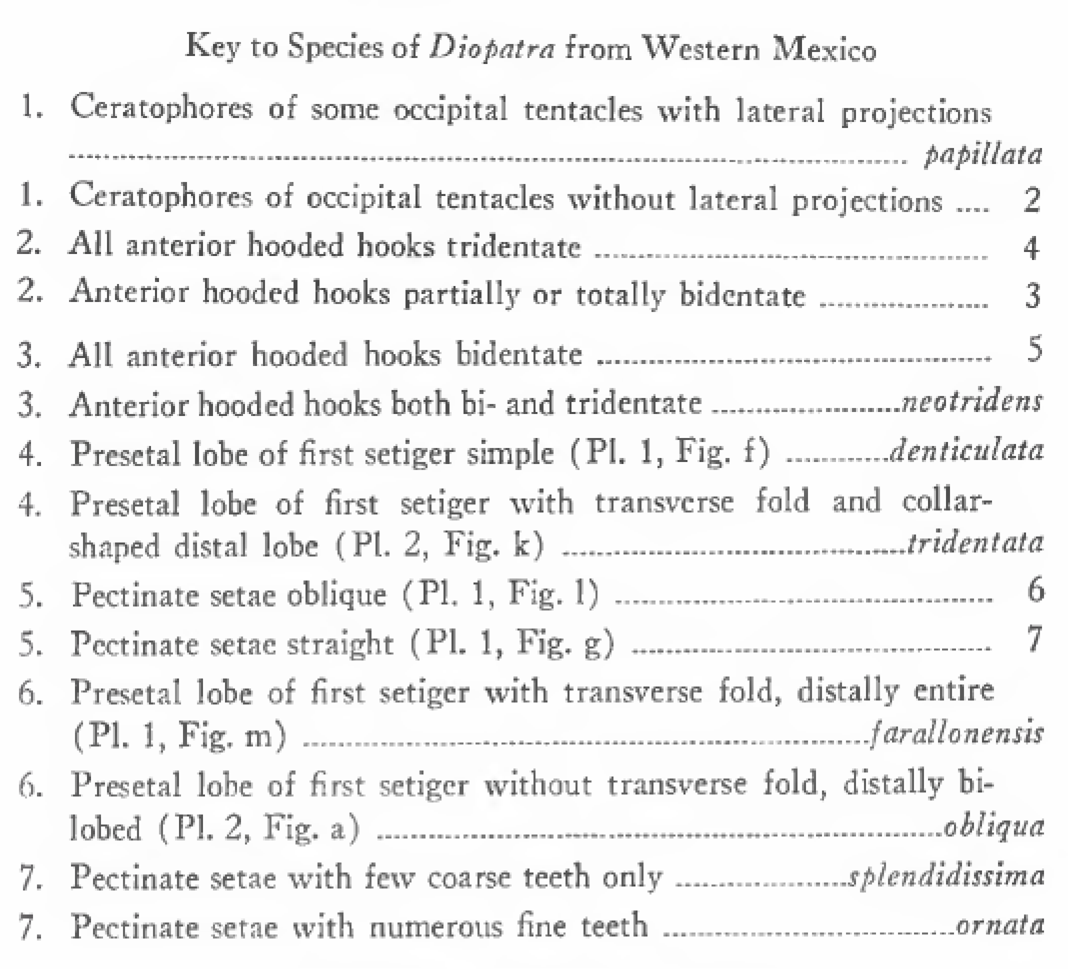
While southern California's coastal communities are famed for their *Macrocystis*-dominated kelp forests, many large areas on the coastal shelf contrast starkly with depauperate sand flats. Much of the animal life is infaunal, living below the water-sediment interface, with the community dominated by diverse assemblages of benthic crustaceans, polychaetes, and molluscs often subject to high rates of predation by a diversity of consumers (VanBlaricom, 1978). Epifaunal organisms that live in this benthic habitat must rely on strategies like camouflage or protection to survive in such an exposed environment. One of the most commonly found invertebrates in these habitats are the tube-building genus of annelids *Diopatra* (Foster & Schiel, 1985).

Diopatra Audouin and Milne Edwards, 1833 is a cosmopolitan genus of tube-building polychaetes in the family Onuphidae (Errantia, Annelida). They inhabit shallow waters worldwide from the tropics to the polar regions. They are noted for their opalescent coloration, spiralled branchiae, and parchment-like tubes that they construct and subsequently adorn with debris from their local environment (Arias et al., 2023).

Diopatra can be identified by a few key traits. Their most notable trait are the large, parchment-like tubes they construct around themselves. While their appearance and characteristics can vary between species, these tubes are generally produced via a mucus they secrete which polymerizes into a hard matrix that adheres to sediment and other debris they come into contact with during formation. *Diopatra* are also commonly known as "decorator worms" due to most species' high selectivity of adhered tube material. While some species keep their tubes bare, many will adorn their tube caps with algae, shell fragments, and other foreign materials. Another morphological characteristic of *Diopatra* are their long spiraled branchiae (gills), which distinguishes the genus from other close relatives like *Onuphis* whose branchiae are shorter and unspiraled. The prostomium is adorned with two short frontal antennae and five long occipital tentacles with ringed ceratophores at their base. Parapodia contain a diverse array of chaetae, ranging from simple needle-like structures to hooks, combs (known as pectinate chaetae), and stouter aciculi. These chaetae often grow in tufts or fascicles (Hartman, 1944).

The eastern Pacific is home to many known species of *Diopatra*. Johan Gustaf Hjalmar Kinberg described *Diopatra splendidissima* from a specimen off the coast of Ecuador in 1865 (Kinberg, 1865), but its range was later expanded by other taxonomists as far north as California (Hartman, 1944). In the early 20th century, John Percy Moore described two more species from the California coast: *Diopatra californica* in 1904 from San Diego (Moore, 1904), and *Diopatra ornata* in 1911 from Monterey (Moore, 1911). Olga Hartman described more species during the Allan Hitchcock expeditions aboard the Velero III in 1944, such as *Diopatra obliqua* and *Diopatra tridentata*. During this expedition, Hartman notably synonymized *Diopatra californica* with *Diopatra splendidissima*, noting their similar morphological characteristics and declaring *Diopatra californica* to not be a valid species (Hartman, 1944).

Species within *Diopatra* can be difficult or impossible to distinguish using large-scale morphology; minor features like differences in chaetae or distribution of the branchiae are generally used instead. Below is a flow chart Kristian Fauchald created to distinguish *Diopatra* species he identified in Western Mexico (Fauchald, 1968):



Key to Species of *Diopatra* from Western Mexico

1. Ceratophores of some occipital tentacles with lateral projections .... *papillata*
1. Ceratophores of occipital tentacles without lateral projections 2
2. All anterior hooded hooks tridentate 4
2. Anterior hooded hooks partially or totally bidentate 3
3. All anterior hooded hooks bidentate 5
3. Anterior hooded hooks both bi- and tridentate .... *neotridens*
4. Presetal lobe of first setiger simple (Pl. 1, Fig. f) .... *denticulata*
4. Presetal lobe of first setiger with transverse fold and collar-shaped distal lobe (Pl. 2, Fig. k) .... *tridentata*
5. Pectinate setae oblique (Pl. 1, Fig. l) 6
5. Pectinate setae straight (Pl. 1, Fig. g) 7
6. Presetal lobe of first setiger with transverse fold, distally entire (Pl. 1, Fig. m) .... *farallonensis*
6. Presetal lobe of first setiger without transverse fold, distally bi-lobed (Pl. 2, Fig. a) .... *obliqua*
7. Pectinate setae with few coarse teeth only .... *splendidissima*
7. Pectinate setae with numerous fine teeth .... *ornata*

Figure 1. Key to *Diopatra* of Western Mexico

While Hartman pointed to the similarities of the pectinate chaetae to justify reclassifying *Diopatra californica* as a junior synonym of *Diopatra splendidissima*, her synonymy was ultimately subjective, largely based on her belief in the theory of cosmopolitanism, the theory that many marine species, especially invertebrates, have wide ranges, spanning entire ocean basins or the globe. With the development and advancement of molecular and genetic identification techniques, cosmopolitan species have become increasingly rare (Hutchings & Kupriyanova, 2018).

Diopatra have been well studied for their significant role in their local environments, and are often referred to as "ecosystem engineers" (Arias et al., 2023). Their tubes stabilize sediment, facilitating the survival and growth of numerous other infaunal and benthic species, as well as increasing general biodiversity in areas they inhabit (Ambrose & Anderson, 1990; Diaz et al., 2003; Woodin, 1981). This includes facilitating the growth of seagrass and macroalgae, which can thrive on their tube-caps in areas otherwise lacking hard substrate (Thomsen & McGlathery, 2005). Their tubes also protect sediment-dwelling organisms from predators (Woodin, 1981). *Diopatra* themselves are an important food source for many marine and intertidal predators, including demersal fish, crabs, and shorebirds (Arias et al., 2023).

One other notable ecological role of *Diopatra* is their ability to host commensal invertebrate communities on their tube-caps. Their imbricate parchment-like tubes and adornments have high surface areas, providing a region for invertebrates and algae to settle in an area generally lacking solid substrate. This structure results in *Diopatra*'s ability to host a wide variety of epifauna. Numerous studies have been conducted examining the biodiversity of tube-cap epifauna and their effects on community structure (Bell & Coen, 1982a, 1982b; Diaz et al., 2003; Dudley et al., 1989; Mangum et al., 1968; Santos & Aviz, 2019; Thomsen et al., 2011). That said, these studies have largely focused on a single species found in the western Atlantic, *Diopatra cuprea*; little to no research has been conducted on the epifauna of *Diopatra* in the northeast Pacific.

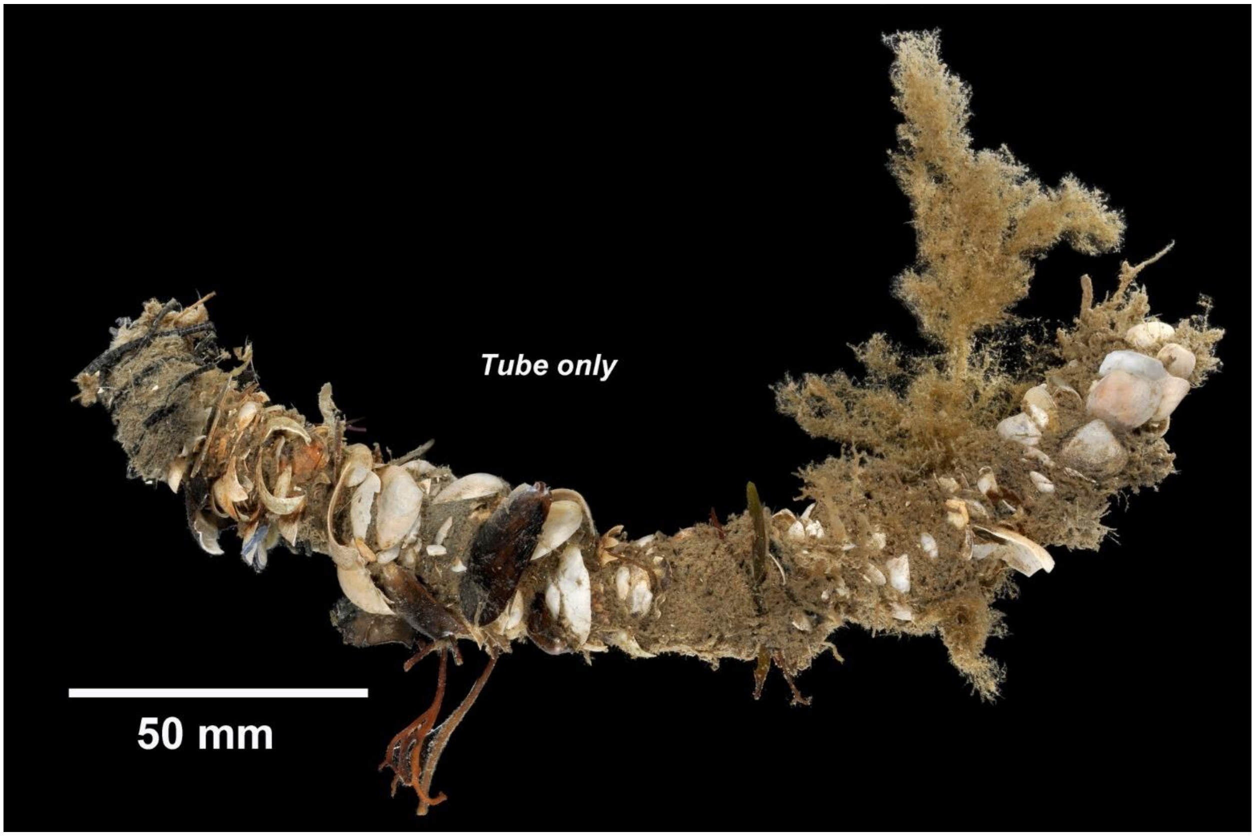


Figure 2. Tube-cap of *Diopatra cuprea* (Source: Lucas, 2020)

Despite their global distribution and generally high densities in shallow soft-bottom environments, *Diopatra* face a number of threats. In Western Europe, *Diopatra* are commonly dug up by fishermen to be used as bait. A study on bait production revealed that active intense harvesting practices were unsustainable, and recommended immediate resource management (Cunha et al., 2005). Bait harvesting is also practiced in other regions of the world, such as Japan, South Africa, and Turkey (Arias et al., 2023). While large-scale exploitation of *Diopatra* is currently limited to small regions of the world, continued overfishing of the world's oceans could increase demand. Climate change also poses a large threat to *Diopatra* populations. A study modeling how increasingly warm and acidic oceans will impact the genus found that these conditions significantly hamper *Diopatra*'s ability to regenerate tissue (Pires et al., 2015). Fluxes in temperatures can also impact feeding and tube production (Myers, 1972). Both bait harvesting and the effects of climate change highlight the need for further study of human impacts on *Diopatra*. We cannot be sure that *Diopatra* are protected until their populations and ecology are better understood.

In this thesis, I address three primary research questions. First, I quantify and characterize the biodiversity of epifaunal communities associated with *Diopatra* tube-caps. Second, I identify the species of *Diopatra* present in the San Diego subtidal zone and examine whether they show a preference for kelp forest or sandy bottom habitats. As part of this, I argue for the resurrection of *Diopatra californica* as a distinct species from *Diopatra splendidissima*. Finally, I compare and contrast the composition of epifaunal communities on tube-caps in kelp forest and sandy bottom habitats in San Diego.

MATERIALS AND METHODS

Preliminary dives were conducted off the San Diego to determine where divers could identify presence of significant numbers of *Diopatra*. Three sites were selected for collection: one in the sandy flats west of the Scripps Institution of Oceanography (“La Jolla sandy site”), one in the north La Jolla kelp forest (“La Jolla kelp site”), and one in the kelp forests west of Point Loma (“Point Loma kelp site”).

The La Jolla sandy site is located at 32.86785° N, 117.26134° W, about 400 m due west of the Scripps Pier in 17 m of depth. It is a soft-bottomed habitat, lacking seagrass, macroalgae, and hard substrate. The La Jolla kelp site is located at 32.83072° N, 117.29152° W, due west of the western terminus of Marine St in 17 m of depth. The site is dominated by a scattered canopy of *Macrocystis pyrifera* and *Pelagophycus porra*, with a rich rocky reef understory algal community of filamentous and coralline red algae, smaller kelps, and other turf. The Point Loma kelp site is located at 32.6995° N, 117.265° W in 21m of depth and is similar ecologically to the La Jolla kelp site.

A temperature and dissolved oxygen recorder (PME miniDO$_2$T) was deployed to monitor physical oceanographic conditions, which continuously sampled temperature and dissolved oxygen concentrations at 1 minute intervals from November 2023 to October 2024. The sensor was periodically swapped out to retrieve data and clean.

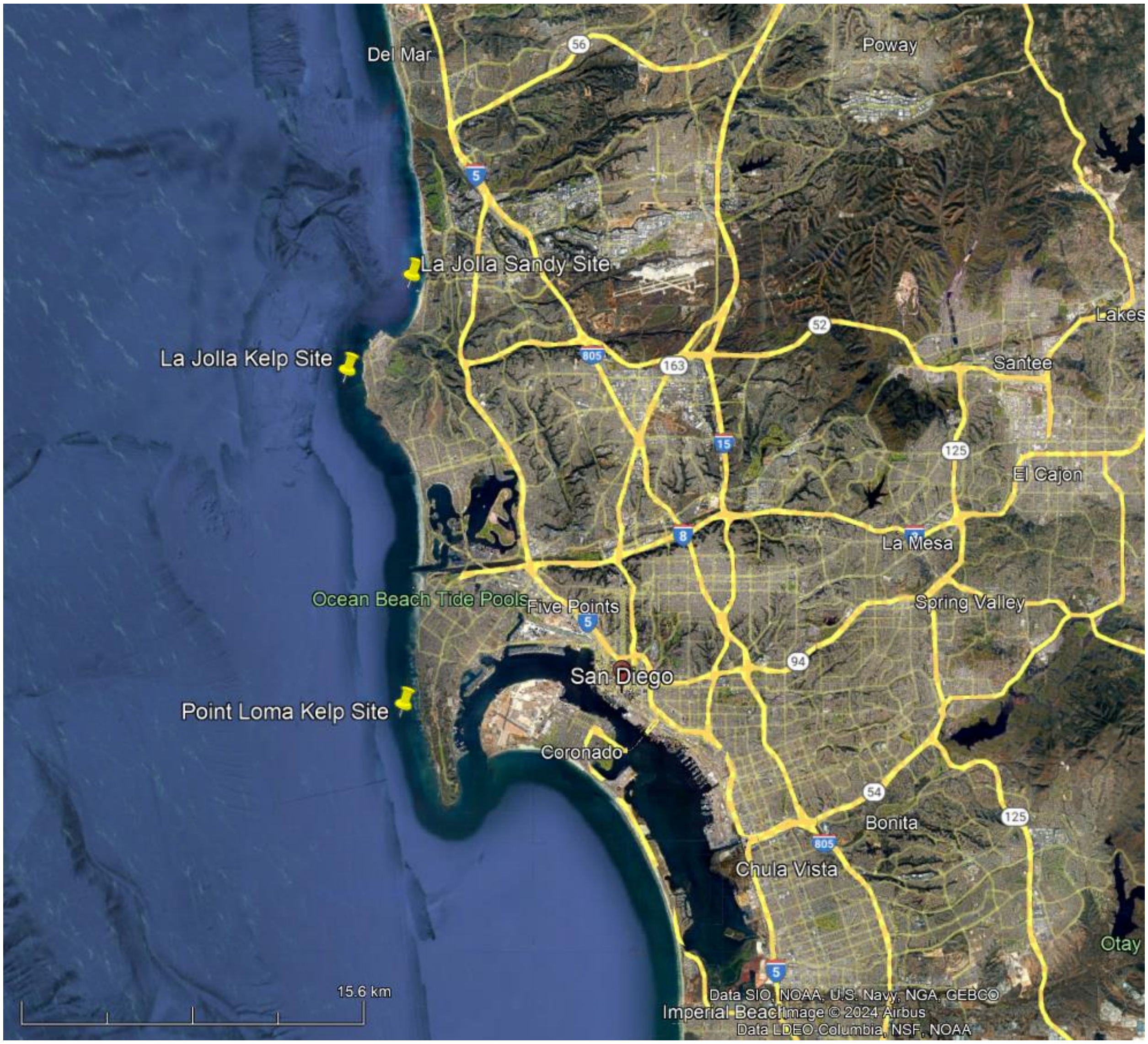


Figure 3. Map of *Diopatra* collection sites in San Diego

Diopatra tube-caps were collected regularly between March and August 2024 at each of the three sites for epifaunal analysis. On each dive, five *Diopatra* tube-caps were identified, cut at the sediment-water interface and placed in separate Falcon tubes. The La Jolla sandy site was visited 6 times (28 tube-caps analyzed); the La Jolla kelp site was visited 5 times (25 tube-caps analyzed); the Point Loma kelp site was visited 1 time (3 tube-caps analyzed).

In addition, adult *Diopatra* were separately collected. Due to their tendency to retreat quickly underground when disturbed, collecting an intact *Diopatra* specimen can be difficult.

Ambush retrieval with a trowel was necessary to collect intact or mostly intact adult *Diopatra* specimens. Adults were collected at the La Jolla sandy site and La Jolla kelp site for species identification.

After collection in the field, tube-caps were brought back to the lab for analysis. To prevent sample degradation and rotting of specimens before identification could take place, tube-cap analysis occurred within 36 hours of collection. Tube-caps were placed in petri dishes and examined under a dissecting scope. Forceps, a scalpel, and dissecting scissors were used to open the tube-cap and manipulate specimens. Macroinvertebrates (anything larger than ~0.3 mm) were identified to the lowest taxonomic level using morphological traits, and animal counts were recorded for each tube cap. Specimens with unknown identities were photographed for later identification. Due to their sheer numbers and relatively small size, abundance and species richness of nematodes and copepods were not quantified. Morphological identification was aided by a taxonomic guide book (Morris et al., 1980) and community identifications on iNaturalist, a citizen science website used for reporting and identifying wildlife.

Some specimens were set aside in vials with 95% ethanol to create vouchers for genetic analysis. An emphasis was put on intact invertebrates that were of unknown identity or did not match a species that had been previously collected. Extraction protocols were conducted to isolate DNA from the vouchers. The COI and 16S regions of the mitochondrial genome were targeted and amplified via PCR. The COI gene is relatively invariable within species but shows a reasonably reliable gap between closely related species, allowing for more accurate species distinction. For the samples that COI sequencing failed, sequencing of the 16S was conducted instead. PCR products were sent to Eurofins Genomics for processing and sanger sequencing. Sequences were subsequently trimmed in Geneious Prime to maximize quality of the read.

Trimmed sequences were compared to existing sequences using the Basic Local Alignment Search Tool (BLAST) function on the National Center for Biotechnology Information (NCBI)'s GenBank to identify samples. Sample sequences were also aligned with other known sequences via multiple alignment using fast Fourier transform (MAFFT) in the program Mesquite for further identification. A combination of these morphological and genetic techniques were conducted to identify and quantify biodiversity. Processing all samples took many months to complete.

Adult *Diopatra* were identified to the species level via morphological and genetic analysis. Parapodia from the 1st and 12th setigers were removed and made into slides to examine their chaetae. Examination of fine-scale morphology using a high-powered light microscope was used to identify species. Vouchers were created by preserving specimens in 95% ethanol, and tissue samples were taken for DNA extraction and COI sequencing. Chaetal morphology and COI sequences were used together to identify each adult *Diopatra* to the species level.

Vouchered specimens were catalogued and stored in the Scripps Institution of Oceanography's Benthic Invertebrate Collection (SIO-BIC). Sequences from the vouchers were made publicly available on GenBank.

Once tube-cap specimens are identified and enumerated, the data was analyzed to compare and contrast the epifaunal biodiversity between the La Jolla kelp site and La Jolla sandy site. Poor ocean conditions, logistics, and other factors limited the ability to visit and collect specimens from the Point Loma kelp site, resulting in a low sample size, so biodiversity from this site was not included in the comparison. Single-factor ANOVAs were calculated for each of the three most abundant phyla observed (Annelida, Arthropoda, and Mollusca) to compare the abundance of epifauna between sites. A Shannon diversity index was also calculated on the distribution of polychaete families to determine the overall biodiversity within the phylum. A Mann-Whitney U test was subsequently run to determine if the biodiversity of epifaunal polychaetes is significantly different between sites.

RESULTS

Diopatra identification

COI sequencing of *Diopatra* samples revealed the presence of two distinct species: *Diopatra ornata* and *Diopatra californica.* The *Diopatra ornata* sequences matched sequences from samples previously collected from Monterey, CA, Washington state, and British Columbia of the same species. Hartman's (1944) species descriptions identified two key characteristics to differentiate *Diopatra ornata* from other *Diopatra* in the Eastern Pacific – bidentate anterior hooded hooks and finely toothed (20+ tines) pectinate chaetae, both of which were present in collected specimens. The *Diopatra californica* sequences were distinct from those of *Diopatra ornata*, but did not match any named existing sequences. Morphology was similarly used to confirm identification. Bidentate anterior hooded hooks and coarsely-toothed pectinate chaetae were observed with a high-powered light microscope (~7-9 tines).

Figure 4. Anterior end of *Diopatra ornata*

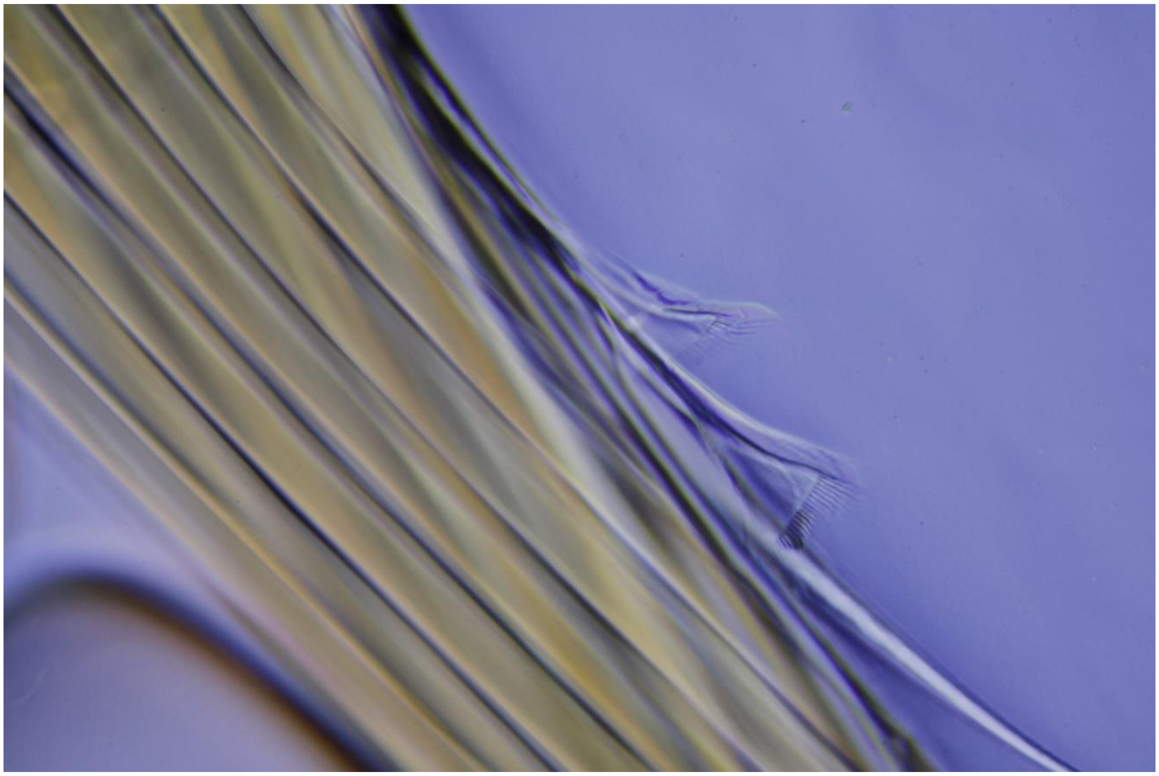


Figure 5. Pectinate chaetae of *Diopatra ornata*

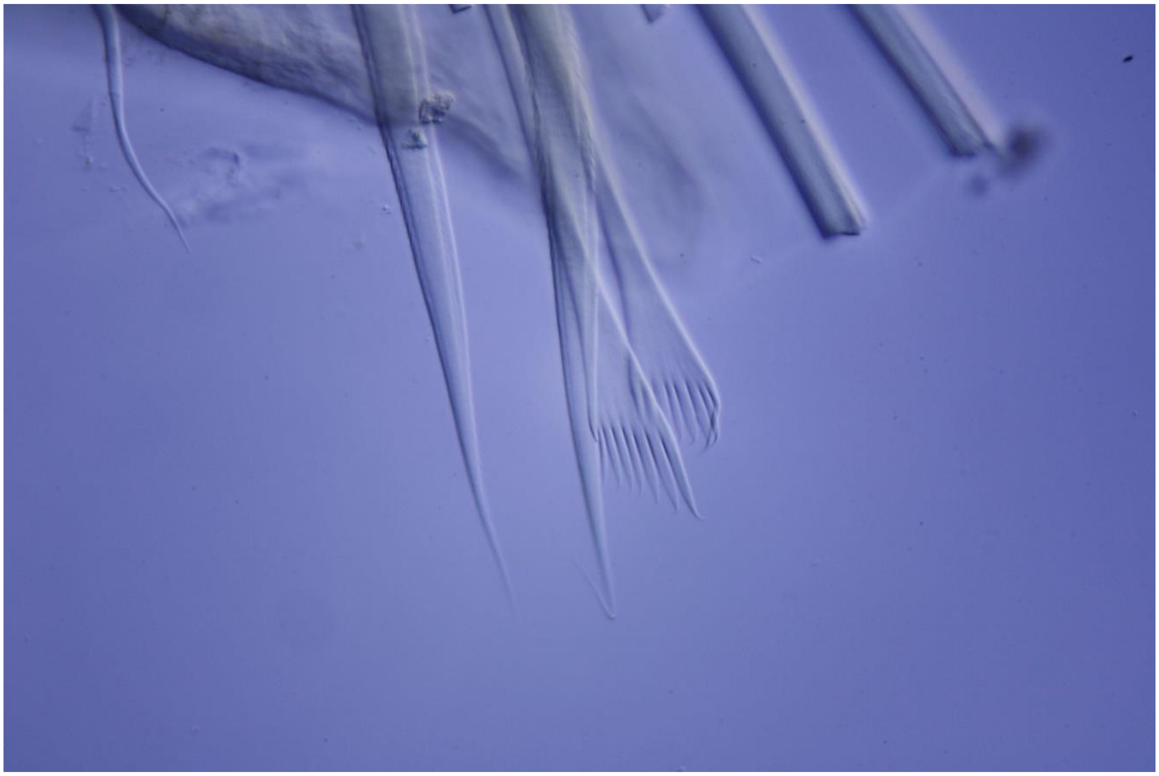


Figure 6. Pectinate chaetae of *Diopatra californica*

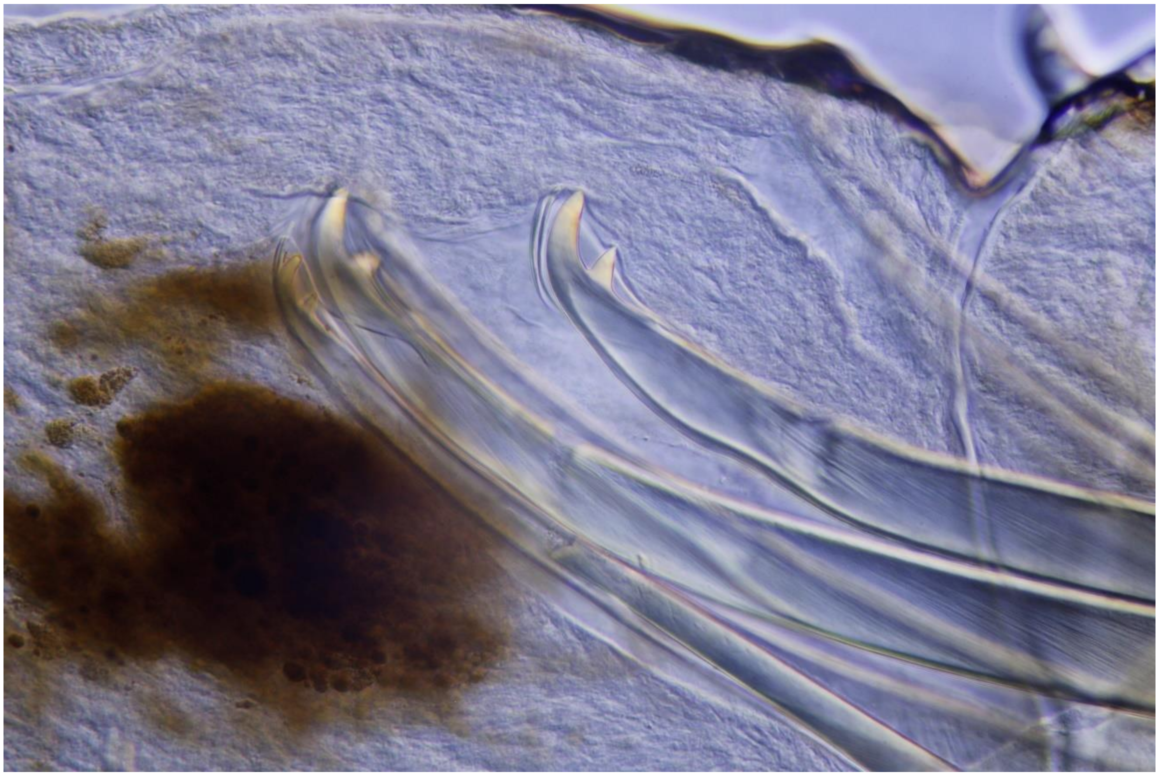


Figure 7. Anterior bidentate hooded hooks of *Diopatra ornata*

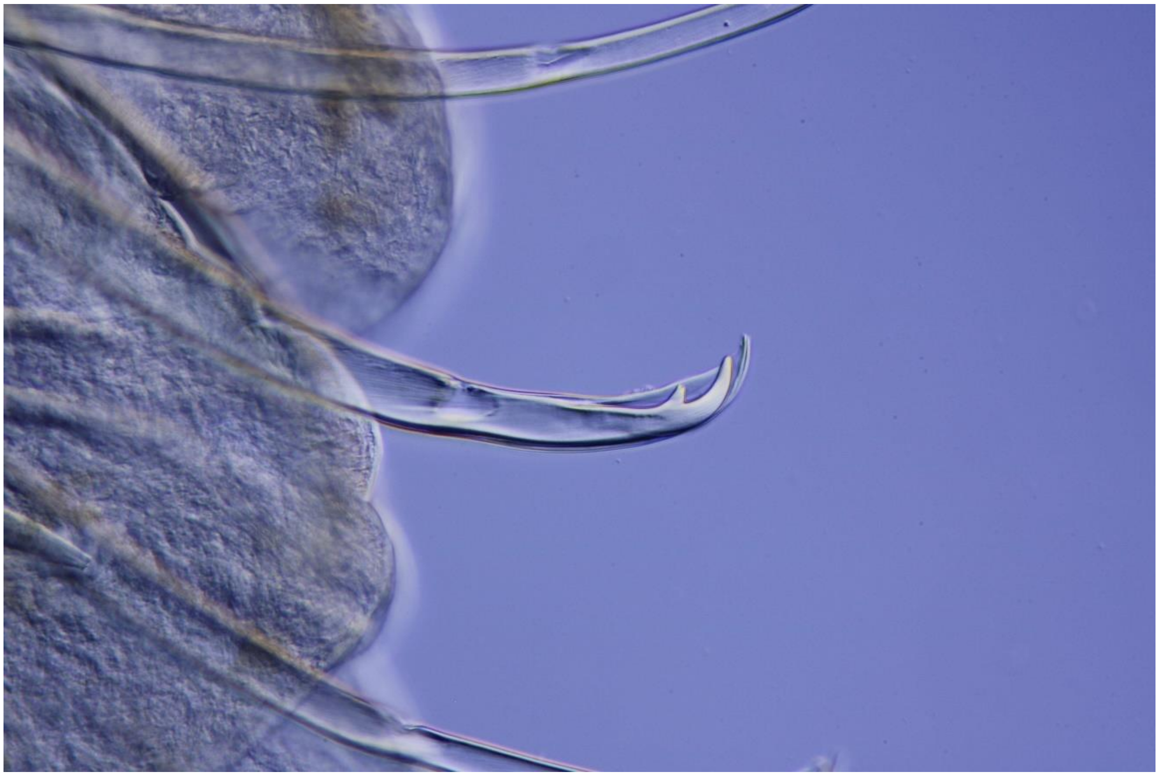


Figure 8. Anterior bidentate hooded hooks of *Diopatra californica*

Of the thirteen adult *Diopatra* samples collected and sequenced, five were identified as *Diopatra californica* and eight were identified as *Diopatra ornata*. They were entirely segregated by habitat, with all *Diopatra californica* samples collected at the La Jolla sandy site, and all *Diopatra ornata* samples collected at the La Jolla kelp site. Epifaunal juvenile *Diopatra* were also found, but only on tube-caps collected at the La Jolla sandy site (from *Diopatra californica*). Of the fourteen epifaunal samples sequenced, only one was identified as *Diopatra ornata* – the rest were identified as *Diopatra californica*.

Multiple qualitative differences were observed between the *Diopatra* found at the La Jolla sandy site and La Jolla kelp site. For example, the overall construction of their tube-caps varied between the two species. *Diopatra ornata*'s tube-caps were generally thicker, adorned with larger shell fragments and pieces of coralline algae. The larger decorations made the tube-caps more rigid overall. On the other hand, *Diopatra californica*'s tube-caps had smaller pieces of sediment and debris, making them more flexible.

Distribution of individual *Diopatra* also starkly contrasted between sites. At the La Jolla sandy site, *Diopatra* were relatively abundant and had a uniform distribution; many individuals could be found per square meter, and they tended to be evenly spaced apart. At the La Jolla kelp site, on the other hand, distribution was much patchier. *Diopatra* tube-caps tended to be found in dense aggregations, typically under rocky ledges near the edges of sandy depressions.

Epifaunal species

Examination of *Diopatra* tube-caps revealed a high diversity of epifaunal arthropods, molluscs, annelids, and other benthic invertebrates. The total epifaunal biodiversity included at least 103 species, including 40 species of annelid, 20 species of arthropod, and 15 species of mollusc. Epifaunal annelid biodiversity included at least sixteen families. Species richness varied between sites. At least 46 epifaunal species were identified at the La Jolla sandy site, while 72 species were identified at the La Jolla kelp site and a further 21 species were identified at the Point Loma kelp site.

Figure 9. Photos of epifaunal annelids with scalebars

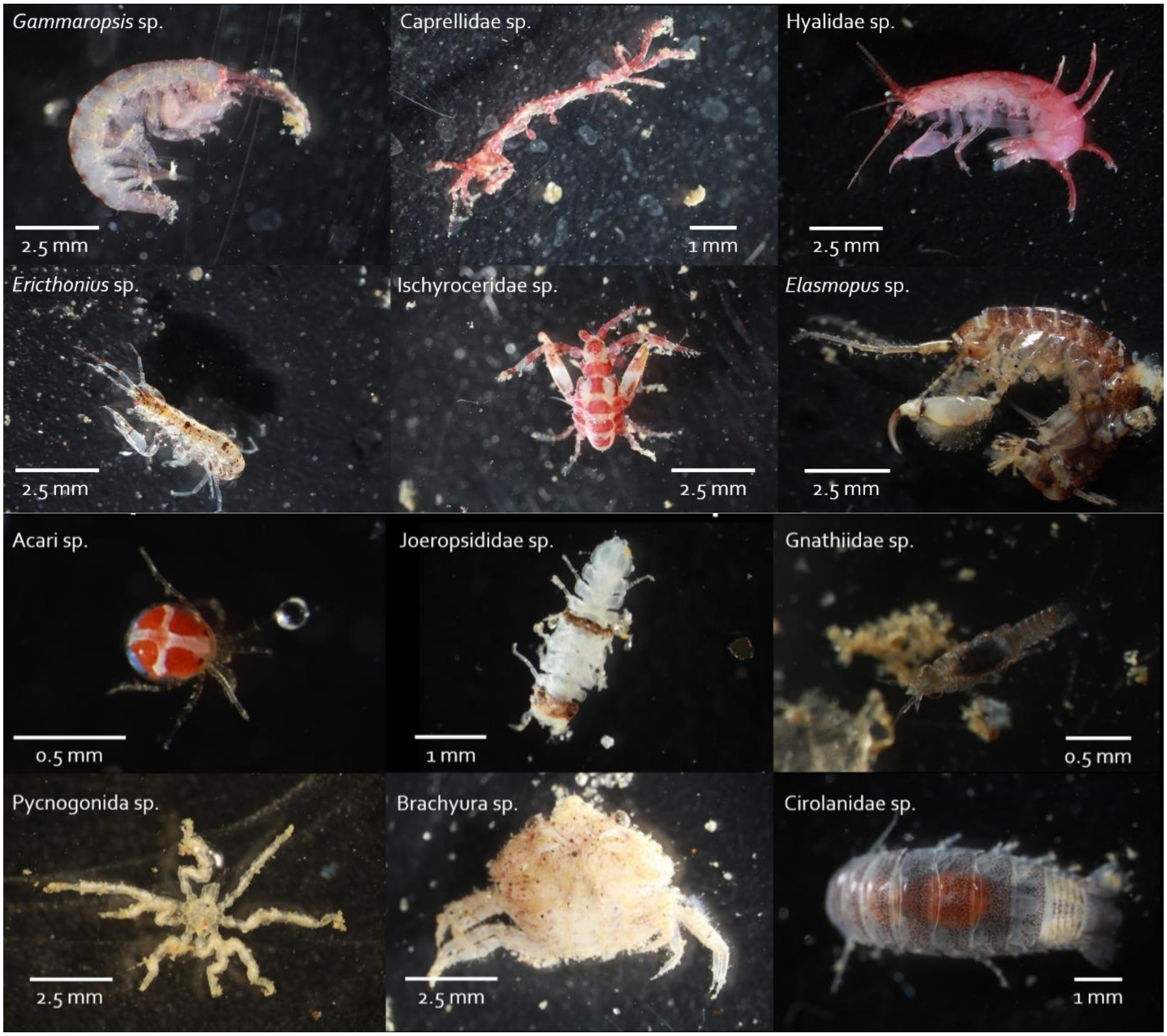


Figure 10. Photos of epifaunal arthropods with scalebars

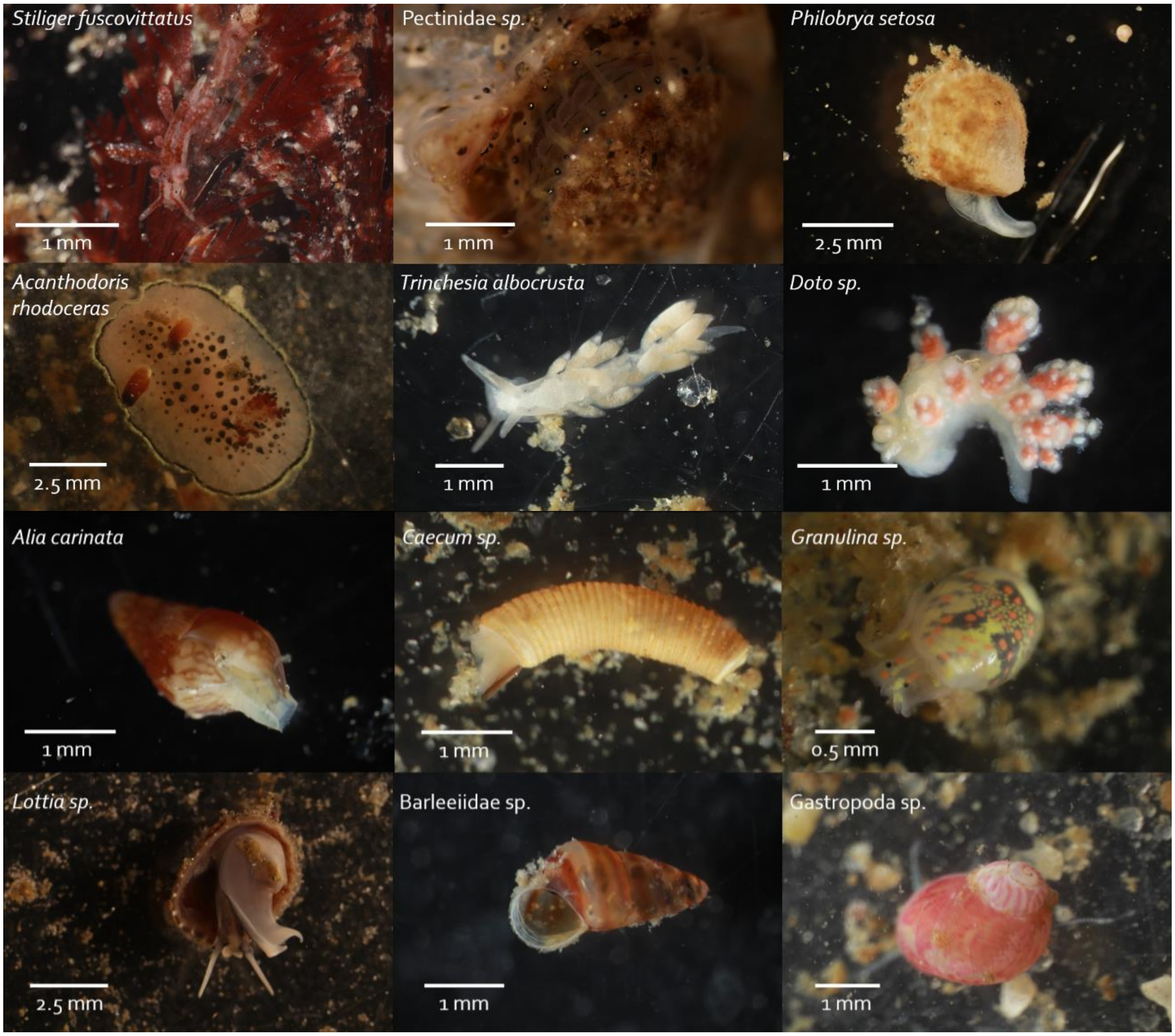


Figure 11. Photos of epifaunal molluscs with scalebars

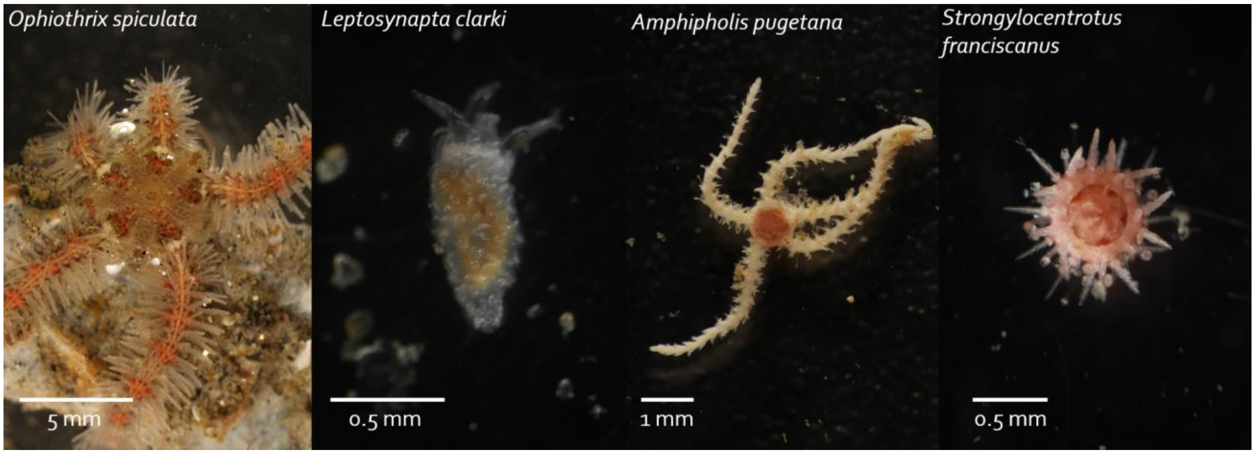


Figure 12. Photos of epifaunal echinoderms with scalebars

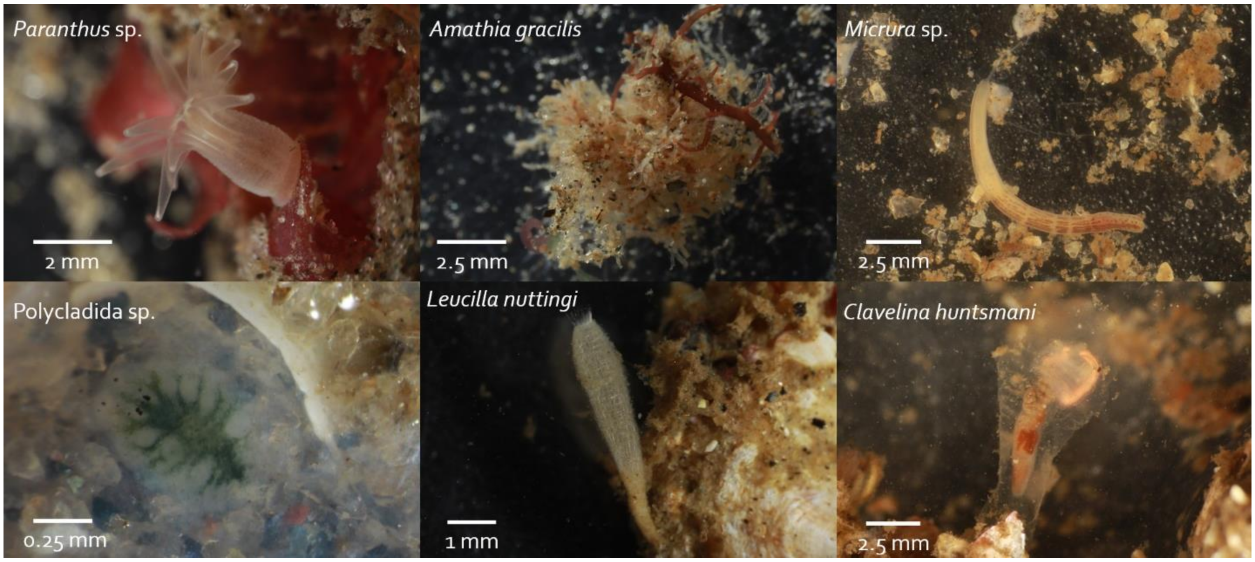


Figure 13. Photos of other epifaunal species with scalebars

Table 1. List of epifaunal invertebrates on Diopatra tube-caps, by phylum

- **Annelida**
  - *Samytha californiensis* Hartman, 1969
  - *Phyllocomus hiltoni* (Chamberlin, 1919)
  - *Chrysopetalum occidentale* Johnson, 1897
  - *Paleanotus bellis* (Johnson, 1897)
  - Cirratulidae sp. Ryckholt, 1851
  - Eunicidae sp. Berthold, 1827
  - *Leodice* sp. Lamarck, 1818
  - *Micropodarke dubia* (Hessle, 1925)
  - *Oxydromus pugettensis* (Johnson, 1901)
  - *Platynereis sp.* Kinberg, 1865
  - *Diopatra californica* Moore, 1904
  - *Diopatra ornata* Moore, 1911
  - *Eteone sp.* Savigny, 1822
  - *Eulalia aviculiseta* Hartman, 1936
  - *Eulalia gracilior* (Chamberlin, 1919)
  - *Eumida sp.* Malmgren, 1865
  - *Nereiphylla sp.* Blainville, 1828
  - *Phyllodoce sp.* Lamarck, 1818
  - *Pterocirrus burtoni* Pleijel, Aguado & Rouse, 2012
  - *Halosydna sp.* Kinberg, 1856
  - *Thormora setosior* (Chamberlin, 1919)
  - *Phragmatopoma sp.* Mörch, 1863
  - *Chone sp.* Krøyer, 1856
  - Serpulidae sp. Rafinesque, 1815
  - *Pholoides asperus* (Johnson, 1897)
  - *Dipolydora giardia* (Mesnil, 1893)
  - *Polydora sp.* Bosc, 1802
  - *Eurysyllis sp.* Ehlers, 1864
  - *Eusyllis sp.* Malmgren, 1867
  - *Exogone sp.* Örsted, 1845
  - *Myrianida sp.* Milne Edwards, 1845
  - *Odontosyllis cf. phosphorea* Moore, 1909
  - *Odontosyllis sp.* Claparède, 1863
  - *Parexogone molesta* (Banse, 1972)
  - *Proceraea sp.* Ehlers, 1864
  - *Pseudosyllis sp.* Grube, 1863
  - *Syllis sp.* Lamarck, 1818
  - *Eupolymnia heterobranchia* (Johnson, 1901)
  - *Pista sp.* Malmgren, 1866
  - *Thelepus sp.* Leuckart, 1849
- **Arthropoda**
  - Acari spp. Leach, 1817
  - Pycnogonida spp. Latreille, 1810
  - Copepoda spp. Milne Edwards, 1840
  - Amphipoda spp. Latreille, 1816
  - *Aoroides columbiae* Walker, 1898
  - *Elasmopus* sp. A. Costa, 1853
  - *Ericthonius* sp. H. Milne Edwards, 1830
  - *Gammaropsis* sp. Lilljeborg, 1855
  - Hyalidae sp. Bulyčeva, 1957
  - *Pariphinotus escabrosus* (J.L. Barnard, 1962)
  - *Photis sp.* Krøyer, 1842
  - *Podoceros* sp. Leach, 1814
  - *Paraconcavus pacificus* (Pilsbry, 1916)
  - Paguroidea sp. Latreille, 1812
  - *Lophopanopeus bellus* (Stimpson, 1860)
  - *Pugettia dalli* Rathbun, 1894
  - *Eualus subtilis* Carvacho & Olson, 1984
  - Anthuroidea Leach, 1814
  - Arcturidae sp. Dana, 1849
  - Cirolanidae sp. Dana, 1852
  - Gnathiidae sp. Leach, 1814
  - Joeropsididae sp. Nordenstam, 1933
  - Munnidae sp. G. O. Sars, 1897
  - Tanaidacea spp. Dana, 1849
  - Ostracoda spp. Latreille, 1802
- **Bryozoa**
  - *Amathia gracilis* (Leidy, 1855)
  - Unidentified Bryozoa sp.
- **Chordata (Tunicata)**
  - *Clavelina huntsmani* Van Name, 1931
- **Cnidaria**
  - *Metridium senile* (Linnaeus, 1761)
  - *Paranthus cf. rapiformis* (Le Sueur, 1817)
  - Actiniaria sp. Hertwig, 1882
  - *Clytia elsaeoswaldae* Stechow, 1914
  - *Xingyurella* sp. Song et al., 2018
- **Echinodermata**
  - *Strongylocentrotus franciscanus* (A. Agassiz, 1863)
  - *Leptosynapta clarki* Heding, 1928
  - *Amphipholis pugetana* (Lyman, 1860)
  - *Ophiothrix spiculata* Le Conte, 1851
- **Mollusca**
  - *Glycymeris cf. septentrionalis* (Middendorff, 1849)
  - *Hiatella arctica* (Linnaeus, 1767)
  - Pectinidae sp. Rafinesque, 1815
  - *Philobrya cf. setosa* (P. P. Carpenter, 1864)
  - *Alia carinata* (Hinds, 18440
  - *Caecum sp.* J. Fleming, 1813
  - *Crepidula naticarum* M. B. Williamson, 1905
  - *Crepidula onyx* G. B. Sowerby I, 1824
  - *Crepipatella lingulate* (A. Gould, 1846)
  - *Lacuna sp.* W. Turner, 1827
  - *Acanthodoris rhodoceras* T. D. A. Cockerell, 1905
  - *Doto sp.* Oken, 1815
  - *Trinchesia albocrusta* (MacFarland, 1966)
  - *Stiliger fuscovittatus* Lance, 1962
  - *Lottia sp.* J. E. Gray, 1833
  - Unidentified Gastropod spp.
- **Nematoda**
  - Nematoda sp. Diesing, 1861
- **Nemertea**
  - *Micrura sp.* Ehrenberg, 1828
  - *Poseidonemertes* sp. Kirsteuer, 1967
  - *Quasitetrastemma nigrifrons* (Coe, 1904)
  - *Tubulanus sp.* (Renier, 1804)
  - Unidentified Nemertea spp.
- **Platyhelminthes**
  - Tricladida spp. Lang, 1884
- **Porifera**
  - *Leucilla nuttingi* (Urban, 1902)

Table 2. List of epifaunal invertebrate species on Diopatra tube-caps at La Jolla sandy site, by phylum

Annelida
- *Samytha californiensis* Hartman, 1969
- *Phyllocomus hiltoni* (Chamberlin, 1919)
- *Paleanotus bellis* (Johnson, 1897)
- Cirratulidae sp. Ryckholt, 1851
- *Micropodarke dubia* (Hessle, 1925)
- *Platynereis sp.* Kinberg, 1865
- *Diopatra californica* Moore, 1904
- *Diopatra ornata* Moore, 1911
- *Eumida sp.* Malmgren, 1865
- *Nereiphylla sp.* Blainville, 1828
- *Phyllodoce sp.* Lamarck, 1818
- *Halosydna sp.* Kinberg, 1856
- *Thormora setosior* (Chamberlin, 1919)
- *Polydora sp.* Bosc, 1802
- *Eusyllis sp.* Malmgren, 1867
- *Myrianida sp.* Milne Edwards, 1845
- *Proceraea sp.* Ehlers, 1864
- *Eupolymnia heterobranchia* (Johnson, 1901)

Arthropoda
- Acari spp. Leach, 1817
- Pycnogonida spp. Latreille, 1810
- Copepoda spp. Milne Edwards, 1840
- Amphipoda spp. Latreille, 1816
- *Ericthonius* sp. H. Milne Edwards, 1830
- *Photis sp.* Krøyer, 1842
- *Paraconcavus pacificus* (Pilsbry, 1916)
- Joeropsididae sp. Nordenstam, 1933
- Munnidae sp. G. O. Sars, 1897
- Tanaidacea spp. Dana, 1849
- Ostracoda spp. Latreille, 1802

Bryozoa
- *Amathia gracilis* (Leidy, 1855)
- Unidentified Bryozoa sp.

Cnidaria
- *Paranthus cf. rapiformis* (Le Sueur, 1817)
- *Clytia elsaeoswaldae* Stechow, 1914

Mollusca
- Pectinidae sp. Rafinesque, 1815
- *Crepidula naticarum* M. B. Williamson, 1905
- *Doto sp.* Oken, 1815
- *Stiliger fuscovittatus* Lance, 1962
- *Lottia sp.* J. E. Gray, 1833
- Unidentified Gastropod spp.

Nematoda
- Nematoda sp. Diesing, 1861

Nemertea
- *Poseidonemertes* sp. Kirsteuer, 1967
- *Tubulanus sp.* (Renier, 1804)
- Unidentified Nemertea spp.

Platyhelminthes
- Tricladida spp. Lang, 1884

Porifera
- *Leucilla nuttingi* (Urban, 1902)

Table 3. List of epifaunal invertebrates on Diopatra tube-caps at La Jolla kelp site, by phylum

Annelida
- *Chrysopetalum occidentale* Johnson, 1897
- *Paleanotus bellis* (Johnson, 1897)
- Eunicidae sp. Berthold, 1827
- *Leodice* sp. Lamarck, 1818
- *Micropodarke dubia* (Hessle, 1925)
- *Oxydromus pugettensis* (Johnson, 1901)
- *Platynereis sp.* Kinberg, 1865
- *Eteone sp.* Savigny, 1822
- *Eulalia aviculiseta* Hartman, 1936
- *Eulalia gracilior* (Chamberlin, 1919)
- *Phyllodoce sp.* Lamarck, 1818
- *Pterocirrus burtoni* Pleijel, Aguado & Rouse, 2012
- *Halosydna sp.* Kinberg, 1856
- *Phragmatopoma sp.* Mörch, 1863
- *Chone sp.* Krøyer, 1856
- *Pholoides asperus* (Johnson, 1897)
- *Dipolydora giardia* (Mesnil, 1893)
- *Eurysyllis sp.* Ehlers, 1864
- *Eusyllis sp.* Malmgren, 1867
- *Exogone sp.* Örsted, 1845
- *Odontosyllis cf. phosphorea* Moore, 1909
- *Odontosyllis sp.* Claparède, 1863
- *Parexogone molesta* (Banse, 1972)
- *Pseudosyllis sp.* Grube, 1863
- *Syllis sp.* Lamarck, 1818
- *Pista sp.* Malmgren, 1866
- *Thelepus sp.* Leuckart, 1849

Arthropoda
- Acari spp. Leach, 1817
- Pycnogonida spp. Latreille, 1810
- Copepoda spp. Milne Edwards, 1840
- Amphipoda spp. Latreille, 1816
- *Aoroides columbiae* Walker, 1898
- *Gammaropsis* sp. Lilljeborg, 1855
- *Pariphinotus escabrosus* (J.L. Barnard, 1962)
- *Podoceros* sp. Leach, 1814
- Paguroidea sp. Latreille, 1812
- *Lophopanopeus bellus* (Stimpson, 1860)
- *Pugettia dalli* Rathbun, 1894
- *Eualus subtilis* Carvacho & Olson, 1984
- Anthuroidea Leach, 1814
- Arcturidae sp. Dana, 1849
- Gnathiidae sp. Leach, 1814
- Joeropsididae sp. Nordenstam, 1933
- Tanaidacea spp. Dana, 1849
- Ostracoda spp. Latreille, 1802

Bryozoa
- Unidentified Bryozoa sp.

Chordata (Tunicata)
- *Clavelina huntsmani* Van Name, 1931

Cnidaria
- Actiniaria sp. Hertwig, 1882
- *Xingyurella* sp. Song et al., 2018

Echinodermata
- *Strongylocentrotus franciscanus* (A. Agassiz, 1863)
- *Leptosynapta clarki* Heding, 1928
- *Amphipholis pugetana* (Lyman, 1860)
- *Ophiothrix spiculata* Le Conte, 1851

Mollusca
- *Glycymeris cf. septentrionalis* (Middendorff, 1849)
- *Hiatella arctica* (Linnaeus, 1767)
- Pectinidae sp. Rafinesque, 1815
- *Alia carinata* (Hinds, 18440
- *Caecum sp.* J. Fleming, 1813
- *Crepidula onyx* G. B. Sowerby I, 1824
- *Crepipatella lingulate* (A. Gould, 1846)
- *Lacuna sp.* W. Turner, 1827
- *Acanthodoris rhodoceras* T. D. A. Cockerell, 1905
- *Trinchesia albocrusta* (MacFarland, 1966)
- Unidentified Gastropod spp.

Nematoda
- Nematoda sp. Diesing, 1861

Nemertea
- *Micrura sp.* Ehrenberg, 1828
- *Quasitetrastemma nigrifrons* (Coe, 1904)
- Unidentified Nemertea spp.

Platyhelminthes
- Tricladida spp. Lang, 1884

Porifera
- *Leucilla nuttingi* (Urban, 1902)

Statistical analysis of biodiversity

Single-factor ANOVAs of the abundance of epifaunal annelids, arthropods, and molluscs revealed significant differences in community composition between the La Jolla sandy site and La Jolla kelp site. While more annelids were found per tube cap at the La Jolla sandy site on average, it was not a statistically significant difference. However, both arthropods ($p < 0.0005$) and molluscs ($p < 0.000005$) were found to be significantly more abundant on La Jolla kelp forest tube-caps.

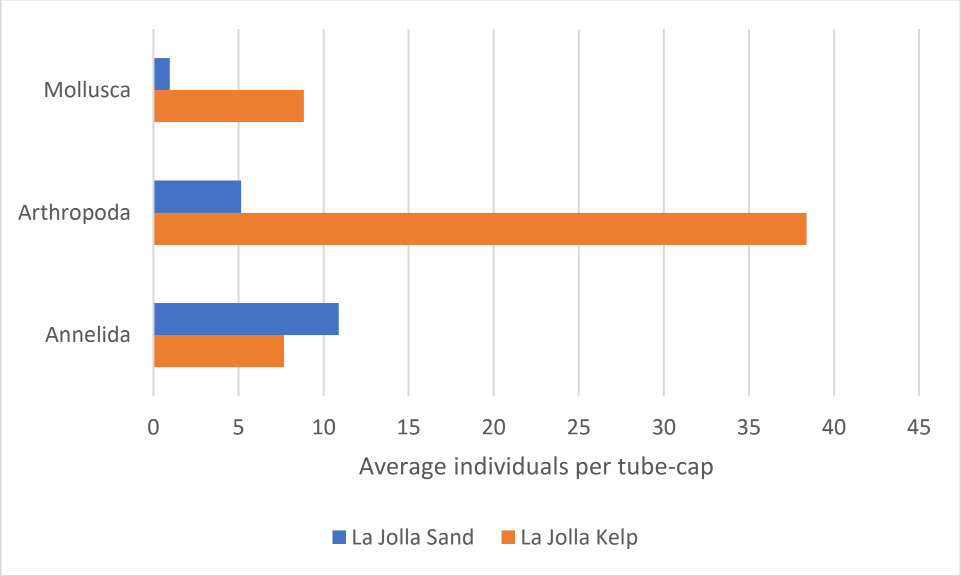


Figure 14. Comparative abundances of epifaunal annelids, arthropods, and molluscs between sandy and kelp forest sites

Abundances of epifaunal annelids by family also varied greatly, both from family to family and between sites. However, the overall biodiversity was not found to be significantly different when examined via Shannon diversity indices.

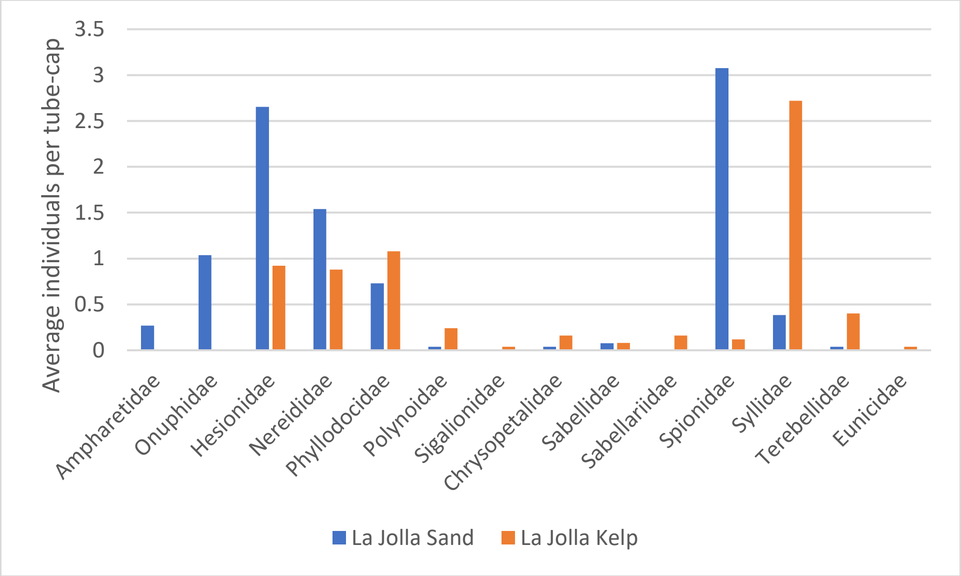


Figure 15. Comparative abundances of epifaunal annelid families between sandy and kelp forest sites

Physical Oceanography

Continuously recorded temperature and dissolved oxygen data from the sensor at the La Jolla sandy site is plotted below. The site exhibited high daily variability in temperature and dissolved oxygen as well as seasonal changes. Notable hypoxic and anoxic events occurred periodically as well.

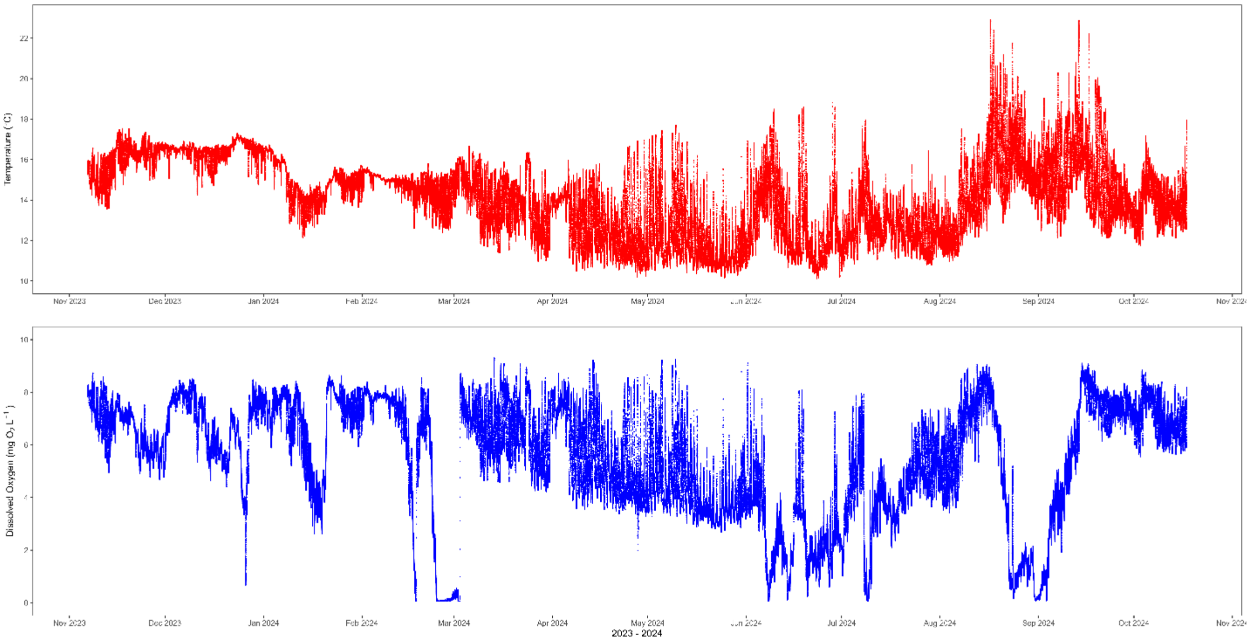
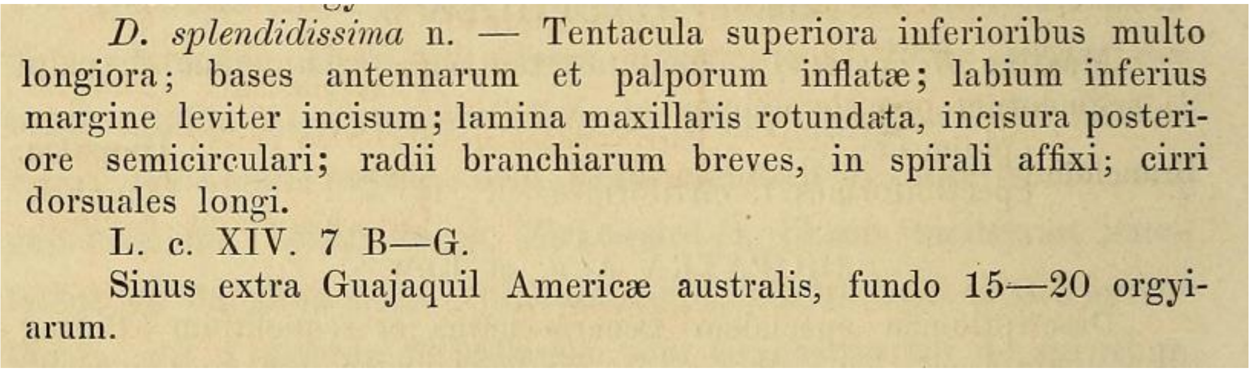


Figure 16. Temperature (°C) and dissolved oxygen (mg O_2 L^{-1}) from the PME miniDO$_2$T, November 2023 – October 2024

DISCUSSION

Multiple arguments can be made for the resurrection of *Diopatra californica* as a valid species. First, Kinberg's original description of *Diopatra splendidissima* is wholly insufficient in describing the species' morphology. It is only a few short phrases long, and fails to mention any chaetal features, let alone pectinate chaetae specifically, which is typically used to distinguish *D. splendidissima* from other members of the genus (Fauchald, 1968; Kinberg, 1865). There are some illustrations in his plates, but the description is still extremely inadequate to justify synonymy from morphology alone.



D. splendidissima n. — Tentacula superiora inferioribus multo longiora; bases antennarum et palporum inflatæ; labium inferius margine leviter incisum; lamina maxillaris rotundata, incisura posteriore semicirculari; radii branchiarum breves, in spirali affixi; cirri dorsuales longi.

L. c. XIV. 7 B—G.

Sinus extra Guajaquil Americæ australis, fundo 15—20 orgyiarum.

Figure 17. Latin description of *Diopatra splendidissima* (Source: Kinberg, 1865)

In Hartman's description of *Diopatra splendidissima* from 1944, she uses these similar morphological characteristics as her reasoning for the synonymy (Hartman, 1944). However, there is another key issue with her discrepancy – she did not utilize any specimens found anywhere near where Kinberg had described the type of *D. splendidissima* in his original description and plates in 1865. Between 1931 and 1941, the Hancock expeditions aboard the Velero III voyaged for thousands of miles around the eastern Pacific Ocean, travelling from California in the north to Peru in the south (Fraser, 1943). While the type specimen of *D. splendidissima* is from Guayaquil, Ecuador, Hartman's only supposed *D. splendidissima* samples were from southern California and Mexico. Hartman's sole justification is the similarity of the pectinate chaetae.

Figure 18. Map of sites in California and Baja California where Olga Hartman identified *Diopatra splendidissima*

While this taxonomic change predated the invention of molecular identification techniques, it is still subjective and problematic. Advancements and increased use of genetic sequencing have repeatedly revealed that “cosmopolitan species” are typically complexes of distinct species (Gómez et al., 2002; Ladner & Palumbi, 2012; Pilar Cabezas et al., 2013). One example can be seen with *Chaetopterus variopedatus*. While long believed to have a global distribution, multiple recent studies have revealed certain populations to in fact be distinct species. The cosmopolitan nature of *C. variopedatus* was long justified due to the longevity of its planktonic larval stage, which allows for broad dispersal. However, studies in and Monterey Bay and San Diego revealed that specimens previously believed to be *C. variopedatus* in fact belonged to separate species, later named *C. pugaporcinus* and *C. dewysee*, respectively (Osborn et al., 2007; Tilic & Rouse, 2020). While some truly cosmopolitan species do exist, such as *Hesionides arenaria* and *Stygocapitella subterranean* (Schmidt & Westheide, 2000), they are believed to be extremely rare. As such, *Diopatra californica*’s status as a species should be reinstated. That said, future studies could compare the COI sequences from my *Diopatra californica* specimens with those of *D. splendidissima* from Ecuador to confirm molecularly. Ultimately, Hartman failed to prove with certainty that *D. splendidissima* and *D. californica* were the same species, so the onus of proof still lies with her.

The clear habitat divide between *Diopatra ornata* and *Diopatra californica* samples warrants further investigation. The differences in tube-cap construction could potentially be a result of species differences, availability of decorating material from the surroundings, or a combination of the two factors. While it was not statistically significant, the higher average abundances of polychaetes on *Diopatra californica*'s tube-caps may have been a result of these structural differences. Many epifaunal polychaetes, including juvenile *Diopatra*, constructed smaller tubes along the outside of *Diopatra californica*'s tube-caps. These accessory tubes were much less common on *Diopatra ornata* samples. A transplant study of the two species, where *Diopatra californica* adults are placed at the kelp forest site while *Diopatra ornata* adults are placed at the sandy site, could determine what causes this variability in tube-cap structure and what role it plays in biodiversity of the epifaunal community.

This question of tube-cap viability for epifauna could also be expanded to include other substrates. Epifauna documentation for this project was limited to invertebrates exclusively found on and in tube-caps. The relationship between epifaunal density and substrate have been well studied (Dahl & Dahl, 2002; Driscoll, 1967; Hardin et al., 1994). A follow-up study could compare biodiversity of tube-cap epifauna to that of surrounding infauna and epifauna on other substrates.

Since temperature and dissolved oxygen were only monitored at the La Jolla sandy site, and temporal change of the tube-cap epifauna was not studied, drawing any conclusions of significance from this physical oceanographic data is difficult. That said, the repeated periods of hypoxia and anoxia likely influenced the tube-cap communities. Significant wave flows and currents were felt during collection dives at both La Jolla sites, and likely played a role in this measured variability. While hypoxic events can cause stress or death to individual animals, it can have broader impacts on the ecosystem, altering food webs, interspecific interactions, and population dynamics (Diaz & Rosenberg, 1995). This could potentially lead to partial or complete defaunation of tube-caps, depending on the severity and longevity of hypoxic and anoxic events. Despite these phenomena, though, *Diopatra* tube-cap communities are known to be resilient, and can epifauna can recolonize these habitats in a matter of hours or days (Bell & Devlin, 1983).

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