Seven new Lacydonia (Annelida: Phylodocida) Species from the Pacific Ocean and Caribbean Sea

Green, Kiirah

2023

UNIVERSITY OF CALIFORNIA SAN DIEGO

Seven new *Lacydonia (*Annelida: Phylodocida*)* Species from the Pacific Ocean and Caribbean Sea

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

in

Marine Biology

by

Kiirah Green

Committee in charge:

Professor Greg Rouse, Chair
Professor Ryan Hechinger
Professor Lisa Levin

2023

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

University of California San Diego

2023

DEDICATION

This thesis is dedicated this to my incredible family, loving partner, and friends. Your unwavering love, encouragement, and support have been the guiding forces that propelled me forward on this challenging journey. Thank you for believing in me, standing by my side, and celebrating every milestone with me. This accomplishment is as much yours as it is mine, and I am forever grateful for your immeasurable impact on the pursuit of my thesis.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my advisor and committee chair, Greg Rouse, for his invaluable guidance and expert knowledge in the field of invertebrates, including *Lacydonia*. His unwavering support and insightful advice throughout my research have been instrumental in shaping this project.

I am also indebted to my committee members, Lisa Levin and Ryan Hechinger, for their remarkable intelligence and invaluable guidance throughout the course of this study. Their constructive feedback and valuable insights have greatly contributed to the success of this research.

I would like to extend a heartfelt thank you to Charlotte Seid, whose assistance in sorting and cataloging all the samples into the benthic invertebrate collection has been truly invaluable. Her wealth of knowledge and constant support have been an endless resource, ensuring the smooth progress of this project.

A special acknowledgment goes to my fellow lab members of the Rouse Lab, Dr. Marina McCowin, Avery Hiley, and Sonja Huč. Their involvement in the collection and sequencing of the organisms, along with their efforts in training me on various laboratory techniques, have been crucial to the completion of this study.

Furthermore, I would like to express my deepest gratitude to my family, partner, and friends for their unwavering support, love, and the cherished memories we have shared throughout my graduate school journey. Their encouragement and understanding have provided me with the strength and motivation to overcome challenges and excel in my research.

Lastly, I would like to extend my sincere appreciation to all the members of the Rouse Lab for their friendship, willingness to share their knowledge, and for providing a safe and nurturing environment for me to grow as a scientist. Their camaraderie and collaborative spirit have truly enriched my experience in the lab.

I am humbled and grateful for the support and contributions of all those mentioned above. Without their assistance, this research would not have been possible.

VITA

2019	Bachelor of Science in Marine Science, Coastal Carolina University
2023	Master of Science in Marine Biology, University of California San Diego

MARINE BIOLOGY

ABSTRACT OF THE THESIS

Seven new *Lacydonia (*Annelida: Phylodocida*)* Species from the Pacific Ocean and Caribbean Sea

by

Kiirah Green

Master of Science in Marine Biology

University of California San Diego, 2023

Professor Greg Rouse, Chair

The deep sea, once considered homogeneous and devoid of life, has undergone a transformative shift in understanding since the 19th-century HMS Challenger expedition, with advancements in technology contributing to our growing knowledge of this vast and biodiverse habitat, which comprises 96% of habitable space on Earth and remains largely unexplored. Within this ecosystem, annelids, particularly those inhabiting chemosynthetic environments, play a significant ecological role. Despite the recognized diversity within the order Phyllodocida, which encompasses families such as Siboglinidae, Dorvilleidae, Polynoidae, Serpulidae, and Phyllodocidae, elucidating their intricate relationships has proven challenging due to the scarcity of comprehensive data. Notably, the family Lacydoniidae, found within the order Phyllodocida, stands out. The genus Lacydonia exhibits an extensive bathymetric distribution, occupying a range of deep-sea habitats. However, our understanding of the phylogenetic relationships within *Lacydonia* as well as aspects such as diet, life cycle, and internal anatomy, remains limited. In this study, we present an updated phylogeny of the genus *Lacydonia* and describe seven novel species based on meticulous morphological examinations and comprehensive molecular sequence analyses. Specimens were collected from methane seeps in the Eastern Pacific Ocean and Caribbean Sea, resulting from recent research expeditions. By elucidating the taxonomy and evolutionary relationships within Lacydonia, our research provides valuable insights into the remarkable diversity and complex evolutionary history of this enigmatic annelid genus, underscoring the significance of deep-sea exploration for unraveling hidden biodiversity and shedding light on the intricate ecological dynamics of these unique ecosystems.

INTRODUCTION

The deep sea is formally characterized by depths greater than 200 meters, as it is at this threshold where the penetrating influence of light ceases to exist. The deep sea was once thought to be homogenous and devoid of life, but those ideas began to change in the 19th century during the HMS Challenger expedition (Ramirez-Llodra et al. 2010). As technology has advanced, our understanding of this environment has also continued to grow. Due in part to the discovery and study of chemosynthetic production from habitats such as hydrothermal vents, methane seeps, and organic falls, today we know that the deep sea is an environment that encompasses some of the largest and most biodiverse habitats on earth, representing 96% of habitable space on earth while also being one of the least explored (DOSI. 2023; Levin et al. 2016; Ramirez-Llodra et al. 2010). Approximately 200,000 species, encompassing both prokaryotic and eukaryotic organisms, have been cataloged from the Earth's oceans. However, estimates suggest that the actual number of species present could exceed 2,000,000 (Mora et al. 2011). Given that the deep sea constitutes most of the ocean's volume, it is highly likely that a significant number of species in the deep sea remain unclassified and undocumented. Though the benthic environment, or the lowest level of a body of water, is host to a variety of organisms, the technical challenges of studying deep-sea life have made it especially difficult to study small-bodied organisms. As a result, most deep-sea research focuses on megafauna that require less specialized equipment, leaving a shortage of data that focus on the descriptions and ecology of macrofauna (microscopic organisms) and meiofauna (organisms smaller than macrofauna) in the deep sea (Levin et al. 2016; Fleming et al. 2022).

Amidst the overlooked macrofauna are many annelid species whose lineages inhabit deep-sea chemosynthetic environments. Recognizing the gap in knowledge, in recent years there have been a countless amount of annelid species descriptions from chemosynthetic based ecosystems and other deep-sea habitats including the families Siboglinidae Caullery, 1914, Dorvilleidae Chamberlin, 1919, Polynoidae Kinberg, 1856, Serpulidae Rafinesque, 1815, and Phyllodocidae Örsted, 1843 (Rouse et al. 2018; Hatch et al. 2020; Yen and Rouse 2020; Rouse and Kupriyanova 2021; Pearson and Rouse 2022). Several families above are included within the order Phyllodocida Dales, 1962, which is widely recognized as one of the most diverse clades among annelids. However, the relationships within this order have proven challenging to decipher due to limited available data (Tilic et al., 2022). Prior studies have revealed that families within Phyllodocida occupy wide bathymetric ranges, and benthic dwelling organisms can survive in extreme environments such as methane seeps and hydrothermal vents (Martin et al., 2021). One such family is Lacydoniidae Bergström, 1914, which, since the description of the first species in 1874, remains understudied, leading to unresolved phylogenetic relationships among its species.

Observations suggest members of *Lacydonia* Marion, 1874 are free-living benthic polychaetes that inhabit sandy bottoms, muddy unconsolidated sediments, rock bottoms, encrusting algae, and coral rubble (Rizzo et al. 2016; Magalhães et al. 2012; Rouse et al. 2022). *Lacydonia* are widespread and have been found in almost every ocean basin. Some species can be found in the shallow subtidal while others are found in deep abyssal zones, allowing for a wide bathymetric range within the genus (Table 1). The currently described species have consistent morphology with small palps, small antennae, a body length of up to 8 mm, a yellowish tint and either the presence or absence of eye spots. Little information has been reported on the diet, life cycle, and internal anatomy of *Lacydonia*, although there is some mention of a proboscis visible through the body wall and a tangle of channels that potentially lead to jaws on the anterior end. Few viable specimens have been efficiently extracted from their environment contributing to a deficit in knowledge of *Lacydonia* physiology and ecology. Additionally, the absence of defining morphological characteristics, like lack of bright pigmentation and overall small size, has also made this species difficult to identify and study (Rouse et al. 2022).

The type species for *Lacydonia*, *Lacydonia miranda* Marion, 1874, was described from the shallow waters, approximately 20 meters deep, within the Mediterranean Sea in the Gulf of Marseille. Upon the initial description of the type species, *Lacydonia* was placed within the family of Phyllodocidae based on morphological similarities. Later, the family name Lacydoniidae was erected by Bergström as he separated *Lacydonia* from Phyllodocidae in 1914 (Universitet 1914; Hookabe et al. 2020; Tilic et al. 2022; WoRMS). While this relationship has been long debated Tilic et al (2022) determined the present placement of *Lacydonia,* thus resolving the relationships within the order of Phyllodocida. The results conclude that *Lacydonia* is a single genus in its own family, Lacydoniidae, that is a sister group to Typhloscolecidae Uljanin, 1878, Lopadorrhynchidae Claparède, 1870, and Phyllodocidae. Together these four families form Phyllodociformia Levinsen, 1883 within the order Phyllodocida.

Fifteen nominal species make up *Lacydonia* including *Lacydonia miranda* Marion, 1874; *Lacydonia mikrops* Ehlers, 1913; *Lacydonia papillate* Uschakov, 1958; *Lacydonia oculata*

Hartman, 1967; *Lacydonia cirrata* Hartman & Fauchald, 1971; *Lacydonia laureci* Laubier 1975; *Lacyonia gorda* Hartmann-Schröder, 1993; *Lacydonia hampsoni* Blake, 1994; *Lacydonia eliasoni* Hartmann-Schröder, 1996; *Lacydonia quadrioculata* Magalhães, Bailey-Brock & Rizzo, 2012; *Lacydonia anapaulae*, *Lacydonia brasiliensis* and *Lacydonia jacki* Rizzo, Magalhães & Santos, 2016, *Lacydonia japonica* Hookabe et al. 2020, and *Lacydonia shohoensis* Hookabe et al. 2022. Some call into question the validity of *L. eliasoni* (Rizzo et al. 2016; Magalhães et al. 2012) because it was originally described without deposition of type material. The description of *L. eliasoni* by Hartmann-Schröder in 1996 is seemingly based on the written descriptions and illustrations found in the publication by Eliason 1962 (Eliason 1962; Magalhães et al. 2012; Rizzo et al. 2016). Here we treat *Lacydonia eliasoni* as a valid name. With only 15 species described and a lack of information surrounding the genus further revisions need to be done to solidify phylogenetic positions within the genus and gain a better understanding of internal morphology.

Using specimens collected on recent expeditions to the Eastern Pacific Ocean and Caribbean Sea, we update the phylogeny of the genus *Lacydonia* and describe seven new species by examination of morphology and molecular sequence data. This includes specimens derived from methane seeps in the Cascadia Margin, Gulf of California, and Costa Rica Margin and new molecular data involving mitochondrial gene Cytochrome Oxidase subunit 1 CO1 and 16S and nuclear data 18S, 28S and HH3.

METHODS

Sample Collection

Lacydonia specimens in this study were collected on several cruises throughout multiple localities in the Pacific Ocean and Caribbean Sea from 2003 to 2020 (see Table 2 and Figure 1). Samples were retrieved by process of HOV (human operated vehicles), ROV (remotely operated vehicles), and SCUBA (self-contained underwater breathing apparatus). Additional material of a *Lacydonia hampsoni* paratype was borrowed from the National History Museum of Los Angeles County for morphological observations.

Live images of some specimens were taken in the field with several cameras including the Canon EOS Rebel T6s, the Canon EOS Rebel T2i, or the Canon PowerShot G9 along with stereo microscopes Leica MZ9.5 stereomicroscope and Leica S8 Apo stereomicroscope.

Specimens were fixed in 95% ethanol to preserve DNA, formalin to preserve the morphological features, or osmium tetroxide for scanning electron microscopy. Some specimens were cut with the anterior preserved in formalin and the posterior preserved in 95% ethanol. All specimens were deposited in the Scripps Institution of Oceanography Benthic Invertebrate Collection.

Molecular Analysis

Several ethanol preserved *Lacydonia* samples underwent DNA extraction. Protocols and reagents were provided by Zymo research miniprep plus kits (Catalog # D4069) and Microprep plus kits (Catalog #D4074), for samples with smaller tissue, were utilized.

The mitochondrial gene Cytochrome Oxidase subunit 1 (CO1) was amplified with applied specifications from Table 4 and approximately 700 base pairs were sequenced to initially delineate species. A single representative from each clade was chosen and the mitochondrial gene 16s rRNA was amplified along with the nuclear genes 18s rRNA, 28s rRNA, and Histone H3 (HH3). DNA amplification details outlining temperature profiles and primers can also be seen in Table 4. PCR purification was done with ExoSAP-IT and corresponding manufacturer protocol (USB, Affymetrix, Ohio).

Sanger Sequencing was completed by Eurofins Genomics (Louisville, KY). Consensus sequences were assembled using the software platform Geneious v.11.1.5 and the "De Novo Assembly" feature in the standard settings. All sequences produced as a result of this study were placed on GenBank (Table 2). Genes from the previously available *Lacydonia eliasoni, Lacydonia japonica*, *Lacydonia* sp. Tosa (Hookabe et al. 2020), *Lacydonia shohoensis*, *Lacydonia laureci*, and Lacydonia sp. MB-2010 (Böggemann 2009) were retrieved from genbank.

Phylogenetic Analysis

CO1 sequence data generated in this study was combined with all CO1 sequence data available on genbank from specimens including: *Lacydonia eliasoni*, *Lacydonia japonica*, *Lacydonia* sp. Tosa, *Lacydonia shohoensis* see Table 2. All sequences were aligned using MUSCLE3.8.31_i86darwin64 attachment via Mesquite version 3.70. Maximum likelihood analysis for CO1 and model selection was generated in the software platform RAxML-NG version RaxmlGUIv.2.0.10. The model selected was TVM+I+G and 10 runs were performed. The tree was rooted with *Notophyllum crypticum* (Nygren et al. 2010) and *Pterocirrus montereyensis* (Hartman 1936). A concatenated maximum likelihood analysis with bootstrap support values for aligned genes CO1, 16S, 18S, 28S, and HH3 was performed in the software platform RAxML-NG version RaxmlGUIv.2.0.10. RAxML-NG also performed the model selection; CO1: TPM3uf+I+G, 16S: TVM+G, 18S: TIM3ef+I+G, 28S: TIM2+G, HH3: TIM1+G. Concatenated analyses were performed with 10 runs and 100 replicates.

Bayesian analysis was performed on aligned concatenated genes CO1, 16S, 18S, 28S, and HH3 using the program MrBayes 3.2.7a arm where best fit models for each gene were selected via JModelTest 2 version jmodeltest 2.1.20 2; CO1: GTR+I+G, 16S: GTR+G, 28S: GTR+G, 18S: SYM+I+G. Sequences were concatenated using Sequence Matrix. Jar and exported as a non-interleaved nexus. There were 20,000,000 generations, every 1000 trees were sampled, and the burnin was set to 10%.

Habitat Evolution and Biogeographical Analysis

Uncorrected pairwise distances for CO1 data were conducted in the program PAUP* version paup4a168_osx. Haplotype networks were generated using PopART version popART 1.7.1 for *Lacydonia kennethi* n. sp, *Lacydonia costaricensis* n. sp, *Lacydonia amanniae* n. sp. Lastly, habitat evolution was assessed using a maximum likelihood ancestral state reconstruction within Mesquite version 3.70. The character of presence on methane seep, wood, and sediment was mapped on to the Concatenated ML phylogenetic tree. If the type species for the organism was described from a methane seep they were categorized as methane seep, if they were found on wood they were categorized as wood, and if the description did not mention either or specifically mentioned sediment, the organism was categorized as sediment.

Morphological Analysis

Dissected parapodia were mounted onto slides with an Aqua-mount mounting medium supplied by Lerner Laboratories or mounted on temporary cavity slides. To analyze larger features or whole specimens a stereo microscope (Leica MZ9.5) was utilized and to analyze smaller features a compound light microscope (Leica DMR HC with differential interference contrast) was utilized. To gain images with much more detail a Scanning Electron Microscope or SEM was used. To prepare specimens for SEM some individuals were treated with 0.1% osmium tetroxide that incubated for 2 hours. All specimens were dried in an ethanol series, diluted to 100% Hexamethyldisilazane (HMDS), and air dried. Dried specimens were mounted on aluminum subs using double sided adhesive tape and coated in a metal sputter coater with gold-palladium.

RESULTS

Species Delimitation

Initial species delimitation involving the mitochondrial gene CO1 revealed the ML tree as seen in Figure 2. The 40 terminals demonstrate seven clades that are distinct from previously described species of *Lacydonia* as indicated by the branch lengths. Uncorrected pairwise distances for CO1 (Table 3) show a maximum intraspecific pairwise variation of less than 2% and a minimum interspecific variation of greater than 6%.

Phylogeny

In the ML analysis all new species are supported by a node with a bootstrap value of 61%. There is a shallow water clade composed of *Lacydonia miranda, Lacydonia moniquae* n. sp*, and Lacydonia justini* n. sp supported by a bootstrap value of 98%; where *Lacydonia miranda* and *Lacydonia justini* n. sp form a clade that is most closely related to *Lacydonia moniquae* n. sp. This shallow water clade is most closely related to a clade composed of *Lacydonia laureci* and *Lacydonia eliasoni*. A deepwater clade supported by a bootstrap value of 56% included *Lacydonia shohoensis, Lacydonia eesharanganiae* n. sp, *Lacydonia paulproctori* n. sp, *Lacydonia kennethi* n. sp*, Lacydonia ckostaricensis* n. sp*, and Lacydonia amanniae* n. sp where *Lacydonia shohoensis* is sister to a clade composed of all other species. Concatenated maximum likelihood (ML) and bayesian analysis Figure 3 were dissimilar in that *Lacydonia eesharanganiae* n. sp and *Lacydonia paulproctori* n. sp switched places.

The Bayesian analysis results indicate a positional interchange between *Lacydonia eesharanganiae* n. sp and *Lacydonia paulproctori* n. sp, in contrast to the maximum likelihood analysis (see Figure 4). However, both species remain within a clade of deep-water organisms that are most closely related to *L. shohoensis*, with all relationships strongly supported by a posterior probability value exceeding 90%.

Habitat Evolution and Biogeography

Ancestral state reconstruction (Figure 8) shows the transition to seep-dwelling organisms is ambiguous. The ancestor that gave rise to clade 3 has a 50% proportional likelihood of living at a seep. However, a clear transition to sediment-dwelling organisms is seen by the circles in figure 8 getting progressively blacker in clade 1 and clade 2. In clade 1 the most recent common ancestor shared between *L. japonica*, *Lacydonia* sp Tosa, and *Lacydonia* sp MB 2010 has a 95% proportional likelihood of living on sediment. In clade 2 the most recent common ancestor shared between all organisms has a 98% proportional likelihood of living on sediment. Lastly, that most recent common ancestor of all the organisms included in this analysis (excluding the outgroup) has a 94% proportional likelihood of potentially coming from sediment.

The biogeography was also assessed for several species that were collected from multiple locations (see Figures 5-7). *Lacydonia kennethi* n. sp has two haplotypes, one representing the collection site in the Gulf of California and the other representing the collection site off the coast of Costa Rica Jaco summit, each 11 base pairs apart from each other (1.6% maximum intraspecific variation). *Lacydonia costaricensis* n. sp has five different haplotypes and 1 hypothesized haplotype from the collection site at Mound 12 (CR) where the colors correspond to two different depths. Of the 6 different haplotypes one is a single haplotype found at both depth distributions, and each of the 6 are three base pairs apart (0% maximum intraspecific variation). *Lacydonia amanniae* n. sp has 14 different haplotypes and three hypothesized haplotypes all corresponding to the sample sites in deep water off the coast of Oregon, Del Mar CA and Costa Rica (Mound11 and Jaco Summit). The maximum intraspecific variation across the entire haplotype is 1.2%.

Systematics

Family Lacydoniidae Bergström, 1914

Genus *Lacydonia* Marion and Bobretzky, 1875

Lacydonia moniquae n. sp

Figure 9

Material Examined/Type Material-

Holotype: A4729*, osmium coated and SEM preparation, Curlew Bank, South of Carrie Bow Cay, Belize, 16.8025°N 88.0817°W, 15 m depth, November 6, 2006 Paratype: A4881, incomplete specimen, Curlew Bank, South of Carrie Bow Cay, Belize, 15 m depth, *indicates posterior was used for DNA extraction

Description/Diagnosis-

Holotype: maximum length 3.0 mm (32 chaetigers), maximum body width 0.37 mm, live specimen slightly transparent, several dorsal small brown pigment spots, yellowish intestines visible through body wall. Preserved specimens generally brown. Following prostomium 1 achaetigerous 1st segment, 3 uniramous chaetigers, 29 biramous chaetigers and a pygidium.

Prostomium: box-shaped, slightly rounded anteriorly, 2x wider than long. Features: 1 pair of digitiform antennae (29 µm), 1 median antenna (22 µm), red eye spots. Ventral: 1 pair short, conical palps near mouth. Nuchal organs: ciliated pits at posterior prostomium ends, right and left.

1st segment achaetigerous, 1 pair short elliptical cirri, length shorter posterior to anterior. Uniramous chaetigers 1-3: neuropodial lobe, 1 dorsal cirrus, 1 ventral cirrus, compound spinigers, then biramous. Notopodia conical, shorter than neuropodia, tapers to point, 1 ovoid dorsal cirrus. Notochaetae with up to 7 finely serrated capillaries. Neuropodia narrow, longer than notopodia, distally pointed, 1 ovoid ventral cirrus. Neurochaetae with up to 16 compound spinigers, coarsely serrated blades. Pygidium with lateral cirri, no pygidial median papilla.

Remarks-

All of the *Lacydonia moniquae* n. sp were hand collected by SCUBA from scrapings of algae and sediment near Carrie Bow Cay off the coast of Belize in the Caribbean Sea (15m). Morphologically *Lacydonia moniquae* n. sp is most similar to *Lacydonia miranda* because of the parallels in the presence of eyes, the shape of the parapodia and dorsal cirrus, the serrations of the neurocheatae, and the shallow water distributions. However, morphological differences can be found in the pygidium as *Lacydonia moniquae* n. sp has no pygidial median papilla which is present in *Lacydonia miranda* and the pronounced on the notochaetae of *Lacydonia miranda* are not observed in *Lacydonia moniquae* n. sp. *Lacydonia moniquae* n. sp was found in the Caribbean Sea where no other *Lacydonia* have been described from. The closest and morphologically most similar *Lacydonia* to be described from this area were collected from Campos Basin, Brazil; Lacydonia *brasiliensis* and *Lacydonia jacki* both have eyes. *Lacydonia brasiliensis* and *Lacydonia jacki* were collected from waters at least 100m deeper than *Lacydonia moniquae* n. sp, and the shape of the parapodia and size of serrations are also different. No genetic data were available for *Lacydonia brasiliensis* and *Lacydonia jacki;* however, concatenated ML results (Figure 3) show *Lacydonia moniquae* n. sp is sister to a clade composed of *Lacydonia miranda* and *Lacydonia justini* n. sp, all shallow-water organisms supported by a bootstrap value of 98%.

Etymology-

Lacydonia moniquae n. sp is named in honor of Monique Browder, the mother of the lead author, in recognition of her unwavering love, support, and the profound influence she has had on the lead author's drive and independence throughout their life.

Lacydonia justini n. sp

Figure 10 - 11

Material Examined/Type Material-

Holotype: A14218*, preserved in 70% ethanol, sediment, SanClemente Island, California, United States, 32.9165°N 118.4641°W, 10 m depth, July 18th, 2017 Paratypes: A14160, A14161* sediment, SanClemente Island, California, United States, 12 m depth; A4321, La Jolla Cove, California, United States, 12 m depth, *indicates posterior was used for DNA extraction

Description/Diagnosis-

Holotype: 4.2 mm length, 34 chaetigers, .65 mm max width. Live: slightly transparent, no dorsal pigment spots, visible yellowish intestines. Preserved: generally brown. Sequence: Prostomium, 1 achaetigerous segment, 3 uniramous chaetigers, 30 biramous chaetigers, pygidium.

Prostomium: box-shaped, slightly rounded anteriorly, 2x wider than long. Features: 1 pair of digitiform antennae (32.4 µm), 1 median antenna (28.4 µm), red eye spots. Ventral: 1 pair short, conical palps near mouth. Nuchal organs: ciliated pits at posterior prostomium ends, right and left.

1st segment achaetigerous, 1 pair short elliptical cirri, length shorter posterior to anterior. Uniramous chaetigers 1-3 (segments 2-4): neuropodial lobe, 1 conical dorsal cirrus, 1 conical ventral cirrus, finely serrated compound spinigers. Chaetiger 4 and following: all biramous. Notopodia rounded, shorter than neuropodia, 1 ovoid dorsal cirrus. Notochaetae up to 10 coarsely serrated capillaries. Neuropodia globular, longer than notopodia, distally pointed, 1 ovoid ventral cirrus. Neurochaetae up to 25 compound spinigers, coarsely serrated blades. Pygidium: lateral cirri present, no pygidial median papilla.

Remarks-

Most of the *Lacydonia justini* n. sp were collected from maerl gravel samples from the Black Caverns on the eastern side of SanClemente Island, California, (10m). Gravel was kept in the lab and live animals were picked through over several days including: 2017-07-18, 2017-07-19, 2017-07-22, 2017-08-02. The single specimen collected from kelp holdfast in La Jolla Cove, California does not have DNA sequenced, but was morphologically similar to the holotype. Morphologically, *Lacydonia justini* n. sp is most similar to *Lacydonia moniquae* n. sp both present with red eye spots, the lack of pygidial median papilla, conical notopodial dorsal cirrus, and the same type of serrations on the chaetae; however, the notopodial and neuropodial lobes are different shapes. It is also important to note that both *Lacydonia moniquae* n. sp and *Lacydonia justini* n. sp are found at similar depths and substrates. Based on the concatenated ML results *Lacydonia justini* n. sp is most closely related to *Lacydonia miranda* though the bootstrap value for this relationship is less than 50%. *Lacydonia miranda* is similar to *Lacydonia justini* n. sp in that they are both found in shallow water and have eye spots, but the shape of the notopodial and neuropodial lobes are different, the serrations on the chaetae on *Lacydonia justini* n. sp are smaller than on *Lacydonia miranda*, and the pygidium does not possess median papilla though it is present in *Lacydonia miranda*.

Etymology-

Lacydonia justini n. sp is named after the lead author's younger brother, Justin Green, for the joy he has provided the lead author throughout her life.

Lacydonia eesharanganiae n. sp

Figure 11

Material Examined/Type Material-

Holotype: A1525A* preserved in 70% ethanol, seep, Mound 11, Costa Rica, 8.9208 °N 84.3054 °W, ~1000m depth, February 25, 2009, HOV Alvin, dive number AD4504 Paratypes: A1354 preserved in 8% of formalin, seep, Mound 11, Costa Rica, ~1000m depth, *indicates posterior was used for DNA extraction

Description/Diagnosis-

Holotype: 0.57 mm length, 5 chaetigers (incomplete), 0.46 mm max width. Preserved: tan.

Sequence: Prostomium, 1 achaetigerous segment, 3 uniramous chaetigers, 1 biramous chaetigers.

Prostomium: rounded, 2x wider than long. Antennae and median antennae not observed (assumed present). No eye spots.

1st segment achaetigerous, 1 pair short elliptical cirri, length shorter posterior to anterior. Uniramous chaetigers 1-3 (segments 2-4): neuropodial lobe, 1 conical dorsal cirrus, 1 conical ventral cirrus, finely serrated compound spinigers. Chaetiger 4 and 5: both biramous, chaetiger 5 partially damaged. Notopodia rounded, longer than neuropodia, 1 ovoid dorsal cirrus. Notochaetae up to approx. 4 finely serrated capillaries. Neuropodia globular, slightly shorter than notopodia, distally pointed, 1 ovoid ventral cirrus. Neurochaetae up to approx. 8 compound spinigers, finely serrated blades.

Remarks-

The two specimens included in the sample A1345 do not have DNA sequenced but were morphologically similar to and collected from the same location as the holotype.

Etymology-

Lacydonia eesharanganiae n. sp is named after the lead author’s lab mate, Eesha Rangani, for her friendship and encouragement throughout the duration of the lead author's thesis.

Lacydonia paulproctori n. sp

Figure 12

Material Examined/Type Material-

Holotype: A9814* preserved in 70% ethanol, wood, Quepos Plateau, Costa Rica, 8.5855°N 84.5484°W, 2184 m depth, October 26, 2018, HOV Alvin, dive number AD4980_W1, *indicates posterior was used for DNA extraction

Description/Diagnosis-

Holotype: 1.4mm length, 23 chaetigers, .37mm max width. Live: slightly transparent, no dorsal pigment spots, visible whitish intestines. Preserved: yellowish tint. Sequence: Prostomium, 1 achaetigerous segment, 3 uniramous chaetigers.

Prostomium: rounded, stunted, 2x wider than long. Features: 1 pair digitiform antennae, 1 median antenna. No eye spots. Palps not observed in preserved specimen (presumed present).

Notopodia lobe: slightly smaller than neuropodial lobe, conical, 1 rounded dorsal cirrus. Notochaetae: up to 14 coarsely serrated capillaries. Neuropodia lobe: slim, tapers to blunt point, 1 conical ventral cirrus at base. Neurochaetae: up to 21 compound spinigers, coarsely serrated blades. Pygidium: lateral cirri.

Remarks-

Lacydonia paulproctori n. sp is a single specimen within its own clade discovered on a piece of wood in sediment within the Quepos Plateau, Costa Rica at a depth of 2184 m. *Lacydonia paulproctori* n. sp demonstrated a close phylogenetic relationship to *Lacydonia eesharanganiae* n. sp, as well as a clade comprising *Lacydonia costaricensis* n. sp, *Lacydonia kennethi* n. sp and *Lacydonia amanniae* n. sp. However, this relationship is supported by a relatively low bootstrap value of 23%. Morphologically, *Lacydonia paulproctori* n. sp exhibits morphological similarities to *L. shohoensis* (another wood-dwelling species), particularly in terms of prostomium shape and parapodial lobe shape.

Etymology-

Lacydonia paulproctori n. sp is named after the lead author's friend and lab mate, Paul Proctor, for his advice and encouragement throughout the duration of the lead author's thesis.

Lacydonia kennethi n. sp

Figure 13

Material Examined/Type Material-

Holotype: A3340*, preserved in 70% ethanol, seep, Guaymas Basin, Gulf of California, Mexico, 27.5875 °N 111.473 °W, ~1500 m depth, April 15th, 2012, ROV Doc Ricketts, dive number D388 Paratypes: A1606*, A1433a, A1433b seep, Jaco Summit Costa Rica, ~740 m depth, *indicates posterior was used for DNA extraction

Description/Diagnosis-

Holotype: 11.5 mm length, 48 chaetigers, 1.4 mm max width. Live: slightly transparent, no pigment spots, visible yellowish intestines. Preserved: tan, pigment spot at median antennae.

Sequence: Prostomium, achaetigerous 1st segment, 3 uniramous chaetigers, 44 biramous chaetigers, pygidium.

Prostomium: box-shaped, slightly rounded anteriorly, 2x wider than long. Features: 1 pair slim digitiform antennae (86 µm), 1 median antenna (86 µm) near horizontal groove on prostomium. No eye spots. Ventral: 1 pair short, cylindrical palps near mouth. Nuchal organs: ciliated pits at posterior prostomium ends, right and left.

1st segment achaetigerous, 1 pair short elliptical cirri, length shorter posterior to anterior. Uniramous chaetigers 1-3 (segments 2-4): neuropodial lobe, 1 conical dorsal cirrus, 1 conical ventral cirrus, finely serrated compound spinigers. Chaetiger 4 and following: all biramous. Notopodia rounded, tapers to a point, 1 conical dorsal cirrus. Notochaetae up to 15 coarsely serrated capillaries. Neuropodia thick, tapers to a point, 1 ventral cirrus. Neurochaetae up to 23 compound spinigers, coarsely serrated blades. Pygidium: lateral cirri, pygidial median papilla.

Variation-

Paratype from Jaco Summit varies from the holotype in that the body is mostly translucent and the yellowish intestines are visible through the body wall.

Remarks-

One *Lacydonia kennethi* n. sp was collected from a methane seep at pinkie's vent in the Guaymas Basin, Gulf of California, Mexico, (1500 m). The single specimen from a seep at the location known as Jaco Summit, Costa Rica, is not only similar to the holotype morphologically but also genetically (See haplotype network in Figure 5). The two specimens possess a maximum intraspecific variation of 0.01668 percent. *Lacydonia kennethi* n. sp is most closely related to *Lacydonia amanniae* n. sp though the two are not morphologically similar. The shape of the notopodial and neuropodial lobe is shorter on *Lacydonia kennethi* n. sp than that of *Lacydonia* *amanniae* n. sp (See Figure 13 and Figure 15). *Lacydonia amanniae* n. sp is also slightly longer in length than *Lacydonia kennethi* n. sp.

Etymology-

Lacydonia kennethi n. sp is named in tribute to Kenneth Marcellus Green, the father of the lead author, in acknowledgement of his lifelong love and support. His enduring encouragement has played a pivotal role in fostering the lead author's passion for science and the ocean.

Lacydonia costaricensis n. sp

Figure 14

Diagnosis-

Material Examined/Type Material-

Holoptype: A1925*, preserved in 70% ethanol, seep, Mound 12, Costa Rica, 8.9308 °N 84.3125 °W, ~1000 m depth, January 9th 2010, HOV Alvin, dive number AD4588, Paratypes: A1319, A1485*, A1926, A9773*, A9774*, A1422, A14223, A14224, A14225, A14226, A14227, A14228, A14229, A14230, A14231, A14232, A14233, Mound 12, Costa Rica, ~1000 m depth; A9880*, Parrita Seep, Costa Rica, ~1400m; A10090*, The Thumb, Costa Rica, ~1072 m depth, *indicates posterior was used for DNA extraction

Description/Diagnosis-

Holotype: 5.2 mm length, 36 chaetigers, 0.54 mm max width. Live: slightly transparent, 2 pigment spots on posterior prostomium dorsal surface, visible proboscis and yellowish intestines. Preserved: tan, pigment spot at median antennae. Sequence: Prostomium, achaetigerous 1st segment, 3 uniramous chaetigers, 32 biramous chaetigers, pygidium.

Prostomium: rounded, 2x wider than long. Features: 1 pair slim digitiform antennae (46.2 µm), 1 median antenna. No eye spots. Ventral: 1 pair short, cylindrical palps near mouth. Nuchal organs: ciliated pits at posterior prostomium ends, right and left.

1st segment achaetigerous, 1 pair short elliptical cirri, length shorter posterior to anterior. Uniramous chaetigers 1-3 (segments 2-4): neuropodial lobe, 1 conical dorsal cirrus, 1 ventral cirrus, finely serrated compound spinigers. Chaetiger 4 and following: all biramous. Notopodia rounded, shorter than neuropodia, 1 conical dorsal cirrus. Notochaetae approx. 6 coarsely serrated capillaries. Neuropodia lobe round, 1 digitiform ventral cirrus. Neurochaetae up to 17 compound spinigers, coarsely serrated blades. Pygidium: lateral cirri, pygidial median papilla.

Remarks-

Most *Lacydonia costaricensis* n. sp are collected from Mound 12, Costa Rica (~1000m) except two specimens, A9880 and A10090 which were collected from sites off Costa Rica that were slightly north of the Mound 12 collection site (see figure 1). All specimen that went into the analysis of this group had a less than 1% maximum intraspecific variation. *Lacydonia costaricensis* n. sp is most closely related to a clade composed of *Lacydonia amanniae* n. sp and *Lacydonia kennethi* n. sp and *Lacydonia paulproctori* n. sp. Though there are morphological differences between all previously mention species *Lacydonia costaricensis* n. sp is morphologically most similar to *Lacydonia kennethi* n. sp. The parapodial lobes are similar in shape and size, and the serrations on the chaetae seem similar also (see Figure 13 and Figure 14). However, the prostomium is rounded on *Lacydonia costaricensis* n. sp where it is boxy in *Lacydonia kennethi* n. sp. *Lacydonia kennethi* n. sp also has a horizontal grove on the prostomium where the median antenna sits which is not present is *Lacydonia costaricensis* n. sp.

Etymology-

Lacydonia costaricensis n. sp was named in honor of the captivating country in Central America, known as Costa Rica, owing to its profound cultural affinity for water and the exclusive occurrence of these worms within the waters bordering this country.

Lacydonia amanniae n. sp

Figure 15-16

Material Examined/Type Material-

Holotype: A2648A, preserved in 70% ethanol, seep, Hydrate Ridge, Oregon, United States, 44.6701°N 125.0987 °W, ~600 m depth, September 4th, 2011, ROV Jason II, dive number JasonII 593 HR4, Paratypes: A1525b, A1525c, A1526, Mound 11, Costa Rica, ~1000 m depth; A1605, Jaco Summit, Costa Rica ~740 m depth; A2022, A2028, A2092, A2094, A2646, A2647, A2648, A2648B A2649, A2650, A2651, Hydrate Ridge, Oregon, ~600 m depth; A12314, Del Mar Seep, California, ~1000 m depth, *indicates posterior was used for DNA extraction

Description/Diagnosis-

Holotype: 3.1 mm length, 30 chaetigers, .24 mm max width. Live specimen: pigmented prostomium, slightly transparent body, no dorsal pigment spots, visible proboscis and yellowish intestines. Preserved: generally brown. Sequence: Prostomium, 1 achaetigerous segment, 3 uniramous chaetigers, all biramous chaetigers, pygidium.

Prostomium: box-shaped, slightly rounded anteriorly, 2x wider than long. Features: 1 pair digitiform antennae (83 µm), 1 median antenna (70 µm). No eye spots. Ventral: 1 pair short, cylindrical palps near mouth. Nuchal organs: ciliated pits at posterior prostomium ends, right and left.

1st segment achaetigerous, 1 pair short elliptical cirri, shorter posterior to anterior. Uniramous chaetigers 1-3 (segments 2-4): neuropodial lobe, 1 globular dorsal cirrus, 1 anteriorly rounded digitiform ventral cirrus, finely serrated compound spinigers. Chaetiger 4 and following: all biramous. Notopodia: pentagonal form, globular, rounded, 1 dorsal cirrus (circular for chaetigers 1-3, progressively more digitiform from chaetiger 4). Notochaetae up to 16 coarsely serrated capillaries (approx. 300 µm). Neuropodia thick, blunt, globular, 1 small digitiform ventral cirrus. Neurochaetae up to 20 compound spinigers, finely serrated blades (approx. 400 µm). Pygidium: lateral cirri, pygidial median papilla.

Variation-

There are slight differences in coloration of the live paratypes found in Oregon, Del Mar, and Costa Rica (See Figure 16). The body length of the paratypes can range from approximately 3 mm to 15 mm.

Remarks-

Lacydonia amanniae n. sp consists of specimens collected from deep-water collection sites off the coast of Oregon, California, and Costa Rica including Hydrate Ridge, Del Mar, Mound 11 and Jaco summit. Not only does CO1 sequence data suggest that the specimens from these locations have a maximum intraspecific variation of 0.01202 percent they are all morphologically similar. *Lacydonia amanniae* n. sp. exhibits similarities with *Lacydonia hampsoni* in terms of location. This resemblance arises from the fact that the holotype of *Lacydonia hampsoni* was originally described from San Francisco, which coincides with the geographical range of *Lacydonia hampsoni* (spanning from Oregon to Costa Rica). Additionally, there is a depth overlap between the two species, as both were discovered in waters exceeding 1000 meters in depth. Though no DNA is available for *Lacydonia hampsoni* a morphological analysis of *Lacydonia hampsoni* and *Lacydonia amanniae* n. sp revealed the two species are not similar in body length and parapodia shape. To be more specific, *Lacydonia amanniae* n. sp is longer in length than *Lacydonia hampsoni* and the shape of neuropodia is thicker and blunt in *Lacydonia amanniae* n. sp as opposed to *L. hampsoni.* The dorsal cirrus of *Lacydonia amanniae* n. sp is more globular and rounded than that of *L. hampsoni.* DNA from *L. hampsoni* is necessary to confirm this relationship.

Etymology-

Lacydonia amanniae n. sp is named after the lead author's sister, Amanni Evans, for her continued guidance and inspiration throughout the lead author's life.

DISCUSSION

Not much genetic data was available for the genus of *Lacydonia* and in prior studies the presence and abundance of eyes spots have been used in identifying species. Using the eye characteristic (Blake and Hilbig 1994) categorized all described *Lacydonia* into three groups 1) species with large eyes, *i.e.*, *Lacydonia miranda*, *Lacydonia oculata* , and *Lacydonia jacki*; 2) species with small eyes *i.e.*, *Lacydonia mikrops*, *Lacydonia quadrioculata* , and *Lacydonia brasiliensis*; 3) species lacking eyes entirely *i.e.*, *Lacydonia papillata*, *Lacydonia cirrata* , *Lacydonia laureci*, *Lacyonia gorda*, *Lacydonia hampsoni*, *Lacydonia eliasoni*, and *Lacydonia anapaulae* *(Magalhães et al. 2012)*. Recent additions to the genus *Lacydonia,* described by Hookabe et al. 2020; Hookabe et al. 2022 (*Lacydonia japonica* and *Lacydonia shohoensis*), also lack eye spots entirely. Though the historic perspective on the taxonomy of this group is still in question because most of the previously described species do not have genetic data available, the present study makes a large contribution to the diversity within the genus *Lacydonia* through the introduction of seven newly discovered species. These species include *Lacydonia moniquae* n. sp and *Lacydonia justini* n. sp, both characterized by large eye spots, as well as *Lacydonia eesharanganiae* n. sp, *Lacydonia paulproctori* n. sp, *Lacydonia kennethi* n. sp, *Lacydonia costaricensis n. sp,* and *Lacydonia amanniae* n. sp, all lacking eye spots. There are no universal cutoffs for species delimitation however, interspecific CO1 distances between pairs of already described *Lacydonia* are noted as being >11% (Hookabe et al. 2022; Hookabe et al. 2020). Though it's important to note that these analyses only include genetic data for *L. eliasoni, L. japonica, L. shohoensis* and *Lacydonia* sp. Tosa. The inclusion of the 7 new species here, as well as *L. miranda,* are well-supported by >7% interspecific CO1 distance values (refer to table 3) and bootstrap values comparable to those observed in Phyllodocids. As such, this study enhances our understanding of the genus Lacydonia and expands its taxonomic diversity.

Using the little genetic data available, Hookabe et al. (2022) proposed Lacydonia sp. Tosa and *Lacydonia japonica* form a clade that is sister to a clade composed of *Lacydonia eliasoni*, *Lacydonia* sp. MB-2010, *Lacydonia shohoensis*, and *Lacydonia laureci* sensu Böggemann (2009) where *L. laureci* is most closely related to *L. shohoensis.* On the contrary the present study shows the previously described *Lacydonia tosa* and *Lacydonia japonica* forming a clade with *Lacydonia* sp. MB-2010, supported by the low ML bootstrap value of 25%. Though the low bootstrap value may indicate that the position of *Lacydonia* sp. MB-2010 may not be accurate, the Bayesian analysis shows this same relationship (see Figure 3 and Figure 4). Furthermore, in this study *L. shohoensis* is found to be most closely related to a deep-water clade consisting of wood and methane seep-associated species, namely *Lacydonia eesharanganiae* n. sp, *Lacydonia paulproctori* n. sp, *Lacydonia kennethi* n. sp, *Lacydonia costaricensis* n. sp, and *Lacydonia amanniae* n. sp (see Figure 3), supported by a robust ML bootstrap value of 98%. Whereas Hookabe et al. (2022) places *L. shohoensis* with *Lacydonia laureci* sensu Böggemann (2009), supported by a bootstrap value of 61%. Further taxonomic revisions, including the incorporation of genetic data derived from many of the previously described species, are imperative to establish definitive relationships within *Lacydonia*.

Haplotype networks can assess biogeographical data with genetic data demonstrating in Figures 5-7 the genetic exchange over wide distances that are orders of magnitude larger than the annelids themselves. Within *Lacydonia amanniae* n. sp organisms collected from Oregon differ by 3 base pairs from those collected from Costa Rica, a 5572 km distance apart. This is similar to *Lacydonia kennethi* n. sp where the organisms included differ by 11 base pairs and are 3463 km apart (Jaco Summit, CR and Guaymas Basin, Mexico). Though the base pair difference is greater for this group, a 1.6% maximum intraspecific variation is like other annelids described within Phyllodocida. The large base pair difference observed here could be attributed to sampling artifacts as only two organisms had genetic data in this analysis, and there could be more *Lacydonia* uncollected that have yet to fill in these gaps. The haplotype network for *Lacydonia costaricensis* n. sp displays a single haplotype that can be found at both depth distributions indicating these benthic organisms, genetically identical by CO1 standards, can be distributed throughout a depth range of 400m.

The biogeographical analysis indicates that there are five sympatric species found in Costa Rica. These five species form a clade of wood-associated and methane seep-associated species most closely related to *L. shohoensis,* also a wood-associated species. The five sympatric species (*Lacydonia eesharanganiae* n. sp, *Lacydonia paulproctori* n. sp, *Lacydonia kennethi* n. sp, *Lacydonia costaricensis* n. sp, and *Lacydonia amanniae* n. sp) have a >6% minimum interspecific variation and a maximum intraspecific variation of <2% meaning the genetic variation of the organisms within the individual clades is smaller than when compared to organisms outside of those clades. Speciation taking place without great geographical distribution is often seen in other annelid groups (Thornhill et al. 2012; Yen and Rouse 2020). In the Dorvilleidae family, a family of annelids closely relate to *Lacydonia,* isotope analysis has proven to be fruitful approach at identifying different niches for sympatric species (Levin et al. 2013). When applied to *Lacydonia* this technique may also prove to be helpful at identifying different species off the Pacific coast of Costa Rica. Nevertheless, due to the consistent morphology of the *Lacydonia* genus the revelation of five distinct species from Costa Rica would have been unlikely in the absence of genetic data.

Ancestral state reconstruction has facilitated the assessment of habitat evolution in these organisms. Although not all described *Lacydonia* species were included in this study, those with available genetic data are found in three distinct habitats, namely sediment, wood, and methane seeps. Figure 8 reveals the ambiguity surrounding the transition to methane seep-dwelling organisms, while indicating a definite transformation to sediment-dwelling organisms. It is highly probable that the organism that gave rise to this entire group originally inhabited sediment. It is important to note that due to the lack of genetic information for all described species in this analysis, the depiction in Figure 8 may not accurately reflect historical events. A more comprehensive understanding of the transition to methane seep and wood-dwelling organisms would be obtained by incorporating additional genetic information from shallow water *Lacydonia* species. Moreover, including genetic data for all species of *Lacydonia* would enhance the confidence in these findings.

CONCLUSION

In this study we generated data to support the erection of seven new species of *Lacydonia* including 1 shallow-water species from the Caribbean Sea, 1 shallow-water species from San Clemente Island and 5 species that show evidence of sympatric speciation in Costa Rica. Though internal anatomy was not analyzed here, an indication of maturity is seen by the presence of eggs or sperm which can be observed in some of the specimens in this study (See Figures 10-11). It is also understood by the population connectivity that there is evidence for genetic exchange that is orders of magnitudes larger than the length of the annelids of this genus. A possible explanation could be larval dispersal or intermediate habitats, both of which would require future work to elucidate. There are several biodiversity assessments indicating *Lacydonia* species may also be present at chemosynthetic hydrothermal vent environments and adjacent wood falls along the California coast (Tunnicliffe et al, 1998; Judge et al, 2016). These indications highlight a notable affinity of *Lacydonia* species with chemosythetic habitats. This contributes to the potential for new species emergence and, further facilitates population connectivity through the intermediate habitat theory. While this paper does not resolve all of the relationships within the genus of *Lacydonia* nor does it answer all questions of internal anatomy and ecology, it does function as an important reminder that DNA can often reveal data, and in this case new species, that may have otherwise been overlooked.

Table 1: Species List. This is a matrix of morphological characters from descriptions and redescriptions of all valid *Lacydonia* species. *Lacydonia* sp Tosa and *Lacydonia* sp MB Bogemann 2010 and available information are also included though not considered valid species.

Species of lacydonia	Type locality	Depth range	Maximum length	umber of chaetigers	Body color	Pigment spots	Prostomium	Eyes	Lateral antennae	Median antenna	Palps
***Lacydonia miranda* Marion & Bobretzky, 1875**	Gulf of Marseille, mediterranean	shallow waters	5mm	36-39	yellowish	not mentioned	wider than long	pair of large eyes	fusiform	only reported in Pleijel & fauchald,1993	similar to antenae
***Lacydonia mikrops* Ehlers, 1913**	Kaiser Wilhelm-II-Land Eastern Antarctica	382-385m	1.6mm	15	not mentioned	two spots on the pygidium	little wider than long	pair of small eyes near lateral edge of prostomium	short	not mentioned, presumably lost or over looked	similar to antenae
***Lacydonia papillata* Uschakov, 1958**	Kuril Trench, NW Pacific	3,352-5,690m	5.5mm	33	light olive green	4 large papilliform dark spots behind chaetiger 1	broad, semicircular anteriorly; wider than long	absent	conical to digitiform	only reported in Boggemann (2009) as inserted in posterior half of prostomium	Slightly shorter than antennae
***Lacydonia oculata* Hartman, 1967**	off Wiencke Island, antarctic Penninsula	Shallow about landing and pier	6-6.5mm	45 in Pleijel & fauchald (1993), 50 in Hartman (1967)	brownish yellow; darker laterally on each segment and on tips of ventral and dorsal cirri	not mentioned	twice as wide as long	pair of large eyes, anteriorly situated	small papilliform	small, rounded, situated posterior to the eyes	similar to antenna; situated in small depressions
***Lacydonia cirrata* Hartman & Fauchald, 1971**	US east coast	2,022m	6mm	29-32	not described	not described	wider than long	absent	not described	not described	very small ventro-lateral (described as antennae)
***Lacydonia laureci* Laubier, 1975**	Malapan trench, Eastern mediterranean	4,690m	5.9mm	39	whitish	only described in Boggeman, 2009 as red-brown pigmented spots on prostomium and first segment	rectangular, wider than long with two lateral lobes on posterior margin	absent	subterminal, digitiform, slender	similar to lateral antennae; positioned in a prostomial indentation	similar ro antennae or slightly shorter than lateral antennae
***Lacyonia gordia* Hartmann Schröder, 1993**	Antarctic Peninsula	165-177m	7.8mm	45	not described	not described	about 3x wider than long	absent	short, oval	short, oval (on second half of prostomium)	not described
***Lacydonia hampsoni* Blake, 1994**	off San Francisco, Western US Coast	985-1990m	4mm	30-35	tan	pigment spots on prostomium and first 3 chaetigers; pigment spots on boarders of parapodia and cirri	Oval-shaped 1.5x wider than long	absent	short digitiform	short digitiform (on anterior one-third of prostomium	simiar to antennae

Table 1: Species List. (Continued)

Species of lacydonia	Type locality	Depth range	Maximum length	umber of chaetigers	Body color	Pigment spots	Prostomium	Eyes	Lateral antennae	Median antenna	Palps
Lacydonia eliasoni Hartmann-Schröder, 1996	Skagerrak	271-487m	3.5mm	20-29	body pale with transverse stripes of brown pigmentation on posterior edges of segments	one pair present on achaetous segment*	Short broad, about 2x wider than long	Absent	missing	short, digitiform	not described
Lacydonia quadrioculata Magalhães, Bailey-Brock & Rizzo, 2012	Oahu, Hawaii, northwestern Pacific	27-65.5m	3.0mm	8-26	Yellowish to brownish	present on prostomium, anterior end of achaeous segment; one pair per segment, near parapodial base, one pair on pygidium	twice wider than long	2 pairs	short digitiform to conical	similar to lateral antennae; on posterior half of prostomium	similar to frontal antennae
Lacydonia jacki Rizzo, Magalhães & Santos, 2016	Campos Basin, Brazil	shallow waters 72-103m	4.1mm	39	brownish	pigmented spots on the prostomium and dorssal region including parapodia and dorsal cirri	twice wider than long. Has two latero-frontal incisions.	one pair, large and black	two lateral dorsal antennae	not seen	two latero-ventral palps
Lacydonia anapaulae Rizzo, Magalhães & Santos, 2016	Campos Basin, Brazil	1272m	2mm	22	yellowish	sometimes with scattered small red-brown pigmented spots	anterior slit slightly pigmented. Twice wider than long	eyes absent	longer than palps	as long as lateral ones	2 palps selnder and filiform. Shorter than the lateral antennae
Lacydonia brasiliensis Rizzo, Magalhães & Santos, 2016	Campos Basin, Brazil	680-1379m	1.4-2mm	17-22	white to yellow in color.	pigmentation with reddish-brown spot pigments maily on anterior region of prostomium	rounded anteriorly, twice wider than long	two pairs and punctiform.	Twice longer than palps (or hallf as long as prostomium)	present	one pair of palps latero-dorssally and laeroventrally inserted
Lacydonia japonica Hookabe, 2020	Off Ofunato, Iwate, northern Honshu, Japan	262-407m	1.2-5.0mm	17-25	yellowish brownish mottled pattern	White spots present on dorsal surface	anteriorly rounded, 1.4 times wider than long, without a slit on median anterior edge	eyespots and eyes absent	Single short pair. Slightly longer than median antenna	single median antenna present, short, conical	Single pair of palps present on ventral side of prostomium, short, conical

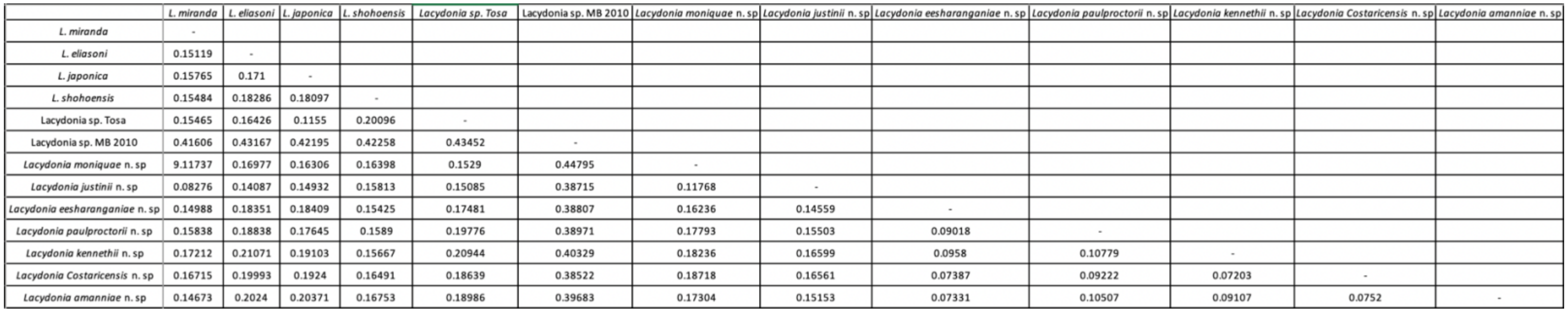
Table 1: Species List. (Continued)

Species of lacydonia	Type locality	Depth range	Maximum length	umber of chaetigers	Body color	Pigment spots	Prostomium	Eyes	Lateral antennae	Median antenna	Palps
Lacydonia sp Tosa *Hookabe, 2020*	Tosa Bay, Kochi, off western Japan	100m	?	?	?	?	?	?	?	?	?
Lacydonia shohoensis Hookable, 2021	Shoho seamount, Nishi-shichito ridge, North-western Pacific Occean	2042m	4.0-4.2mm	33	Generally tansparent; pale yellow intestine visible through integument	pale orange pigment spots present	width is twice the length. Anteriorly rounded. With a groove on the median anterior edge	no eyes	paired. Same length as the median antennae	single median antenna present. Same length as the lateral.	a pair of palps on ventral side of prostomium, short and conical. Same length as paird lateral antennae.
Lacydonia sp MB Bögemann2010	Abyssal SE Atlantic, near the Guinea Basin	5443-5244	?	?	?	?	?	?	?	?	?
Lacydonia moniquae n. sp	Carrie Bow Cay, Caribbean Sea , Belize	15m	3.0339mm	32	slightly transparent; yellowish intestines visible through the body wall	brown pigment spots on the dorsal side	Width is twice the length. Rounded anteriorly	eye spots	paired slim digitiform.	present	Single pair of palps present on ventral side of prostomium, short, conical
Lacydonia justinii n. sp	San Clemente Island, Pacific Ocean	12m	4.23mm	34	slightly transparent; yellowish intestines visible through the body wall	not observed	Width is twice the length. Rounded anteriorly	eye spots	paired digitiform.	present	Single pair of palps present on ventral side of prostomium, short, conical
Lacydonia eesharanganiae n. sp	Costa Rica Margin, Pacific Ocean	1000m	.5749mm (for the 1st 6 cheatigers)	5 (incomplete specimen)	Preserved specimen was tan	not observed	Width is twice the length. Rounded anteriorly	no eyes	not observed in holotype	Assumed present (not observed)	single pair of palps present on the ventral side of prostomium, short, digitiform.
Lacydonia paulproctorii n. sp	Costa Rica Margin, Pacific Ocean	2000m	1.4209mm	23	slightly transparent; whitish intestines visible through the body wall	not observed	Stunted. Width is twice the length	no eyes	paired.	present	not observed
Lacydonia kennethii n. sp	Guaymas Basin, Gulf of California, Mexico	740-1600m	11.4523mm	48	slightly transparents; yellowish intestines visible through the body wall	not observed	Width is twice the length. With a groove at the base of the median antenna. Rounded anteriorly	no eyes	paired slim digitiform.	present	single pair of palps present on the ventral side of prostomium, short, digitiform.
Lacydonia costaricensis or costaicanthus n. sp	Costa Rica Margin, Pacific Ocean	1000-1400m	5.1752mm	36	slightly transparent; proboscis and yellowish intestines visible through the body wall	two brown pigment spots on the posterior end of the dorsal surface of the prostomium	Width is twice the length. Rounded anteriorly	no eyes	paired slim digitiform.	present	Single pair of palps present on ventral side of prostomium, short, conical
Lacydonia amanniae n. sp	Cascadia Margin, Hydrate ridge, Pacific Ocean	>1000m	15mm	30	prostomium pigmented, body slightly transparent, yellowish intestines visible through the body wall.	not observed	Width is twice the length. Rounded anteriorly	no eyes	paired digitiform.	present	single pair of palps present on the ventral side of prostomium, short, digitiform.

Table 2: New specimens utilized alongside the relevant species information sourced from GenBank. The provided information includes voucher numbers, collection site details (including site coordinates and depth), and GenBank numbers.

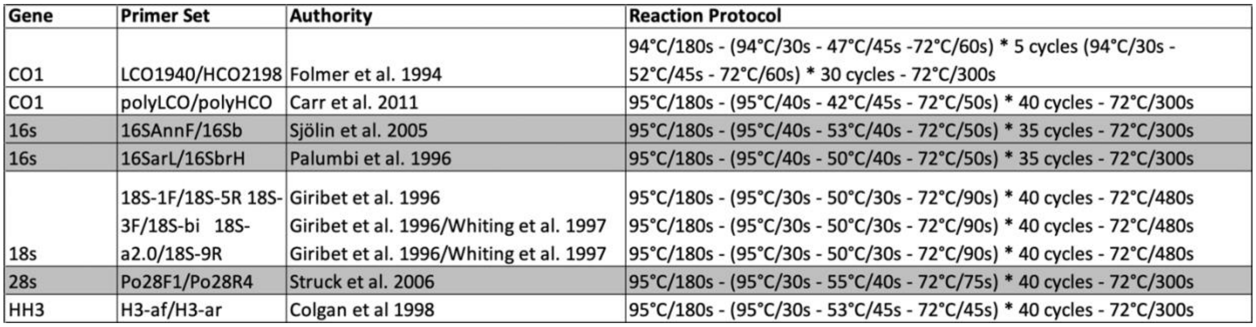
Lacydonia	**Voucher**	**Site**	**Coordinates**	**Depth (m)**	**CO1**	**16s**	**18s**	**28s**	**HH3**
L. miranda	SIO-BIC A4261	Formiche di Vivara, Italy	40.7448°N; 13.9783°E	15					
L. eliasoni	SIO-BIC A14170	Trondheimsfjord, Norway	63.4767°N; 10.0011°W	200					
L. eliasoni	SMNH90987	Trondheimsfjord, Norway	63.4767°N; 10.0011°W	200	AY996120	AY996061	MG254416		
L. japonica	NSMT-Pol-H-810	Ofunato, Japan	6.7054°N; 6.4487°E	262	LC520110	-	LC520118	LC520119	
Lacydonia sp. MB 2010	ZMH P25287 A	Guinea Basin, West Africa	0°40.50'N 5°29.7'W	5000	GQ426678	GQ426617	GQ426580	-	
Lacydonia sp. Tosa		Tosa Bay, Japan	18.452°N; 35.829°E	100	LC520109	LC520111	LC520109	LC520120	
L. shohoensis	NSMT-Pol H-844	Shoho Seamount, Japan	20.854°N; 39.472°E	2042	MZ702655	MZ700230		MZ700232	
Lacydonia moniquae n. sp	SIO-BIC A4729	Carrie Bow Cay, Belize	16.8025°N; 88.0817°W	15					
Lacydonia moniquae n. sp	SIO-BIC A4881	Carrie Bow Cay, Belize	16.7885°N; 88.0763°W	15					
Lacydonia justini n. sp	SIO-BIC A14161	San Clemente Island, California	32.9165°N; 118.4641°W	12					
Lacydonia justini n. sp	SIO-BIC A14218	San Clemente Island, California	32.9165°N; 118.4641°W	10					
Lacydonia eesharanganiae n. sp	SIO-BIC A1525A	Mound 11, Costa Rica	8.9208°N; 84.3054°W	1000					
Lacydonia paulproctori n. sp	SIO-BIC A9814	Quepos Plateu, Costa Rica	8.5855°N; 84.5484°W	2184					
Lacydonia kennethi n. sp	SIO-BIC A1606	Jaco Summit, Costa Rica	9.1723°N; 84.7987°W	740					
Lacydonia kennethi n. sp	SIO-BIC A3340	Guaymas Basin, Mexico	27.5875°N; 111.473°W	1600					
Lacydonia costaricensis n. sp	SIO-BIC A1485	Mound 12, Costa Rica	8.93°N; 84.3135°W	1000					
Lacydonia costaricensis n. sp	SIO-BIC- A10090	The Thumb, Costa Rica	9.0486°N; 84.3945°W	1000	MZ562520				
Lacydonia costaricensis n. sp	SIO-BIC-A9774	Mound 12, Costa Rica	8.9303°N; 84.3123°W	1000					
Lacydonia costaricensis n. sp	SIO-BIC-A9880	Parrita Seep, Costa Rica	9.0318°N; 84.6205°W	1401					
Lacydonia costaricensis n. sp	SIO-BIC-A9773	Mound 12, Costa Rica	8.9303°N; 84.3123°W	1000					
Lacydonia costaricensis n. sp	SIO-BIC-A1925	Mound 12, Costa Rica	8.9303°N; 84.3125°W	1000					
Lacydonia amanniae n. sp	SIO-BIC-A1526A	Mound 11, Costa Rica	8.9208°N; 84.3054°W	1000					
Lacydonia amanniae n. sp	SIO-BIC-A2648	Hydrate Ridge, Oregon	44.6701°N; 125.0987°W	600					
Lacydonia amanniae n. sp	SIO-BIC-1605	Jaco Summit, Costa Rica	9.1723°N; 84.7987°W	740					
Lacydonia amanniae n. sp	SIO-BIC-A2651	Hydrate Ridge, Oregon	44.6701°N; 125.0987°W	600					
Lacydonia amanniae n. sp	SIO-BIC-A2649B	Hydrate Ridge, Oregon	44.6701°N; 125.0987°W	600					
Lacydonia amanniae n. sp	SIO-BIC-A2648A	Hydrate Ridge, Oregon	44.6701°N; 125.0987°W	600					
Lacydonia amanniae n. sp	SIO-BIC-A2649	Hydrate Ridge, Oregon	44.6701°N; 125.0987°W	600					
Lacydonia amanniae n. sp	SIO-BIC-A2646	Hydrate Ridge, Oregon	44.6701°N; 125.0987°W	600					
Lacydonia amanniae n. sp	SIO-BIC-A1526B	Mound 11, Costa Rica	8.9208°N; 84.3054°W	1000					
Lacydonia amanniae n. sp	SIO-BIC-A1525C	Mound 11, Costa Rica	8.9208°N; 84.3054°W	1000					
Lacydonia amanniae n. sp	SIO-BIC-A1525B	Mound 11, Costa Rica	8.9208°N; 84.3054°W	1000					
Lacydonia amanniae n. sp	SIO-BIC-A1526C	Mound 11, Costa Rica	8.9208°N; 84.3054°W	1000					
Lacydonia amanniae n. sp	SIO-BIC-A2649A	Hydrate Ridge, Oregon	44.6701°N; 125.0987°W	600					
Lacydonia amanniae n. sp	SIO-BIC-A2647	Hydrate Ridge, Oregon	44.6695°N; 125.0981°W	600					
Lacydonia amanniae n. sp	SIO-BIC-A2650	Hydrate Ridge, Oregon	44.6701°N; 125.0987°W	600					
Lacydonia amanniae n. sp	SIO-BIC-A12314A	Del Mar Seep, California	32.9043°N; 117.7823°W	1020					
Lacydonia amanniae n. sp	SIO-BIC- A12314B	Del Mar Seep, California	32.9043°N; 117.7823°W	1020					

Table 3: Interspecific genetic distance % among *Lacydonia* based on approximately 600 bp of CO1 sequences.



	L. miranda	*L. eliasoni*	*L. japonica*	*L. shohoensis*	*Lacydonia sp. Tosa*	Lacydonia sp. MB 2010	*Lacydonia moniquae* n. sp	*Lacydonia justinii* n. sp	*Lacydonia eesharanganiae* n. sp	*Lacydonia paulproctorii* n. sp	*Lacydonia kennethii* n. sp	*Lacydonia Costaricensis* n. sp	*Lacydonia amanniae* n. sp
L. miranda	-												
L. eliasoni	0.15119	-											
L. japonica	0.15765	0.171	-										
L. shohoensis	0.15484	0.18286	0.18097	-									
Lacydonia sp. Tosa	0.15465	0.16426	0.1155	0.20096	-								
Lacydonia sp. MB 2010	0.41606	0.43167	0.42195	0.42258	0.43452	-							
Lacydonia moniquae n. sp	9.11737	0.16977	0.16306	0.16398	0.1529	0.44795	-						
Lacydonia justinii n. sp	0.08276	0.14087	0.14932	0.15813	0.15085	0.38715	0.11768	-					
Lacydonia eesharanganiae n. sp	0.14988	0.18351	0.18409	0.15425	0.17481	0.38807	0.16236	0.14559	-				
Lacydonia paulproctorii n. sp	0.15838	0.18838	0.17645	0.1589	0.19776	0.38971	0.17793	0.15503	0.09018	-			
Lacydonia kennethii n. sp	0.17212	0.21071	0.19103	0.15667	0.20944	0.40329	0.18236	0.16599	0.0958	0.10779	-		
Lacydonia Costaricensis n. sp	0.16715	0.19993	0.1924	0.16491	0.18639	0.38522	0.18718	0.16561	0.07387	0.09222	0.07203	-	
Lacydonia amanniae n. sp	0.14673	0.2024	0.20371	0.16753	0.18986	0.39683	0.17304	0.15153	0.07331	0.10507	0.09107	0.0752	-

Table 4: cycler specifications, genes, primers, and protocols used in this study.



Gene	Primer Set	Authority	Reaction Protocol
CO1	LCO1940/HCO2198	Folmer et al. 1994	94°C/180s - (94°C/30s - 47°C/45s -72°C/60s) * 5 cycles (94°C/30s - 52°C/45s - 72°C/60s) * 30 cycles - 72°C/300s
CO1	polyLCO/polyHCO	Carr et al. 2011	95°C/180s - (95°C/40s - 42°C/45s - 72°C/50s) * 40 cycles - 72°C/300s
16s	16SAnnF/16Sb	Sjölin et al. 2005	95°C/180s - (95°C/40s - 53°C/40s - 72°C/50s) * 35 cycles - 72°C/300s
16s	16SarL/16SbrH	Palumbi et al. 1996	95°C/180s - (95°C/40s - 50°C/40s - 72°C/50s) * 35 cycles - 72°C/300s
18s	18S-1F/18S-5R 18S-3F/18S-bi 18S-a2.0/18S-9R	Giribet et al. 1996 Giribet et al. 1996/Whiting et al. 1997 Giribet et al. 1996/Whiting et al. 1997	95°C/180s - (95°C/30s - 50°C/30s - 72°C/90s) * 40 cycles - 72°C/480s 95°C/180s - (95°C/30s - 50°C/30s - 72°C/90s) * 40 cycles - 72°C/480s 95°C/180s - (95°C/30s - 50°C/30s - 72°C/90s) * 40 cycles - 72°C/480s
28s	Po28F1/Po28R4	Struck et al. 2006	95°C/180s - (95°C/30s - 55°C/40s - 72°C/75s) * 40 cycles - 72°C/300s
HH3	H3-af/H3-ar	Colgan et al 1998	95°C/180s - (95°C/30s - 53°C/45s - 72°C/45s) * 40 cycles - 72°C/300s

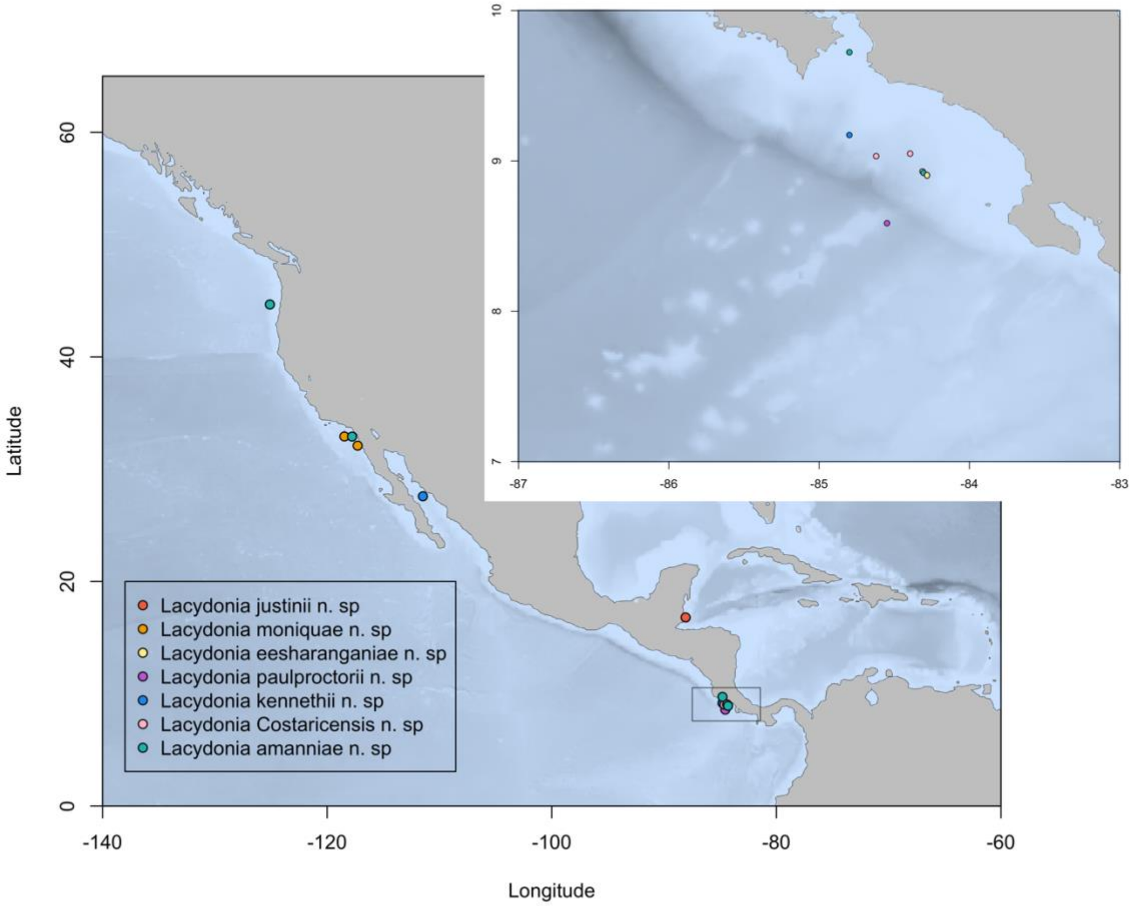


Figure 1: A map of the collection sites for all new specimens listed in Table 1 and Table 2 generated using the R package marmap R package (Pante & Simon-Bouhet 2013) and modified with Adobe Illustrator (Adobe Inc. 2019).

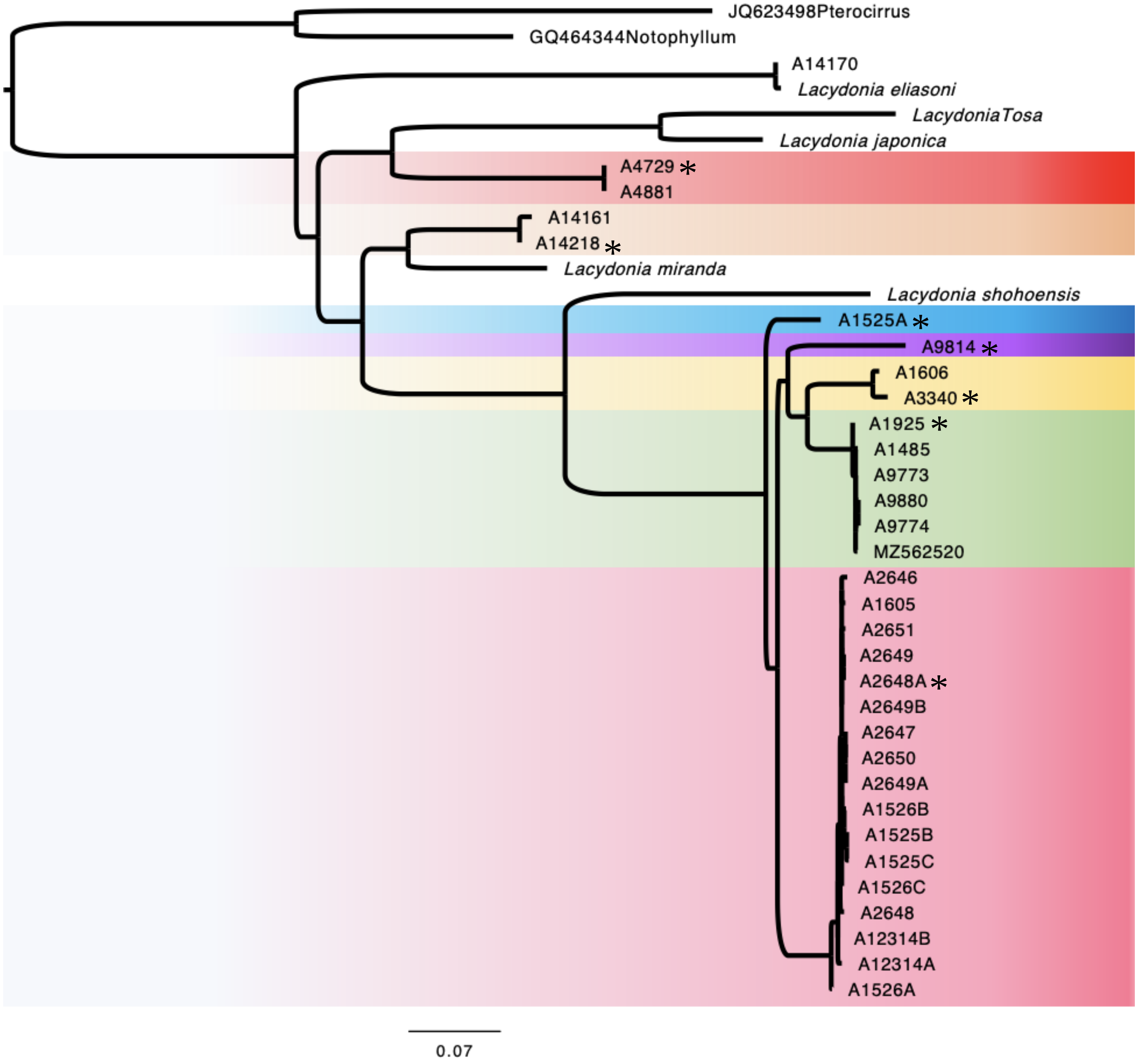


Figure 2: Maximum likelihood CO1 tree displaying seven distinct clades and the specimens that make up those clades including newly collected organisms labled with the Scripps Institution of Oceanography Benthic Invertebrate Collection voucher numbers and CO1 sequences of previously described species available on GenBank (*L. eliasoni*, *Lacydonia* sp Tosa, *L. japonica*, *L. miranda*, *L. shohoensis*). The corresponding GenBank accession numbers can be found in Table 2. The tree was constructed using the software platform RAxML-NG (version RaxmlGUIv.2.0.10) with the TVM+I+G model. A total of 10 runs were performed, and the resulting image was further modified using Adobe Illustrator (Adobe Inc., 2019). * indicate holotypes.Colors are to distinguish between clades.

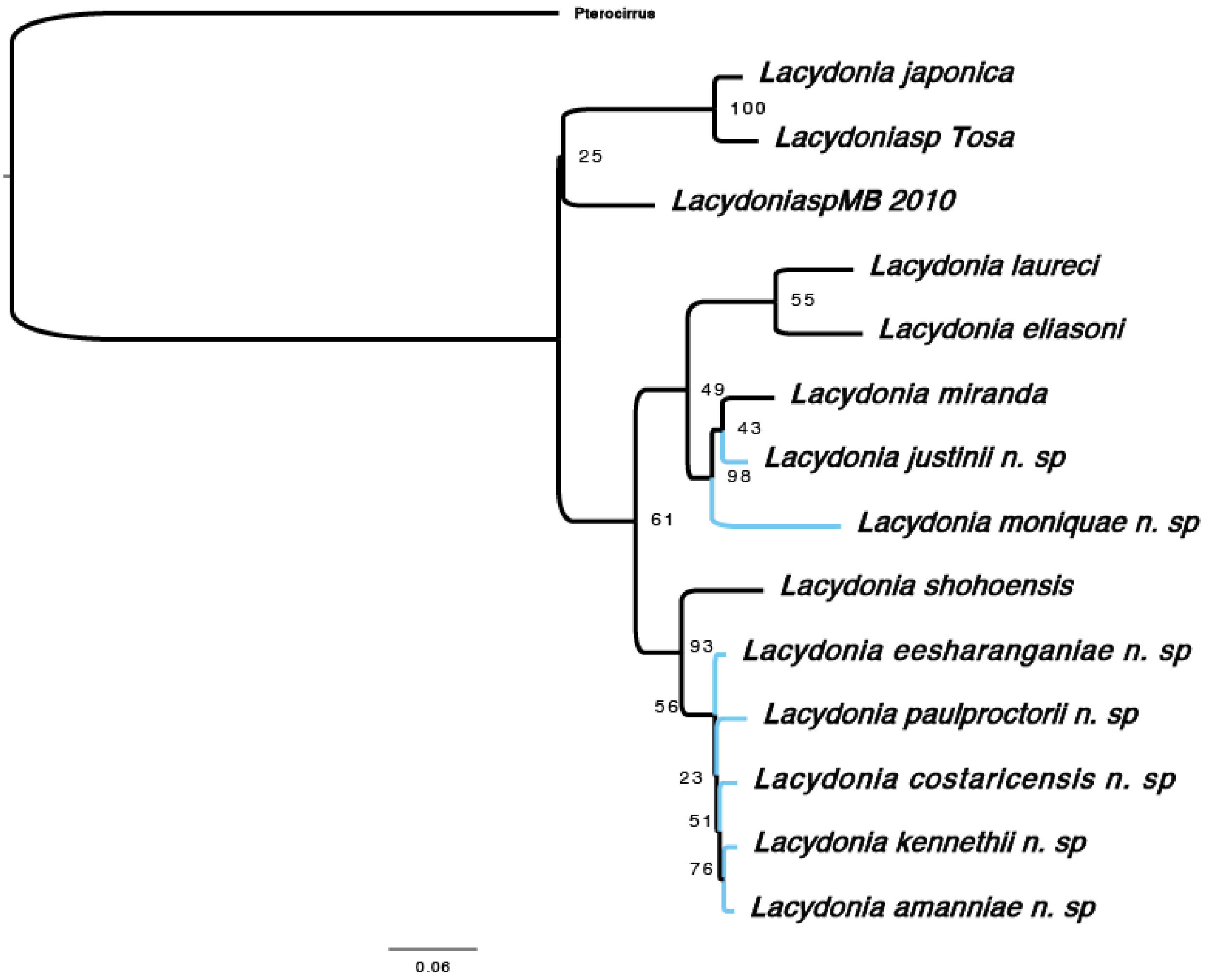


Figure 3: Concatenated Maximum likelihood analysis generated using sequences CO1, 16S, 18S, 28S and HH3. Colored branches indicate newly described species as a result of the present study. Numbers at the nodes indicate bootstrap values (%). The program utilized was RAxML-NG (version RaxmlGUIv.2.0.10) which was also used for model selection. Concatenated analyses were performed with 10 runs and 100 replicates. The resulting image was further modified using Adobe Illustrator (Adobe Inc., 2019).

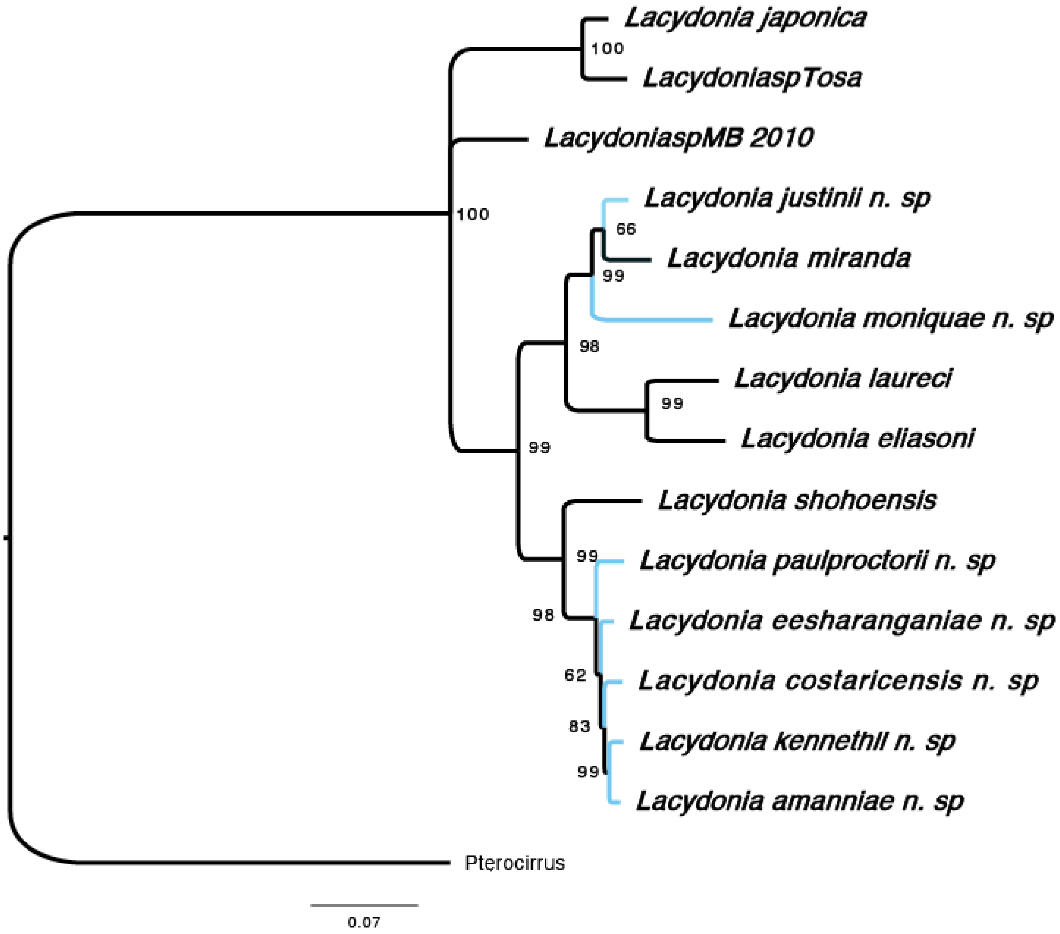


Figure 4: Bayesian analysis on concatenated genes CO1, 16S, 18S, 28S, HH3. Colored branches indicate newly described species as a result of the present study. Numbers at the nodes indicate posterior probability values greater than 50%. The program utilized was MrBayes 3.2.7a and best fit models for each gene were selected via JModelTest 2 version jmodeltest 2.1.20 2. Analysis included 20,000,000 generations, every 1000 trees were sampled, and the burnin was set to 10%. The resulting image was further modified using Adobe Illustrator (Adobe Inc., 2019).

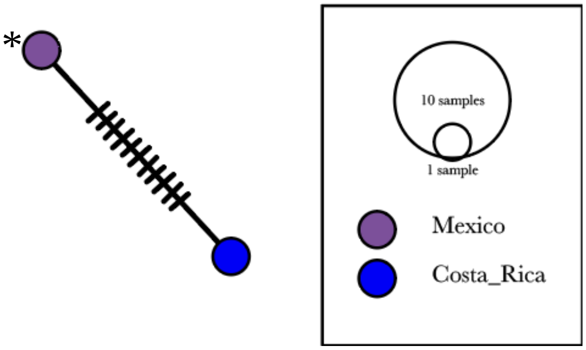


Figure 5: CO1 haplotype network for *Lacydonia kennethi* n. sp. The circles represent haplotypes, the size of the circle is an indication of how many samples are included in 1 haplotype, the colors represent locations, and the hatch marks represent base pair differences. *=Holotype

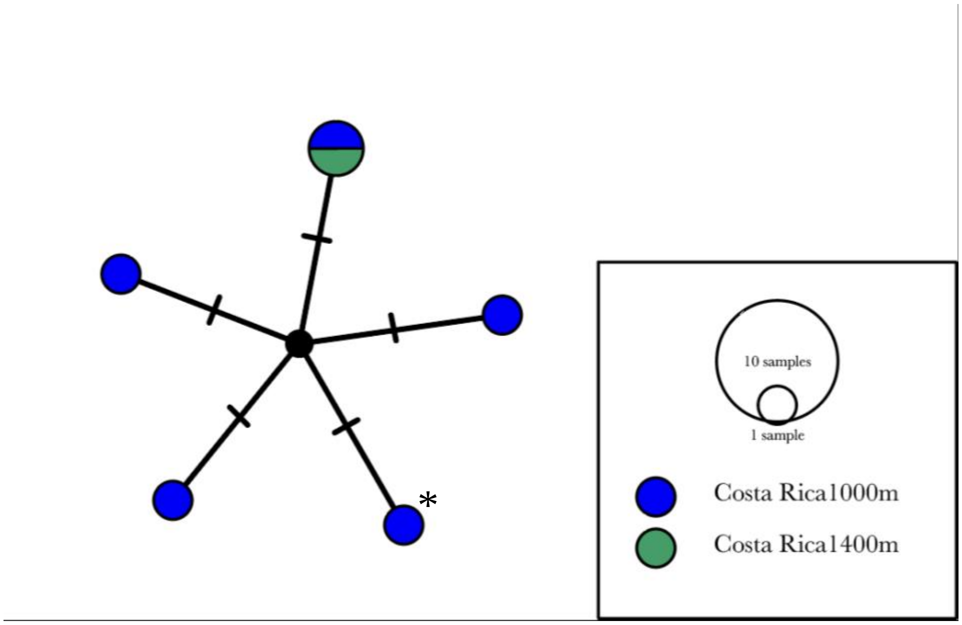


Figure 6: CO1 haplotype network for *Lacydonia costaricensis* n. sp. The circles represent haplotypes, the size of the circle is a indication of how many samples are included in 1 haplotype, the colors represent depths at the mound 12 collection site off the coast of Costa Rica, and the hatch marks represent base pair differences. *=Holotype

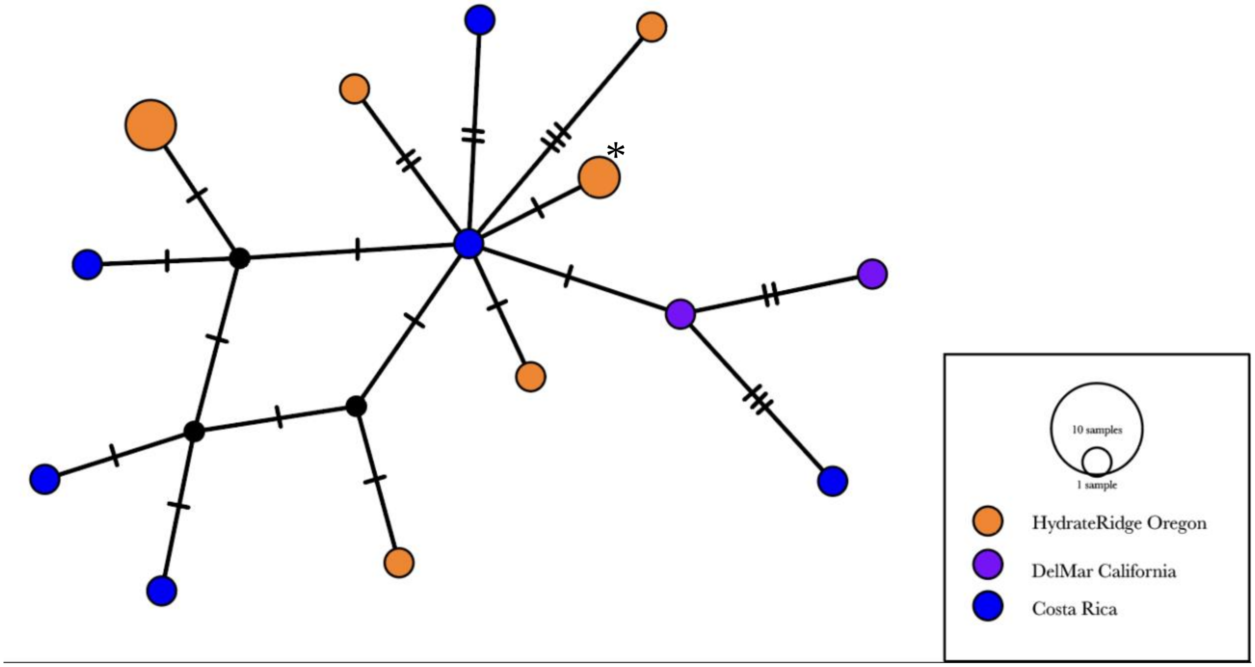


Figure 7: CO1 haplotype network for *Lacydonia amanniae* n. sp. The circles represent haplotypes, the size of the circle is a indication of how many samples are included in 1 haplotype, the colors represent locations, and the hatch marks represent base pair differences. *=Holotype

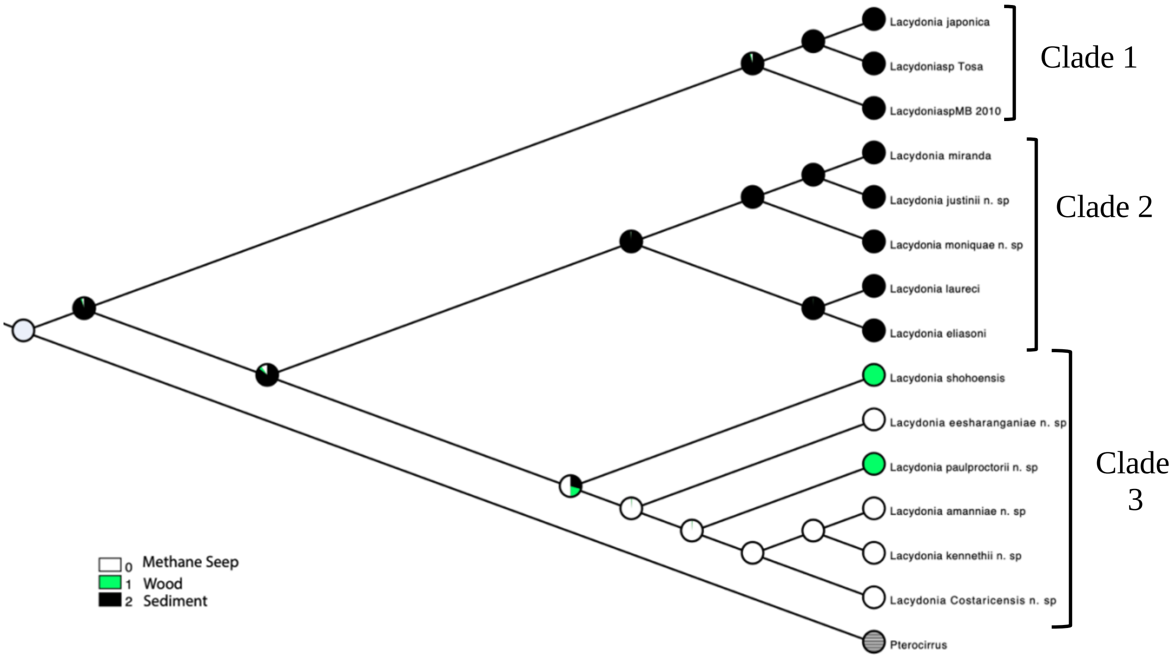


Figure 8: Habitat evolution analysis generated using the character states of presence on methane seep, wood, or sediment and the results from the concatenated maximum likelihood analysis. The program utilized was Mesquite version 3.70 and the resulting image was further modified using Adobe Illustrator (Adobe Inc., 2019).

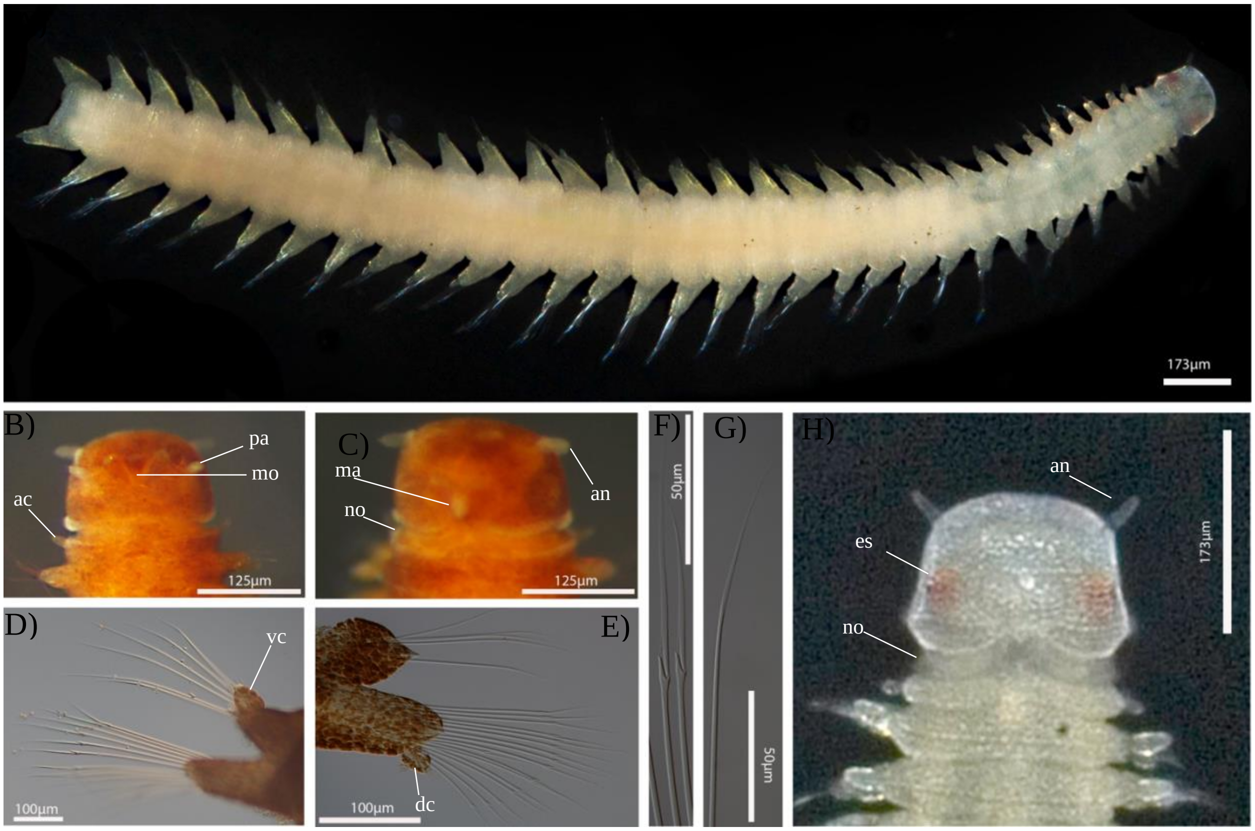


Figure 9: *Lacydonia moniquae* n. sp. holotype A4729 A) whole body dorsal view; B) ventral view of prostomium (pa, palps; mo, mouth; as, achaetus 1st segments); C) dorsal view of prostomium (ma, median antennae; no, nuchal organ; an, antennae); D) cheatiger 22 showing simple and compound cheatae, and the shape of shape of parapodial lobes and dorsal cirrus (vc, ventral cirrus); E) cheatiger 21 showing simple and compound cheatae, and the shape of shape of the parapodial lobes and ventral cirrus (dc, dorsal cirrus); F) neurochaetae of mid body; G) notochaetae of mid body; H) live dorsal view of anterior (es, eye spots).

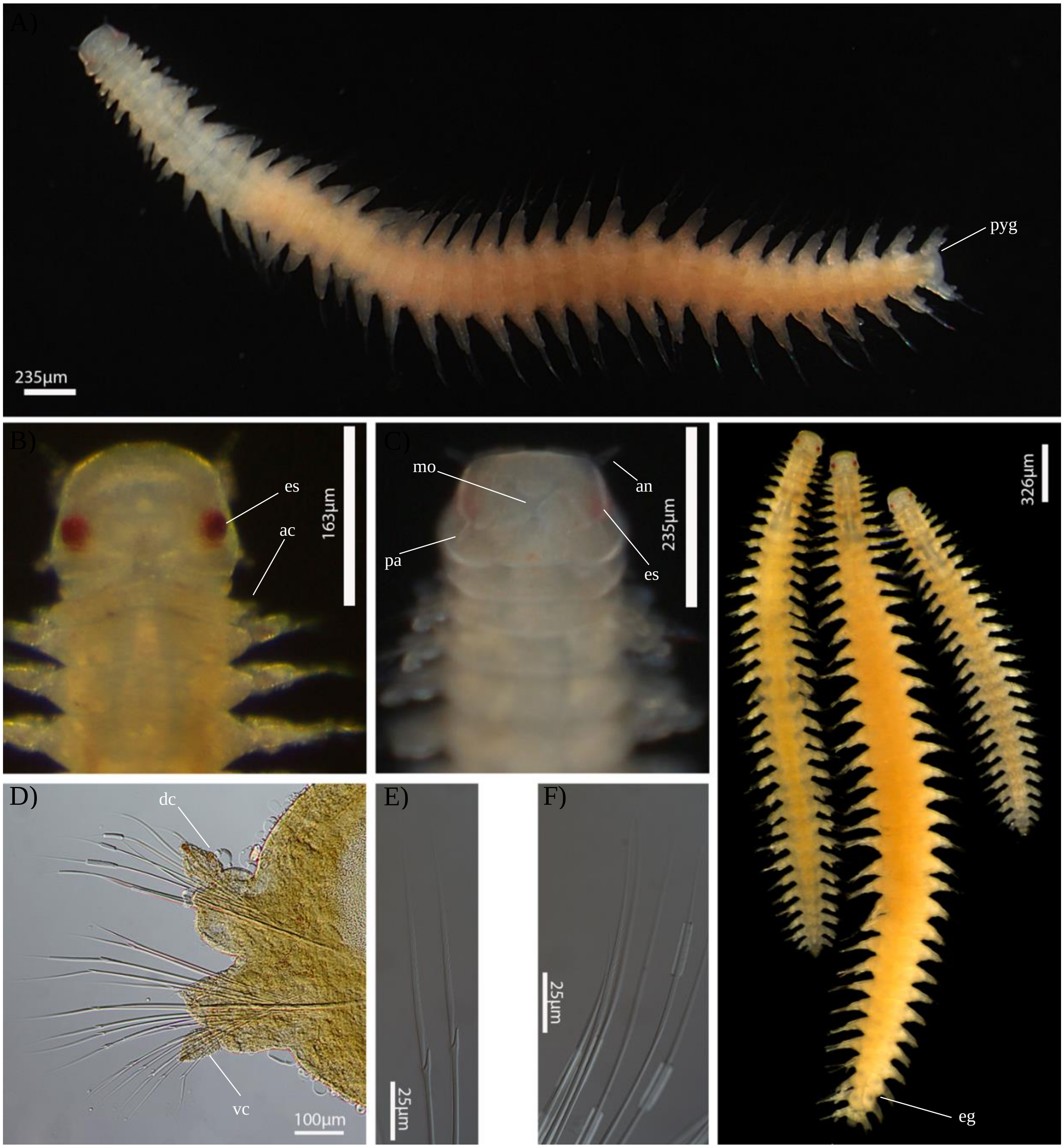


Figure 10: *Lacydonia justini n. sp.* A) whole body dorsal view of *Lacydonia justini n. sp*. holotype A14218 (pyg, pygidium); B) live dorsal view of anterior paratype A14161B (es, eyes; ac, achaetus 1st segment); C) live ventral view of anterior paratype A14218 (mo, mouth; pa, palp; an, antennae); D) cheatiger 23 showing simple and compound cheatae and the shape of shape of the parapodial lobes and cirrus paratype 14160, (dc, dorsal cirrus; vc, ventral cirrus); E) neurochaetae of mid body paratype 14160; F) notochaetae of mid body paratype 14160; G) whole body dorsal view *Lacydonia justini n. sp*. paratypes A14161A, A14161B, A14161C (eg, eggs).

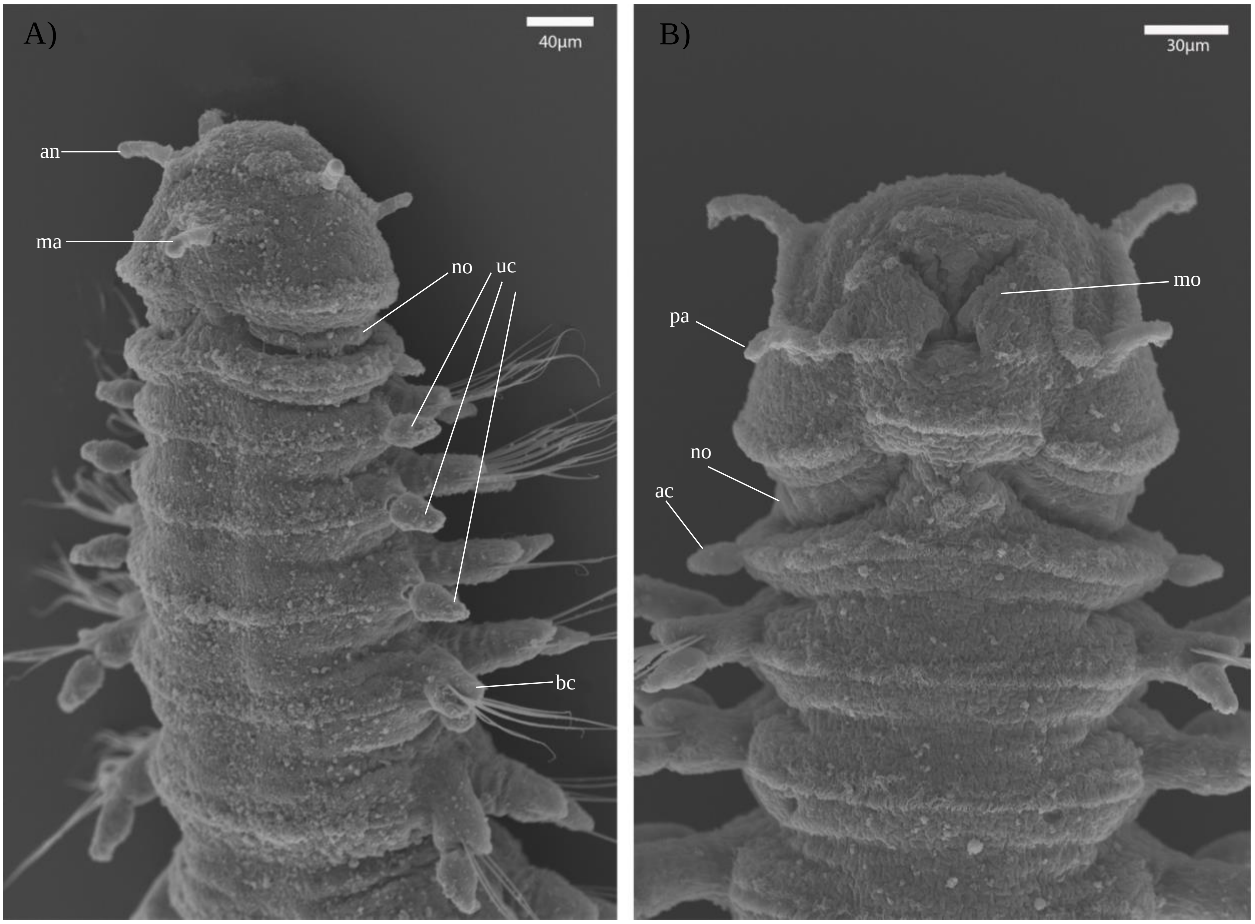


Figure 11: *Lacydonia justini n. sp.* A) SEM dorsal view of anterior paratype A14161b (an, antennae; ma, median antennae; no, nuchal organ; uc, uniramous chaetiger; bc, biramous chaetiger); B) SEM ventral view of anterior paratype A14161a (mo, mouth; pa, palps; ac, achaetus 1st segment).

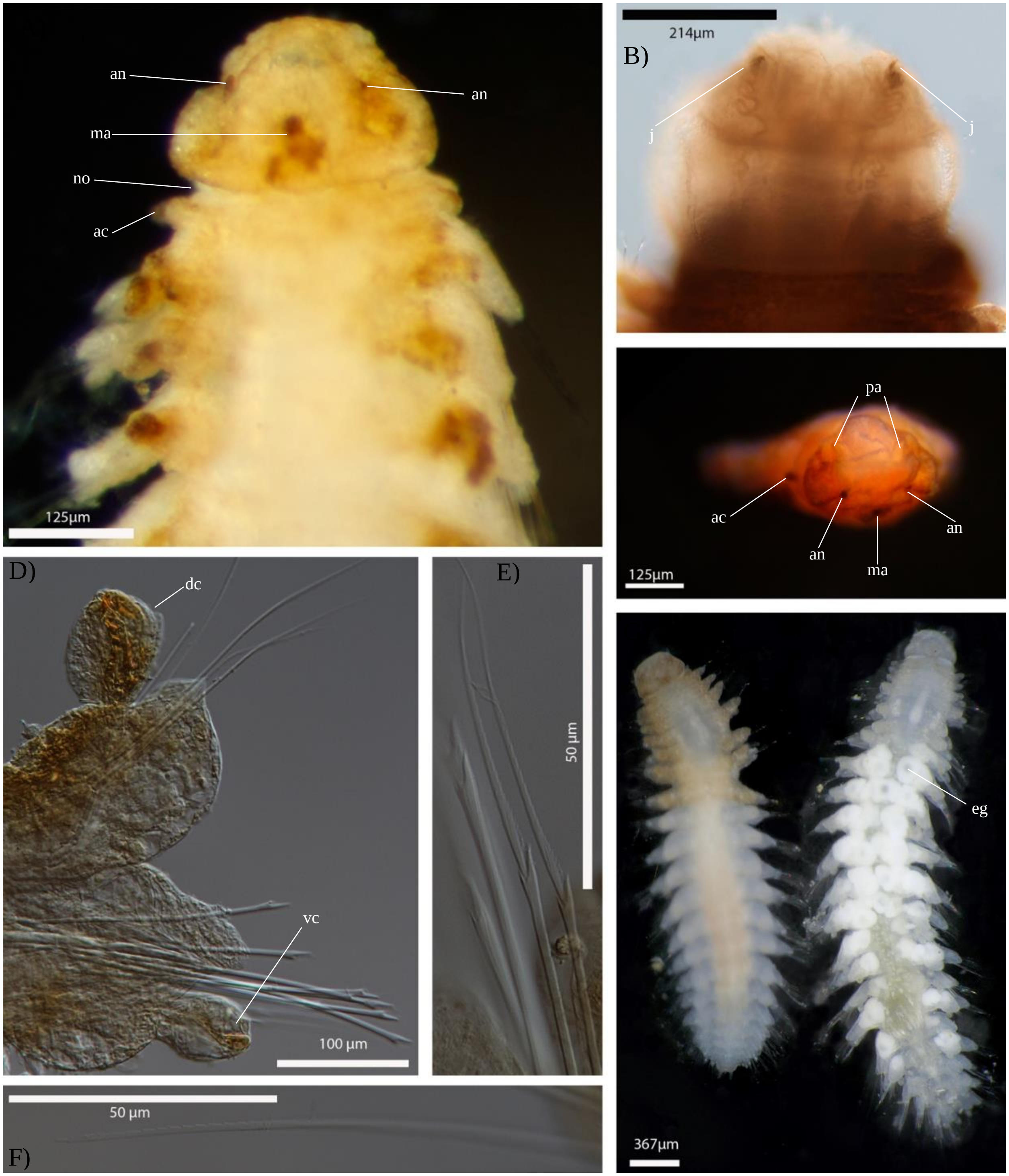


Figure 12: *Lacydonia eesharanganiae* n. sp. A) dorsal view of anterior holotype A1525a (an, antennae; ma, median antennae; no, nuchal organ; ac, achaetus 1st segment); B) everted proboscis paratype A1354a (j, potential jaws); C) mounted along the transverse plane holotype A1525a; D) cheatiger 6 showing simple and compound cheatae and the shape of the parapodial lobes and cirrus holotype A1525a, (dc, dorsal cirrus; vc, ventral cirrus); E) neurochaetae of upper body holotype A1525a; F) notochaetae of upper body holotype A1525a; G) whole body dorsal view of *Lacydonia eesharanganiae* n. sp. paratypes A1354a, A1354b (eg, eggs).

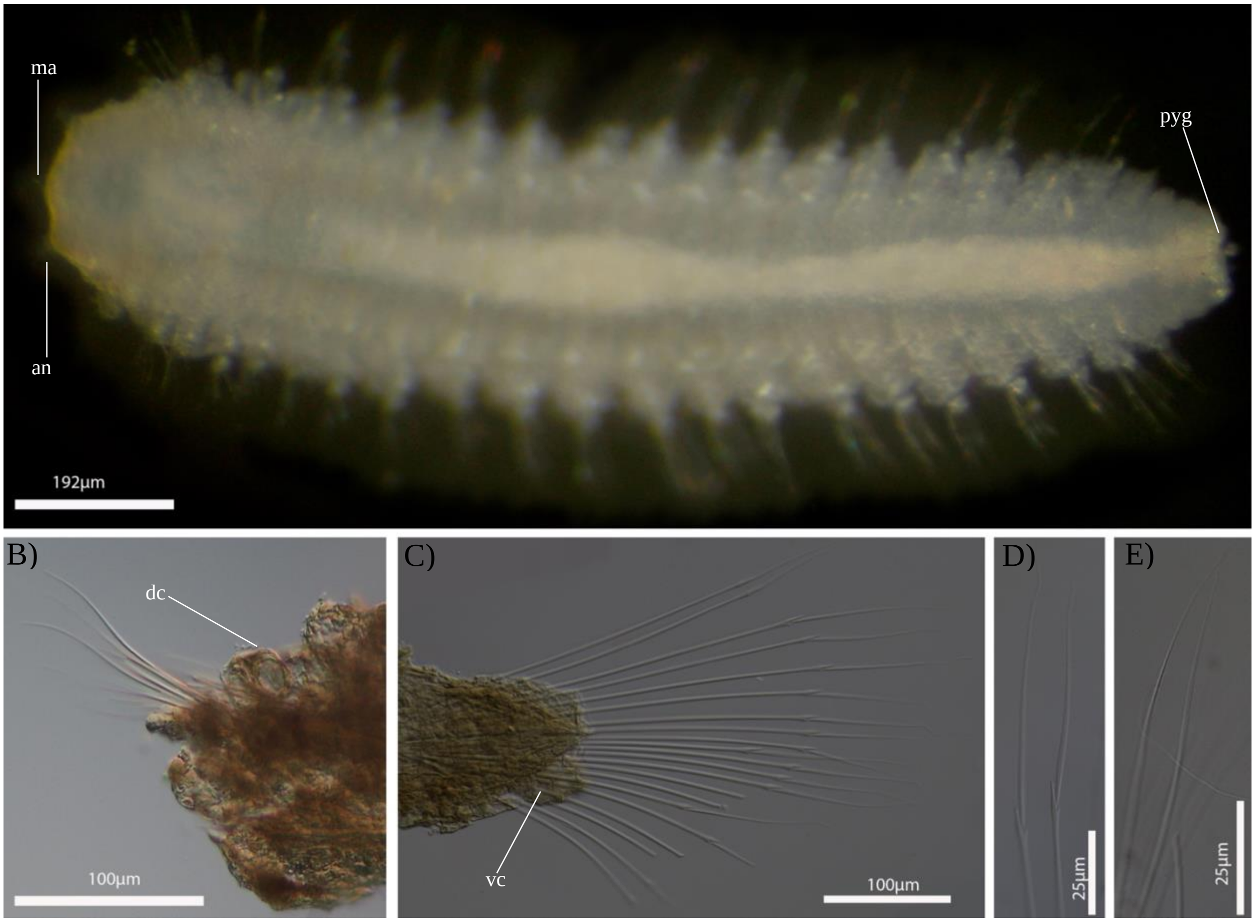


Figure 13: *Lacydonia paulproctori* n. sp. holotype A9814; A) whole body dorsal view of holotype A9814 (ma, median antennae; an, antennae; pyg, pygidium); B) upper body notopodial lobe with simple chaetae and dorsal cirrus (dc, dorsal cirrus); C) upper body neuropodial lobe with compound chaetae and ventral cirrus (vc, ventral cirrus); D) neurochaetae of upper body;E) notochaetae of upper body.

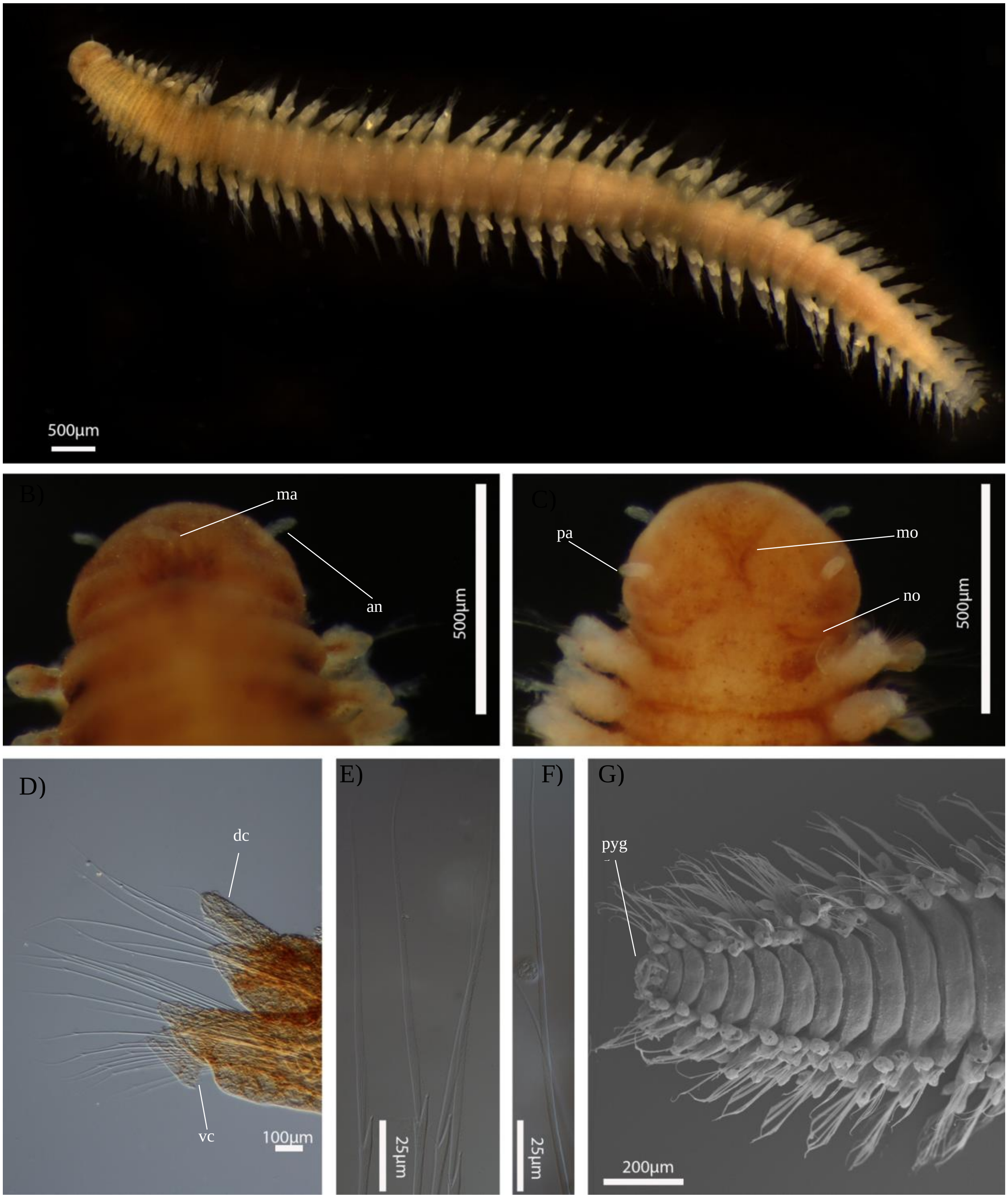


Figure 14: *Lacydonia kennethi* n. sp. A) whole body dorsal view of holotype A3340 B) dorsal view of prostomium (ma, median antennae; an, antennae); C) ventral view of prostomium (pa, palp; mo, mouth; no, nuchal organ); D) chaetiger 24 showing simple and compound chaetae and the shape of the parapodial lobes and cirrus of holotype of holotype A3340; E) neurochaetae of mid body holotype A3340; F) notochaetae of mid body holotype A3340; G) SEM dorsal view of posterior paratype A1433.

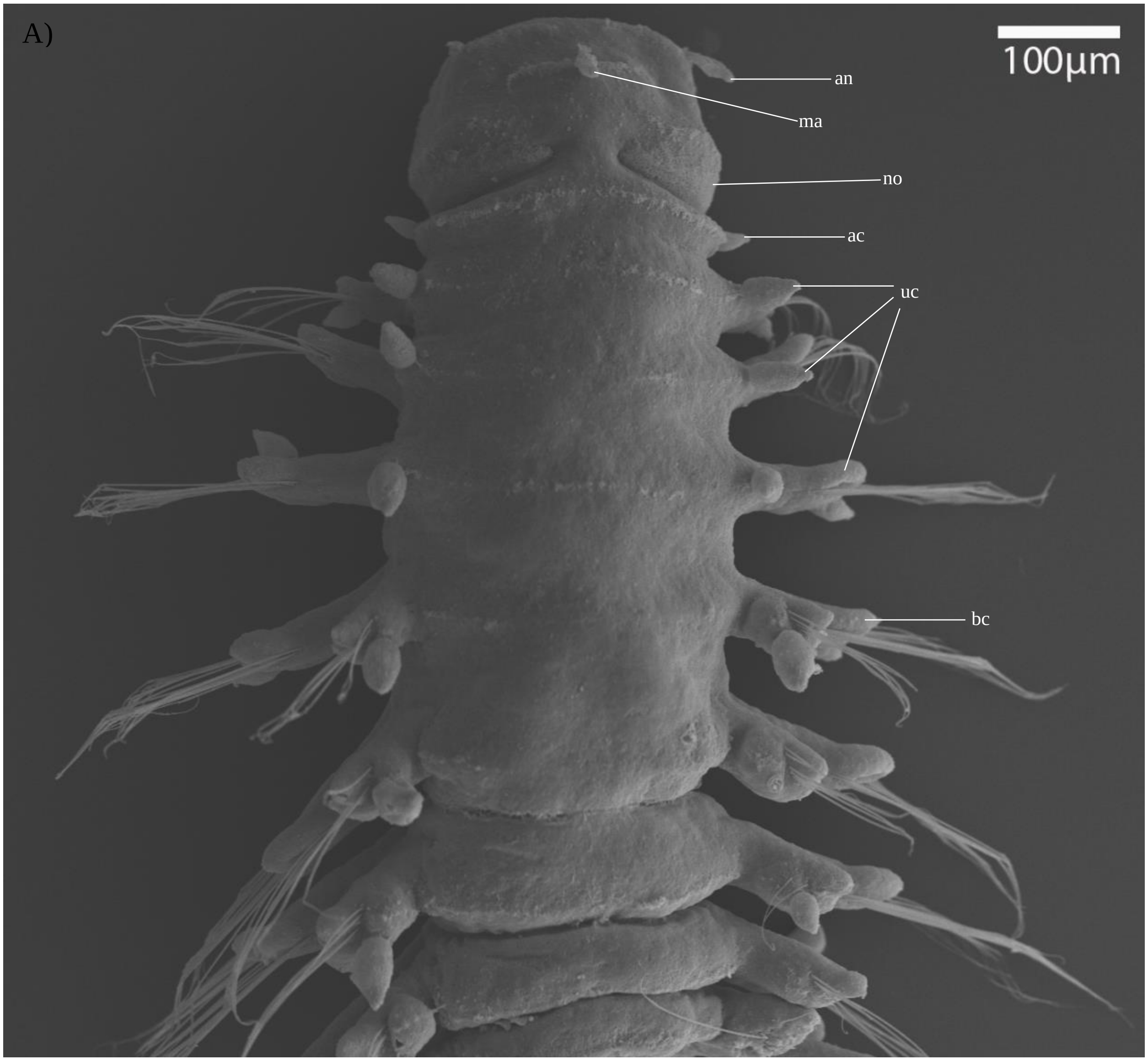


Figure 15: *Lacydonia kennethi* n. sp. A) SEM dorsal view of anterior paratype A1433 (an, antennae; ma, median antennae; no, nuchal organ; ac, achaetus 1st segment; uc, uniramous chaetigers; bc, biramous chaetiger).

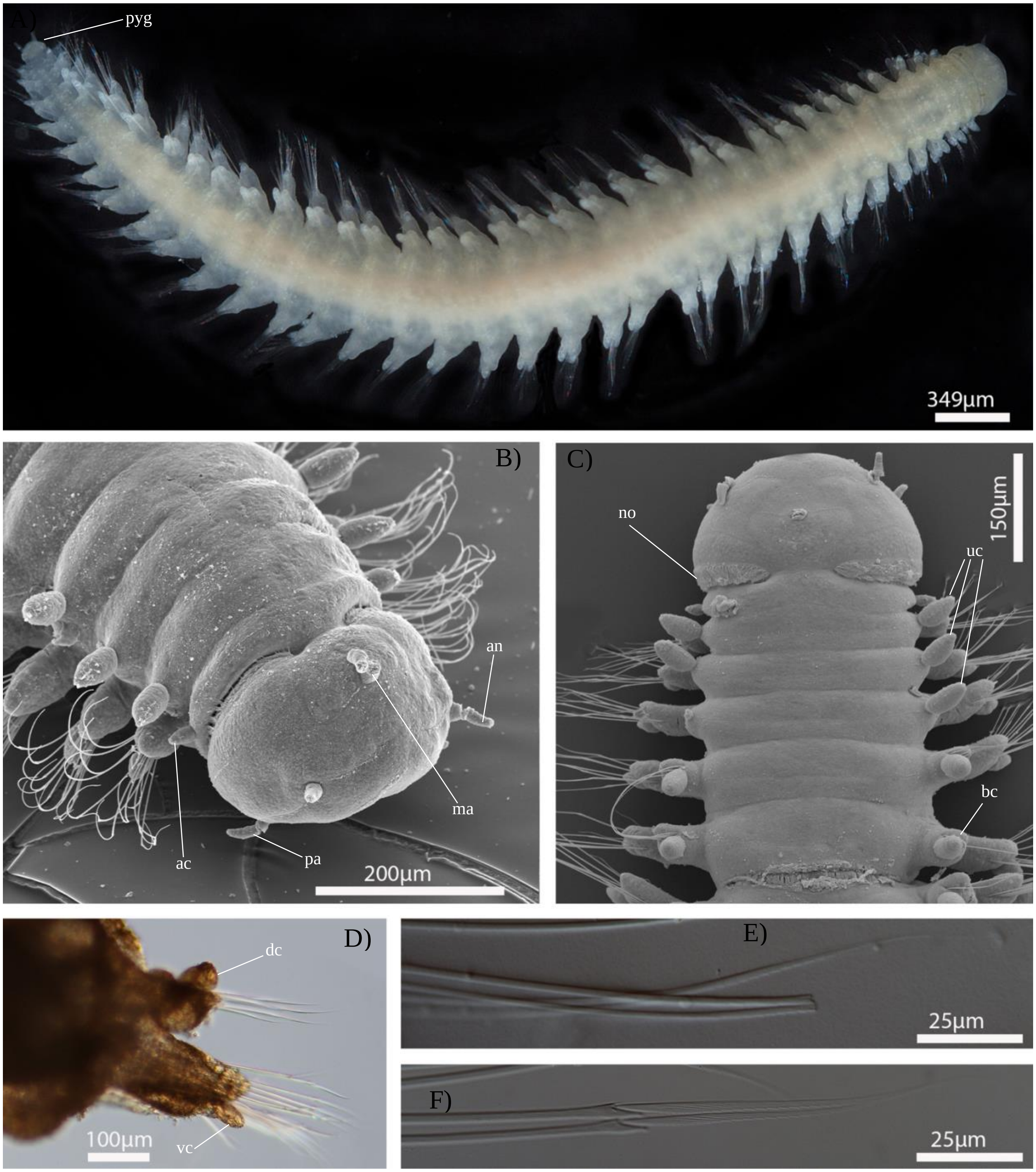


Figure 16: *Lacydonia costaricensis* n. sp. A) whole body dorsal view of holotype A1925 (pyg, pygidium); B) SEM dorsal view of anterior paratype A1926 (ac, achaetus 1st segment; pa, palp; ma, median antennae; an, antennae); C) SEM dorsal view of anterior paratype A1926 (no, nuchal organ; uc, uniramous chaetiger; bc, biramous chaetiger;); D) chaetiger 15 showing simple and compound chaetae and the shape of the parapodial lobes and cirrus of paratype A14232 (dc, dorsal cirrus; vc, ventral cirrus); E) notochaetae of mid body paratype A9774; F) neurochaetae of mid body paratype A9774.

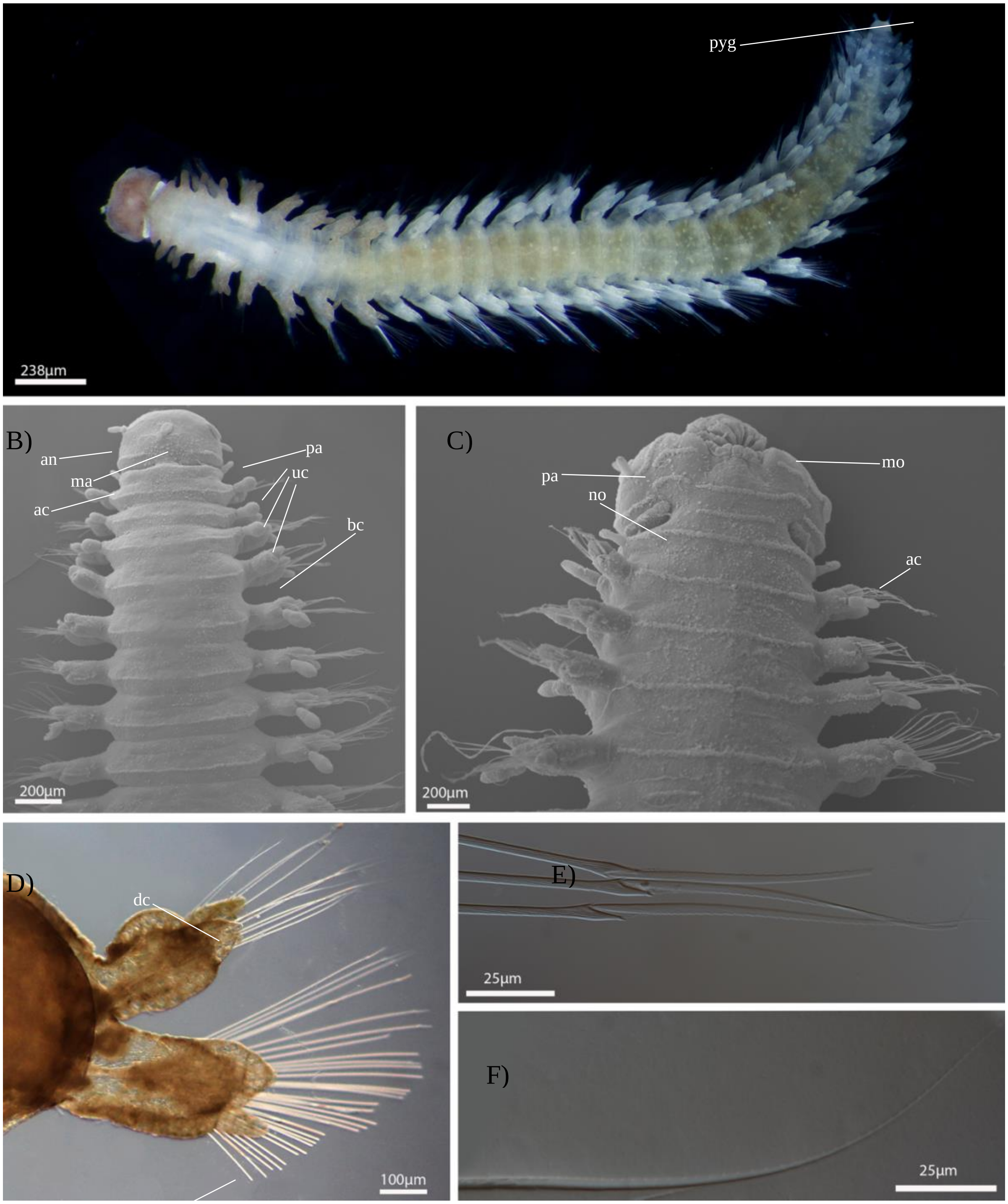


Figure 17: *Lacydonia amanniae* n. sp. A) whole body dorsal view of holotype A2648a (pyg, pygidium); B) SEM dorsal view of anterior paratype A12314c (an, antennae; ma, median antennae; ac, achaetus 1st segment; pa, palp; uc, uniramous chaetiger, bc, biramous chaetiger); C) SEM ventral view of anterior paratype A12314d (no, nuchal organ); D) midbody chaetiger showing simple and compound chaetae and the shape of the parapodial lobes and cirrus of paratype A12314a (dc, dorsal cirrus; vc, ventral cirrus); E) notochaetae of mid body paratype A12314a; F) neurochaetae of mid body paratype A12314a.

Figure 18: *Lacydonia amanniae* n. sp. A) whole body dorsal view of paratypes A2650, A2651 (eg, eggs); B) whole body dorsal view of paratypes A12314a, A12314b, A12314c, A12314d; C) whole body dorsal view of paratype A1605.

REFERENCES

Bergström, Erik. (1914). Zur Systematik der Polychætenfamilie der Phyllodociden. Zoologiska bidrag från Uppsala. 3: 37-224, plates I-V., available online at http://biodiversitylibrary.org/page/36924250

Blake, J. A., & Hilbig, B. (1994). Taxonomic Atlas of the Benthic Fauna of the Santa Maria Basin and Western Santa Barbara Channel: The Annelida: Oligochaeta and Polychaeta: Phyllodocida (Phyllodocidae to Paralacydoniidae). Santa Barbara Museum of Natural History.

Böggemann, M. (2009). Polychaetes (Annelida) of the abyssal SE Atlantic. *Organisms, Diversity & Evolution*, *9*(4), 251–428.

Carr, Christina M., Sarah M. Hardy, Tanya M. Brown, Tara A. Macdonald, and Paul D. N. Hebert. 2011. “A Tri-Oceanic Perspective: DNA Barcoding Reveals Geographic Structure and Cryptic Diversity in Canadian Polychaetes.” *PLoS ONE* 6(7). doi: 10.1371/journal.pone.0022232.

Caullery, M. 1914. Sur les Siboglinidae, type nouveau d'invertébrés receuillis par l'expédition du Siboga. Comptes rendus hebdomadaires des séances de l'Académie des sciences. 158: 2014-2017., available online at http://biodiversitylibrary.org/page/7159983
page(s): 2014

Chamberlin, Ralph V. (1919). The Annelida Polychaeta [Albatross Expeditions]. Memoirs of the Museum of Comparative Zoology at Harvard College. 48: 1-514., available online at http://www.biodiversitylibrary.org/ia/memoirsofmuseumo4801harv
page(s): 338

Claparède, Édouard. (1870). Les Annélides Chétopodes du Golfe de Naples. Supplément. Mémoires de la Société de physique et d'histoire naturelle de Genève. 20(2): 365-542 [separate pagination at page top, continuous pagination at bottom]., available online at https://biodiversitylibrary.org/page/2094031
page(s): 462

Colgan, D. J., McLauchlan, A., Wilson, G. D. F., Livingston, S. P., Edgecombe, G. D., Macaranas, J., Cassis, G., & Gray, M. R. (1998). Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology*, *46*(5), 419–437.

Dales, R.P. 1962. The polychaete stomodeum and the inter-relationships of the families of Polychaeta. Proceedings of the Zoological Society of London 139(3): 389-428.

Darriba D., G. L. Taboada, R. Doallo, D. Posada. 2012. “jModelTest 2: more models, new heuristics and parallel computing.” Nature Methods 9(8):772.

Deep Ocean Stewardship Initiative. DOSI. (2023, July 4). https://www.dosi-project.org/

Edgar, Robert C. 2004. “MUSCLE: multiple sequence alignment with high accuracy and high throughput.” *Nucleic Acids Research* 32(5):1792–1797. doi.org/10.1093/nar/gkh340.

Ehlers, E. 1913. Die Polychaeten-Sammlungen der deutschen Südpolar- Expedition, 1901-1903. Deutsche Südpolar-Expedition 1901-1903 im Auftrage des Reichsamtes des innern herausgegeben von Erich von Drygalski Leiter Expedition, 13(4): 397-598, plates XXVI-XLVI., available online at https://www.biodiversitylibrary.org/page/2139283

Eliason, A. (1962). *Die Polychaeten der Skagerak-Expedition 1933*.

Fleming, B. F. M., Beaulieu, S. E., Mills, S. W., Gaggiotti, O. E., & Mullineaux, L. S. (2022). Ecological connectivity in Pacific deep-sea hydrothermal vent metacommunities. *Marine Ecology Progress Series*, *MFC*. https://doi.org/10.3354/meps14182

Folmer, O., M. Black, W. Hoeh, R. Lutz, and Robert C. Vrijenhoek. 1994. “DNA Primers for Amplification of Mitochondrial Cytochrome c Oxidase Subunit I from Diverse Metazoan Invertebrates.” Australian Journal of Zoology 3(5):294–99. doi: 10.1071/ZO9660275.

Giribet, Gonzalo, Salvador Carranza, Jaume Baguñà, Marta Riutort, and Carles Ribera. 1996. “First Molecular Evidence for the Existence of a Tardigrada + Arthropoda Clade.” *Molecular Biology and Evolution* 13(1): 76–84. doi: 10.1093/oxfordjournals.molbev.a025573.

Hartman, O.; Fauchald, K. (1971). Deep-water benthic polychaetous annelids off New England to Bermuda and other North Atlantic Areas. Part II. Allan Hancock Monographs in Marine Biology. 6: 1-327., available online at http://hdl.handle.net/10088/3458
page(s): 128

Hartman, Olga. (1967). Polychaetous annelids collected by the USNS Eltanin and Staten Island cruises, chiefly from Antarctic Seas. Allan Hancock Monographs in Marine Biology. 2: 1-387.
page(s): 134-135

Hartmann-Schröder, G. (1993). Die Polychaeten der "Polarstern" -Reise ANTX/1b zur Antarktischen Halbinsel und Isla de los Estados (Feuerland, Argentinien) 1991. Teil 1: Polynoidae bis Iphitimidae. Mitteilungen aus den Hamburgischen Zoologischen Museum und Institut. 90: 127-150.
page(s): 132

Hartmann-Schröder, G. (n.d.). Annelida, Borstenwürmer, Polychaeta [Annelida, bristleworms, Polychaeta]. The Fauna of Germany and Adjacent Seas with Their.

Hatch, A. S., Liew, H., Hourdez, S., & Rouse, G. W. (2020). Hungry scale worms: Phylogenetics of Peinaleopolynoe (Polynoidae, Annelida), with four new species. *ZooKeys*, *932*, 27–74.

Hookabe, N., Jimi, N., Tsuchida, S., Fujiwara, Y., & Kajihara, H. (2020). Lacydoniidae (Annelida) Off the Coast of North-eastern Japan: A Description of Lacydonia japonica sp. nov.

Zoological Studies , *59*, e28.

Hookabe, N., Jimi, N., Yokooka, H., Tsuchida, S., & Fujiwara, Y. (2022). Lacydonia shohoensis (Annelida, Lacydoniidae) sp. nov.--a new lacydonid species from deep-sea sunken wood discovered at the Nishi-Shichito Ridge, North-western Pacific Ocean. *Journal of the Marine Biological Association of the United Kingdom. Marine Biological Association of the United Kingdom*, 1–7.

Judge, J., & Barry, J. P. (2016). Macroinvertebrate community assembly on deep-sea wood falls in Monterey Bay is strongly influenced by wood type. *Ecology*, *97*(11), 3031–3043.

Kinberg, J.G.H. (1856). Nya slägten och arter af Annelider, Öfversigt af Kongl. Vetenskaps-Akademiens Förhhandlingar Stockholm, 12 (9-10), 381-388 [read 1855; printed 1856]., available online at https://www.biodiversitylibrary.org/page/15970133
page(s): 383

Kozlov, Alexey M., Diego Darriba, Tomáš Flouri, Benoit Morel, and Alexandros Stamatakis. 2019. "RAxML-NG: A fast, scalable, and user-friendly tool for maximum likelihood phylogenetic inference." *Bioinformatics* 35(21): 4453-4455.

Laubier, L. (1975). Lacydonia laureci sp. n., annélide polychète nouvelle de l'étage abyssal de méditerranée orientale. Vie et milieu , 25(1), 75–82.

Leigh, Jessica W. and David Bryant. 2015. "POPART: Full-Feature Software for Haplotype Network Construction." *Methods in Ecology and Evolution* 6(9): 1110–1116. doi: 10.1111/2041-210X.12410.

Levin, L. A., Ziebis, W., F. Mendoza, G., Bertics, V. J., Washington, T., Gonzalez, J., Thurber, A. R., Ebbe, B., & Lee, R. W. (2013). Ecological release and niche partitioning under stress: Lessons from dorvilleid polychaetes in sulfidic sediments at methane seeps. *Deep-Sea Research. Part II, Topical Studies in Oceanography*, *92*, 214–233.

Levin, L. A., Baco, A. R., Bowden, D. A., Colaco, A., Cordes, E. E., Cunha, M. R., Demopoulos, A. W. J., Gobin, J., Grupe, B. M., Le, J., Metaxas, A., Netburn, A. N., Rouse, G. W., Thurber, A. R., Tunnicliffe, V., Van Dover, C. L., Vanreusel, A., & Watling, L. (2016). Hydrothermal Vents and Methane Seeps: Rethinking the Sphere of Influence. *Frontiers in Marine Science*, *3*. https://doi.org/10.3389/fmars.2016.00072

Levinsen, G.M.R. (1883). Systematisk-geografisk Oversigt over de nordiske Annulata, Gephyrea, Chaetognathi og Balanoglossi. Videnskabelige Meddelelser fra Dansk naturhistorisk Forening i Köbenhavn, 1882. 160-251., available online at http://biodiversitylibrary.org/page/34979059
page(s): 180

Maddison, W. P. and D. R. Maddison. 2023. "Mesquite: a modular system for evolutionary analysis." Version 3.80. http://www.mesquiteproject.org.

Magalhães, W. F., Bailey–brock, J. H., & Rizzo, A. E. (2012). Lacydonia quadrioculata, a new lacydoniid (Polychaeta: Phyllodocida) from Oahu, Hawaii. *Zootaxa*, *3589*(1), 65–76.

Marion, A.F. (1874). Sur les Annélides du Golfe de Marseille. Comptes Rendus de l'Académie des Sciences, Paris. 79: 398-401., available online at
https://biodiversitylibrary.org/page/36304057
page(s): 399-400

Martin, D., Aguado, M. T., Fernández Álamo, M.-A., Britayev, T. A., Böggemann, M., Capa, M., Faulwetter, S., Fukuda, M. V., Helm, C., Petti, M. A. V., Ravara, A., & Teixeira, M. A. L. (2021). On the Diversity of Phyllodocida (Annelida: Errantia), with a Focus on Glyceridae, Goniadidae, Nephtyidae, Polynoidae, Sphaerodoridae, Syllidae, and the Holoplanktonic Families. *Diversity*, *13*(3), 131.

Mora, C., Tittensor, D. P., Adl, S., Simpson, A. G. B., & Worm, B. (2011). How Many Species Are There on Earth and in the Ocean? *PLoS Biology*, *9*(8), e1001127.

Örsted, A.S. (1843). Annulatorum danicorum conspectus. Auctore A.S. Örsted. Fasc. I. Maricolæ. (Quæstio ab universitate Hafniensi ad solvendum proposita et proemio ornata). , available online at http://www.biodiversitylibrary.org/bibliography/11849
page(s): 25

Palumbi, Stephen R. 1996. "Nucleic Acids II: The Polymerase Chain Reaction." Pp. 205–247 in *Molecular Systematics*, edited by D. M. Hillis, C. Moritz, and B. K. Mable. Sunderland: Sinauer & Associates Inc.

Pearson, K. A. M., & Rouse, G. W. (2022). Vampire Worms; A revision of Galapagomystides (Phyllodocidae, Annelida), with the description of three new species. *Zootaxa*, *5128*(4), 451–485.

Rafinesque, C. S. (1815). Analyse de la nature ou Tableau de l'univers et des corps organisés. [Book]. 1-224, (self-published) Palermo., available online at
http://gallica.bnf.fr/ark:/12148/bpt6k98061z.r=rafinesque.langEN page(s): 136

Rambaut, A. 2009. "FigTree: Tree Figure Drawing Tool." Available at:
http://tree.bio.ed.ac.uk/software/figtree/.

Rambaut, Andrew, Alexei J. Drummond, Dong Xie, Guy Baele, and Marc A. Suchard. 2018. "Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7." Systematic Biology 67 (5): 901–4. doi.org/10.1093/sysbio/syy032.

Ramirez-Llodra, E., Brandt, A., Danovaro, R., De Mol, B., Escobar, E., German, C. R., Levin, L. A., Martinez Arbizu, P., Menot, L., Buhl-Mortensen, P., Narayanaswamy, B. E., Smith, C. R., Tittensor, D. P., Tyler, P. A., Vanreusel, A., & Vecchione, M. (2010). Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. *Biogeosciences* , *7*(9), 2851–2899.

Rizzo, A. E., Magalhães, W. F., & Santos, C. S. G. (2016). Lacydoniidae Bergström, 1914 (Polychaeta) in the South Atlantic: morphology, three new species and five new records. *Journal of the Marine Biological Association of the United Kingdom. Marine Biological Association of the United Kingdom*, *96*(6), 1265–1285.

Ronquist, Fredrik, Maxim Teslenko, Paul van der Mark, Daniel L. Ayres, Aaron Darling, Sebastian Höhna, Bret Larget, Liang Liu, Marc A. Suchard, and John P. Huelsenbeck. 2012. “MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space.” Systematic Biology 61(3): 539–42. doi.org/10.1093/sysbio/sys029.

Rouse, G., Pleijel, F., & Tilic, E. (2022). *Annelida*. https://doi.org/10.1093/oso/9780199692309.001.0001

Rouse, G. W., Goffredi, S. K., Johnson, S. B., & Vrijenhoek, R. C. (2018). An inordinate fondness for Osedax (Siboglinidae: Annelida): Fourteen new species of bone worms from California. *Zootaxa*, *4377*(4), 451–489.

Rouse, G. W., & Kupriyanova, E. K. (2021). Laminatubus (Serpulidae, Annelida) from eastern Pacific hydrothermal vents and methane seeps, with description of two new species. *Zootaxa*, *4915*(1), zootaxa.4915.1.1.

Struck, Torsten H., Günter Purschke, and Kenneth M. Halanych. 2006. “Phylogeny of Eunicida (Annelida) and Exploring Data Congruence Using a Partition Addition Bootstrap Alteration (PABA) Approach.” *Systematic Biology* 55(1): 1-20. doi.org/10.1080/10635150500354910.

Sjölin, E., Erséus, C., & Källersjö, M. (2005). Phylogeny of Tubificidae (Annelida, Clitellata) based on mitochondrial and nuclear sequence data. *Molecular Phylogenetics and Evolution*, *35*(2), 431–441.

Thornhill, D. J., Struck, T. H., Ebbe, B., Lee, R. W., Mendoza, G. F., Levin, L. A., & Halanych, K. M. (2012). Adaptive radiation in extremophilic Dorvilleidae (Annelida): diversification of a single colonizer or multiple independent lineages? *Ecology and Evolution*, *2*(8), 1958–1970.

Tilic, E., Stiller, J., Campos, E., Pleijel, F., & Rouse, G. W. (2022). Phylogenomics resolves ambiguous relationships within Aciculata (Errantia, Annelida). *Molecular Phylogenetics and Evolution*, *166*, 107339.

Tunnicliffe, V., McArthur, A. G., & McHugh, D. (1998). A Biogeographical Perspective of the Deep-Sea Hydrothermal Vent Fauna. In J. H. S. Blaxter, A. J. Southward, & P. A. Tyler (Eds.), *Advances in Marine Biology* (Vol. 34, pp. 353–442). Academic Press.

Uljanin, B. (1878). Sur le Genre Sagitella N. Wagner. Archives de zoologie expérimentale et générale. Ser. 1, 7: 1-32, 4 plates., available online at https://www.biodiversitylibrary.org/page/5628173 page(s): 27

Universitet, U. (1914). *Zoologische Beiträge Aus Uppsala*. Almqvist & Wiksells.

Uschakov, P. V. 1958. Dra novykh vida mnogoshchetinkovykh chervei iz semeistra Phyllodocidae (Polychaeta) s abissal'nykh glubin Kurilo-Kamchatskor padiny [Two new species of polychaetes belonging to the family Phyllodocidae from the abysses of the Kurile-Kamchatka depression]. Trudy Instituta Okeanologia, Akademia nauk SSSR 27: 204-207.

Whiting, Michael F., James C. Carpenter, Quentin D. Wheeler, and Ward C. Wheeler. 1997. "The Stresiptera Problem: Phylogeny of the Holometabolous Insect Orders Inferred from 18S and 28S Ribosomal DNA Sequences and Morphology." *Systematic Biology* 46(1): 1. doi: 10.2307/2413635.

WoRMS Editorial Board (2023). World Register of Marine Species. Available from https://www.marinespecies.org at VLIZ. Accessed 2023-07-16. doi:10.14284/170

Yen, N. K., & Rouse, G. W. (2020). Phylogeny, biogeography and systematics of Pacific vent, methane seep, and whale-fall Parougia (Dorvilleidae : Annelida), with eight new species. *Invertebrate Systematics, 34*(2), 200–233.