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Systematics of Deep-Sea Nereididae (Annelida) from Vents, Seeps & Whalefalls

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Systematics of Deep-Sea Nereididae (Annelida) from Vents, Seeps & Whalefalls

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

in

Marine Biology

by

Eesha Ravi Rangani

Committee in charge:

Professor Greg Rouse, Chair
Professor Ron Burton
Professor Lisa Levin

2023

The Thesis of Eesha Ravi Rangani 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 to my grandmother and my supportive family, who have played a critical role in the success and pursuit of my master's degree.

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ACKNOWLEDGMENTS

I want to express my gratitude to Professor Greg Rouse for being my advisor and committee chair. His guidance, expertise, and interest in Nereidids have been invaluable to me. I also want to thank Professors Lisa Levin and Ron Burton, who were also members of my committee and provided valuable guidance and support. I am grateful to the lab members who helped with sample collection, sequencing, and publication, including Professor Greg Rouse, Avery Hiley, and Dr. Marina McCowin. Dr. Charlotte Seid was a great help in sorting and cataloging samples and provided ideas and support. Avery Hiley, Dr. Marina McCowin, and Sonja Huc provided training in lab logistics, sequencing techniques, and bioinformatics.

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Chapter 1 is currently being prepared for submission for publication of the material. Rangani, Eesha R., and Rouse, Greg W are the authors. This material was researched and written by the thesis author.

VITA

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ABSTRACT OF THE THESIS

Systematics of Deep-Sea Nereididae (Annelida) from Vents, Seeps & Whalefalls

by

Eesha Ravi Rangani

Master of Science in Marine Biology

University of California San Diego, 2023

Professor Greg Rouse, Chair

Nereididae is one of the most diverse families of polychaetes, containing 700 species and 45 genera. Nereidids are commonly found in shallow-water marine biomes, but also occur in deep-sea environments, freshwater habitats, and estuaries. Several species of nereidids have been described from the deep sea; however, only a few species belonging to the genera *Nereis* have been collected from chemosynthetic-based habitats and two species of *Neanthes* from a whalefall. New nereidid samples were collected from Costa Rica (seeps), Gulf of California (seeps and vents), California (seeps and whalefalls), Oregon (seeps), and from the North Fiji and Lau Basins (vents), representing three new species. Also, representatives of *N. sandersi* and *N. piscesae* from their type localities were obtained, as were specimens of the type species of *Nereis (N. pelagica)* and *Neanthes (N. vaali).* A concatenated gene tree was built using mitochondrial and nuclear genes in order to understand the taxonomic position of the new samples collected. Additionally, mitogenomes were generated for all these terminals and analyzed with existing mitogenome data for Nereididae, allowing for the clear delineation of *Nereis* and *Neanthes*. It appears there has been at least two origins of chemosynthetic-associated Nereididae.

Chapter 1: Systematics of Deep-Sea Nereididae (Annelida) from Vents, Seeps & Whalefalls

INTRODUCTION:

Nereididae Blainville, 1818 is one of the most diverse groups among families of polychaetes, with over 700 described species and 45 genera. Nereidids are ordinarily found in shallow-water biomes, including freshwater habitats and estuaries (Beesley et al., 2000). Their widespread occurrence has resulted in various species being harvested commercially as fish bait (Bakken and Wilson 2005) and for laboratory research (Fischer et al. 2010). *Hediste diversicolor* is commonly found in European mudflats and has a significant economic value for feeding fish and crustaceans in aquaculture units and fishing (Daniela et al. 2016). For similar reasons, *Platynereis dumerilli* has been used for research in development, toxicology, and neurobiology (Fischer et al. 2010). Nereididae can also be found in all three major chemosynthetic habitats of the deep sea: hydrothermal vents, methane seeps, and organic falls (Beesley et al., 2000).

The deep sea refers to areas that are deeper than 200 m, making it the largest biome on Earth (Costello et al. 2015; Costa et al. 2020). Although the Earth's oceans make up 71% of the planet's surface area and are considered home to a large number of undescribed species, marine species account for only 16% of all species described (Costello and Chaudhary 2017; Costa et al. 2020). Over the past few decades, several new geomorphological features have been discovered in the deep sea. These include cold seeps, hydrothermal vents, seamounts, canyons, brine pools, and polymetallic nodule fields (Levin and Sibuet 2012). The discovery of chemosynthetic habitats (hydrothermal vents, methane seeps, and organic falls) changed the traditional definition of necessary conditions for life on Earth (Vrijenhoek 2013). Unlike photosynthetic ecosystems, life in these habitats thrives at high pressure, extreme temperatures, and in the presence of dissolved gases that are pernicious to most life (Vrijenhoek 2013). Although these limitations exist, the biomass of benthic organisms in chemosynthetic systems is astonishingly high - 500 to 1000 times greater than the area around these habitats on the sea floor (Tunnicliffe 1991; Tunnicliffe 1992; Nakajima et al. 2014). Several studies and surveys have suggested that the biodiversity of polychaetes in chemosynthetic ecosystems is largely under-evaluated (Glover et al. 2002; Alalykina).

The first descriptions of deep-sea nereidids came from Blake in 1985; a new species named *Nereis sandersi* was described from the Galápagos Rift hydrothermal vents at 0° collected from a depth of 2500 m (Blake 1985). Later, Blake and Hilbig described *Nereis piscesae* from the Juan de Fuca (JDF) Ridge collected between 1983 and 1986 (Blake and Hilbig 1990). *N. piscesae* was collected from the ASHES Vent field at a depth of ~ 1500 m (Blake and Hilbig 1990). Then came the description of a nereidid from a whale-fall community from the base of São Paulo Ridge, 4204 m deep in the Southwest Atlantic; the species was placed in the genus *Neanthes* under the name *Neanthes shinkai* (Shimabukuro et al. 2017). In 2022, a new species, *Nereis tricirrata* was described from cold seeps in the South China Sea (Lin et al. 2022). More recently, three new species of nereidids were identified and described from a whalefall at a depth of 1000 m off Byron Bay, Australia (Georgieva et al. 2023), namely: *Neanthes adriangloveri*, *Neanthes visicete,* and an unnamed species of *Nereis* (Georgieva et al. 2023). Out of the 770 different species of nereids, a mere 47 have been identified below the 200 m mark (WoRMS - world register of marine spec...; Shimabukuro et al. 2017; Blake 1985; Blake and Hilbig 1990; Drennan et al. 2021; Lin et al. 2022; Georgieva et al. 2023), constituting a paltry 9% of the entire nereidid family. Surprisingly, only 7 of those 47 species have been found in chemosynthetic environments, accounting for only 0.9% of all Nereididae (WoRMS - world register of marine species; Shimabukuro et al. 2017; Blake 1985; Blake and Hilbig 1990; Drennan et al. 2021; Lin et al. 2022; Georgieva et al. 2023).

Nereididae is one of the most speciose and well-studied families of marine annelids (Alves et al. 2023). The family in question still poses challenges in terms of its taxonomic classification (Alves et al. 2023). Previous attempts at understanding its evolution have relied heavily on morphology, leading to differing hypotheses (Bakken and Wilson 2005; Fitzhugh 1987; Glasby 1991; Santos et al. 2006). The subfamily Nereididinae Blainville, 1818, which includes the family's type species, *Nereis pelagica*, is defined by the presence of paragnaths and/or papillae on their pharynx, as well as biramous parapodia (Alves et al. 2023). There is a lack of molecular phylogenetic analyses on nereidids, and those that exist do not cover the entire group (Alves et al. 2023). The use of the most broadly used molecular markers (i.e COI, 16S, 18S) has not been applied broadly across the group (Hall et al. 2004; Ruta et al. 2007; Magesh et al. 2012; Liu et al. ; Drennan et al. 2021). Alves et al., 2023 used the aforementioned markers to examine relationships within the group. Their results suggested the existence of three clades within established subfamilies. Within Nereididinae, most of the recognized genera appear to be non-monophyletic, including the genera of *Neanthes, Platynereis*, and *Pseudonereis* (Alves et al. 2023). Although this study was successful in using both nuclear and mitochondrial markers, it lacked the molecular representation of the type species, *Neanthes vaalii*. Present molecular studies include broadly shallow water taxa but lack representatives of their deep-sea counterparts, owing to the difficulty of collecting specimens.

From 2001 to 2021, multiple expeditions gathered samples of nereidids from the deep sea chemosynthetic habitats. These basins contained habitats such as hydrothermal vents, methane seeps, and whalefalls. Our study presents three new species of nereidids, each found in different habitats. Additionally, we offer a detailed analysis of Nereididnae using both molecular markers and whole mitochondrial genomes to strengthen our findings. The use of mitochondrial genomes for phylogenetics is a relatively new tool (Weigert et al. 2016). Mitochondrial genomes hold valuable phylogenetic information, both in terms of gene sequences and gene order (Alves et al. 2020). When studying the evolution and phylogenetics of annelids, it has been determined that analyzing and contrasting the organization of mitochondrial genes can be an extremely efficient approach (Alves et al. 2020; Weigert et al. 2016). Since the relationships between nereidid genera are not well understood, we utilized new molecular data from complete mitochondrial sequences to investigate the evolutionary history of Nereididae. We present phylogenetic analysis using molecular markers (COI, 16s, and 18s) and mitogenomes.

MATERIALS AND METHODS:

Sample collection:

Specimens for this study were collected over several years in multiple locations during research cruises using ROVs, or the HOV Alvin, between 2005 and 2021 (Table 1). Specimens from Costa Rica were collected under permits SINAC-CUS-PI-R-035-2017 and SINAC-CUSBSE-PI-R-032-2018. Specimens from Mexico were collected under permits PPFE/DGOPA-200/18 and PPFE/DGOPA-090/21. Samples were fixed in 95% ethanol or 5% formalin in seawater. Some samples were cut in half, with the anterior fixed in formalin and the posterior in ethanol. Live specimen pictures were taken on the field using Leica MZ9.5 or Leica S8 Apo stereo microscopes with Canon EOS Rebel cameras. Nereididae samples were collected from the following regions: Costa Rica (seeps), the Gulf of California (seeps and vents), California (seeps and whalefalls), Oregon (seeps), and the North Fiji and Lau Basins (vents). Also, representatives of *N. sandersi* and *N. piscesae* from their type localities were obtained. Specimens were deposited at the Scripps Institution of Oceanography Benthic Invertebrate Collection (SIO-BIC), La Jolla, California, USA. Type specimens of newly described species from Costa Rica and Mexico will be deaccessioned from SIO-BIC and deposited with the Instituto de Ciencias del Mar y Limnología, Estación Mazatlán UNAM, Universidad Nacional Autónoma de México (ICML-EMU). Samples collected from the Galapagos Rift were stored in the Museum of Comparative Zoology, Department of Invertebrate Zoology (MCZ:IZ). The National Museum of Victoria (NMV) in Melbourn, Australia, provided additional material for *Neanthes vaalii*.

Morphological Analysis:

The preserved specimens were analyzed through stereomicroscopy (Leica MZ12.5) and compound light microscopy (Leica DMR). Temporary wet mounts were used to observe the parapodia, with slides prepared using DI water and coverslips. Canon EOS Rebel T6i and Rebel T5i cameras were used to capture images. The parapodia were then stored in 2 ml vials containing 95% ethanol and deposited at SIO-BIC.

DNA Extraction, Amplification, and Sequencing:

Specimens that were fixed in 95% ethanol were used for DNA extraction, and the mid-body of each specimen was subsampled and used for DNA extraction. The Zymo Research DNA-Tissue Miniprep and Microprep kits were used depending on the size of the tissue, and the manufacturer's protocol was followed (Irvine, California, USA). The mitochondrial fragment -

COI, was amplified using polyLCO (5'-GAYTATWTTCAACAAATCATAAAGATATTGG-3') and polyHCO (5'-TAMACTTCWGGGTGACCAAARAA TCA-3') primers. Samples were amplified using a master mix of 8.5 uL of water, 12.5 uL of Apex (Genesee Scientific, San Diego, California, USA) or Lamda Taq (Lamda Biotech, Ballwin, Missouri, USA), 1.0 uL of each primer (forward - PolyLCO, and reverse - PolyHCO), and 2.0 uL of eluted DNA. The rest of the PCR was carried out in the Eppendorf thermocycler (Eppendorf, Hamburg, Germany) with settings as follows: Initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 40 seconds, annealing at 42°C for 45 seconds, elongation at 72°C for 50 seconds, and lastly the final extension at 72°C for 5 minutes. The PCR products were purified using 2.0 uL of ExoSAP-IT corresponding to the manufacturer's protocol (USB, Affymetrix, Ohio). Eurofins Genomics (Louisville, KY, USA) performed Sanger sequencing on the resulting PCR products. Consensus sequences were assembled using Geneious version 11.0.15 with the "De Novo Assembly" option (Kearse et al. 2012) under default settings.

Molecular Analysis:

Alignment of the sequences was conducted using Muscle (Edgar., 2004) IQ-Tree (Nguyen et al., 2015) was installed as a package on the program Mesquite v3.8 (Maddison and Maddison, 2023) and used to conduct a maximum likelihood (ML) analysis (Nguyen et al., 2015) on the barcoding gene COI only. Following this, complete 16s sequences were generated from the assembled mitogenomes of six species of Nereididae. For more information on genome assembly and annotation, please refer to the relevant section. Nuclear genes for *Nereis sandersi* (18s)*, Nereis piscesae*(18s)*, Neanthes vaalii*(18s), *Nereis kiirahae* n. sp. (18s)*, Nereis moana* n. sp. (18s)*, Neanthes jyothiae* n. sp. (18s) were assembled on Geneious using genome skimming data (see next section). In order to produce a high-quality consensus sequence in Geneious, reads were interleaved using MitoBIM v.1.9.1 (Hahn et al. 2013), mapped to a list of 18s genes from close relatives using minimap2 (Li, 2018), and then aligned using samtools (Danecek et al. 2021).

Sequences generated from this study and existing data for the Nereididae taxa were combined into a single dataset (Table 2). The tree was rooted with the outgroup of representatives from Hesionidae, and Chrysopetalidae based on previously published data (Alves et al. 2023).

Not all species had the representation of all genes. (Table 2). Individual gene sequences were imported to Mesquite v. 3.8 (Maddison and Maddison 2023) where they were aligned using Muscle (Edgar, 2004) for COI and 16s, while MAFFT (Katoh & Standley, 2013) was utilized for aligning the rRNA gene 18s with default settings. Aligned files were uploaded to RAxML GUI v2.0 (Edler et al. 2020) where three gene partitions were generated. We conducted a maximum likelihood (ML) analysis using RAxML-NG (Kozlov et al. 2019), and obtained node support values through bootstrapping with 1,000 pseudoreplicates. The trees produced through these analyses were displayed using FigTree (Rambaut, 2009) and improved using Adobe Illustrator (Adobe Inc. 2019). Untrimmed COI alignments were used to compute interspecific and intraspecific pairwise distances using PAUP* v. 4.0a168 (Swofford, 2002). PopART v1.7 (Leigh and Bryant 2015) was used to create haplotype networks using COI data for *N. sandersi* and *N. piscesae*, utilizing the TCS network (Clement et al. 2000) option.

Whole Genome Sequencing:

Due to financial and temporal limitations, certain representative organisms were selectively chosen for genome skimming. The aim was to select nereidid species that had not yet been genetically characterized and to incorporate unclassified species from vents, seeps, and whalefalls. Extracted genomic DNA (gDNA) for *Nereis sandersi, Nereis piscease, Neanthes vallii,* and three new species of nereidids were chosen for next-generation sequencing. Extracted DNA underwent quality control using 1.5% agarose gel and was quantified using the Qubit dsDNA HS Assay Kit (ThermoFisher, Pittsburgh, PA, USA). The required amount of DNA was then prepared for shipping to Novogene (Sacramento, CA, USA) for DNA sample quality control and Whole Genome Sequencing (WOBI). The process was carried out by utilizing the Illumina NovaSeq6000 platform, which produced paired-end aligned reads of 150 base pairs (bp). The reads underwent cleaning and trimming through the use of Trimmomatic v.0.39.

Genome assembly and annotation:

To assemble the mitogenomes, Mitofinder v.14 (Allio et al. 2020) was used on the UC San Diego Research Cluster (research-it.ucsd.edu). The 13 protein-coding genes were translated using the Invertebrate Mitochondrial Code (NCBI; transl_table = 5). All of the RefSeq annelid mitogenomes that are available on NCBI GenBank were included in the reference file used for Mitofinder. We used the MitoFinder pipeline integrated with MEGAHIT v.1.2.9 (Li et al. 2016) and Arwen v.1.2.3 (Laslett & Canbäck 2008) parameters to annotate the assembled mitochondrial genomes. To ensure the completeness and accuracy of annotations, the MITOS Web Server (Bernt et al. 2013a) was utilized. For any required modifications, Geneious Prime v.11.0.14 (Kearse et al. 2012) was used to modify annotated assemblies manually. Additionally, nucleotide sequences of genes were extracted, and protein-coding genes were translated into amino acids using the Invertebrate Mitochondrial Code (NCBI; transl_table = 5). The length of assembled genomes is reported in Table 7.

Mitogenomic phylogenetics:

All taxa in Table 3 were used, including published sequences from GenBank. The published records of Gymnonereidinae mitogenomes were used to root the tree, with *Tylorrhynchus heterochaetus* acting as the outgroup. The dataset utilized 13 protein-coding genes and 2 rRNA genes (12s & 16s rRNA). The Mesquite v.3.7 (Maddison & Maddison 2023) software was used to align individual genes using MAFT (Katoh & Standley 2013) with default settings for rRNA genes and Muscle (Edgar 2004) with default settings for other protein-coding genes. The RAxML GUI v2.0 (Edler et al. 2020) was used to generate gene partitions and perform a maximum likelihood (ML) analysis with RAxML-NG (Kozlov et al. 2019). Through bootstrapping with 1,000 pseudoreplicates, node support was evaluated. FigTree (Rambaut 2009) and Adobe Illustrator (Adobe Inc. 2019) were used to magnify the mitogenome ML tree's visualization.

RESULTS:

Biogeography:

Haplotype networks were used to assess the biogeography of two previously described species, *Nereis sandersi,* and *Nereis piscesae*. *Nereis sandersi* was observed to have four haplotypes, each belonging to the Galapagos Rift, the East Pacific Rise at 9° N and 28° S, and specimens from the Alarcón Rise and Pescadero Basin sharing a haplotype (Figure 1). The genetic distance between the Galapagos Rift and the East Pacific Rise at 38° S is the greatest, with haplotypes varying by 8 base pairs (Figure 1). The maximum intraspecific variation calculated among these haplotypes is 0%.

Nereis piscesae has six distinct haplotypes, with specimens from the Del Mar seeps and the Guyamas Basin forming a single haplotype (Figure 2). Similarly, haplotypes collected from

Hydrate Ridge, Oregon, and the Monterey Bay whalefall form a single haplotype (Figure 2). The greatest genetic distance in the corresponding haplotypes was observed between Costa Rica and the haplotypes of Oregon and Monterey Bay, which differed by 4 base pairs (Figure 2). The calculated maximum intraspecific variation was 0%. *Nereis piscesae* was collected from its type locality on the Juan de Fuca Ridge and distributed all the way to seeps in Costa Rica (Figure 2).

Phylogenetics using mitochondrial (16s & COI) and nuclear gene (18s):

In the maximum likelihood analysis using the mitochondrial genes COI and 16s and the nuclear gene 18s, we see a clade that is formed of deep-sea nereidids. Within the clade, we see *Neanthes shinkai, Nereis kiirahae* n. sp.*, Nereis sandersi, Nereis sp., Nereis moana* n. sp.*, Nereis piscesae* and *Nereis tricirrata* emerging from a common ancestor (Figure 3). The bootstrap support value for this node is 75% suggesting a strong placement of the clade (Figure 3). The results also display *Neanthes shinkai* grouping with other species of *Nereis* and being sister to *Nereis kiirahae* n. sp.*,* which provides us with evidence that *Neanthes shinkai* should be renamed *"Nereis" shinkai* (Figure 3). The maximum interspecific distance between the two was calculated to be 9% (Table 4). *Nereis sandersi* is observed to be closely related to the species described with the Eastern Australian whalefall *Nereis sp.*; the interspecific distance calculated between the two was 13% (Table 4). *Nereis moana* n. sp., is closely related to both *Nereis sandersi* and *Nereis piscesae* with interspecific distances of 11% and 14%, respectively (Table 4). *Nereis piscesae* is monophyletic, with *Nereis tricirrata* supported by a bootstrap value of 56% (Figure 3) and an interspecific distance of 17% (Table 4). Lastly, *Neanthes jyothiae* n. sp.*,* is sister to *Nicon maculata* and forms a clade with the type species *Neanthes vaalii* (Figure 3). The interspecific distance between the type and *Neanthes jyothiae* n. sp., was calculated to be 20% (Table 4).

Mitogenomic phylogeny:

The results presented in the concatenated gene tree were validated further by the mitogenome tree (Figure 4). The mitogenome tree also shows the formation of a deep-water clade with *Nereis piscesae, Nereis sandersi, Nereis kiirahae* n. sp., and *Nereis moana* n. sp. sharing a common ancestor (Figure 4). The clade received strong support, with a bootstrap value of 93%. Within this clade, we find *Nereis kiirahae* n. sp. and *Nereis moana* n. sp., as sister groups that have a bootstrap value of 96% (Figure 4). The monophyletic status of *Nereis sandersi* and *Nereis piscesae* is supported by a bootstrap value of 100% (Figure 4). *Neanthes jyothiae* n. sp. is observed to be sister to *Pseudonereis varigata* and is placed within the same clade as the type species, *Neanthes vaalii,* as supported by a bootstrap value of 95% (Figure 4).

RANGE EXTENSIONS:

Nereis sandersi

Figure 1

Type locality. Specimens were only known from the Galápagos Rift, Ecuador

New specimens. Alarcón Rise, Gulf of California; Pescadero Bain, Gulf of California; East Pacific Rise, Gulf of California.

Remarks. A new Eastern-Pacific record indicates range extension from the Alarcon Rise at 23°N in the Gulf of California all the way up to the East Pacific Rise at 38°S (Figure 1), displaying connectivity of over 6000 km in the Gulf of California. The samples were obtained from the Galápagos Rift at a depth of 2599 m, which aligns with the holotype and paratype that were described from a depth range of 2400 to 2600 m. Morphological analysis indicated specimens that lacked eyes, had two frontal antennae, had conical paragnaths in a vertical cluster, and had short, denticulate falcigers. The diagnosis of *Nereis sandersi* was confirmed by consistent observations. Samples were gathered from the Alarcón Rise and Pescadero Basin in the Gulf of California, covering depths ranging from 2300 m to 3700 m (Figure 1). The collection from both locations indicates an overlap in depth ranges. COI sequences revealed four unique haplotypes (Figure 1).

Nereis piscesae

Figure 1

Type locality. Specimens were previously only known from the hydrothermal vents of the Northeastern Pacific, Juan de Fuca Ridge.

New specimens. Hydrate Ridge, Oregon; Monterey Bay, California; Del Mar seeps, California; Guyamas Basin, Gulf of California; and Costa Rica seeps, Costa Rica.

Remarks. *Nereis piscesae* has been found in a wider range than previously recorded, from the Juan De Fuca Ridge at 45° N to Costa Rica seeps at 9° N in the Eastern Pacific (Figure 2). These observations reveal connectivity in the Eastern-Pacific region spanning over 5000 km. The samples gathered from Juan De Fuca's type locality were found at a depth of 1500 m, which matches the holotype and paratypes. Specimens collected displayed conical paragnaths with large rows arranged vertically and elongated, thick falcigers. The diagnosis of *Nereis piscesae* was confirmed by these observations. Samples were collected from vents (Juan De Fuca - 2200 m), seeps (Hydrate Ridge - 800 m, Del Mar - 1000m , Guaymas Basin - 1300 m, Costa Rica - 1000 m), and a Whalefall (2900 m) off Monterey Bay (Figure 2). COI results revealed six unique haplotypes with a maximum intraspecific distance of 0%.

TAXONOMY:

FAMILY NEREIDIDAE Blainville, 1818

Genus *Nereis* Linnaeus, 1758

Type species: ***Neries pelagica*** Linnaeus, 1758

Generic diagnosis: Prostomium with a complete anterior margin, one pair of antennae,none pair of biarticulated palps with conical palpostyles, and four pairs of tentacular cirri. Peristomium apodous, greater than the length of chaetiger 1. Eyes present or absent. Conical paragnaths present or absent. Size of notopodial dorsal ligule similar in anterior and posterior chaetigers or markedly reduced on posterior chaetigers. Prechaetal notopodial lobe present or absent, smaller than dorsal notopodial ligule on anterior chaetigers, usually reduced or absent posteriorly. Dorsal cirrus is basally attached to the notopodial dorsal ligule in all chaetigers but lacks a basal cirrophore. Notoaciculae are absent in chaetigers 1 and 2. Notochaetae: homogomph spinigers and homogomph falcigers present. Dorsal fascicle neurochaetae: homogomph spinigers present, heterogomph falcigers on anterior chaetigers present or absent, posterior chaetigers present. Ventral fascicle neurochaetae: heterogomph spinigers present or absent, heterogomph falcigers present or absent.

FAMILY NEREIDIDAE Blainville, 1818

Genus *Nereis* Linnaeus, 1758

Nereis kiirahae **n. sp.**

Figure 5 & 6

Materials Examined: Holotype: A10014*, JaichMaa Vent Field, Pescadero Basin, 23.9416°N 108.8558°W, 3677 m depth (COI, Whole mitochondrion). Paratype: A14210, A14207, Ruby Whalefall, Monterey Submarine Canyon, California, 2898 m depth (COI). A13985, A10195, A14073, A14082, and A14083 Auka Vent Field, Pescadero Basin, 3600 m depth (COI). Specimens fixed in 70% ethanol.

Diagnosis: (1) No eyes; (2) 3 pairs enlarged anterior cirri (tentacular cirri) instead of 4; (3) Prostomium wider than long; (4) Homogomph falcigers united, 10-12 teeth; (5) Ventral cirrus increases anteriorly.

Description: Photographed A10195, complete, 1.50 mm long, 0.07 mm wide (incl. parapodia), 65 chaetigers (Figure 5A). Pharynx not inverted; observed alive - yellow coloration (Figure 5A).

Specimen A10014*: Incomplete (Figure 5B-F), width 0.5 mm (incl. parapodia). Prostomium trapezoid, wider than long (Figure 5B). Robust palps (0.1 mm), cylindrical palpophores, smaller oval palpostyle (Figure 5B-C). Eyes not observed (Figure 5B). 3 pairs enlarged anterior cirri (tentacular cirri), largest 0.44 mm (Figure 5B-C). 1 pair shorter than prostomium, 2 pairs extend beyond (Figure 5B-C). Tentacularphores - cylindrical, triangular pointed ends. Pharynx not everted, no visible jaws.

First 2 chaetigers uniramous (Figure 5D), followed by biramous (Figure 5E-F). Dorsal cirri (uniramous) slightly longer than ventral cirri (Figure 5D). Dorsal & ventral ligules same size, triangular tips (Figure 5D). In biramous parapodia, ventral cirrus longer than dorsal cirrus (Figure 5E-F). Ventral cirrus size increases anteriorly (Figure 5E-F). Chaetiger 27: ventral cirrus approx. twice the length of dorsal cirrus (Figure 5.1A). Dorsal & ventral ligule approx. same size, median ligule larger (Figure 6A). Notochaeutal: 5 falcigers, 10 spinigers. Neurochaetal: 2 falcigers, 1 spiniger (Figure 6). Spinigers: Finely serrated triangular tips, unidentate joints (Figure 6B-C). Falcigers unidentate, 10-12 teeth (Figure 6D-E).

Distribution: The holotype, A10014* and five paratypes A13985, A10195, A14073, A14082, and A14083, were collected by the ROV SuBastian between 3655 - 3684 m in the Pescadero Basin Vents, Gulf of California. The ROV Doc Ricketts collected two paratypes, identified as A14210 and A14207, from a whalefall called 'Ruby'. The whalefall was located at a depth of 2898 m in the Monterey Submarine Canyon, California.

Etymology: *Nereis kiirahae* n. sp. was named after the lead author's lab member, Kiirah Rachel Green. Latin feminine name ending with -ae.

Remarks: *Nereis kiirahae* n. sp. is a benthic Nereididae without eyes. Live specimens were collected by ROV *SuBastian* and photographed following the collection event. The holotype was sequenced for COI, and the entire mitochondrial genome and each paratype were sequenced for COI only. The maximum intraspecific pairwise distance between the eight available sequences was only 0.01% (Table 5). *Nereis kiirahae* n. sp. was identified as the sister species to *Neanthes shinkai* using ML analysis, with a 62% bootstrap value (Figure 3). The interspecific distance between the two was as low as 9% (Table 4). *Nereis kiirahae* n. sp. is a member of a monophyletic clade of chemosynthetic species that includes *Nereis sandersi and Nereis piscesae* (Figure 3). For the aforementioned species, the interspecific pairwise distance was 12% and 15%, respectively (Table 4). Based on the mitogenome tree, we found *Nereis kiirahae* n. sp. within the clade of deep water nereidids and sister to *Nereis moana* n. sp. with 96% support (Figure 4). *Nereis kiirahae* n. sp. can be distinguished from *Neanthes shinkai* by the number of pairs of enlarged anterior cirri (tentacular cirri); *Nereis kiirahae* n. sp. has three pairs, whereas *Neanthes shinkai* has four. Prostomium in *Nereis kiirahae* n. sp. is wider than long, whereas it is not in *Neanthes shinkai.*

FAMILY NEREIDIDAE Blainville, 1818

Genus *Nereis* Linnaeus, 1758

***Nereis moana* n. sp.**

Figure 7 & 8

Materials Examined: Holotype: A4643* Fiji Basin, West Pacific vents, Fiji, 16.99°S 173.9158°E, 1991 m depth (COI). Paratypes: A4668, Lau Back-Arc Basin, West Pacific vents, Tonga, 1906 m depth (COI). A14217, Lau Back-Arc Basin, West Pacific vents, Tonga, 2657 m depth (COI, Whole mitochondrion). Specimens fixed in 70% ethanol.

Diagnosis:(1) 4 pairs enlarged anterior cirri (tentacular cirri); (2) No dense paragnath clusters; (3) Biramous chaetae - dorsal cirrus larger than ventral cirrus; (4) Falcigers rounded, unserrated tips.

Description: A4643*: Posteriorly incomplete (Figure 7), 0.6 mm long, 0.4 mm wide (incl. parapodia). Trapezoidal prostomium, 0.002 mm palps, 4 pairs of enlarged anterior cirri (tentacular cirri), longest up to 0.02 mm (Figure 7A-B). Lacks eyes/eye spots (Figure 7A-B). Pharynx not inverted, no dense paragnath clusters (Figure 7A-B).

Chaetigers: 1st uniramous, following biramous (Figure 7C-D). Dorsal cirrius larger than ventral cirrus (Figure 7C-D). Dorsal & ventral ligule similar, median ligule smaller (Figure 7C-D). In biramous chaetae, dorsal cirrus smaller ventral cirrus (Figure 7C-D). Dorsal, median & ventral ligules same size (Figure 7C-D). Dorsal and ventral ligules conical with triangular tips (Figure 7C-D). Articles from prechaetal & postchaetal lobes (Figure 8A-C).

Neurochaete: 6-7 falcigers, 4-5 spinigers (Figure 8A-C). Acicula: thick blades with pointed edges (Figure 8D). Chaetae: falcigers & spinigers (Figure 8E-F). Falciger blades unidentate, rounded tip (Figure 8E). Spinigers with fine serrations & narrow pointed tips (Figure 8F).

Distribution: The holotype, A4643* was retrieved from the Fiji Basin in Fiji using the ROV Jason II at a depth of 1991 m. Paratypes A4668 and A14217 were retrieved from the Lau Back-Arc Basin, Tonga, at a depth of 1906 m and 2657 m, respectively. *Nereis moana* n. sp. is a species that is unique to this region and yet to be found elsewhere.

Etymology: The new species has been named "moana," which refers to the ocean in Tongan and symbolizes the Tongan people's deep connection with the sea.

Remarks: *Nereis moana* n. sp. is a part of the *Nereis* clade that all belong to chemosynthetic environments (Figure 3). The holotype, A4643* and the paratype A4668 was sequenced for COI. The paratype A14217 was sequenced for COI and its whole mitochondrial. All of the COI sequences available are identical, with a maximum pairwise intraspecific distance of 0.00% (Table 6). *Nereis moana n. sp* was observed to be sister to the clade with both *Nereis piscesae* and *Nereis sandersi* (Figure 3) with an interspecific maximum pairwise distance of 14% and 11% respectively (Table 4). It is also observed to be monophyletic with *Nereis kiirahae* n. sp. (described during this study), with an interspecific distance of 10% (Table 4). This is further supported by the mitogenome tree, where *Nereis moana* n. sp. is sister to *Nereis kiirahae* n. sp. with a high support value of 96% (Figure 4). The placement of *Nereis moana n. sp* within a clade of other deep-sea nereidids is further supported by this tree with a bootstrap value of 87% (Figure 4).

Nereis moana n. sp. differs from *Nereis kiirahae* n. sp. by having four pairs of enlarged anterior cirri (tentacular cirri) instead of three. The dorsal cirrus is larger than the ventral cirrus in biramous chaetae, with a larger median ligule, whereas the opposite is true for *Nereis kiirahae* n. sp. Falciger tips are rounded with no serrations; *Nereis kiirahae* n. sp. has serrated falcigers with teeth.

FAMILY NEREIDIDAE Blainville, 1818

Genus *Neanthes* Kinberg, 1865

Type species: ***Neanthes vaalii*** Kinberg, 1865

Generic diagnosis: Prostomium with complete anterior margin, one pair of antennae, one pair of biarticulated palps with conical palpostyles, and four pairs of tentacular cirri with distinct cirrophores. Eyes present or absent. One apodous anterior segment, greater than length of chaetiger 1. Conical paragnaths in the pharynx: present or absent in Areas IIV; smooth bar-like paragnaths present or absent in Area IV. Oral ring, conical paragnaths: Areas V and VI present as separate groups or not; VVIII, present or absent. Dorsal notopodial ligule is present, and it is either the same size on anterior and posterior chaetigers or significantly smaller on posterior chaetigers. Notopodial prechaetal lobe present or absent, smaller than dorsal notopodial ligule on anterior chaetigers, usually reduced or absent posteriorly, present throughout all chaetigers or restricted to a few anterior chaetigers. Acicular process present or absent; present on anterior chaetigers, decreasing in size posteriorly. Dorsal cirrus attached to dorsal notopodial ligule basally or mid-dorsally to subterminally on posterior chaetigers; lacks basal cirrophore. Neuropodial postchaetal lobe is absent or present. Notoaciculae are absent in chaetigers 1 and 2. Notochaetae: homogomph spinigers. Neurochaetae, dorsal fascicle: heterogomph spinigers present or absent, homogomph spinigers present, heterogomph falcigers on anterior chaetigers present, on posterior chaetigers present or absent. Neurochaetae, ventral fascicle: heterogomph spinigers present or absent, homogomph spinigers present or absent, heterogomph falcigers present.

FAMILY NEREIDIDAE Blainville, 1818

Genus *Neanthes* Kinberg, 1865

***Neanthes jyothiae* n. sp.**

Figure 9 & 10

Materials Examined: Holotype: A3333*, South of Pinkie's "Vent", Guaymas Basin, Gulf of California, 27.5781° N, 111.4498° W, 1892 m depth (COI and Whole Mitochondrial). Specimen fixed in 70% ethanol.

Diagnosis: (1) 2 pairs of eyes posterior larger, oval; anterior rounded; (2) One pair of elongated branchiae; (3) Paragnaths: Triangular, less dense.

Description: Holotype: Complete, pinkish, iridescent when alive (Figure 9A). 20 chaetigers: up to 0.15 mm long, 0.04 mm wide (incl. parapodia) (Figure 9B-C). Prostomium (Figure 9B): Wider than long, distinct palps (0.01 mm apart), cylindrical palpophore, rounded palpostyle. Elongated antennas (0.006 mm) parallel to palps (Figure 9B). 4 pairs of enlarged anterior cirri (tentacular cirri), posterodorsal longest (up to 0.04 mm) (Figure 9B-C). 2 pairs of eyes - posterior larger, oval; anterior rounded, slightly closer (Figure 9A). Pharynx: Smooth brown jaws, 7-8 teeth (Figure 9D). Paragnaths: Triangular, less dense & fewer than other species (Figure 9D).

Parapodia: 1 uniramous - dorsal cirrus 2x ventral cirrus, single noto-acicula (Figure 9E). Parapodia 25 & 27 biramous - longer ventral cirri, 2 aciculae (Figure 9F-G). Dorsal ligule larger ventral ligule (Figure 10A). Notochaetae: 9-10 homogomph spinigers, 3-4 homogomph flacigers (Figure 10B-E). Neurochaetae: 8-9 homogomph spinigers, 3 homogomph flacigers (Figure 10B-E). Spiniger shape: triangular, sharp end; flaciger shape: triangular, rounded end (Figure 10B-C).

Median ligule: notochaetetal spinigers & flacigers (Figure 10B-E). Ventral ligule: neurochaetal articles (Figure 10B-E).

Distribution: *Neanthes jyothiae* n. sp. was collected from the South of Pinkie's "Vent", Guaymas Basin, Gulf of California, at a depth of 1800 m using the ROV Doc Ricketts. Species yet to be found elsewhere.

Etymology: *Neanthes jyothiae* n. sp. was named after the lead author's grandmother, Jyothi Devi Rangani, whose love provided the lead author with encouragement and motivation for success. "Jyothi" in Sanskrit means light, this species was observed to have eyes; Latin feminine ending -ae.

Remarks: *Neanthes jyothiae* n. sp. is sister to *Nicon maculata* an Antarctica species of benthic nereidid, this placement is supported by a 100% bootstrap value (Figure 3), with a maximum pairwise interspecific distance of 16% (Table 4). This species is also within the clade of the type *Neanthes vaalii* with 50% support (Figure 3) and an interspecific distance of 20% (Table 4). In the mitogenome tree, *Neanthes jyothiae* n. sp. is sister to *Pseudonereis varigata* and is monophyletic with the type species *Neanthes vaalii* with a support value of 95% (Figure 4).

DISCUSSION:

The haplotype network analysis revealed that *Nereis sandersi* and *Nereis piscesae* have extended their range, with connectivity observed across the Eastern Pacific. Nereis sandersi species is found both in the Alarcón Rise and Pescadero Basin hydrothermal vents found in the Gulf of California (GoC). The GoC marks the boundary between the Pacific and North American plates, and is characterized as a transtensional plate boundary. Around 3.5 million years ago, the separation of the Baja Peninsula from mainland Mexico initiated seafloor spreading in this area

(Lizarralde et al. 2007; Paduan et al. 2018). The Alarcón Rise is situated in the northernmost region of the East Pacific Rise (EPR), approximately 2300 m deep (Goffredi et al. 2017). The vents in this location are recognized for their sulfide chimneys that are supported by basalt (Goffredi et al. 2017). The Pescadero Basin is situated in the southern part of the Gulf of California, just 75 km from the Alarcón Rise (Goffredi et al. 2017; Spelz et al. 2015). It is located at a depth of 3700 m within a sediment basin that contains a number of carbonate chimneys (Goffredi et al. 2017). A study conducted by Goffredi et al. in 2017 found that out of 116 macrofaunal species collected, only three were found in both the Alarcón rise and Pescadero Basin. One of the three species that overlapped was *Nereis sandersi* (Goffredi et al. 2017), which aligns with the results presented in the study. According to Goffredi et al.'s 2017 research, larval dispersal does not appear to be the main factor causing community differentiation between venting systems. Instead, it seems that the suitability of the habitat plays a larger role in supporting species differentiation (Goffredi et al. 2017). It is possible that *Nereis sandersi* is more adapted to live in vents at varying depths, separated by around 1300 m.

Anaerobic metabolism creates reducing environments that support chemoautotrophic activity by using inorganic chemicals as fuel (Bernardino et al. 2012). Such metabolic processes are commonly found at hydrothermal vents, methane seeps, and large organic whalefalls (Bernardino et al. 2012) . The distribution of hydrothermal vents, methane seeps, and whalefalls is uneven, with the highest occurrence near tectonic plates and ocean margins (German et al. 2010; Bernardino et al. 2012; German et al. 2011; Levin and Sibuet 2012) . While these environments may share similar chemical activity, the number of species that overlap between them is limited (Tunnicliffe et al. 2003). However, there is a greater occurrence of overlap at the level of genera and families in these reducing habitats (Tunnicliffe et al. 2003; Bernardino et al. 2012). There are fewer than five common species found among vents, seeps, and whalefalls (Tunnicliffe et al. 2003). However, species belonging to the following taxa - vestimentiferans, bathymodiolid mussels, and vesicomyid clams have been observed at all three sites (Tunnicliffe et al. 2003; Smith et al. 2002; Smith and Baco 1998). All of the aforementioned fauna are known to have symbionts (Baco et al. 1999; Tunnicliffe et al. 2003). This study reveals that the polychaete *Nereis piscesae* has been found in all three extreme environments, suggesting that it may be more adaptable to varying environmental conditions compared to other similar fauna.

The phylogenetic analysis using concatenated genes displayed the formation of a chemosynthetic clade of species belonging to the genera of *Nereis.* Within this clade, we also see the inclusion of *Neanthes shinkai*, which is observed to be sister to the new species described in this study *Nereis kiirahae* n. sp. The two records of *Neanthes* from chemosythetic environments are observed to have eye spots; here we see *Neanthes jyothiae* n. sp. with four pairs of eyes and *Neanthes andriangloveri* is also observed to have eyes (Georgieva et al. 2023). In contrast, all species belonging to *Nereis* that have been described from these environments lack eye spots. Both molecular and morphological analysis indicate that *Neanthes shinkai* was misidentified and placed within Neanthes, and it should be renamed as *"Nereis" shinkai.*

The observation of a chemosynthetic clade in the concatenated gene tree remained consistent with the results produced using mitogenomes. Nereis is commonly cited as one of the most problematic taxa however (Alves et al. 2023; Alves et al. 2020); here, we see a distinct monophyly forming with chemosynthetic *Nereis*. Our results further demonstrated that there have been at least two origins of chemosynthetic-associated Nereididae with *Neanthes jyothiae n. sp* forming a separate clade. A further possibility of investigating these two origins could look at the absence and presence of eyes in deep sea Nereididae.

Nereidids, as one of the most species-rich annelid families found below the 500 m mark, have been the focus of this study (Shimabukuro et al. 2017). Our research has provided supporting data for the identification of three new Nereididae species originating from vents, seeps, and whalefalls. These findings highlight the importance of chemosynthetic communities in sustaining diverse fauna through the high productivity facilitated by microbial communities (Tunnicliffe et al. 2003). Nereididae, in particular, relies on the functioning of these ecosystems to thrive in extreme conditions (Salcedo et al. 2019).

Notably, some chemosynthetic systems have suffered damage due to human activities, while others are now targeted for exploitation (Levin et al. 2016; Ramirez-Llodra et al. 2011). Understanding the biodiversity of these ecosystems holds significant implications for informing future policies, especially concerning mining activities (Levin et al. 2016). Despite the widespread distribution of Nereididae within various chemosynthetic habitats, questions remain regarding their dispersal mechanisms.

While this study does not fully resolve all relationships within Nereididae or answer all inquiries about the origins of deep-sea nereidids, it serves as a crucial reminder of the power of DNA analysis. Through this approach, we have uncovered new species that might have otherwise been overlooked. By continuing to explore and study the taxonomic diversity within Nereididae and other deep-sea taxa, we enhance our understanding of these intricate ecosystems and their conservation needs. As we gain more insights into the relationships and distribution patterns of deep-sea species, we can better inform conservation strategies and resource management for these vulnerable and unique habitats.

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Chapter 1 is currently being prepared for submission for publication of the material. Rangani, Eesha R., and Rouse, Greg W are the authors. This material was researched and written by the thesis author.

TABLES:

Table 1: New specimens collected for this study, including voucher, site, depth, and collection information. Holotype = *

Species	Voucher	Site	Coordinates	Depth (m)	Date	Dive
Nereis sandersi	SIO-BIC A1738	38S German Flats South EPR, Pacific Antarctic Ridge Hydrothermal Vents	37.79° S, 110.91° W	2220	Mar. 24, 2005	A4090
	SIO-BIC A6161	Alarcon Rise, Gulf of California	23.35° N, 108.53° W	2299	Apr. 20, 2015	D752
	SIO-BIC A6149					
	SIO-BIC A13659	9N, Marker 28, East Pacific Rise	9.83° N, 104.29° W	2525	Apr. 11, 2019	A5020
	SIO-BIC A14019	Dianne's Vent, Auka Vent Field, Gulf of California	23.95° N, 108.86° W	3654	Nov. 2, 2021	S0474
	MCZ:IZ 68096	Galápagos Rift, Eucador	0.9694° N 85°, 52.790° W	2599.3	Jun. 20, 2015	H1433
Nereis piscesae	SIO-BIC A1361	Mound 11, Costa Rica	8.91° N, 84.30° W	1045	Feb. 26, 2009	AD4505
	SIO-BIC A8258	Mound 12, Costa Rica	8.93° N, 84.31° W	1001	Mar. 24, 2005	AD4906
	SIO-BIC A8291		8.93° N, 84.31° W	1004	May. 25, 2017	AD4910
	SIO-BIC A2117 A	Hydrate Ridge, Oregon	44.67° N, 125.09° W	603	Aug. 4, 2010	AD4632
	SIO-BIC A2117 B		44.67° N, 125.09° W	603	Aug. 4, 2010	AD4632
	SIO-BIC A2046 A		44.56° N, 125.15° W	795	Aug. 7, 2010	AD4635
	SIO-BIC A14244	Juan de Fuca Ridge, Oregon	46.0° N, 130.0° W	1542	Aug. 31, 2021	J2-1383
	SIO-BIC A14245					
	SIO-BIC A7727 A		45.98° N, 130.02° W	1550	Aug. 1, 2016	D876
	SIO-BIC A7727 B		45.98° N, 130.02° W	1550	Aug. 1, 2016	D876
	SIO-BIC A12292	Del Mar, California	32.90° N, 117.78° W	1023	Feb. 5, 2020	D1246
	SIO-BIC A14213		32.90° N, 117.78° W	1036	May. 19, 2013	D472
	SIO-BIC A16344	Guaymas Basin, Gulf of California	26.75° N, 111.17° W	1314	Apr. 16, 2012	D390
	SIO-BIC A14134		27.01° N, 111.41° W	2012	Nov. 13, 2009	A4558
	SIO-BIC A14209	Monterey Canyon, California	36.77° N, 122.08° W	1018	Nov. 17, 2009	D95
	SIO-BIC A14210 A		36.61° N, 122.43° W	2898	Nov. 19, 2009	D98
	SIO-BIC A14207		36.61° N, 122.43° W	2893	May. 11, 2005	D10
	SIO-BIC A11397		36.77° N, 122.08° W	1032	Nov. 7, 2005	T916
	SIO-BIC A10014*	Pescadero Basin, Mexico	23.94° N, 108.85° W	3677	Nov. 20, 2018	S0199
Nereis kiirahae n. sp.	SIO-BIC A14210	Monterey Submarine Canyon, California	36.61° N, 122.43° W	2898	Nov. 19, 2009	D98
	SIO-BIC A14207		36.61° N, 122.43° W	2893	Mar. 11, 2005	D10
	SIO-BIC A13985	Pescadero Basin, Mexico	23.95° N, 108.86° W	3655.9	Oct. 29, 2021	S0470
	SIO-BIC A10195		23.94° N, 108.85° W	3684.8	Nov. 16, 2018	S0195
	SIO-BIC A14073		23.95° N, 108.86° W	3655.1	Nov. 8, 2021	S0480
	SIO-BIC A14082		23.94° N, 108.85° W	3670.6	Nov. 9, 2021	S0481
	SIO-BIC A14083		23.94° N, 108.85° W	3668.3	Nov. 9, 2021	S0481
Nereis moana n. sp.	SIO-BIC A14217*	Lau Back Arc Basin, Tonga	20.05° S, 176.13° W	2657	May. 18, 2005	J2-141
	SIO-BIC A4668		22.53° S, 176.71° W	1906	May. 23, 2005	J2-146
	SIO-BIC A4643	Fiji Basin, Fiji	16.99° S, 173.91° E	1991	May. 29, 2005	J2-150
Neanthes vaalii	NMV F303022	Foster Beach, Australia	38.40° S, 146.10° E	1	Feb. 2, 2015	N/A
	NMV F303023		38.40° S, 146.10° E	1	Feb. 2, 2015	N/A
	NMV F303024		38.40° S, 146.10° E	1	Feb. 2, 2015	N/A
Neanthes jyothiae n. sp.	SIO-BIC A3333*	Guaymas Basin, Gulf of California	27.57° N, 111.44° W	1802	Apr. 13, 2012	D385

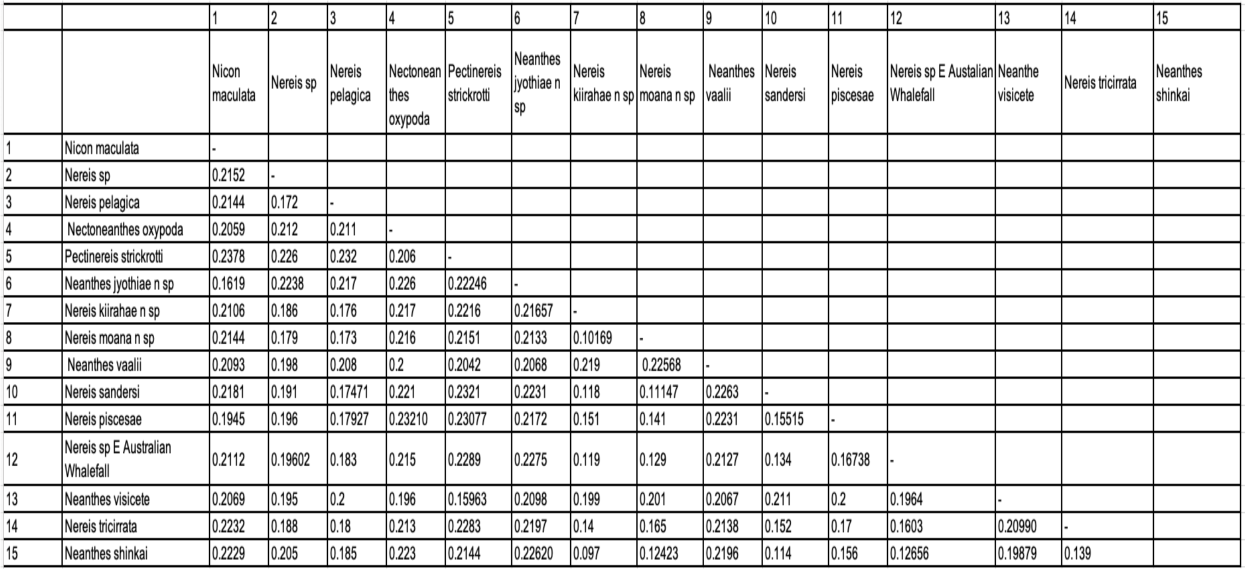
Table 2: Species with GenBank/BOLD numbers for sequences used to generate the concatenated tree.

Family	**Species**	**COI**	**16s**	**18s**	**Location**	**Authority**
Nereididae	*Alitta succinea* (Leuckart, 1847)	MW825350	MW826068	MW826081	Brazil	(Alves et al., 2023)
	Ceratocephale abyssorum (Hartman & Fauchald, 1971)	GQ426683	GQ426618	GQ426585	Guinea Basin	(Böggemann, 2009)
	Ceratocephale loveni Malmgren, 1867		DQ442614	DQ442616	Sweden	(Ruta et al., 2007)
	Ceratonereis longiceratophora Hartmann-Schröder, 1985	AY583701		AB106251	Australia	(Colgan et al., 2006)
	Ceratonereis sp.	LIPOL031-08			Australia	BOLD direct submission
	Cheilonereis cyclurus (Harrington, 1897)	MF538532	MF538532		South Korea	(Park et al., 2017)
	Gymnonereis crosslandi (Monro, 1933)	DISA414-18			USA	BOLD direct submission
	Gymnonereis sp.	KY805815	KY704332		India	(Vijapure et al., 2019)
	Hediste atoka Sato & Nakashima, 2003	LC323006	LC323043	LC323072	Japan	(Tosuji et al., 2019)
	Hediste diadroma Sato & Nakashima, 2003	KX499500	KX499500		South Korea	(Kim et al., 2016)
	Laeonereis culveri (Webster, 1879)	MW825351	MW826069	MW826082	Brazil	(Alves et al., 2023)
	Namalycastis abiuma (Grube, 1872)	KU351089	KU351089		China	(Lin et al., 2016)
	Namalycastis indica (Southern, 1921)	MG759522	MG759523		Myanmar	(Bolotov et al., 2018)
	Namalycastis jaya Magesh et al., 2012	HQ456363	HM138706	JX483867	India	(Magesh et al., 2012)
	Namanereis hummelinc ki (Augener, 1933)	KT235957			Montserrat/ UK	(Shoobs et al., 2016)
	Neanthes ceciliae Steiner & Santos, 2004	MW825352	MW826070	MW826083	Brazil	(Alves et al., 2023)
	Neanthes glandicincta (Southern, 1921)	KY094478	KY094478		China	(Lin et al., 2017)
	Neanthes meggitti (Monro, 1931)	MF958994	MF959006		Montserrat/UK	(Bolotov et al., 2018)
	Nectoneanthes oxypoda (Marenzeller, 1879)	HZPLY588- 13			China	BOLD direct submissionl
	Nereis pelagica Linnaeus, 1758		AY340470	AY340438	Sweden	(Rousset et al., 2007)
	Nereis sp.	MF960765	MF960765		South Korea	(Kim et al., 2017)
	Nicon maculata Kinberg, 1865	MW825353	MW826071		Antarctic sea	(Alves et al., 2023)
	Paraleonnates uschakovi Chlebovitsch & Wu, 1962	KX462988	KX462988		South Korea	(Park et al., 2016)
	Perinereis aibuhitensis (Grube, 1878)	KF611806	KF611806		South Korea	(Kim et al., 2015)
	Perinereis anderssoni Kinberg, 1866	MW825354	MW826072	MW826084	Brazil	(Alves et al., 2023)
	Perinereis cultrifera (Grube, 1840)	MN812983	MN812983	OQ732688	France	(Alves et al., 2020)
	Perinereis nuntia (Lamarck, 1818)	JX644015	JX644015		South Korea	(Won et al., 2013)
	Perinereis sp.	MN823962	MN823971	OQ732689	Panama	(Alves et al., 2020)
	Platynereis australis (Schmarda, 1861)	MN830367	MN830367	OQ732690	Chile	(Alves et al., 2020)
	Platynereis bicanaliculata (Baird, 1863)	MN812984	MN812984	OQ732691	USA	(Alves et al., 2020)
	Platynereis dumerilii (Audouin & Milne Edwards, 1833)	AF178678	AF178678		Unknown	(Boore & Brown, 2000)
	Platynereis massiliensi s (Moquin- Tandon, 1869)	MN812985	MN812985	OQ732692	Wales	(Alves et al., 2020)
	Platynereis sp1	MN830365	MN830365	OQ732693	Brazil	(Alves et al., 2020)
	Platynereis sp2	MW825355	MW826073	MW826085	Brazil	(Alves et al., 2023)
	Pseudonereis palpata (Treadwell, 1923)	MW825356	MW826074	MW826086	Brazil	(Alves et al., 2023)
	Pseudonereis variegata (Grube, 1857)	MN855134	MN855213	OQ732694	South Africa	(Alves et al., 2020)
	Simplisetia cf. erythraensi s (Fauvel, 1918)	EU835670			Australia	(Metcalfe & Glasby, 2008)
	Tambalagamia fauveli Pillai, 1961	HZPLY601-13			China	BOLD direct submission
	Tylorrhynchus heterochaetus (Quatrefages, 1866)	KM111507	KM111507		China	(Chen et al., 2016)
	Nereis sp.	OQ801422	OQ820960	OQ803224	Australia	(Georgieva et al., 2023)
	Neanthes visicete	OQ801421	OQ820958	OQ803223	Australia	(Georgieva et al., 2023)
	Neanthes shinkai	LC331618			Brazil	(Shimabukuro et al., 2017)
	Nereis tricirrata	OP292645	OP292646	OP292647	China	(Lin et al., 2022)
	Pectinereis strickrotti	OL782600			Costa Rica	SIO-BIC
	Nereis sandersi (Blake, 1985)	SIO-BIC A13659	SIO-BIC A13659	SIO-BIC A13659	East Pacific Rise	Present study
	Nereis pisceae (Blake, 1990)	SIO-BIC A12292	SIO-BIC A12292	SIO-BIC A12292	USA	Present study
	Neanthes vaalii (Kinberg, 1865)	NMV F303024	NMV F303024	NMV F303024	Australia	Present study
	Nereis moana n. sp	SIO-BIC A14217	SIO-BIC A14217	SIO-BIC A14217	Tonga	Present study
	Nereis kiirahae n. sp	SIO-BIC A10014	SIO-BIC A10014	SIO-BIC A10014	Gulf of California	Present study
	Neanthes jyothiae n. sp	SIO-BIC A3333	SIO-BIC A3333	SIO-BIC A3333	Gulf of California	Present study
Chrysopetalidae	*Arichlidon gathofi* Watson Rusell,2000	MN855127		OQ732695	Panama	(Alves et al., 2020)
Hesionidae	*Oxydromus pugettensi* s Johnson, 1901	MN855132	MN855211	OQ732696	USA	(Alves et al., 2020)

Table 3: Specimens selected for whole mitogenome analyses are listed in, with the new whole mitochondrion indicated by bold sequence.

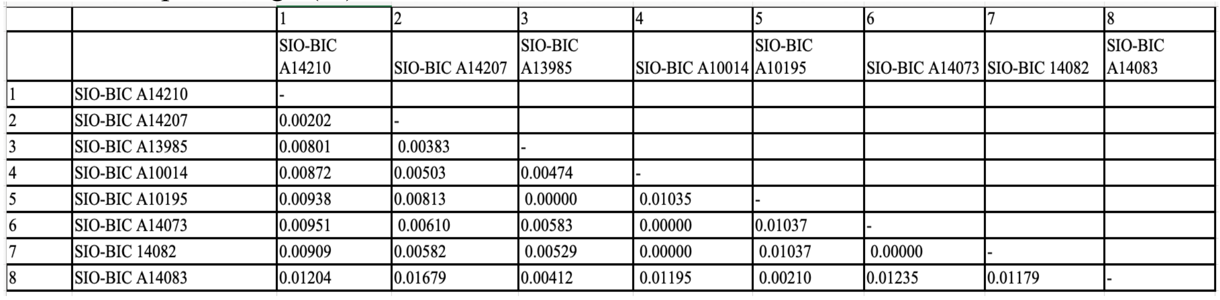
Species	Specimen Voucher or Source	Collection Site	GenBank Accession Numbers
Alitta succinea (Leuckart, 1847)	Alves et al. (2020)	USA: Florida, Cedar Key	MN812981
Arichlidon gathofi Watson Russell, 2000 1	Alves et al. (2020)	USA: North Carolina	MN855126 (COX1), MN855135 (COX2), MN855144 (COX3), MN855116 (CYTB), MN855107 (ATP6), MN855196 (ND5), MN855188 (ND4L), MN855178 (ND4), MN855154 (ND1), MN855171 (ND3), MN855164 (ND2)
Arichlidon gathofi Watson Russell, 2000 2	Alves et al. (2020)	Panama: Bocas del Toro, Hospital Point	MN855127 (COX1), MN855136 (COX2), MN855145 (COX3), MN855205 (ND6), MN855117 (CYTB), MN855108 (ATP6), MN855197 (ND5), MN855179 (ND4), MN855155 (ND1), MN855172 (ND3), MN855165 (ND2)
Bhawania goodei Webster, 1884	Alves et al. (2020)	Panama: Bocas del Toro, Punta Caracol	MN855128 (COX1), MN855146 (COX3), MN855118 (CYTB), MN855198 (ND5), MN855189 (ND4L), MN855180 (ND4), MN855208 (16S), MN855156 (ND1), MN855173 (ND3), MN855166 (ND2)
Cheilonereis cyclurus (Harrington, 1897)	Park et al. (2017)	Korea: Gangwondo, Goseong-gun	MF538532
Dendronereis chipolini Hsueh, 2019	Zhen et al. (2022)	China: Beibu Gulf, Maowei Sea	MW532084
Hediste diadroma Sato & Nakashima, 2003	Kim et al. (2016)	Korea: Masan Harbour	KX499500
Hediste japonica (Izuka, 1908)	Park et al. (2020)	South Korea: Incheon, Ganghwa-gun, Hwado-myeon, Yeocha-ri (Ganghwa Mudflat Center)	MN876864
Hesionides sp.	Alves et al. (2020)	Panama: Bocas del Toro, Hospital Point	MN855129 (COX1), MN855137 (COX2), MN855147 (COX3), MN855119 (CYTB), MN855109 (ATP6), MN855199 (ND5), MN855190 (ND4L), MN855181 (ND4), MN855157 (ND1), MN855174 (ND3), MN855167 (ND2)
Laeonereis culveri (Webster, 1879)	Seixas et al. (2016)	Brazil: Rio de Janeiro, Marica Lagoon	KU992689
Microphthalmus listensis Westheide, 1967 1	Alves et al. (2020)	Germany: Sylt, List	MN855130 (COX1), MN855138 (COX2), MN855148 (COX3), MN855206 (ND6), MN855120 (CYTB), MN855110 (ATP6), MN855182 (ND4), MN855209 (16S), MN855158 (ND1)
Microphthalmus listensis Westheide, 1967 2	Alves et al. (2020)	Germany: Sylt, List	MN855139 (COX2), MN855149 (COX3), MN855121 (CYTB), MN855111 (ATP6), MN855200 (ND5), MN855191 (ND4L), MN855183 (ND4), MN855210 (16S), MN855159 (ND1), MN855175 (ND3)
Microphthalmus similis Bobretzky, 1870	Alves et al. (2020)	Germany: Sylt, List	MN855131 (COX1), MN855140 (COX2), MN855150 (COX3), MN855122 (CYTB), MN855112 (ATP6), MN855201 (ND5), MN855192 (ND4L), MN855184 (ND4), MN855160 (ND1), MN855168 (ND2)
Namalycastis abiuma (Grube, 1872)	Lin et al. (2016)	China: Xiamen, Yuandang Lake	KU351089
Neanthes glandicincta (Southern, 1921)	Lin et al. (2017)	China: Xiamen, Yuandang Lake	KY094478
Nectoneanthes oxypoda (Marenzeller, 1879)	SIO-BIC A13109	Japan: Southern Osaka, Tanagawa-Tanigawa, Osaka Bay	OL782599
Pectinereis strickrotti sp. nov.	SIO-BIC A9836	Costa Rica: Mound 12 West	OL782600
Nereis pelagica Linnaeus, 1758	SIO-BIC A6054	Norway: Trondheimsfjord, Biologisk Stasjon	OL782598
Nereis sp.	Kim et al. (2017)	Korea: Dok-do Island	MF960765
Nereis zonata Malmgren, 1867	Nam et al. (2021)	Beaufort Sea	MT980928
Oxydromus pugettensis (Johnson, 1901)	Alves et al. (2020)	USA: Washington, Snug Harbor	MN855132 (COX1), MN855141 (COX2), MN855151 (COX3), MN855123 (CYTB), MN855113 (ATP6), MN855202 (ND5), MN855193 (ND4L), MN855184 (ND4), MN855211 (16S), MN855161 (ND1), MN855176 (ND3)
Oxydromus sp.	Alves et al. (2020)	Panama: Bocas del Toro	MN855133 (COX1), MN855142 (COX2), MN855152 (COX3), MN855124 (CYTB), MN855114 (ATP6), MN855203 (ND5), MN855194 (ND4L), MN855186 (ND4), MN855212 (16S), MN855162 (ND1), MN855177 (ND3), MN855169 (ND2)
Paraleonnates uschakovi Chlebovitsch & Wu, 1962	Park et al. (2016)	Korea: Ganghwa Island	KX462988
Perinereis aibuhitensis (Grube, 1878)	Kim et al. (2015)	Korea: Ganghwa Island	KF611806
Perinereis cultrifera (Grube, 1840)	Alves et al. (2020)	France: Arcachon	MN812983
Perinereis nuntia (Lamarck, 1818)	Won et al. (2013)	Korea: Yeosu	JX644015
Perinereis sp.	Alves et al. (2020)	Panama: Bocas del Toro	MN823962 (COX1), MN823963 (COX2), MN823964 (COX3), MN823970 (ND6), MN823961 (CYTB), MN823960 (ATP6), MN823969 (ND5), MN823968 (ND4L), MN823967 (ND4), MN823972 (12S), MN823971 (16S), MN823965 (ND1), MN823966 (ND2)
Platynereis bicanaliculata (Baird, 1863)	Alves et al. (2020)	USA: Washington, Lopez Island	MN812984
Platynereis cf. *australis*	Alves et al. (2020)	Chile: Chonchi, Isla de Chiloe	MN830367
Platynereis dumerilii (Audouin & Milne Edwards, 1833)	Boore & Brown (2000)	Europe	AF178678
Platynereis massiliensis (Moquin-Tandon, 1869)	Alves et al. (2020)	Wales: Pembroke, West Angle Bay	MN812985
Platynereis sp. 1	Alves et al. (2020)	Brazil: Ceara, Cascavel	MN830365
Platynereis sp. 2	Alves et al. (2020)	Brazil: Rio de Janeiro, Cabo Frio	MN830366
Pseudonereis variegata (Grube, 1857)	Alves et al. (2020)	South Africa: Western Cape, Fish Hoek	MN855134 (COX1), MN855143 (COX2), MN855153 (COX3), MN855207 (ND6), MN855125 (CYTB), MN855115 (ATP6), MN855204 (ND5), MN855195 (ND4L), MN855187 (ND4), MN855214 (12S), MN855213 (16S), MN855163 (ND1), MN855170 (ND2)
Tylorrhynchus heterochetus (Quatrefages, 1866)	Chen et al. (2016)	South China: Nalong River estuary	KM111507
***Nereis sandersi* (Blake, 1985**	**SIO-BIC A13659**	**East Pacific Rise**	**This study (unpublished)**
***Nereis pisceae* (Blake, 1990**	**SIO-BIC A12292**	**USA**	**This study (unpublished)**
Nereis moana n. sp	**SIO-BIC A14217**	**Tonga, West Pacific**	**This study (unpublished)**
Nereis kilrahae n. sp	**SIO-BIC A10014**	**Pescadero, Gulf of California**	**This study (unpublished)**
Neanthes jyothiae n. sp	**SIO-BIC A3333**	**Guyamas, Gulf of California**	**This study (unpublished)**
***Neanthes vaalii* (Kinberg, 1**	**NMV F303024**	**Australia**	**This study (unpublished)**

Table 4: Uncorrected interspecific COI pairwise distances for Nereididae. Pairwise distances in percentage (%).



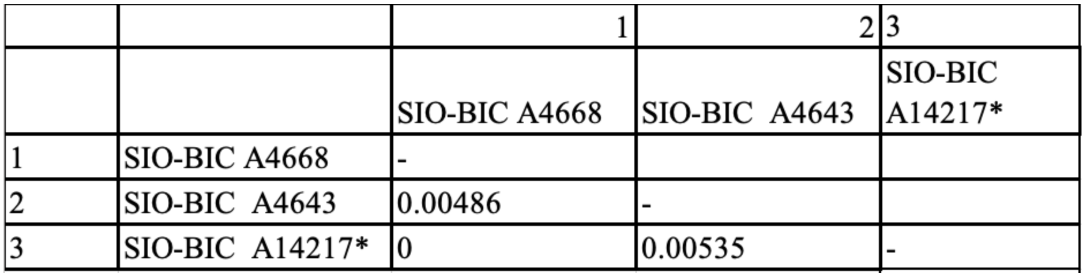
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Nicon maculata	Nereis sp	Nereis pelagica	Nectoneanthes oxypoda	Pectinereis strickrotti	Neanthes jyothiae n sp	Nereis kiirahae n sp	Nereis moana n sp	Neanthes vaalii	Nereis sandersi	Nereis piscesae	Nereis sp E Austalian Whalefall	Neanthe visicete	Nereis tricirrata	Neanthes shinkai
1	Nicon maculata	-														
2	Nereis sp	0.2152	-													
3	Nereis pelagica	0.2144	0.172	-												
4	Nectoneanthes oxypoda	0.2059	0.212	0.211	-											
5	Pectinereis strickrotti	0.2378	0.226	0.232	0.206	-										
6	Neanthes jyothiae n sp	0.1619	0.2238	0.217	0.226	0.22246	-									
7	Nereis kiirahae n sp	0.2106	0.186	0.176	0.217	0.2216	0.21657	-								
8	Nereis moana n sp	0.2144	0.179	0.173	0.216	0.2151	0.2133	0.10169	-							
9	Neanthes vaalii	0.2093	0.198	0.208	0.2	0.2042	0.2068	0.219	0.22568	-						
10	Nereis sandersi	0.2181	0.191	0.17471	0.221	0.2321	0.2231	0.118	0.11147	0.2263	-					
11	Nereis piscesae	0.1945	0.196	0.17927	0.23210	0.23077	0.2172	0.151	0.141	0.2231	0.15515	-				
12	Nereis sp E Australian Whalefall	0.2112	0.19602	0.183	0.215	0.2289	0.2275	0.119	0.129	0.2127	0.134	0.16738	-			
13	Neanthes visicete	0.2069	0.195	0.2	0.196	0.15963	0.2098	0.199	0.201	0.2067	0.211	0.2	0.1964	-		
14	Nereis tricirrata	0.2232	0.188	0.18	0.213	0.2283	0.2197	0.14	0.165	0.2138	0.152	0.17	0.1603	0.20990	-	
15	Neanthes shinkai	0.2229	0.205	0.185	0.223	0.2144	0.22620	0.097	0.12423	0.2196	0.114	0.156	0.12656	0.19879	0.139	

Table 5: Uncorrected intraspecific COI pairwise distances for *Nereis kiirahae* n. sp. Pairwise distances in percentage (%).



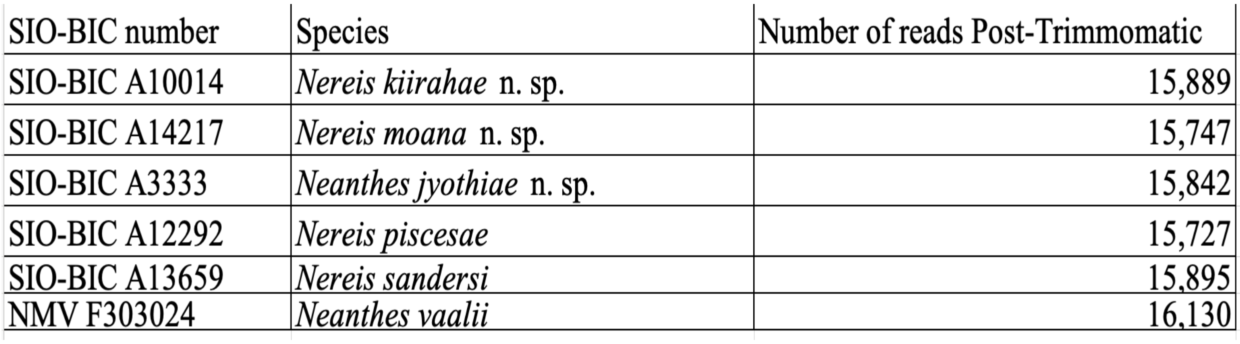
		1	2	3	4	5	6	7	8
		SIO-BIC A14210	SIO-BIC A14207	SIO-BIC A13985	SIO-BIC A10014	SIO-BIC A10195	SIO-BIC A14073	SIO-BIC 14082	SIO-BIC A14083
1	SIO-BIC A14210	-							
2	SIO-BIC A14207	0.00202	-						
3	SIO-BIC A13985	0.00801	0.00383	-					
4	SIO-BIC A10014	0.00872	0.00503	0.00474	-				
5	SIO-BIC A10195	0.00938	0.00813	0.00000	0.01035	-			
6	SIO-BIC A14073	0.00951	0.00610	0.00583	0.00000	0.01037	-		
7	SIO-BIC 14082	0.00909	0.00582	0.00529	0.00000	0.01037	0.00000	-	
8	SIO-BIC A14083	0.01204	0.01679	0.00412	0.01195	0.00210	0.01235	0.01179	-

Table 6: Uncorrected intraspecific COI pairwise distances for *Nereis moana* n. sp. Pairwise distances in percentage (%).



		1	2	3
		SIO-BIC A4668	SIO-BIC A4643	SIO-BIC A14217*
1	SIO-BIC A4668	-		
2	SIO-BIC A4643	0.00486	-	
3	SIO-BIC A14217*	0	0.00535	-

Table 7: Total number of genome skimming reads retained after using Trimmomatic.



SIO-BIC number	Species	Number of reads Post-Trimmomatic
SIO-BIC A10014	*Nereis kiirahae* n. sp.	15,889
SIO-BIC A14217	*Nereis moana* n. sp.	15,747
SIO-BIC A3333	*Neanthes jyothiae* n. sp.	15,842
SIO-BIC A12292	*Nereis piscesae*	15,727
SIO-BIC A13659	*Nereis sandersi*	15,895
NMV F303024	*Neanthes vaalii*	16,130

FIGURES:

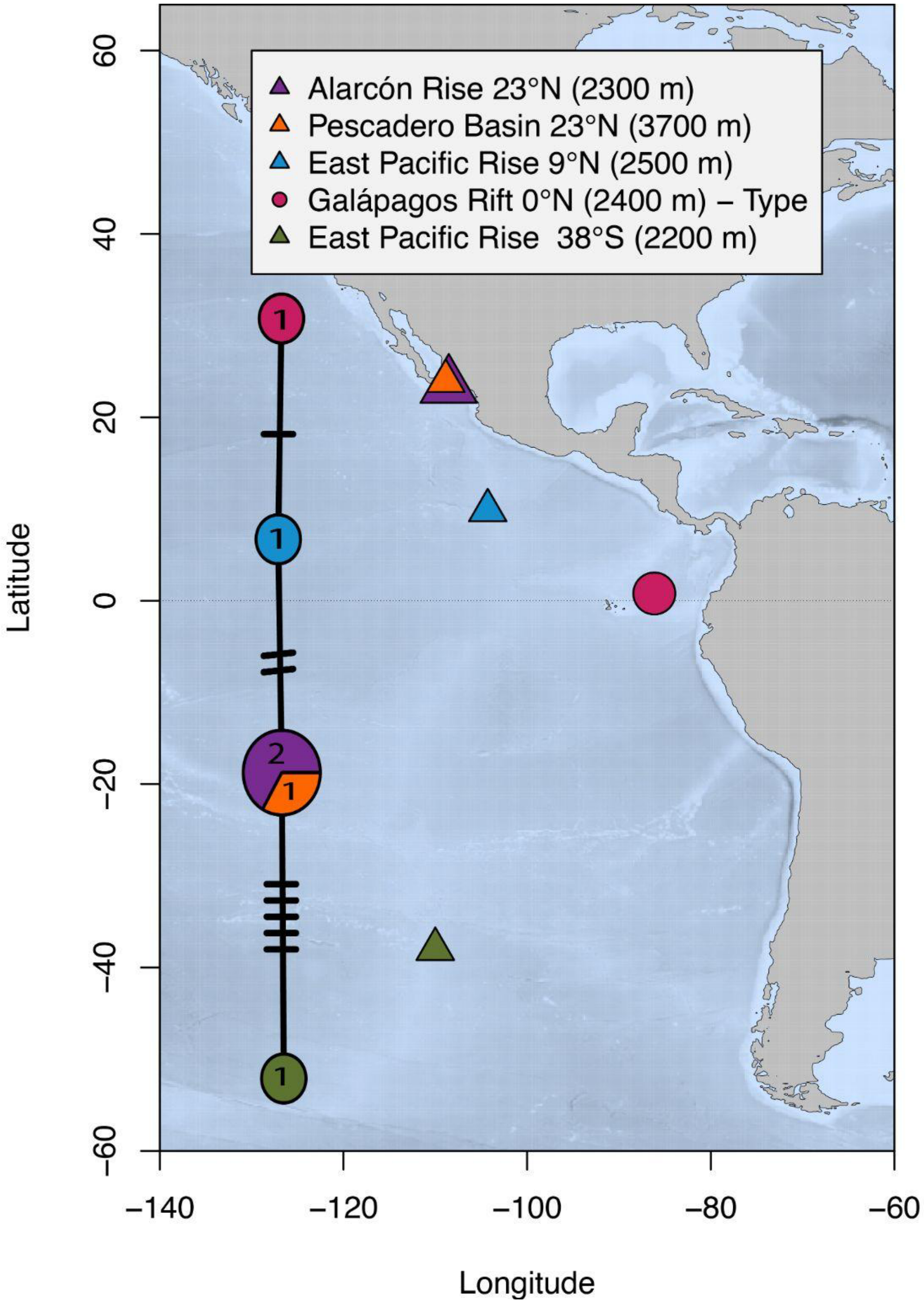


Figure 1: Map displays the range extension of *Nereis sandersi*, with its distribution shown on the map spanning from 23°N to 28°S. Map created using the R package marmap (Pante & Simon-Bouhet 2013) and modified using Adobe Illustrator (Adobe Inc. 2019).

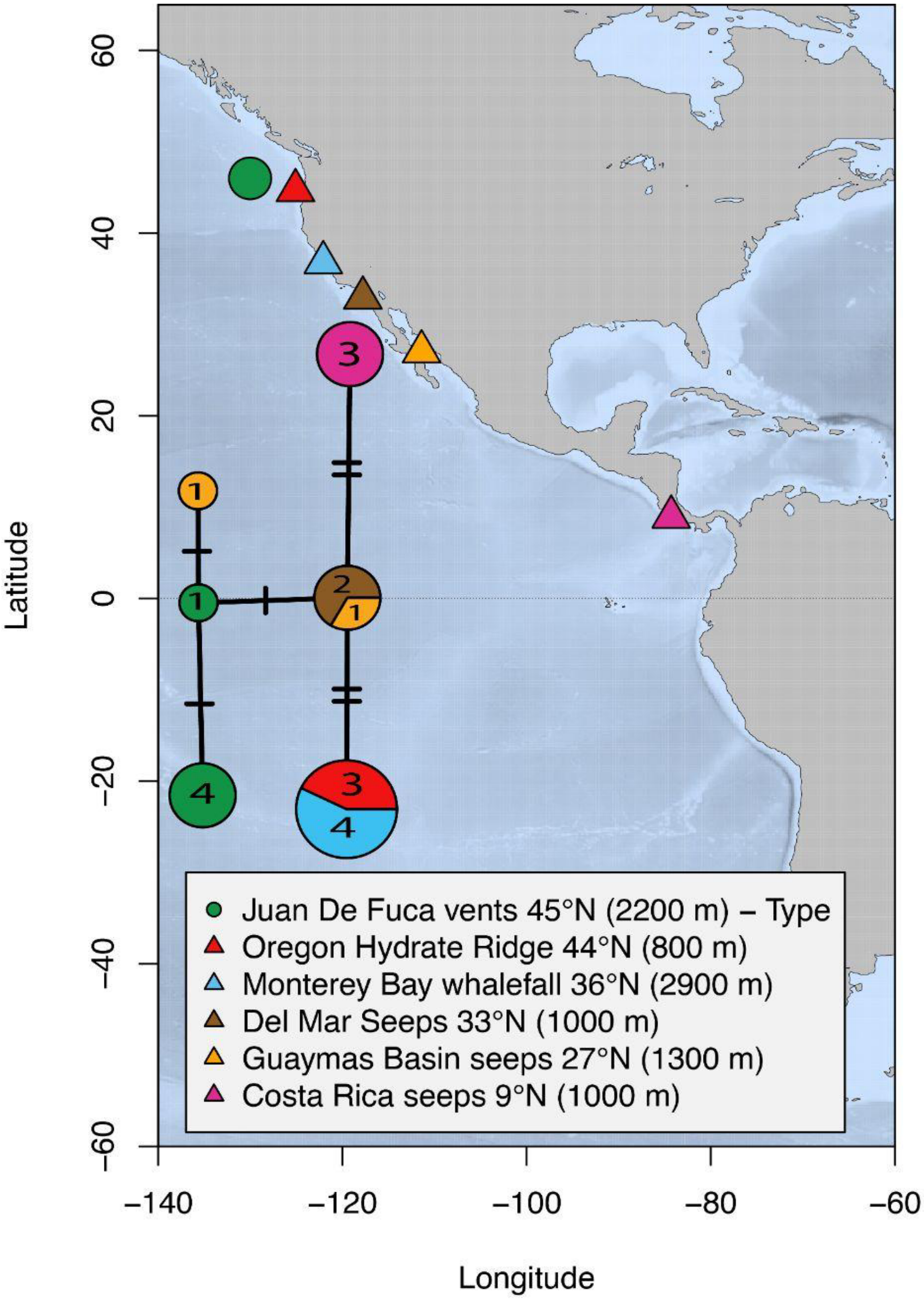


Figure 2: Map illustrates the range extension of *Nereis piscesae*, with its distribution spanning from 45°N to 9°N. Map created using the R package marmap (Pante & Simon-Bouhet 2013) and modified using Adobe Illustrator (Adobe Inc. 2019).

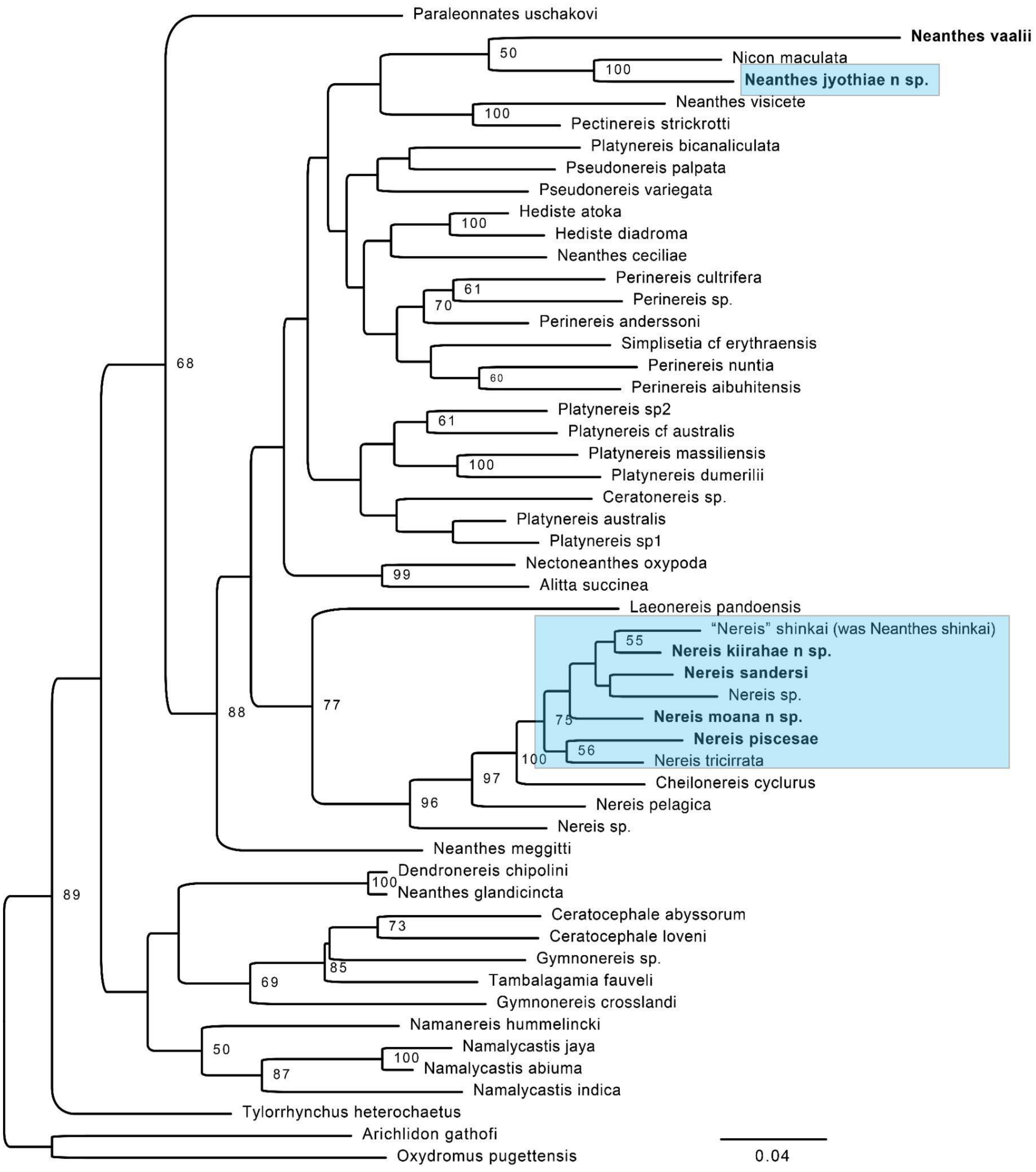


Figure 3: Maximum likelihood tree created by concatenating the mitochondrial genes COI and 16s with the nuclear gene 18s. The names highlighted in bold include the species whose molecular data was produced during this study. Names in blue boxes indicate Nereididae from chemosynthetic habitats. Bootstrap values (%) are represented by the numbers at the nodes. The analysis was carried out over 10 runs and 100 replicates, with the resulting file being modified in Adobe Illustrator (Adobe Inc. 2019).

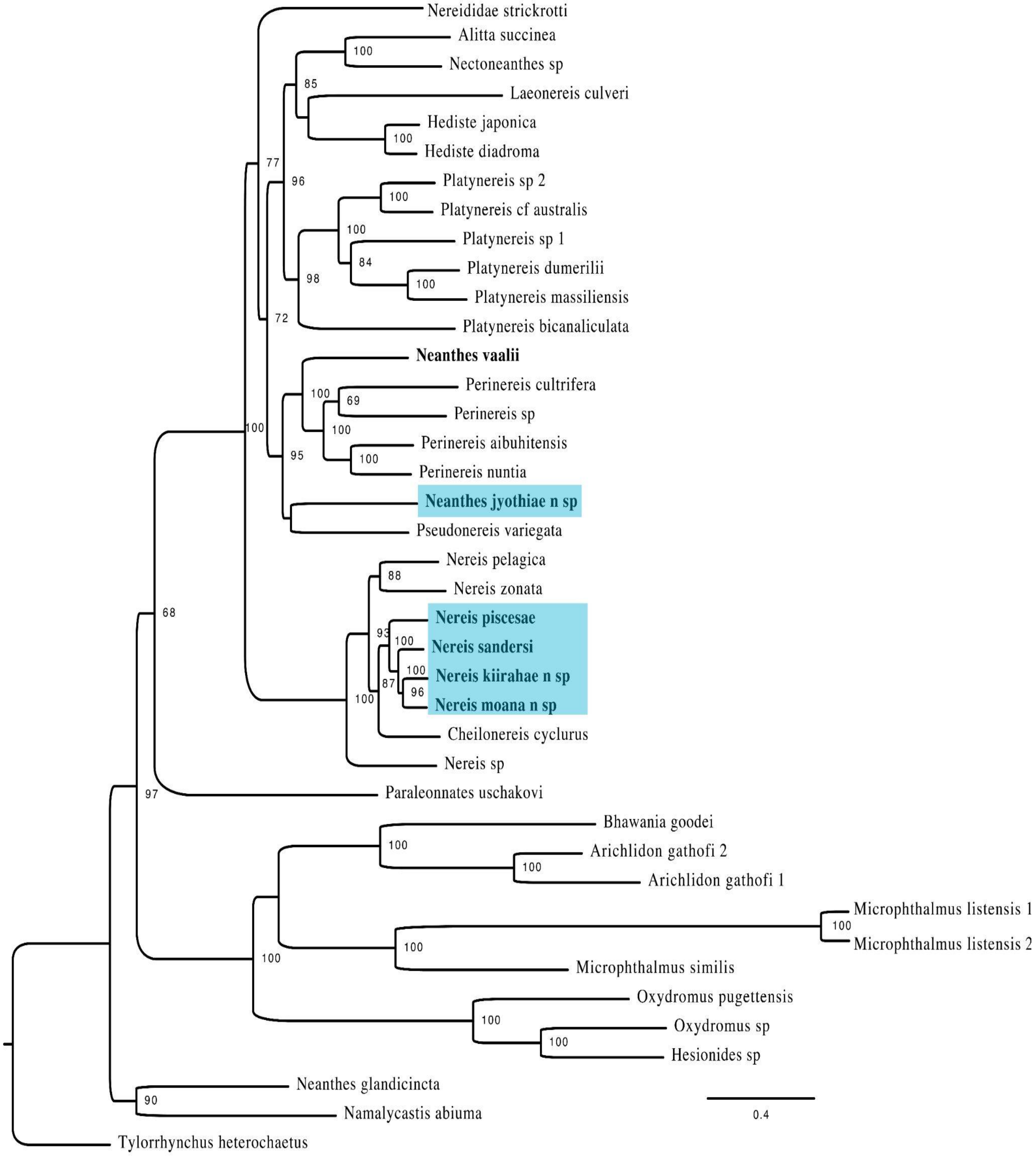


Figure 4: Maximum likelihood Nereididae mitogenome phylogeny of 15 concatenated genes as nucleotides with Gymnonereidinae as the outgroup. The names in bold indicate the species whose genomes were analyzed in this study. Blue boxes represent chemosynthetic taxa. The numbers at the nodes represent the bootstrap values in percentages. The analysis was conducted in 100 runs and 1000 replicates, and the modified file was created using Adobe Illustrator (Adobe Inc. 2019).

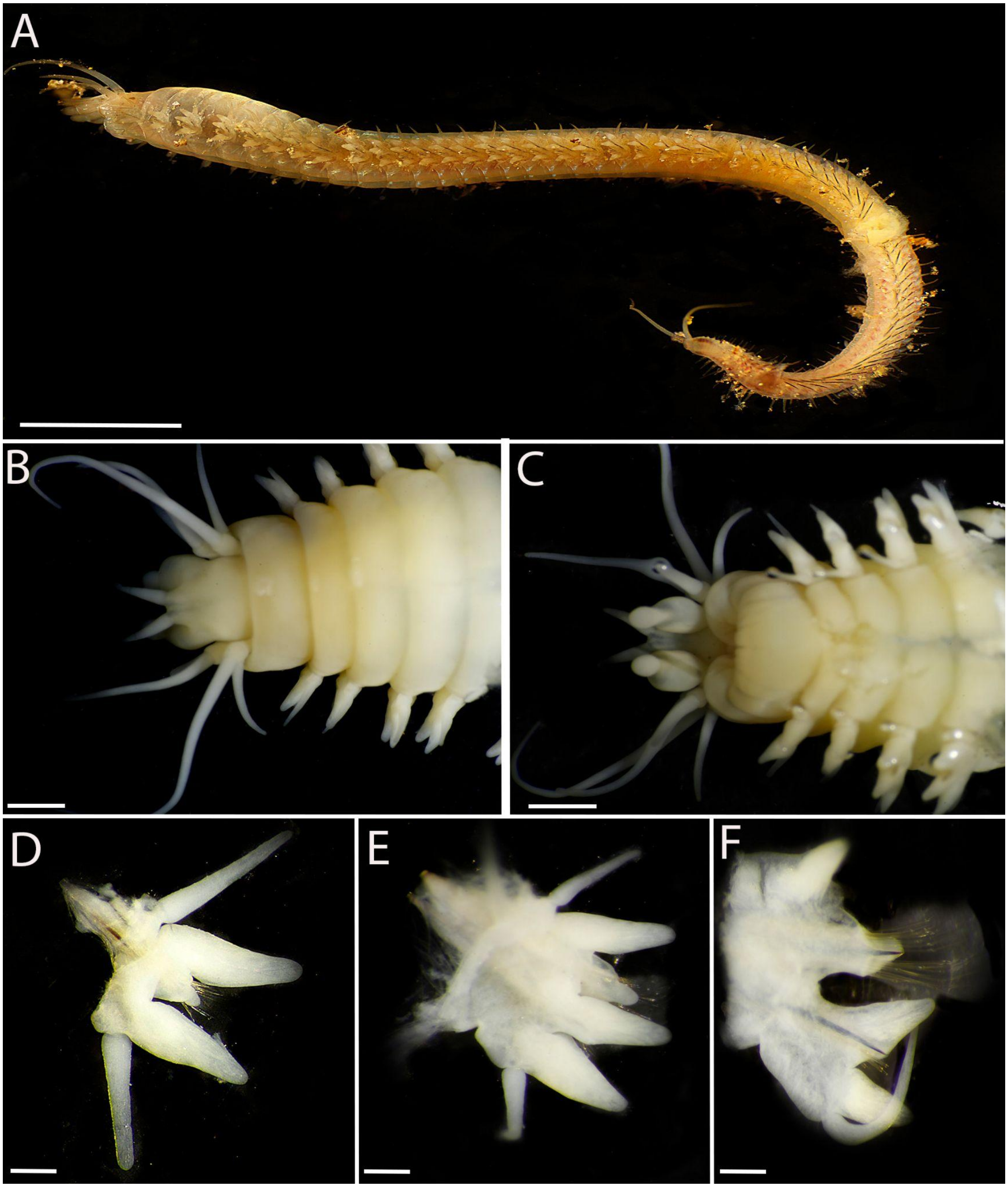


Figure 5: *Nereis kiirahae* n. sp. A) Live picture of *Nereis kiirahae* n. sp. paratype A10195; scale bar is 200 µm; B) Prostomium in dorsal view, scale bar is 100 µm; C) Prostomium in ventral view, scale bar is 100 µm; D-F) Chaetigers 1, 7, 27, posterior view, scale bar is 50 µm.

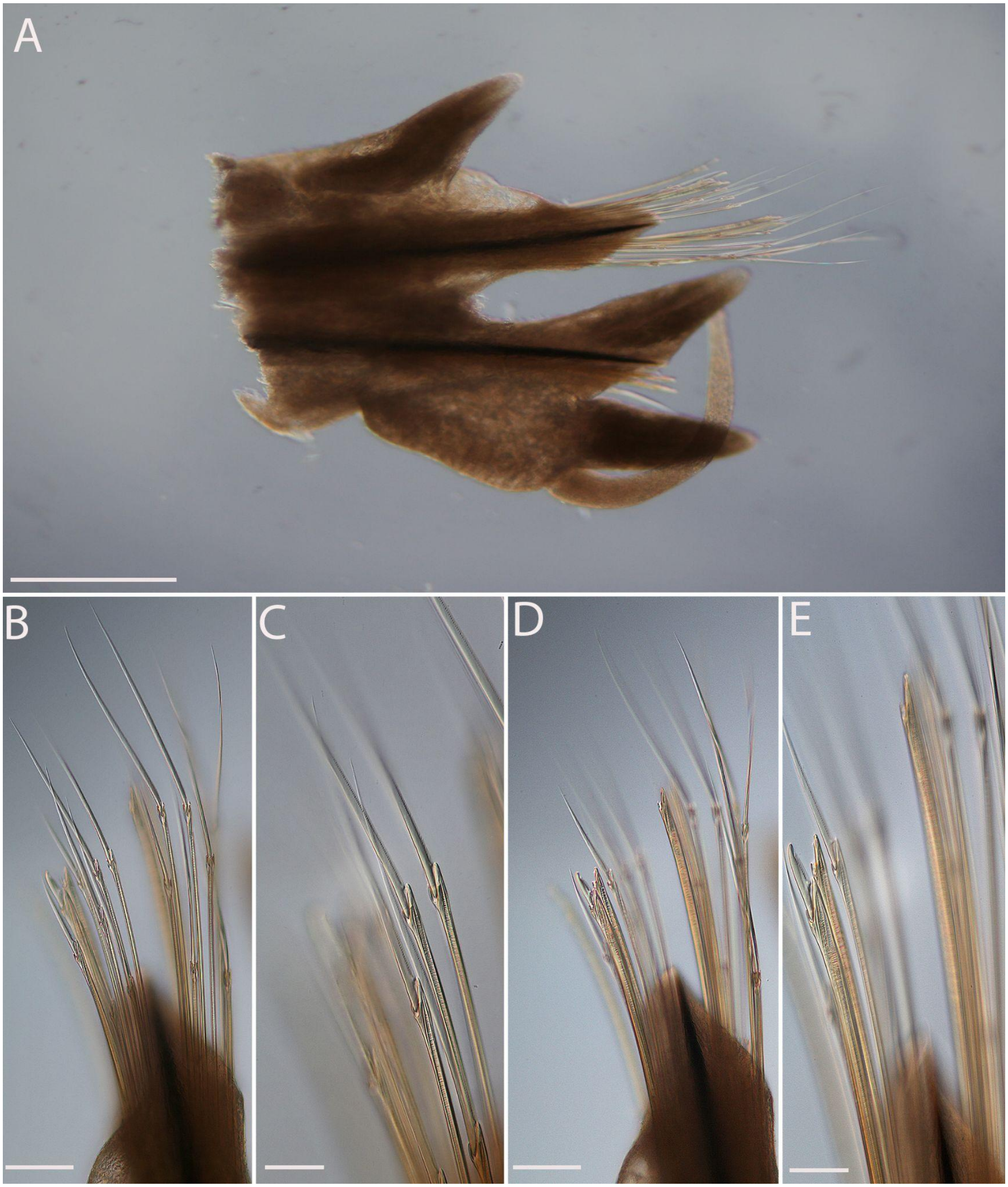


Figure 6: *Nereis kiirahae* n. sp segment 27, holotype, A10014* A) *Nereis kiirahae* n. sp chaetiger 27, scalebar is 500 µm; B) Spiniger, scalebar is 50 µm; C) Spiniger joint, scale bar is 20 µm; D) Falciger, scalebar is 50 µm E) Falciger joint, scale bar is 20 µm.

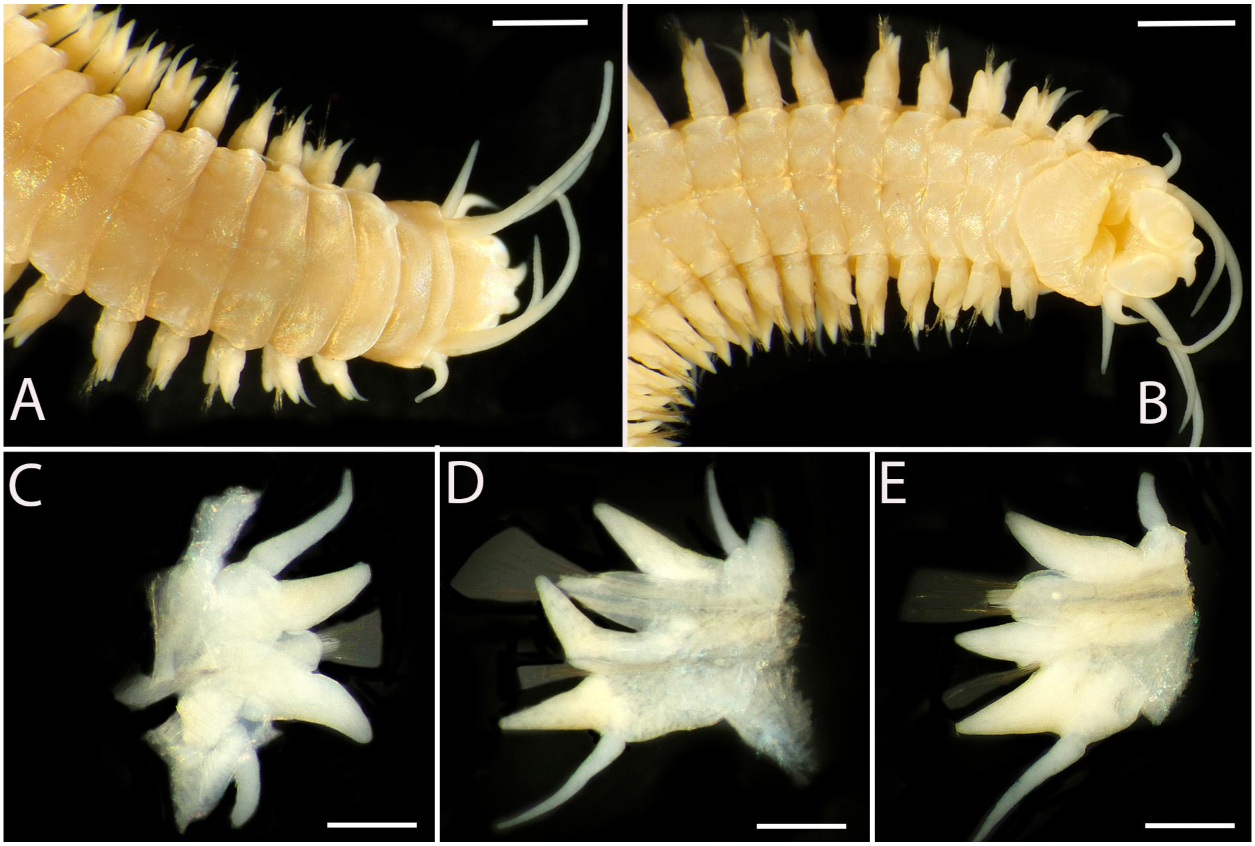


Figure 7: *Nereis moana* n. sp. holotype A4643* A) Prostomium in dorsal view, scale bar is 10 µm; B) Prostomium in ventral view, scale bar is 10 µm; C-E) Chaetigers 1, 6, 36, posterior view, scale bar is 10 µm.

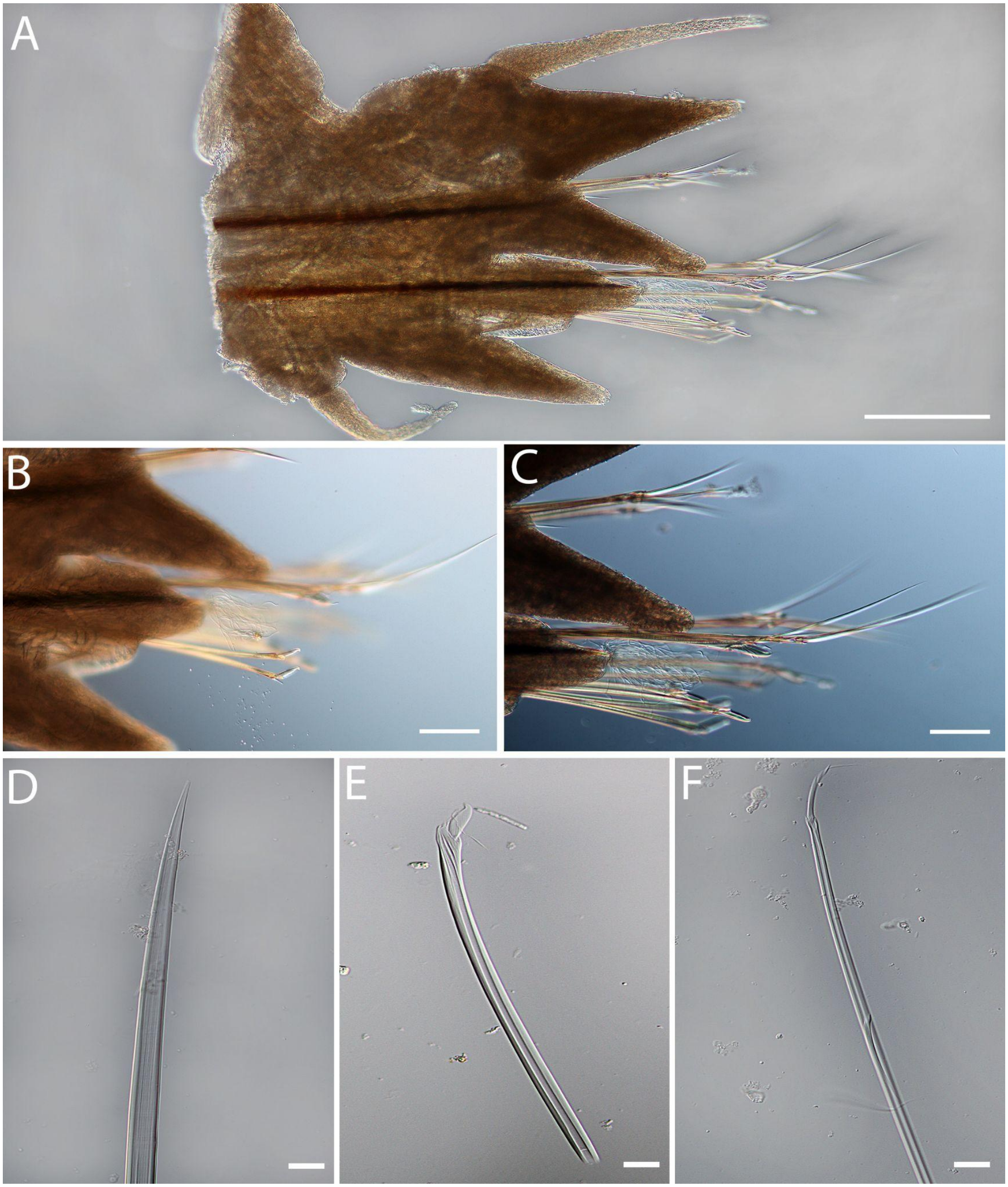


Figure 8: *Nereis moana* n. sp. segment 36, holotype, A4643*A) *Nereis moana* n. sp chaetiger 36 holotype A4643*, scalebar is 200 µm; B-C) Neurocheate falcigers and spiniger, scale bar is 50 µm; D-F) Accicula, falciger, spiniger; scale bar is 30 µm.

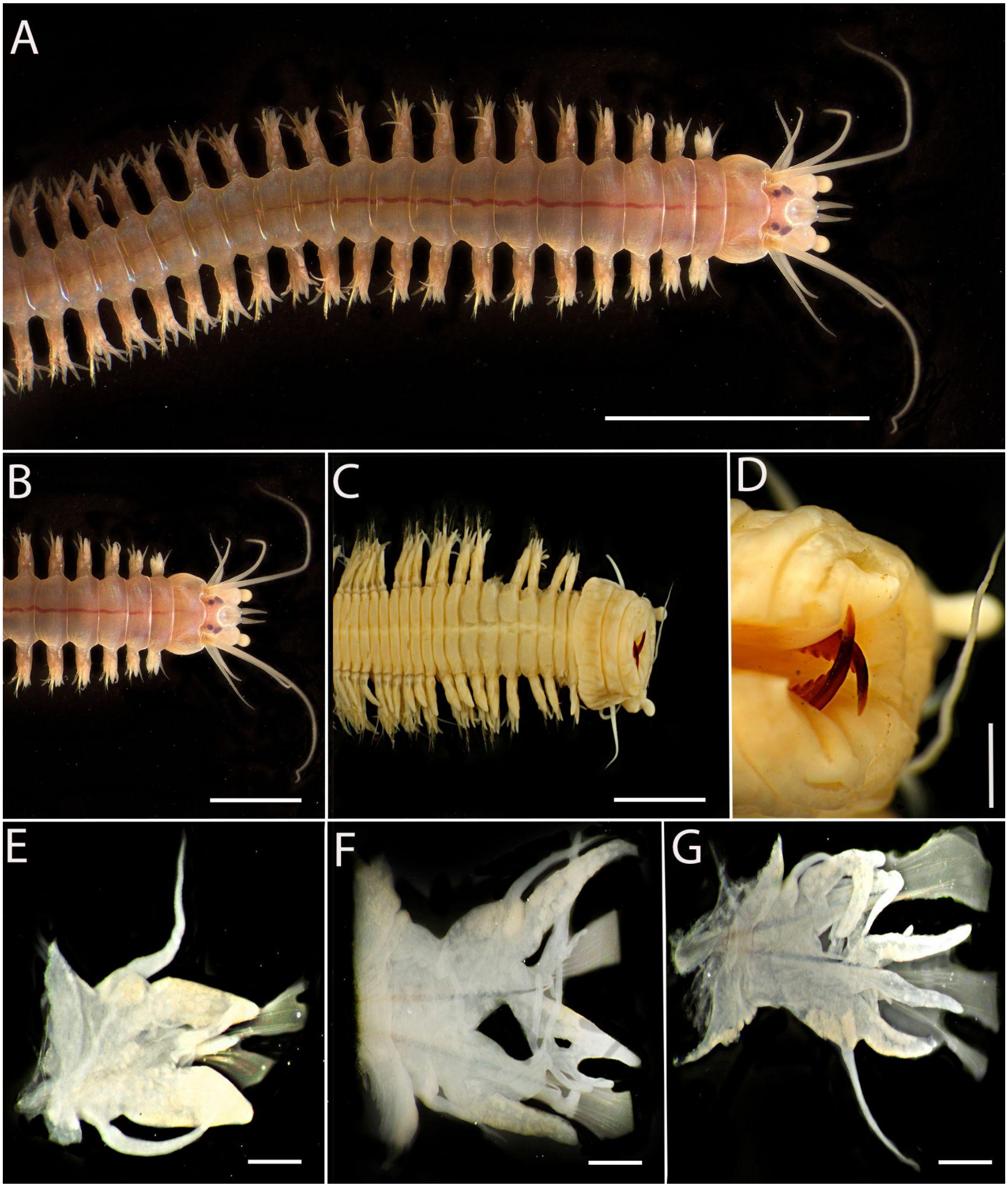


Figure 9: *Neanthes jyothiae* n. sp. holotype A3333* A) Whole specimen live dorsal view, scale bar is 50 µm; B) Prostomium in dorsal view, scale bar is 20 µm; C) Prostomium in ventral view, scale bar is 20 µm; D) Jaws ventral view, scale bar is 5 µm; E-G) Chaetigers 1, 25, 27, posterior view, scale bar is 2 µm.

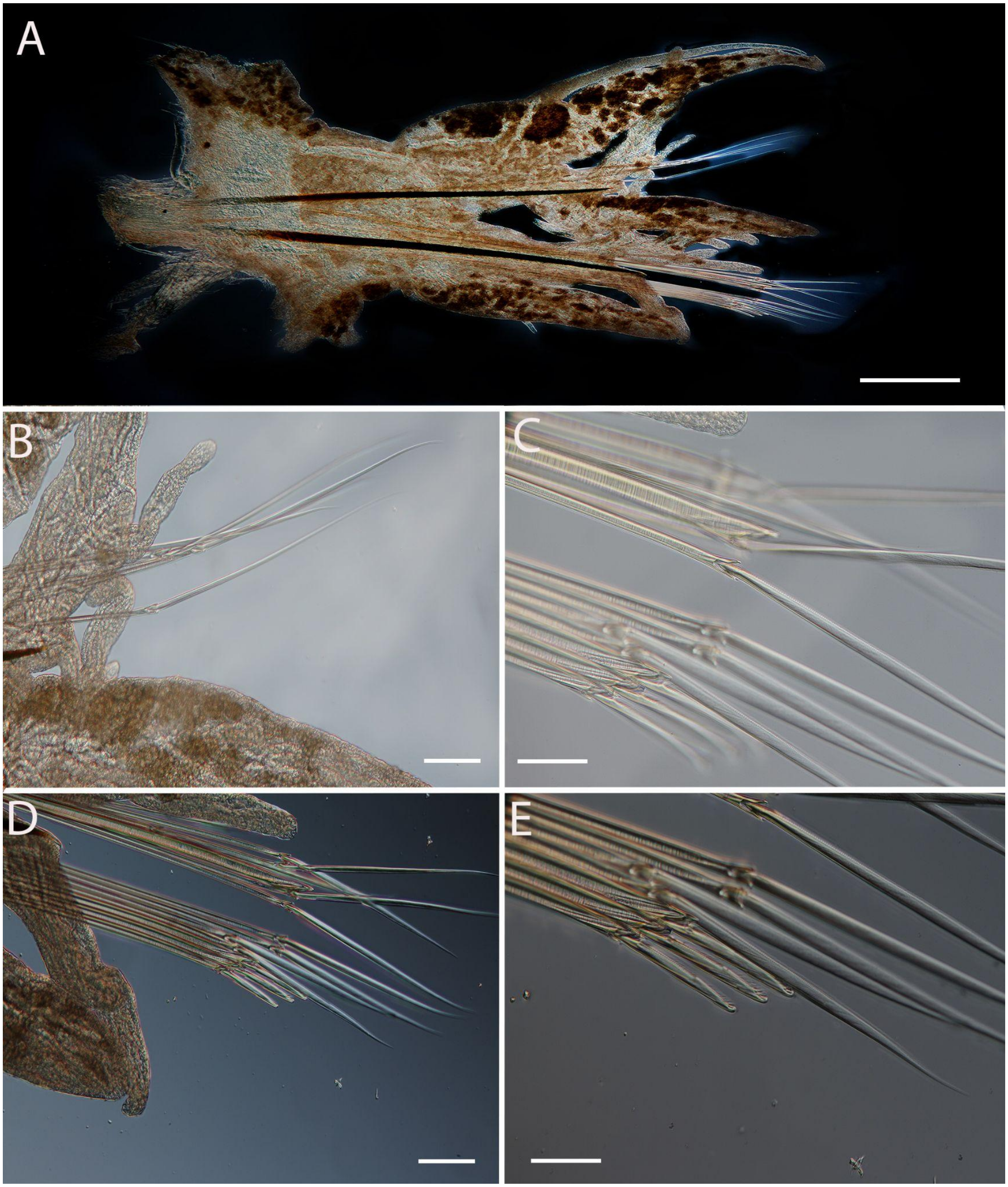


Figure 10: *Neanthes jyothiae* n. sp. segment 27, holotype, A3333* A) *Neanthes jyothiae* n. sp chaetiger 27 holotype A3333*, scale bar is 300 µm; B) Neurocheate spiniger scale bar is 100 µm; C) Notocheate spiniger joint, scale bar is 50 µm; D) Falciger, scale bar is 100 µm; E) Falciger joint, scale bar is 50 µm.

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