Fisheries Journal, 15 (2), 530-544 (2025)
http://doi.org/10.29303/jp.v15i2.1327

IDENTIFICATION OF PORIFERA USING EDNA IN THE CORALS OF TAKAT, SUMENEP, EAST JAVA

Identifikasi Porifera Dengan Menggunakan Edna Di Karang Takat Sumenep Jawa Timur

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(Received December 16th 2024; Accepted April 27th 2025)

ABSTRACT

Karang Takat is a body of water in Sumenep, Madura which has beauty and various kinds of flora and fauna, one of which is Porifera. Porifera are organisms that have an important role in coral reef ecosystems. Porifera live attached to coral reefs and stick to hard coral, because hard coral is a hard substrate for Porifera to stick to. Habitats with hard substrates will be places where porifera grow well, because only 10% of porifera can live. This research was conducted to provide an accurate and latest method for detecting the presence and diversity of Porifera. The eDNA metabarcoding method is suitable for identifying flora and fauna diversity, such as Porifera, with identification based on DNA residues left in their habitat. DNA species can be detected from species that release mitochondria, mucus, and excretory waste products into the environment. The results of Porifera DNA research in Karang Takat found 10 families, namely the *Clionaide* family, *Niphatidae* family, *Tethyidae* family, *Spirastrellidae* family, *Desmacellida* family, *Chalinidae* family, *Microcionidae* family, *Astroscleridae* family, *Geodiidae* family, and *Halisarcidae* family. The result was 11 species of Porifera from the same class. Known species are *Spheciospongia semilunaris, Amphimedon sp, Tethya irisae, Spirastrella sp, Desmacella cf, Haliclona toxia, Clathria reinwardti, Stromatospongia vermicola, Haliclona amboinensis, Geodia sp., and Halisarca caerulea.*

Keywords: eDNA, Coral Reef, Porifera

ABSTRAK

Karang Takat adalah perairan di Sumenep Madura yang memiliki keindahan dan berbagai macam flora dan fauna, salah satunya adalah *Porifera*. *Porifera* merupakan organisme yang memiliki peran penting dalam ekosistem terumbu karang. *Porifera* hidup menempel pada terumbu karang dan menempel pada hard coral, karena hard coral merupakan susbstrat keras untuk tempat menempel *Porifera*. Habitat dengan substrat yang keras akan menjadi tempat porifera tumbuh dengan baik, karena hanya 10% porifera yang dapat hidup. Penelitian ini

dilakukan untuk memberikan metode yang akurat dan terbaru dalam mendeteksi keberadaan dan keanekaragaman *Porifera*. Metode eDNA *metabarcoding* cocok untuk mengidentifikasi keanekaragaman flora dan fauna, seperti *Porifera* dengan mengidentifikasi berdasarkan sisa DNA yang ditinggalkan di habitatnya. DNA spesies dapat dideteksi didapatkan dari spesies melepaskan mitokondria, lendir, dan buangan hasil ekskresi ke lingkungan. Hasil penelitian DNA *Porifera* di Karang Takat menemukan 10 Family yaitu famili *Clionaide*, famili *Niphatidae*, famili *Tethyidae*, famili *Spirastrellidae*, famili *Desmacellida*, famili *Chalinidae* , famili *Microcionidae*, famili *Astroscleridae*, famili *Geodiidae*, dan famili *Halisarcidae*. Hasil terdapat 11 spesies *Porifera* dari 1 kelas yang sama. Spesies yang diketahui adalah *Spheciospongia semilunaris, Amphimedon sp, Tethya irisae, Spirastrella sp, Desmacella cf, Haliclona toxia, Clathria reinwardti, Stromatospongia vermicola, Haliclona amboinensis, Geodia sp.,* dan *Halisarca caerulea*.

Kata Kunci: eDNA, Terumbu Karang, Porifera

INTRODUCTION

Indonesia is a maritime country that has a wider distribution of waters than land. Karang Takat is one example of Indonesian waters located in Sumenep, Madura, East Java and is located between two islands, namely Gili Labak and Gili Genting islands. Gili Labak and Gili Genting islands have an ecology such as many marine ecosystems such as coral reef ecosystems. According to Efendy & Firman (2018), the coral reefs in Gili Labak are only 48.7% in good condition and 51.3% are dead. Gili Labak Island is an island famous for its marine tourism activities, while Gili Genting Island is mostly only for resident activities and a little for marine tourism activities. Karang Takat, which is located between Gili Labak and Gili Genting Islands, is famous for its coral reef ecosystem, therefore it is suspected that there are Porifera species in these waters.

Porifera are organisms that play an important role in coral reef ecosystems. Porifera live attached to coral reefs and hard corals, because hard coral is a hard substrate for Porifera to attach to. Habitats with hard substrates will be a place for Porifera to grow well, because only 10% of Porifera can live. This study was conducted to provide an accurate and up-to-date method for detecting the presence and diversity of Porifera (Fastawa *et al*., 2018). Detecting the presence or absence of Porifera in waters requires a DNA test.

Environmental DNA is a molecular method that can be used to detect the presence of a species in the environment. Samples from the environment used to examine DNA are water, soil or air samples. The advantage of this E-DNA is that it can detect the diversity in the sea, including bacteria, vertebrates, invertebrates, including Porifera. Porifera DNA detection can be done using the Metabarcoding method. (Johnson *et al*., 2023). Karang Takat is famous for its coral reef ecosystem and it is certain that there are many species of Porifera in it and Porifera detection using Environmental DNA has never been done, therefore Porifera identification using Environmental DNA in Karang Takat, Sumenep, East Java is the background to this research.

RESEARCH METHODS

The study entitled "Identification of Porifera Using eDNA in Karang Takat Sumenep East Java" was conducted from August to December 2024. Sampling was carried out in the waters of Karang Takat Sumenep, Madura East Java. Sample extraction and analysis were carried out at the Integrated Laboratory of Diponegoro University. DNA sequencing was carried out at Genetika Scince Indonesia. The map of the sampling location can be seen in Figure 1 below.

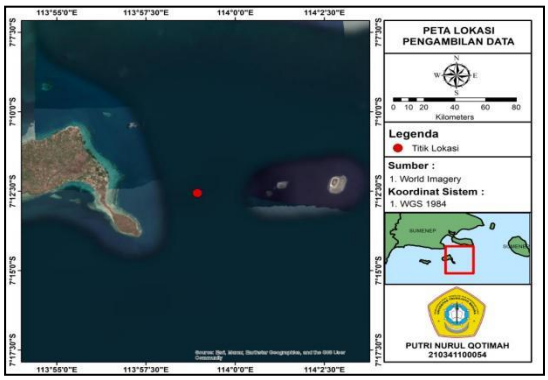


Figure 1**.** Research Sampling Map (Karang Takat Waters)

The tools used in this study were GPS, Boat, Coolbox, Scuba Diving, Feeding Back, Sterifact, Freezer, PCR Gradient, Vortex, Gel Doc, Centrifuge, Micropippet and Nanodrop. The materials used in this study were ATL Buffer, AE Buffer, AW Buffer, AL Buffer, Agarose Gel, Primer, ddH2O and PCR Kit.

Detection of Porifera using eDNA can be done by taking samples in the form of seawater. Seawater samples are put into a 1 liter feeding back, the feeding back tool is opened in the waters containing coral reefs to take sample water, then closed and brought to the surface. The seawater sample that has been obtained in the feeding back is connected to the steringfact. The steringfact is opened to drain the sample water. The water that is drained will pass through the steringfact tube containing filter paper which functions to filter the water. The sample water trapped in the tube containing filter paper is then given an additional ATL Buffer of 720 microliters and then put into a coolbox and taken to the Laboratory to detect micro particles containing DNA, including the remains of organisms such as porifera. ATL Buffer functions to preserve and capture cells that have been released by the species (Ariyanti & Sianturi, 2019).

The extraction process begins by taking 450 microliters of sample water, added with 10 microliters of AL buffer and 10 microliters of ethanol. The sample is then homogenized and transferred to a 750 microliter spinciagen tube. The next process is to centrifuge for 2 minutes at 8000 rpm. The centrifuge functions to separate the sediment from the supernatant. The supernatant obtained is then transferred into a collection tube and added with 500 µl of AW1 buffer, then vortexed for 2 minutes at 8000 rpm. The supernatant is transferred into a new collection tube and added with 500 µl of AW2 buffer and centrifuged for 3 minutes at 14000 rpm, this step is done twice with the same speed and time. The supernatant is transferred to a new tube and added with 1.5 ml of AE buffer, stored at room temperature for 7 minutes, then centrifuged for 2 minutes at 8000 rpm. The DNA sample results were transferred into a new tube and stored in a freezer at a temperature of -20°C to maintain the structure of the DNA obtained. (Setiawan *et al.*, 2020).

DNA before PCR testing needs to be tested for DNA quality first. DNA quality testing can be done by electrophoresis. DNA quality testing is done by taking a DNA sample with a loading buffer and then inserting it into the gel well on the electrophoresis device. Apply 100 volts of electricity for 30 minutes. The next step that can be done is special coloring and then observing using UV light. This coloring can capture the wavelength of the DNA band under UV light exposure, this step is done by inserting the Gel into the UV Transilluminator to observe the wavelength of the DNA band and the length of the Base Pair (Puspitaningrum *et al*., 2018).

DNA Concentration Testing is important to do before proceeding to the PCR stage. The Nanodrop method is used to measure its concentration with a certain absorbance. DNA

concentration measurements are carried out using a nanodrop test with a wavelength of 260 to 280 nm (Sophian & Yustina, 2023).

PCR is a stage used to amplify target DNA segments. This process requires adding primers to specifically amplify target genes. The primer used in this process is COI (Cytochrome oxidase I). The PCR reaction is carried out to amplify target DNA segments. In this process, the target DNA in the sample is denatured or separated into two strands, the primer then undergoes annealing or attaches to the complementary strand, and DNA polymerase undergoes extension or extends the primer to form a new DNA segment (Setyawati & Zubaidah, 2021). The PCR results that have been carried out can be continued to the Sequencing stage.

The DNA sequence produced after going through the data processing process through the QIMEY2 software and to analyze the DNA sequence using the FASTQ Software. There is also DADA2 Software which functions to perform quality filters, cutting, de-noising, merging forward and reverse sequence data. Species abundance analysis is presented in tabular form and explained descriptively.

Data Analysis here is used to calculate the Abundance of species. The abundance of species is the total number of individuals of different species in an environment. The calculation of the relative abundance of Porifera species is done by measuring the comparison between the number of individuals of a species with the total number of individuals of the species found. Analysis of the relative abundance of Porifera species can be measured using the following formula (Latuconsina *et al.*, 2012):

$$KS = \frac{ni}{N} X\ 100\%$$

Information:
KS : relative abundance of Porifera species (%)
Ni : total individuals of each Porifera species
N : total individuals of all Porifera species

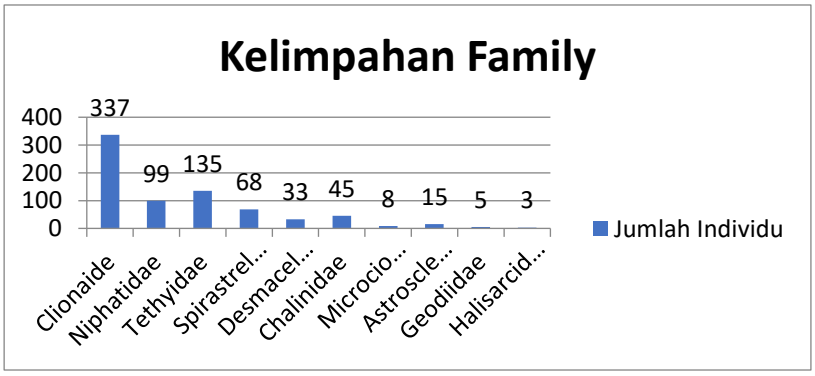
RESULT

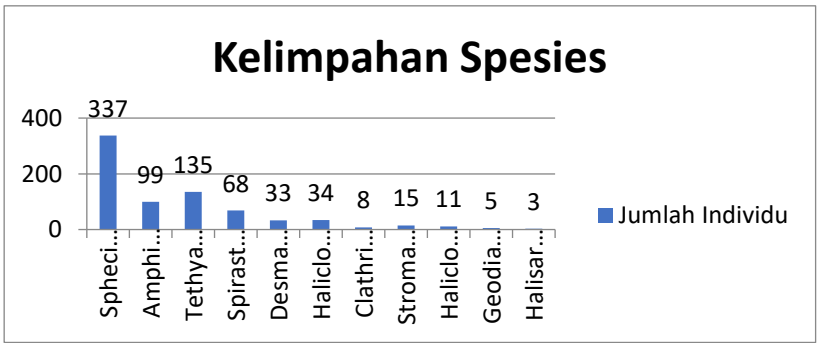
1. Table 1. Results of Porifera Species Diversity in Takat Reef Waters

Kingdom	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
Eukaryota	Porifera	Demospongiae	Clionaida	Clionaide	Spheciospongia	*Spheciospongia semilunaris*
Eukaryota			Haplosklerida	Niphatidae	Amphimedon	*Amphimedon* sp
Eukaryota			NA	NA	NA	*Tethya irisae*
Eukaryota			NA	NA	NA	*Spirastrella* sp. *SPS.2*
Eukaryota			Heterosclero morpha	Desmacellida	Desmacella	*Desmacella cf. anexa PP-2022*
Eukaryota			Haplosclerida	Chalinidae	Haliclona	*Haliclona toxia*

Animalia			Poecilosclerida	Microcionidae	Clathria	*Clathria reinwardti*
Unassigned			Agelasida	Astroscleridae	Stromatospongia	*Stromatospongia vermicola*
Unassigned			Haplosclerida	Chalinidae	Haliclona	*Haliclona amboinensis*
Eukaryota			Tetractinellida	Geodiidae	Geodia	*Geodia* sp.
Unassigned			NA	Halisarcidae	Halisarca	*Halisarca caerulea*

2. Family Abundance and Species Abundance





3. Table 2. Abundance of Individuals and Relative Abundance of Porifera Species

NO	Spesies	Number of Species	Relative Abundance of Porifera Species in Takat Reef (%)
1	*Spheciospongia semilunaris*	337	38,74
2	*Amphimedon* sp	99	11,38
3	*Tethya irisae*	135	15,52
4	*Spirastrella* sp.	68	7,82
5	*Desmacella cf.*	33	3,79

6	*Haliclona toxia*	34	3,91
7	*Clathria reinwardti*	8	0,92
8	*Stromatospongia vermicola*	15	1,72
9	*Haliclona amboinensis*	11	1,26
10	*Geodia* sp.	5	0,57
11	*Halisarca caerulea*	3	0,34
TOTAL		870	100

DISCUSSION

1. Diversity of Porifera Species in Takat Coral Waters

The results of the data in the Takat Coral Waters found 11 Porifera Species. These species come from 10 different Families and 1 class, namely Demospongiae. There are two species of Porifera whose classification is in the Order, Family, Genus, and there is one species of Porifera whose classification is not identified (NA) during the sequencing process, so that unidentified data will be BLASTed (Basic Local Alignment Search Tools) manually to obtain the classification of species that were not identified during the sequencing process. The species found are:

a. *Spheciospongia semilunaris*

The results of the sequence analysis on the sample code are DNA sequences that have similarities with the *Spheciospongia semilunaris* species. The classification according to (Van der wint *et al*., 2020) is as follows:

Kingdom : Eukariota
Phylum : Porifera
Class : Demospongiae
Order : Clionaida
Family : Clionaidae
Genus : Spheciospongia
Species : *Spheciospongia semilunaris*

Figure 2. *Spheciospongia semilunaris*
(Van der wint *et al.*, 2020)

Spheciospongia semilunaris is a species that is included in the Porifera phylum and the Demospongiae class. This species' habitat is in shallow and calm waters without being affected by waves. The morphology of this species is shaped like brown fingers and has a hard skeleton.

b. *Amphimedon* sp.

The results of the sequence analysis on the sample code, namely the DNA sequence sequence that has similarities to the genus *Amphimedon* sp. The classification according to (Shady *et al*., 2021) is as follows:

Kingdom : Eukariota
Phylum : Porifera
Class : Demospongiae
Order : Haplosclerida
Family : Niphatidae
Genus : *Amphimedon*

Figure 3. *Amphimedon* sp.
(Shady *et al*., 2021)

Amphimedon sp. is a genus that is included in the phylum Porifera and class Demospongiae. This genus's habitat is in tropical and subtropical waters. The morphology of this genus is massive, fingered, and some are shaped like tubes.

c. *Tethya irisae*

The results of the Sequence analysis on the sample code are sequences that have similarities with the *Tethya irisae* species. The classification of this species based on the sequencing results obtained N/A results for the Order, Family and Genus sections. The complete classification according to (Sorokin *et al*., 2019) is as follows:

Kingdom : Eukariota
Phylum : Porifera
Class : Demospongiae
Order : Tethyida
Family : Tethyidae
Genus : *Tethya*
Species : *Tethya irisae*

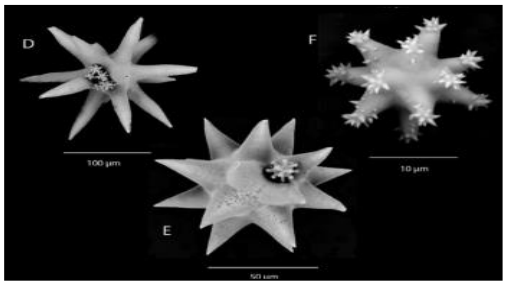


Figure 4. *Tethya irisae*
(Sorokin *et al*., 2019)

Amphimedon sp. is a species that is included in the Porifera phylum and the Demospongiae class. This species' habitat is in shallow waters. The morphology of this genus is globular or ball-like and spiny.

d. *Spirastrella sp. SPS.2*

The results of the Sequence analysis on the sample code are DNA sequences that have similarities with the Genus *Spirastrella* sp. SPS.2. The classification of this species based on the sequencing results obtained N / A results for the Ordo, Family and Genus sections. The complete classification according to (Dat *et al*., 2018) is as follows:

Kingdom : Eukariota
Phylum : Porifera
Class : Demospongiae
Order : Dictyoceratida
Family : Spirastrellidae
Genus : *Spirastrella*

Figure 5. *Spirastrella* sp. *SPS.2*
(Dat *et al.*, 2018)

Spirastrella sp. *SPS.2* is a genus that is included in the phylum Porifera and class Demospongiae. This genus's habitat is in tropical and subtropical waters. The morphology of this genus is to have a body with striking colors such as red and orange.

e. *Desmacella cf. anexa PP-2022*

The results of the sequence analysis on the sample code are DNA sequences that have similarities with the *Desmacella cf. anexa PP-2022* species. The classification according to (Morrow *et al*., 2012) is as follows:

Kingdom : Eukariota
Phylum : Porifera
Class : Demospongiae
Order : Poecilosclerida
Family : Desmacellidae
Genus : *Desmacella*
Species : *Desmacella cf. anexa*

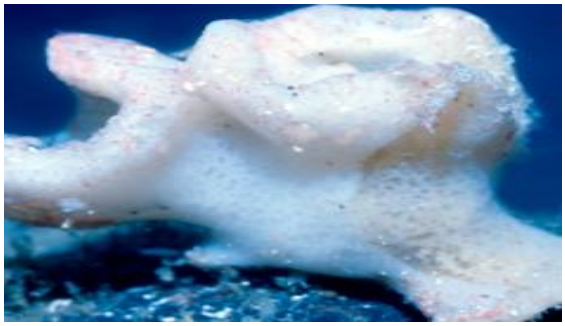


Figure 6. *Desmacella cf. anexa PP-2022.*
(Morrow *et al.*, 2012)

Desmacella cf is a genus that is included in the phylum Porifera and class Demospongiae. The habitat of this species is on the seabed. The morphology of this species is to have a soft body with needle-shaped spicules.

f. *Haliclona toxia*

The results of the Sequence analysis on the sample code are DNA sequences that have similarities with the *Haliclona toxia* species. The classification according to (Schellenberg *et al*., 2019) is as follows:

Kingdom : Eukariota
Phylum : Porifera
Class : Demospongiae
Order : Haplosclerida
Family : Chalinidae
Genus : *Haliclona*
Species : *Haliclona toxia*

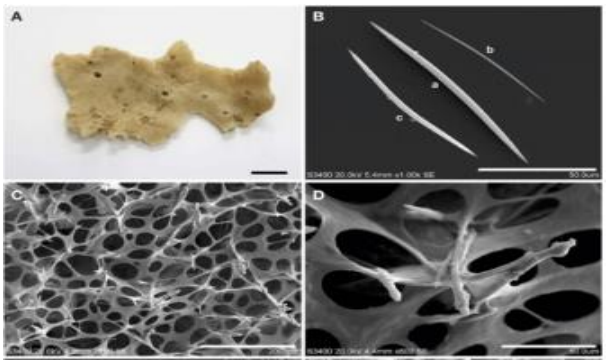


Figure 7. *Haliclona toxia*
(Schellenberg *et al.*, 2019)

Haliclona toxia is a species that is included in the Porifera phylum and the Demospongiae class. The habitat of this species is in shallow waters to medium bottoms. The morphology of this species is to have a branched body and has a smooth surface texture.

g. *Clathria reinwardti*

The results of the sequence analysis on the sample code are DNA sequences that have similarities with the *Clathria reinwardti* species. The classification according to (Trang *et al*., 2022) is as follows:

Kingdom : Eukariota
Phylum : Porifera
Class : Demospongiae
Order : Poecilosclerida
Family : Microcionidae
Genus : *Clathria*
Species : *Clathria reinwardti*

Figure 8. *Clathria reinwardti*
(Trang *et al.*, 2022)

Clathria reinwardti is a species that is included in the phylum Porifera and class Demospongiae. The habitat of this species is in tropical and subtropical waters, its habitat is attached to hard coral reef substrates. The morphology of this species is composed of complex spicules and has a variety of colors.

h. *Stromatospongia vermicola*

The results of the sequence analysis on the sample code are DNA sequences that have similarities with the *Stromatospongia vermicola* species. The classification according to (Willens & Hartman, 1989) is as follows:

Kingdom : Eukariota
Phylum : Porifera
Class : Demospongiae
Order : Agelasida
Family : Stromatospongiidae
Genus : Stromatospongia
Species : *Stromatospongia vermicola*

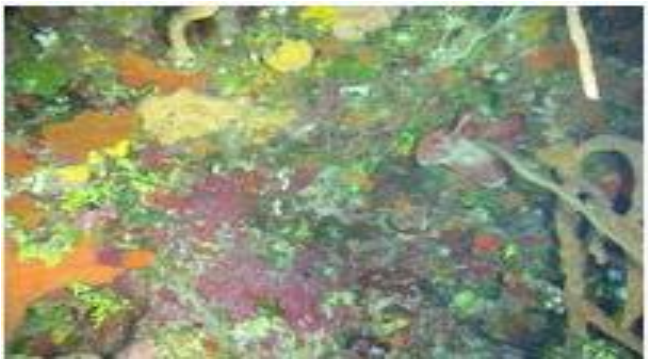


Figure 9. *Stromatospongia vermicola*
(Willens & Hartman, 1989)

Stromatospongia vermicola is a species that is included in the phylum Porifera and class Demospongiae. The habitat of this species is in deeper waters or can be found on rocky seabed substrates. The morphology of this species is arranged like a group or lump that forms a complex structure with an uneven surface or there are protrusions.

i. *Haliclona amboinensis*

The results of the Sequence analysis on the sample code are DNA sequences that have similarities with the *Haliclona amboinensis* species. The classification according to (Schellenberg *et al*., 2019) is as follows:

Kingdom : Eukariota
Phylum : Porifera
Class : Demospongiae
Order : Haplosclerida
Family : Chalinidae
Genus : *Haliclona*
Species : *Haliclona ambionensis*

Figure 10. *Haliclona ambionensis*
(Schellenberg *et al.*, 2019)

Haliclona ambionensis is a species that is included in the phylum Porifera and class Demospongiae. The habitat of this species is in shallow waters with clear water conditions. The morphology of this species is arranged like chunks that form quite large colonies.

j. *Halisarca caerulea*

The results of the Sequence analysis on the sample code are DNA sequences that have similarities with the Halisarca caerulea species. The classification of this species based on the sequencing results obtained N/A results for the Ordo section. The complete classification The classification according to (De Goeij *et al*., 2009) is as follows:

Kingdom : Eukariota
Phylum : Porifera
Class : Demospongiae
Order : Halisarcida
Family : Halisarcidae
Genus : Halisarca
Species : *Halisarca caerulea*

Figure 11. *Halisarca caerulea*
(De Goeij *et al.*, 2009)

Halisarca caerulea is a species that is included in the phylum Porifera and class Demospongiae. The habitat of this species is in the intertidal zone which still experiences ebb and flow. The morphology of this species has a smooth or soft texture and the body does not have spicules.

k. *Geodia* sp.

The results of the sequence analysis on the sample code are DNA sequences that have similarities with the genus *Geodia* sp. The classification according to (Koutsouveli *et al*., 2020) is as follows:

Kingdom : Eukariota
Phylum : Porifera
Class : Demospongiae
Ordo : Tetractinellida
Family : Geodiidae
Genus : *Geodia* sp

Figure 12. *Geodia* sp.
(Koutsouveli *et al.*, 2020)

Geodia sp. is a genus that is included in the phylum Porifera and class Demospongiae. The habitat of this genus is in deep waters to the subtropical waters of the north pole. The morphology of this genus has hard skin and looks like a rock because it is a form of self-protection from predators.

2. Abundance of Porifera Family and Species Abundance

The first bar chart in result number 2 above shows the data representation of families in the Porifera phylum that have been successfully identified. There are a total of 10 families with

varying abundances. The identified families in the Karang Takat waters include the Clionaide family with an abundance value of 337, the Niphatidae family with an abundance value of 99, the Tethyidae family with an abundance value of 135, the Spirastrellidae family with an abundance value of 68, the Desmacellida family with an abundance value of 33, the Chalinidae family with an abundance value of 45, the Microcionidae family with an abundance value of 8, the Astroscleridae family with an abundance value of 15, the Geodiidae family with an abundance value of 5, the Halisarcidae family with an abundance value of 3. The second bar chart above shows the data representation of Species in the Porifera phylum that have been successfully identified. There are a total of 11 species with varying abundances. Takat Reef Waters The identified species include Spheciospongia semilunaris species with an abundance value of 337, Amphimedon sp species with an abundance value of 99, Tethya irisae species with an abundance value of 135, Spirastrella sp. SPS.2 with an abundance value of 68, Desmacella cf. anexa PP-2022 species with an abundance value of 33, Haliclona toxia species with an abundance value of 34, Clathria reinwardti species with an abundance value of 8, Stromatospongia vermicola species with an abundance value of 15, Haliclona amboinensis species with an abundance value of 11, Geodia sp. species with an abundance value of 5, Halisarca caerulea species with an abundance value of 3.

3. Abundance of Individuals and Relative Abundance of Porifera Species

The table in the results of number 3 above shows that there are 11 species of porifera with different abundances. The species Spheciospongia semilunaris has an individual abundance of 337 with a relative abundance of 38.74%. Amphimedon sp. has an individual abundance of 99 with a relative abundance of 11.38%. The species Tethya irisae has an individual abundance of 135 with a relative abundance of 15.52%. The species Spirastrella sp. has an individual abundance of 68 with a relative abundance of 7.82%. The species Desmacella cf. has an individual abundance of 33 with a relative abundance of 3.79%. The species Haliclona toxia has an individual abundance of 34 with a relative abundance of 3.91%. The species Clathria reinwardti has an individual abundance of 8 with a relative abundance of 0.92%. The species Stromatospongia vermicola has an individual abundance of 15 with a relative abundance of 1.72%. The species Haliclona amboinensis has an individual abundance of 11 with a relative abundance of 1.26%. The species Geodia sp. has an individual abundance of 5 with a relative abundance of 0.57%. The Halisarca caerulea species has an individual abundance of 3 with a relative abundance of 0.34%. The highest abundance is in the Spheciospongia semilunaris species with a relative abundance of 38.74% and the lowest abundance is in the Halisarca caerulea species with a relative abundance of 0.34%. The number of Porifera species found in the Karang Takat Waters is 870 species. This can happen because the water parameters in the Karang Takat Waters are good, thus affecting the growth of Porifera.

CONLUSION

The conclusion of this study is that there are 10 families found in the waters of Karang Takat, including the Clionaide family, Niphatidae family, Tethyidae family, Spirastrellidae family, Desmacellida family, Chalinidae family, Microcionidae family, Astroscleridae family, Geodidae family, and Halisarcidae family. There are 11 species of the Porifera phylum found in the waters of Karang Takat, *namely Spheciospongia semilunaris, Amphimedon* sp, *Tethya irisae, Spirastrella* sp, *Desmacella cf, Haliclona toxia, Clathria reinwardti, Stromatospongia vermicola, Haliclona amboinensis, Geodia* sp., and *Halisarca caerulea*. The Spheciospongia semilunaris species is the dominant species among the other 11 species. The species with the highest relative

abundance is the species Spheciospongia semilunaris with 38.74% and the species with the lowest relative abundance is Halisarca caerulea with 0.34%.

ACKNOWLEDGEMENT

Research on the identification of Porifera Species that has been conducted in the Karang Takat Waters, we would like to thank the Institute for Research and Community Service, Trunojoyo University, Madura for financial assistance for this research, and also to Diponegoro University.

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