

**STUDY ON SEVERAL REPRODUCTIVE ASPECTS OF SOFT CORAL  
*SINULARIA FLEXIBILIS* QUOY & GAIMARD IN BARRANG LOMPO ISLAND,  
SPERMONDE ARCHIPELAGO, MAKASSAR CITY**

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**ABSTRACT**

*Sinularia flexibilis* is one species of soft coral having high economic value due to its pharmacologically active contents. This research was aimed to analyze several reproductive aspects of this soft coral using direct observation in its natural habitat, periodically collecting samples of body tissues, and then preparing and observing its histological preparation. Reproductive aspects found in this research were analyzed descriptively. Results of this research showed that the sexuality type of soft coral *S. flexibilis* was simultaneous hermaphrodite that reproduced by *spawning*, its reproductive pattern was hermaphrodite followed by *spawning*. It spawned from May-September, and the development of its sperm and oocyte was divided into four stages with different characters between one to another, particularly differed in shape, presence of organelles, and organelle colors.

Key words: *Sinularia flexibilis*, reproduction, Barrang Lompo Island

**STUDI BEBERAPA ASPEK REPRODUKSI KARANG LUNAK  
*SINULARIA FLEXIBILIS* QUOY & GAIMARD DI PULAU BARRANG LOMPO,  
KEPULAUAN SPERMONDE, KOTA MAKASSAR**

**ABSTRAK**

*Sinularia flexibilis* adalah salah satu jenis karang lunak yang memiliki nilai ekonomis karena mengandung senyawa bersifat farmakologik. Penelitian ini bertujuan untuk menganalisis beberapa aspek reproduksi karang lunak tersebut dengan metode pengamatan secara langsung di alam, mengambil sampel jaringan tubuhnya secara berkala, kemudian membuat dan mengamati sediaan histologiknya. Aspek-aspek reproduksi yang didapatkan pada penelitian ini dianalisis secara deskriptif. Hasil penelitian menunjukkan bahwa tipe seksualitas karang lunak *S. flexibilis* adalah hermafrodit simultan yang bereproduksi dengan cara *spawning*, pola reproduksinya adalah hermafrodit yang diikuti oleh *spawning*, memijah pada bulan Mei-September, dan perkembangan sperma dan oositnya terbagi atas empat tahap dengan karakter yang berbeda antara satu dengan yang lainnya, terutama perbedaan bentuk, keberadaan organel-organel, dan warna organel-organelnya.

Kata kunci: *Sinularia flexibilis*, reproduksi, Pulau Barrang Lompo

**INTRODUCTION**

*Sinularia flexibilis* is one species of soft corals having a high economical value from coral reef ecosystem. Secondary metabolites contained in this soft coral have been known to have pharmaceutical characters. Compounds produced by this soft coral are sinulariolyde, sinularin, dihydrosinularin (Tursch *et al.*, 1978; Michalek and Bowden, 1997), sandensolide monoacetate, flexibolide (Anjaneyulu *et al.*, 1997), cembranoid diterpenes, sinuflexolide,

dihydrosinuflexolide, sinuflexibilin (Duh *et al.*, 1998), diterpenes flexibilide (Aceret *et al.*, 1998), 11-epi-sinulariolide acetate, 11-dehydrosinulariolide, sinulariolide (Hsieh *et al.*, 2003). These compounds have activity as antimicrobe, cytotoxic character, and potential to be used as anticancer compounds.

Beside its significant economical potency to be developed, its population has been threatened worldwide, because most of its habitats i.e. coral reefs have experienced a serious degradation and in terrific condition.

According to Wilkinson (1993) in Tomascik *et.al.* (1997) approximately 10% of global coral reefs have been damaged, and about 30% of these damaged coral reefs will loss within 10-40 years, if level of anthropogenic pressures, population and association of the causal factors are continuously increase, while in Indonesia, according to Suharsono (1999), from 416 locations in 43 areas of Indonesian waters, there were only 6.49% in very good condition, 24.28% in good condition, 28.61% in moderate condition and 40.62% in bad condition.

In order to control high rate of habitat destruction and overexploitation, particularly for utilization as a source of bioactive compounds and production of particular bioactive compounds from technological engineering, it is urgent to provide controlling efforts, especially those related to their culture development. This culture development is focused to produce raw extract and large-scale or industrial-scale of extract fraction and to supply seed for restocking at damaged coral reef areas.

Culture development for production of raw extract and extract fraction is only achieved if reproductive biology aspects of this soft coral have been well understood. Aspects of reproductive biology that should be intensively studied are: sexuality, reproductive style, reproductive pattern, sperm and development stages. The objective of this research was to analyze several reproductive aspects of soft coral *Simularia flexibilis* i.e. sexuality, reproductive style, reproductive pattern, sperm and development stages.

## MATERIALS AND METHODS

Study location and time overall stages in this study were conducted approximately nine months from March to November 2006. Observation and sample collection were done in the vicinity of coral reef of Barrang Lompo Island, Makassar City following lunar cycle. Observation of reproductive behavior was done based on one lunar cycle, while collection of histological samples for determination of sexuality, method and pattern of reproduction, stages of gonad development were done in two lunar cycles. For determination of peak of

reproductive season, collection of histological samples was done for five lunar cycles.

## Materials and Tools

Materials and tools in this research were used in the field, laboratory and hatchery Listed in Table 1.

Table 1. Materials and tools used in this research.

No.	Materials/Tools	Utility
1.	Soft Coral <i>Simularia flexibilis</i>	Tested organism
2.	Formalin 5-10%	Preservative solution
3.	Sample bottles	Store tissue samples
4.	12 N HCl 10%	Decalcified solution
5.	Alcohol 70%-100%	Preservative solution and dehydration
6.	NaOH 10%	Solution for whitening the skeleton of coral's larvae
7.	<i>Xylol</i>	Clearing solution
8.	Parafin	Make tissue blocks
9.	Microtom and microtom blade	Incise the tissue (coral polyp)
10.	Object and cover glasses	Stick the histological incision
11.	Hematoxylin and Eosin	Coloring solution for histological preparation
12.	Light microscope	Observe the coral gonads
13.	Photomicroscope and film negative	Take photomicrograph
14.	<i>Box glass object</i>	Box to store the histological preparation
15.	Scuba diving Set	For diving during observation coral spawning time
16.	<i>Water Quality Checker</i>	Measure temperature and salinity

## Research Procedure

Observation to know sexuality, reproductive style and strategy and level of gonad development were done based on lunar cycle (Glynn *et al.* 1994) for two months (two lunar cycles). This observation was done twice for each lunar phase started from the 1<sup>st</sup> of this phase and 3-4 next days. Sample collection was commenced and ended with new month. The schedule of sample collection was presented in Table 2.

Fragments of sampled branches were fixated by putting them into fixative solution (formalin 5-10% in seawater) for a week, then

were decalcified with 12N HCl 10% solution (solved in aquadest) for 4-6 hours or more (Wallace 1985, Glynn *et al.*, 1991; 1994). Polyps that had been decalcified were stored in specific container (tissue cassette) and were rinsed at tap water for 24 hours to discharge HCl at the tissue surface. These polyps then were temporarily stored in 70% alcohol solution (Fadlallah & Pearse 1982, Glynn *et al.*, 1994) before doing histological preparation. This step followed process of standard tissue technique (Wallace 1985, Kiernan 1990, Glynn *et al.* 1991; 1994).

Table 2. Schedule of field observation and collection of histological samples for observation of sexuality, reproductive style, reproductive pattern, and characteristics of sperm and oocyt development according to the lunar months.

Research activity	Monthly week/day							
	Moon cycle I				Moon cycle II			
	1	2	3	4	1	2	3	4
collection of histological samples for observation of sexuality, reproductive style, reproductive pattern, and characteristics of sperm and oocyt development	x	x	x	x	x	x	x	x

Determination of sexuality type of corals followed Richmond & Hunter (1990); Harrison & Wallace (1990); & Richmond (1997), reproductive style of corals was analyzed based on the results of the field survey when the corals were reproducing and during the experiment of artificial spawning, or from results of observation of the histological preparation. Reproductive style of corals followed Harrison & Wallace (1990); Richmond & Hunter (1990); dan Richmond (1997), whereas, reproductive pattern was determined from data of observation on sexuality type and style.

Cell size and morphology were used to determine stages of sperm and oocyt development (Szmant, 1986). Color character occurred after fixation of Hematoxylin and Eosin was also used to determine these stages. Development of sperm and oocyt was divided

into four stages, as follows: I, II, III, and IV stages based on criterion of Glynn *et al.* (1991, 1994); and Stoddart (1984a, b) in Ward (1992).

### Data Analysis

Sexuality, reproductive style, reproductive pattern and characters of each sperm and oocyt development stage of soft coral *S. flexibilis* were presented as tables and figures to be analyzed descriptively.

## RESULTS AND DISCUSSION

### Sexuality, Reproductive Style and Pattern

Results of histological observation showed that sexuality of *S. flexibilis* was simultaneous hermaphrodite, where eggs and sperms developed at the same polyp or at different polyp but in the same colony, and achieved maturity at the same time. This result was supported by observation of spawning both in natural condition and in hatchery where the mature eggs and sperms were spawned simultaneously within the same colony. Based on this fact, it can be inferred that sexuality type of soft coral *S. flexibilis* is simultaneous hermaphrodite, its reproductive style is spawning, its reproductive pattern is hermaphrodite followed by spawning, and spawned during May-September.

Hermaphrodite sexuality type and spawning reproductive pattern as found at *S. flexibilis* in Barrang Lompo Island is different from those observed at soft coral *Briareum asbestinum* (Brazaeu and Lasker, 1990 in Seo *et al.*, 2008) in Panama, *Corallium rubrum* (Allemand, 1992 in Seo *et al.*, 2008) in Mediterranean, *Pseudoplexaura porosa* (Kapela and Lasker, 1999) in Caribbean, *Fannyella spinosa* (Orejas *et al.*, 2007) in Antarctic whose sexuality is gonochoric brooding reproductive style. Similar hermaphrodite sexuality type was also found at stony corals from families Acroporidae, Faviidae, Merulinidae, Mussidae, Pectinidae, and Pocilloporidae as studied by Harrison and Wallace (1990).

Soft coral *S. flexibilis* in Barrang Lompo Island spawned from May-September, while stony corals *Porites lobata*, *Acropora squarrosa*, and *Montipora foveola* in Central Pacific



spawned during July (Richmond and Hunter, 1990), *Goniastrea palauensis*, *Goniastrea retiformis*, and *Platygyra lamellina* in Great Barrier Reef spawned during October-August (Babcock *et al.*, 1986 in Richmond *et al.*, 1990), *Pavona varians* and *Pavona maldivensis* in Hawaii spawned during June (Kolinski and Cox, 2003), and *Madracis senaria*, *M. mirabilis*, *M. decactis* and *M. pharensis* in Curaçao spawned from April-December (Vermeij *et al.* (2003).

### Characteristics of Sperm Development

Characteristics of each sperm development stage of soft coral *S. flexibilis* could be seen at Table 4, whereas, characters of each stage could be viewed from photomicrograph of histological preparation at Figure 1.

From the results of histological observation to the colony of the soft coral *S. heflexibilis*, its sperm type could be determined as well. Soft coral *S. flexibilis* has sperm type pier-like or ovoid at head and middle parts. Its center shape was ovoid or bullet-like, and its upper end had a zone with less compact material. At the midpiece of sperm, there was mitochondrial sheet or group of single mitochondria, lipid body and complex centriol structure, and cytoplasm at neck of front sperm. This type was similar to that introduced by (Harrison 1985 in Harrison and Wallace 1990) explaining that sperm type like this was commonly found at scleractinian corals having hermaphrodite sexuality type. Further, Benayahu (1991); Gutiérrez-Rodríguez and Lasker (2004) described that at soft corals,

sperm was developed within gastrodermal mesentery, particularly in ventral and lateral parts, and attached to the mesentery during maturation process.

Sperm diameter of *S. flexibilis* at all stages (I-IV) was varied in size ranges and mean size. Sperm had size ranges and mean size 25-100  $\mu\text{m}$  and 66.531  $\mu\text{m}$  (Stage I), 62.5-200  $\mu\text{m}$  and 118.218  $\mu\text{m}$  (Stage II), 150-350  $\mu\text{m}$  and 197.290  $\mu\text{m}$  (Stage III), and 225-500  $\mu\text{m}$  and 327.437  $\mu\text{m}$  (Stage IV) (Table 4). Sperm diameter differences between soft coral *S. flexibilis* and species *A. dimorpha* was caused by difference in polyp size and difference in size of gastrovascular cavity at mesentery between these two soft corals.

### Characteristics of Oocyt Development

Based on modification of Glynn *et. al* (1994), characteristics of oocyt development of soft coral *S. flexibilis* was divided into four oocyt development stages as presented at Table 5 with structure as shown at Figure 2.

Oocyt character I of soft coral *S. flexibilis* found during this research (Table 4) was relatively different from oocyt characters I, II, III, and IV of coral *Caulastrea furcata* and *Lobophyllia corymbosa* as studied by Kawareo *et.al.* (2007). According to Kawareo *et.al.* (2007), oocyt characteristics I of coral *C. furcata* and *L. corymbosa* had relatively large nucleus and the oocyt tend to occur singly. Cortical wall was still very thin, and contain less

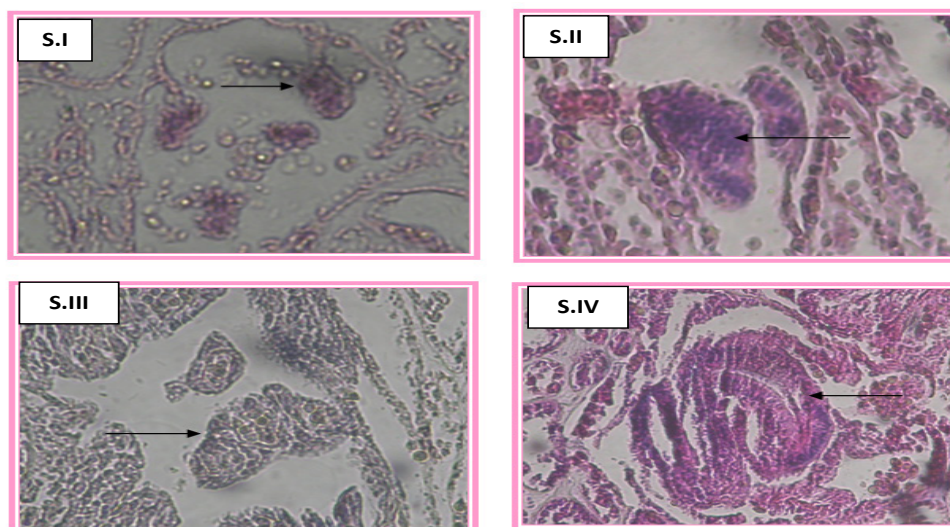


Figure 1. Sperm structure at soft coral *S. flexibilis* obtained from photomicrograph of histological preparation

Table 3. Characteristics of each sperm development stage of soft coral *S.flexibilis* based on Photomicrograph of Histological preparation

Sperm Maturation Stage	Characteristics of <i>S. flexibilis</i> Sperm	Sperm Diameter ( $\mu\text{m}$ )	
		Range	Mean $\pm$ (SE)
S.I	Like a dumbbell, small and clumped, filled by interstitial cells in mesoglea, dark bluish red color	25 - 100	66.531 $\pm$ 1.626
S.II	Its shape started to prolong and spherical, showed a distinct sperm border, extended to mesoglea, bluish violet color	62.5 - 200	118.218 $\pm$ 2.616
S.III	Prolonged, there was distinct lumen and tail, more solid spermatocyt nuclei with dark color (blue/red)	150 - 350	197.290 $\pm$ 5.061
S.IV	Prolonged (spherical oval), there was solid lumen and decreased cytoplasm volume and had distinct tail, dark blue/red color	225 - 500	327.437 $\pm$ 8.583

Table 4. Characteristics of Oocyt Development at soft Coral *S. flexibilis* Based on Results of Histological Observation

Oocyt Maturation Stage	Charac-teristics of <i>S. flexibilis</i> Oocyt	Oocyt Diameter ( $\mu\text{m}$ )		Nucleus Diameter ( $\mu\text{m}$ )		Diameter ratio ( $\mu\text{m}$ )	
		Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	Ran-ge	Mean $\pm$ SE
Oocyt I	Originated from extension of interstitial cells, rounded and small, red and pale in color, unclear wall and nucleus	25 - 87.5	38.6 $\pm$ 1.4	0	0 $\pm$ 0	0	0
Oocyt II	Rounded (oval), larger, red, unclear nucleus, thick wall had been formed	37.5 - 150	84.0 $\pm$ 3.5	0	0 $\pm$ 0	0	0
Oocyt III	Rounded (tend to ellipse), there were more solid fatty granules, distinct nucleus and wall, bluish red in color	100 - 275	150.3 $\pm$ 6.9	25 - 62.6	40.6 $\pm$ 10.3	3.7	1
Oocyt IV	More oval in shape, larger and more solid size, its position moved to the edge, dark red in color, there were sub-nucleus within several nucleus	187.5 - 625	277.0 $\pm$ 12.5	37.5 - 150	52.1 $\pm$ 8.4	5.3	1

cytoplasm and fatty granules. Oocyt character II had increased cytoplasm volume and egg size. Sub nucleus has been differentiated from the nucleus. Vitellogenesis has occurred, however, numbers of fatty granules was still limited with relatively large diameter. Oocyt character III had high cytoplasm volume and high content of fatty granules; however, cortical membrane has not well developed yet. Oocyt character

IV was mature oocyt. Cortical membrane has compressed and clearly observed compact and solid fatty granules.

Oocyt development of soft coral was also developed at mesentery at polyp autozoid calyx, similar to sperm development. After maturation, oocyt moved from mesentery to gastrovascular cavity. Immature eggs hanged at mesentery, and gradually mature eggs detached from the

mesentery (Kawaroe *et.al.*, 2007; Seo *et al.*, 2008). Autozoid or fertile polyps had separate gonads (male and female). Gonad was located at each mesentery. Reproductive processes included egg and sperm releasing by each polyp into water column. External fertilization occurred outside the coral body. Larvae formed had cilia, and then freely swim to look for suitable hard substrates for attachment before forming polyp or new colony (Manuputty, 2002).

Oocyt diameter of *S. flexibilis* at various stages (I-IV) had different size ranges and mean size. Oocyt stage I had size range between 25-87.5  $\mu\text{m}$  and mean size was 38.6  $\mu\text{m}$ . Oocyt stage II had size range between 37.5-150  $\mu\text{m}$  and mean size was 84.0  $\mu\text{m}$ . Oocyt stage III had size range between 100-275  $\mu\text{m}$  and mean size was 150.3  $\mu\text{m}$ , and Oocyt stage IV had size range between 187.5-625  $\mu\text{m}$  and mean size was 277.0  $\mu\text{m}$  (Table 5). Besides difference in size range and mean diameter, oocyt of *S. flexibilis* had also difference in size range and mean size of nucleus, especially at oocyt stage

III and IV, while oocyt I and II have no clear nucleus.

As a comparison, oocyt diameter of soft coral *A. dimorpha* as studied by Seo *et al.* (2008) had relatively different size ranges and mean size compared to *S. flexibilis* at all stages of oocyt development. Seo *et al.* (2008) found oocyt I of coral *A. dimorpha* had size <51  $\mu\text{m}$  and mean diameter of 43  $\mu\text{m}$ . Oocyt II had diameter range of 51-175  $\mu\text{m}$  and mean diameter 114  $\mu\text{m}$ . Oocyt III had diameter range between 176-275  $\mu\text{m}$  and mean diameter was 211  $\mu\text{m}$ . Oocyt IV had diameter range between >276  $\mu\text{m}$  and mean diameter was 359  $\mu\text{m}$ , while Kawaroe *et.al.* (2007) studied oocyt development of coral *Caulastrea furcata* and found mean diameter of oocyt I by 0.275  $\mu\text{m}$ , oocyt II by 0.533  $\mu\text{m}$ , oocyt III by 1.508  $\mu\text{m}$ , oocyt IV by 2.424  $\mu\text{m}$ , and at species *Lobophyllia corymbosa*, they found mean diameter of oocyt I, II, III and IV as much as 0.185  $\mu\text{m}$ , 0.456  $\mu\text{m}$ , 1.092  $\mu\text{m}$ , and 1.558  $\mu\text{m}$ , respectively.

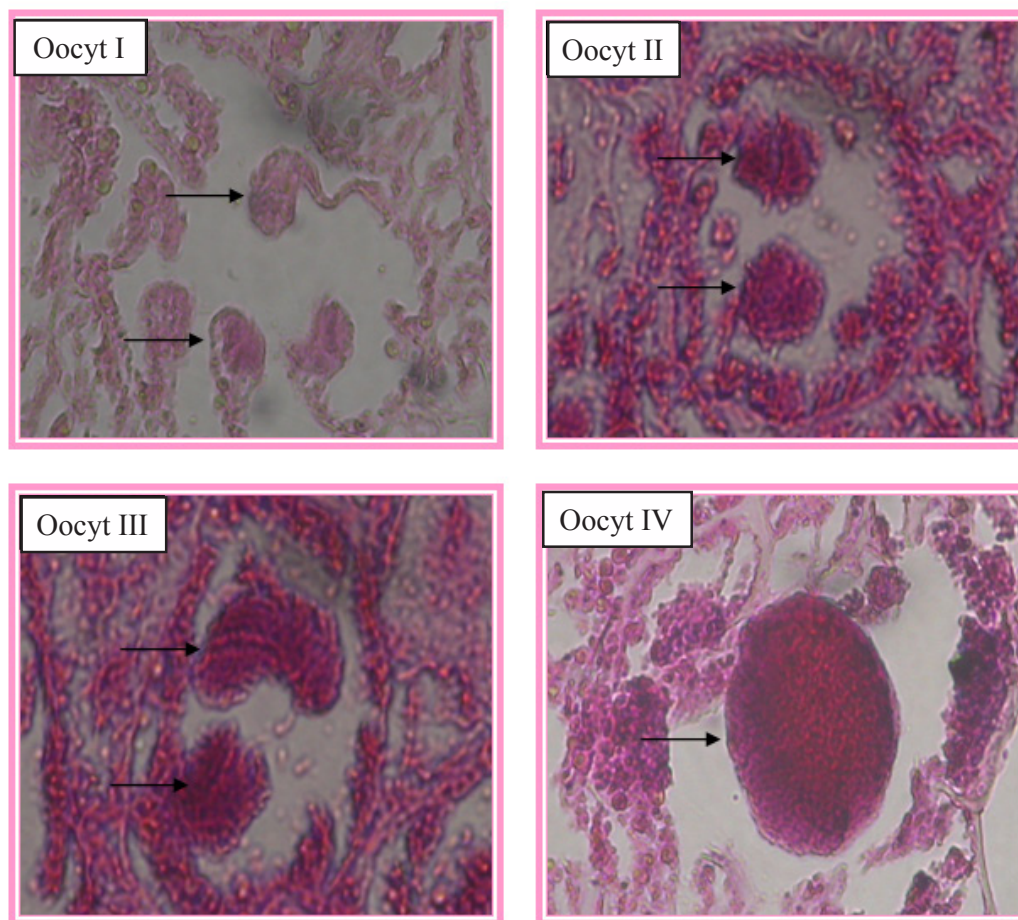


Figure 2. Oocyt structure (I-IV) of soft coral *S. flexibilis* obtained from photomicrograph of histological preparation



## CONCLUSION

Sexuality type of soft coral *S. flexibilis* was simultaneous hermaphrodite reproducing spawning, and its reproductive pattern was hermaphrodite followed by spawning, while sperm and oocyt maturity was divided into four stages each, where each stage has different characters compared to others, particularly in shape and presence of organelles.

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