

## Morphology and Anatomy of Corallimorpharian *Metarhodactis* aff. *boninensis* from Thailand

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### ABSTRACT

Corallimorpharians are sessile cnidarians that are widely distributed from tropical to the polar region. Corallimorpharians exhibit a high level of intraspecific morphological variation and this causes problems in identification and uncertainty of the species boundary. *Metarhodactis* is a monotypic genus of the family Discosomidae and widely distributed in Thai waters. However there were a few studies on the detailed morpho-anatomical structure of *Metarhodactis* species in Thailand. This study aimed to examine the morphological and anatomical characteristic of *Metarhodactis* aff. *boninensis*, and to confirm the taxonomic status of this species. Our specimens collected from Prachuap Khiri Khan Province, Thailand, exhibited an oval-shaped oral disc with curled edge and produced only unbranched papilliform tentacles. Zooxanthellae densely inhabit in the tentacle gastrodermal tissue. *M. aff. boninensis* bears only one nematocyst in which six cnidae types were found. Holotrichs III and microbasic *p*-mastigophores were the most abundant cnidae, followed by holotrichs I. Male and female reproductive organs were observed in separate individuals. It was obvious that the *M. aff. boninensis* in this study was clearly distinct from the original description of *M. boninensis* from Japan on the basis of tentacle shape, cnidae type, and its locality. We suggest that further study to clarify the taxonomic position of *M. aff. boninensis* is required considering a combined data of morpho-anatomical characteristics and molecular markers.

**Keywords:** Corallimorpharian, Cnidarian, Histology, *Metarhodactis* aff. *boninensis*, Morphology, Anatomy, Taxonomy

### INTRODUCTION

Corallimorpharians are marine invertebrates of the phylum Cnidaria. Their internal morphology is similar to that of corals, but the aragonite skeleton, muscles, siphonoglyphs on stomodaeum and ciliated lobes on mesenterial filaments are absent. Its external morphological structure: pedal disc, oral disc, column, and tentacles are similar to the sea anemones. Moreover, the molecular phylogenetic analyses showed a closer relationship between

corallimorpharians and corals than sea anemones (Fautin, 1992; Daly *et al.*, 2007).

Corallimorpharians live as a single polyp or colony adhering to underwater substrates in coral reefs. They are commonly found in shallow reefs and some species formed in the deeper area (Fautin, 2011). Tropical corallimorpharians occur together forming large groups by asexual reproduction to increase the number and domination of space.

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Colorful and polymorphic corallimorpharians are also popular in marine invertebrate aquarium business. Taxonomists recorded 65 species of Corallimorpharia, afterward the list of acceptable identification is 46 as valid species (Fautin *et al.*, 2007). Supporting data for taxonomic classification of corallimorpharians are minimal, therefore, it is difficult to identify the species. The generic nomenclature of the name *Metarhodactis* Carlgren, 1943 was described as the type species, *M. boninensis* Carlgren, 1943. However, the morphological characteristics of our corallimorpharian's morphotypes differed from *M. boninensis* that Carlgren (1943) and Cha (2007) described. Therefore, the objective of this research was to study and describe the morphological and anatomical characteristics of *M. aff. boninensis* in details aiming to apply for the taxonomical study of Corallimorpharian in the future.

## MATERIALS AND METHODS

*Metarhodactis* aff. *boninensis* samples were collected from a giant clam culture pond at Prachuap Khiri Khan Coastal Aquaculture Research and Development Center, Department of Fisheries, Prachuap Khiri Khan province, and delivered to the Ornamental Fish and Aquatic Plants Research and Technology Transfer Center, Faculty of Fisheries, Kasetsart University, Bangkhen Campus, Bangkok. The living specimens were cultured in one metric ton fiber glass tank with 30 ppt artificial sea water with a temperature of 27–29°C. Recirculating system (10 % flow rate), protein skimmer, bubble aeration by sand air stones and biological filtration were used for controlling water quality under a transparent roof with 70 % sunlight. Acclimatization was done for three weeks before the study started.

### Morphological study

Thirty living specimens (n=30) were sampled for morphological study. Coloration, size and tentacle characteristics were recorded. Oral disc, oral orifice, column, and pedal disc diameter, including column height were measured with a

caliper to the nearest 1 mm. Tentacle characteristics such as shape and their arrangement were studied following Cha (2007).

### Anatomical study

Anatomical study of *M. aff. boninensis* included histology and cnidae cytology. In addition, the histological technique was applied for describing the microscopic anatomy of tissues and positioning of internal organs. Cnidae cytology is a part of the anatomical tissue that deals with capsule size and reversible tubule.

The studied histological protocols from Luna (1968) was applied in this study. Whole *M. aff. boninensis* specimens without substrate were preserved in Bouin's solution for 24 hours. The target tissue was trimmed to 4 mm<sup>2</sup> and 1 mm in thickness. Tissue samples were immersed in a serial isopropanol, xylene and finally embedded in paraplast. Transverse and longitudinal serial sections were cut to 5 µm in thickness using a rotary microtome, stained with hematoxylin and eosin (H&E stain). After staining, the sections were mounted with a glass coverslip and observed under a microscope for comparing and contrasting cells. The cells and structural components of tissues were noted.

Cnidae cytological study was observed from fresh cnidae in different tissues of *M. aff. boninensis* and viewed under a light microscope. Squash preparations for cnidae study followed the method of Östman (1991). The undischarged cnidae were collected from different parts of the polyp, discal tentacles, marginal disc, column, pedal disc and mesenterial filament, and were observed under a microscope. Cnidae were classified following Östman (2000) and Fautin *et al.* (2009) that dealt with characteristics of the capsule size and coiling tubule.

Histological tissues and cytological cnidae were recorded by a camera. The width and length of undischarged cnidae were measured utilizing a Dino Capture 2.0 version 1.4.2.D program of the versatile digital microscope.

## RESULTS

Our results are comprised into two parts, including morphology and anatomy of *Metarhodactis* aff. *boninensis*. Morphological investigations exhibit the details of different parts of a body and anatomical investigations include histology of different parts of the body and cnidae cytology.

### Morphology

The body of *M. aff. boninensis* consists of an oral disc, column, and pedal disc (Figure 1). The oral and pedal discs are discoidal in form. The length from oral disc to pedal disc ranges from 8–24 mm.

The detailed morphology of oral disc, oral orifice, tentacle, column and pedal disc are further described as follows:

The oral disc is located in the utmost part of the body with a circular and curly edge (Figure 1a). Its size ranges from 25–130 mm wide, with 1–4 mm in thickness. The oral disc is divided into complete and incomplete radial rows. Complete radial rows are arranged from the oral edge to the oral orifice, consisting of 12–36 rows. The incomplete radial rows lie from the oral edge but do not reach the oral orifice, possessing 24–324 rows. Each row consists of short tentacles. The oral disc colors are reddish brown, orange, scarlet and crimson. The aboral disc, positioned opposite of the oral disc, is mostly brown in color (Figure 1b).

The oral orifice is located at the center of the oral disc with 1–3 mm height and 1–3 mm in diameter. Mostly there is only one oral orifice in the center of the oral disc, accounting for 93.33% of total examined specimen, but 2–3 oral orifices could also be found on the same oral disc, accounting for 6.67% (Figure 1c–d). The color of an oral orifice is

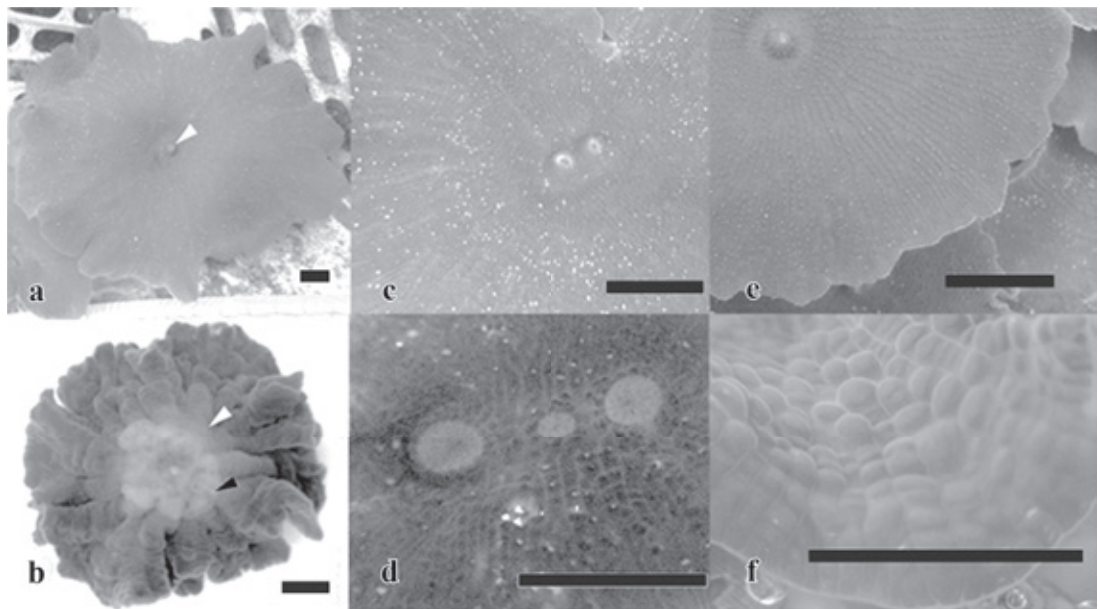


Figure 1. External morphology of *Metarhodactis* aff. *Boninensis*: (a) the oral orifice at the center of oral disc (white arrow); (b) pedal disc (white arrow) and column (black arrow) at the center of aboral; (c) two oral orifices at asexual reproduction state; (d) three oral orifices state of asexual reproduction; (e) tentacle at the oral disc surface; (f) papilliform tentacles. Bar = 1 cm.

the same as the color of the oral disc. The opening end of the oral orifice is an upside down cone in shape. Stomodaeum contains radially arranged white cilia, which attach to the inner edge of the oral orifice.

Tentacles are papilliform, 1–2 mm height and width, with no branches. Tentacles are only found on the oral disc (Figure 1e) and arranged along the radial row of the endocoel and exocoel (Figure 1f).

The column is the part that connects the oral disc and the pedal disc. It is cylindrical, 5–15 mm long and 7–24 mm in diameter, with a smooth surface and brown in color. The pedal disc is the

bottom part of the body that attaches to the substrate. The shape depends on the substrate formation. Size of pedal disc is typically smaller than the oral disc, approximately 10–50 mm in diameter and 1–3 mm in thickness.

### Anatomy

The main structure of *M. aff. boninensis* tissue contains 2 layers (Figure 2a): epidermis and gastrodermis with mesoglea in between these two layers (Figure 2b). The epidermis is a simple permanent tissue consisting of epitheliomuscular cells arranged in a single layer with uncertain shapes and sizes. The nucleus is elliptical in shape,

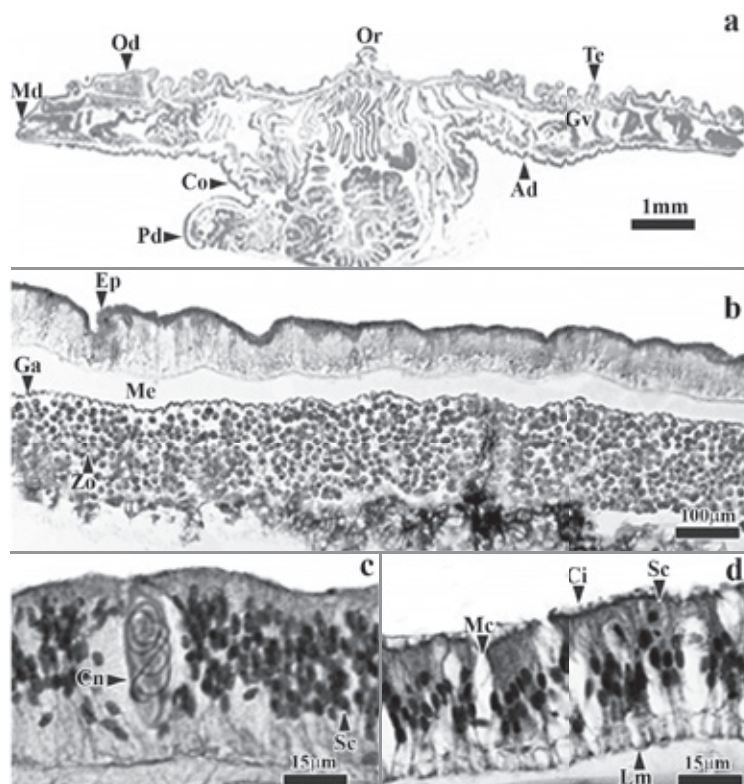


Figure 2. Internal anatomy and histology of *Metarhodactis aff. boninensis*: (a) vertical section of whole specimen showing all parts of the body; (b) vertical section of oral disc showing the main structure; (c) and (d) close-up views of the epidermis showing cnidocytes and mucous cells. Od = oral disc; Pd = pedal disc; Or = oral orifice; Ad = aboral disc; Co = column; Md = marginal disc; Te = tentacle; Ep = epidermis; Ga = gastrodermis; Me = mesoglea; Zo = zooxanthellae; Cn = cnidocyte; Lm = longitudinal muscle fibrils; Mc = mucous cell; Sc = supporting cell; Ci = cilia of cell; Gv = gastrovascular cavity



4.16–5.62  $\mu\text{m}$  in length and 1.86–2.24  $\mu\text{m}$  in width, located close to the basement of the cell. All epidermal cells are positioned at the same basement membrane. The basement membrane persists and acts as the longitudinal muscle. Some epidermal cells possess short cilia (Figure 2d). In the epidermal tissue, there are many different types of cells, for example, cnidocytes (Figure 2c), supporting cells (Figure 2d), and mucous cells (Figure 2d).

The mesoglea is a clear jelly-like substance separating the epidermal and gastrodermal tissues. A fusiform-shaped sensory cell is found scattered throughout the mesoglea (Fig. 3a). The thickness of the mesoglea depends on the tissue structure of the different body parts. The gastrodermis is composed of columnar epithelial cells with the base extending into myofibrillar. This tissue voluminously contains zooxanthellae, the photosynthetic algae (Figure 3b).

The oral disc comprises of two tissue layers, namely, epidermis and gastrodermis, and the gastrovascular cavity. The epidermis is 54.83–114.38  $\mu\text{m}$  in thickness, and contains cnidocytes, supporting cells, and mucous cells. Gastrodermal cells are arranged in a radial manner and form

a mesenterial filament. Mesenterial filaments are elongated and contain the mesoglea. These filaments typically occur in pairs, with approximately 36–180 pairs. Mesenterial filaments are divided into two types: complete and incomplete mesenteries (Figure 4a).

Both complete and incomplete mesenteries occur in pairs. Complete mesentery extends from outer oral disc mesoglea to stomodaeum mesoglea, resulting in the connection of oral disc and stomodaeum mesoglea, whereas incomplete mesentery runs from the outer oral disc, but not reaches to stomodaeum. The gastrovascular cavity of the paired mesenteries is called the endocoel, and the cavity between the paired mesenteries is called exocoel (Figure 4a).

Tentacles consist of epidermis and gastrodermis (Figures 4b and 4c). The epidermis is 18.46–96.84  $\mu\text{m}$  in thickness. The tentacles are composed of cnidocytes, supporting cells, and mucous cells. Mesoglea is about 2.71–28.36  $\mu\text{m}$  in thickness. The gastrovascular forms a cavity in the center of the tentacle. Zooxanthellae reside in the gastrovascular tissue of tentacles (Figure 4c).

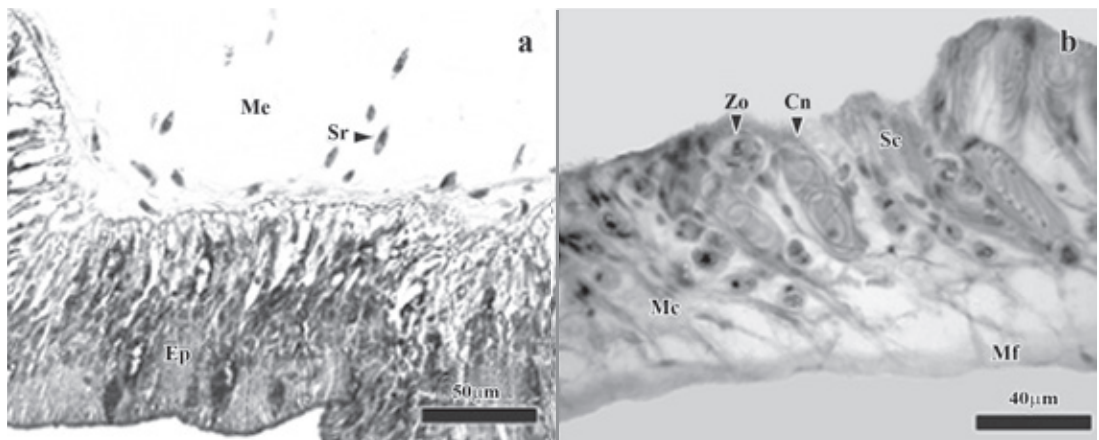


Figure 3. The mesoglea and gastrodermis of *Metarhodactis* aff. *boninensis*: (a) mesoglea of aboral disc showing the sensory cells; (b) gastrodermis showing the cnidocytes, supporting cells, and mucous cells. Sr = sensory cell; Ep = epidermis; Me = mesoglea; Zo = zooxanthellae; Cn = cnidocyte; Sc = supporting cell; Mf = myofibril; Mc = mucous cell

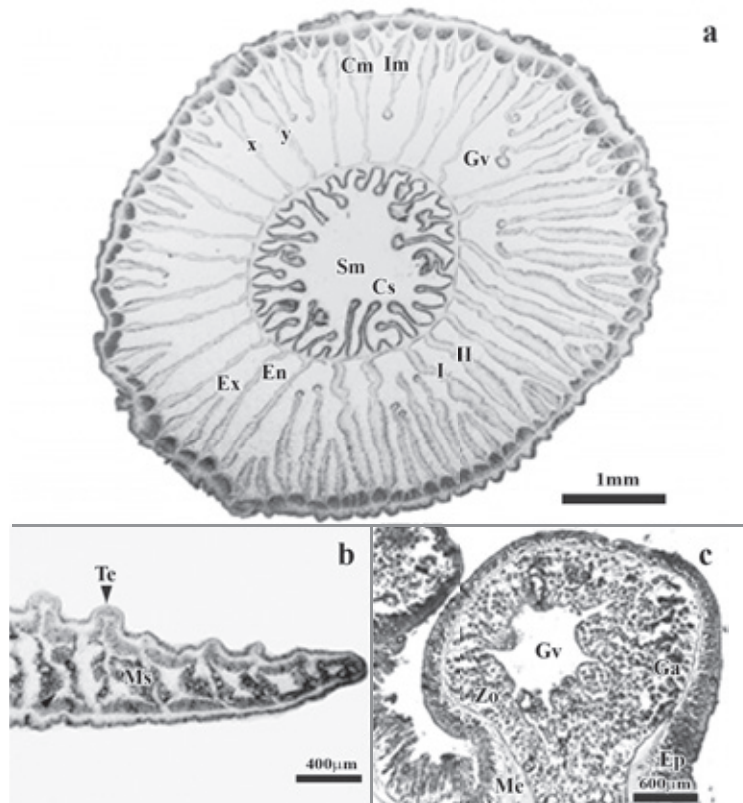


Figure 4. Histological section of oral disc: (a) horizontal section of oral disc showing the cilia inner stomodaeum and radial partition by mesenterial filaments; (b) vertical section of oral disc showing a tentacle; and, (c) vertical section of a tentacle showing two tissue layers and gastrovascular cavity including zooxanthellae in gastrodermis. Ep = epidermis; Ga = gastrodermis; Me = mesoglea; Te = tentacle; Ms = mesenterial filament; Zo = zooxanthellae; En = endocoel; Ex = exocoel; Sm = stomodaeum; Cs = cilia of stomodaeum; Gv = gastrovascular cavity; Cm (I&II and x&y) = complete mesenteries; Im = incomplete mesenteries

The stomodaeum is about 1–4 mm in length, accounting for approximately one-fourth of total body height (Figure 2a). Epidermal cells develop into cilia arranged in a radial manner. The cilia are attached to the inner edge of the stomodaeum and the tip of the cilia extend proximally toward the center of the stomodaeum. The core of each cilium contains mesoglea. Cilia contain three major cell types, i.e. cnidocytes, supporting cells, and mucous cells.

The stomodaeum can be divided into three parts (Figure 5). The anterior stomodaeum is 469.32–796.44  $\mu\text{m}$  in diameter. Cilia are 212.38–

329.62  $\mu\text{m}$  in length and 187.53–230  $\mu\text{m}$  in width. The tip of cilia is directed toward the center of stomodaeum (Figure 5a). The middle stomodaeum has a diameter of 834.33–1,287.87  $\mu\text{m}$ . Cilia length and width are 465.67–942.49  $\mu\text{m}$  and 150.32–245.32  $\mu\text{m}$ , respectively. The tip of each cilium is directed apart from the center of stomodaeum (Figure 5b). The posterior stomodaeum is located close to the column. This stomodaeum is approximately 1,364  $\mu\text{m}$  in diameter. Cilia length and width are 421.82–638.45  $\mu\text{m}$  and 120.45–190.63  $\mu\text{m}$ , respectively. The posterior part begins to form a non-circular stomodaeum shape. The base of cilia was not connected to the ending part of the mesenterial filament (Figure 5c).

The column comprises of two layers: epidermis and gastrodermis (Figure 6). Each tissue contains cnidocytes, supporting cells and mucous cells. The diameter of the column is 8.20–9.65 mm (Figure 6a). The epidermis is 24.23–54.38  $\mu\text{m}$  in thickness and arranged in a circular manner. There is no stomodaeum in the column. Zooxanthellae reside in the space between the edges of the gastrodermis and the mesoglea. The gastrodermis is arranged in a radial manner and form mesenterial filaments. The mesenteries are constricted at the base, inflated at the middle, and gradually taper toward the tip (Figure 6b). The middle mesentery contains a thick mesoglea, at 16.85–24.33  $\mu\text{m}$  in thickness. The distal end of the mesoglea is dichotomously branched to border mesentery and acontia, a thread-like epidermal tissue at the end of the mesenterial filaments (Figure 6b).

*M. aff. boninensis* is dioecious. Reproductive organs are formed in mesoglea of mesentery in the column. For female reproductive organ, the ovaries contain the female gamete cell called oocytes. Oocytes are spherical, 134.52–267.42  $\mu\text{m}$  in diameter, with nucleus positioned in the center of oocyte surrounded by yolk granules (Figure 6c). For male reproductive organ, sperm pocket consists of spermatids (Figure 6d), which are subsequently transformed into sperm cells. Shape and size of sperm pocket are uncertain.

The pedal disc is composed of epidermis and gastrodermis (Figure 7). Each tissue contains cnidocytes, supporting cells, and mucous cells.

The pedal disc has a diameter of 6.24–10.36 mm (Figure 7a). The epidermal cell of the edge of the pedal disc is 32.54–119.87  $\mu\text{m}$  in thickness, and the bottom part is 8.32–14.46  $\mu\text{m}$  in thickness (Figure 7b). Mesoglea of the epidermal cell is 45.32–70.62  $\mu\text{m}$  in thickness. Zooxanthellae inhabit in the connected area of gastrodermis and mesoglea. Gastrodermis arranges in a radial manner and form mesenterial filaments. Mesenteries are constricted at the base, inflated at the middle and gradually tapering toward the end. Middle mesenteries contain thick mesoglea, with 50.54–120.32  $\mu\text{m}$  in thickness. The distal end of mesoglea is dichotomously branched to separate mesentery and acontia. Acontia is relatively large, cured and almost occupied the gastrovascular cavity of the pedal disc.

### Cytology of cnidae

All the cnidae of *M. aff. boninensis* have nematocysts and could be classified into six types: holotrichs I, holotrichs II, holotrichs III, microbasic *b*-mastigophore, microbasic *p*-mastigophore, and hoplotelic microbasic *p*-mastigophore (Figure 8). Details of the width and length of each type of cnidae capsules are presented in Table 1.

Holotrichs I is characterized by a tubular-shaped structure furnished with barbs. The tubules are coiled into three to four of the 8 figures in the capsule (Figure 8a). The capsule length and width are 121.58–146.73  $\mu\text{m}$  and 43.41–72.78  $\mu\text{m}$ , respectively. This cnidae type is found specifically around the mesenterial filament.

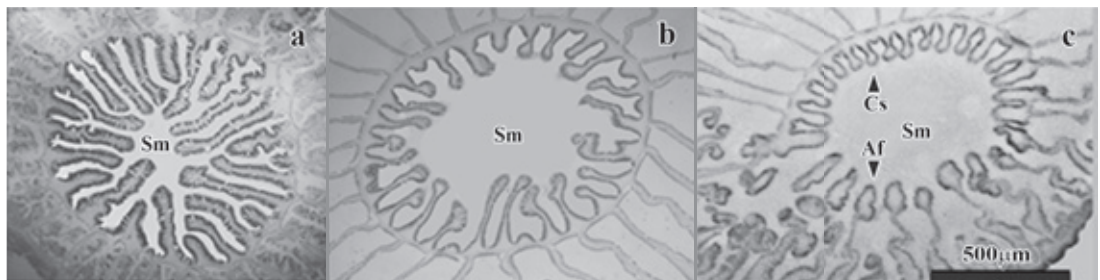


Figure 5. Vertical section of stomodaeum showing three parts: (a) anterior stomodaeum; (b) middle stomodaeum; and, (c) posterior stomodaeum. Sm = stomodaeum; Af = acontia filament; Cs = cilia of stomodaeum.

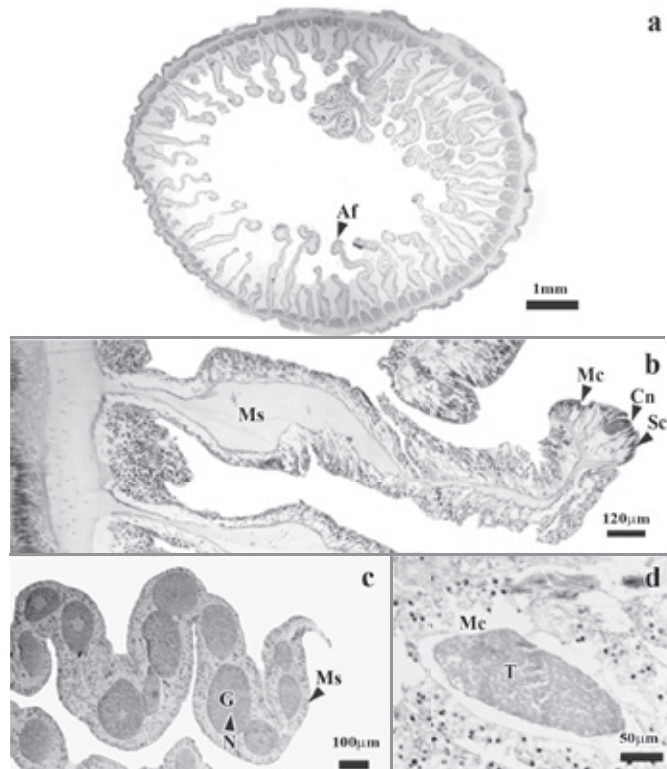


Figure 6. Histological section of a column: (a) vertical section of a column showing acontia filament and reproductive organs; (b) horizontal section of mesenterial filament showing ovary and oocytes; (c) ovary and oocytes; and, (d) sperm pocket in mesoglea of mesenterial filament. Af = acontia filament; Me = mesoglea; Mc = mucous cell; Cn = cnidocyte; Sc = supporting cell; G = granule; N = nucleus; Ms = mesenterial filament; T = testis

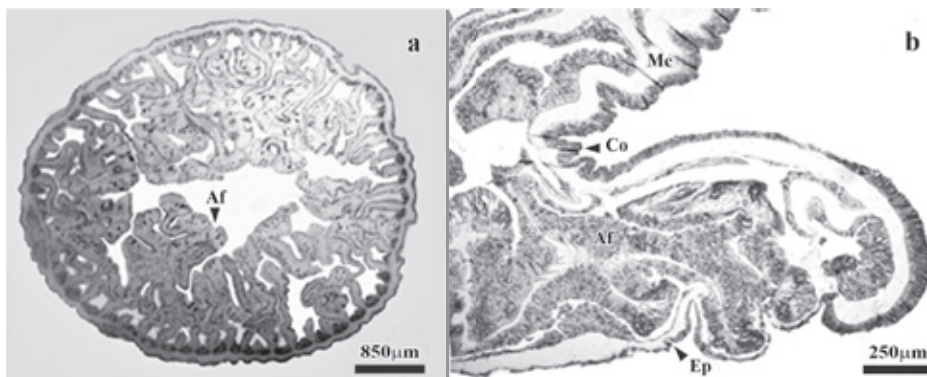


Figure 7. Histological section of pedal disc: (a) vertical section of pedal disc showing acontia filaments in gastrovascular cavity; (b) horizontal section of pedal disc showing acontia filaments. Af = acontia filament; Me = mesoglea; Co = column; Ep = epidermis.



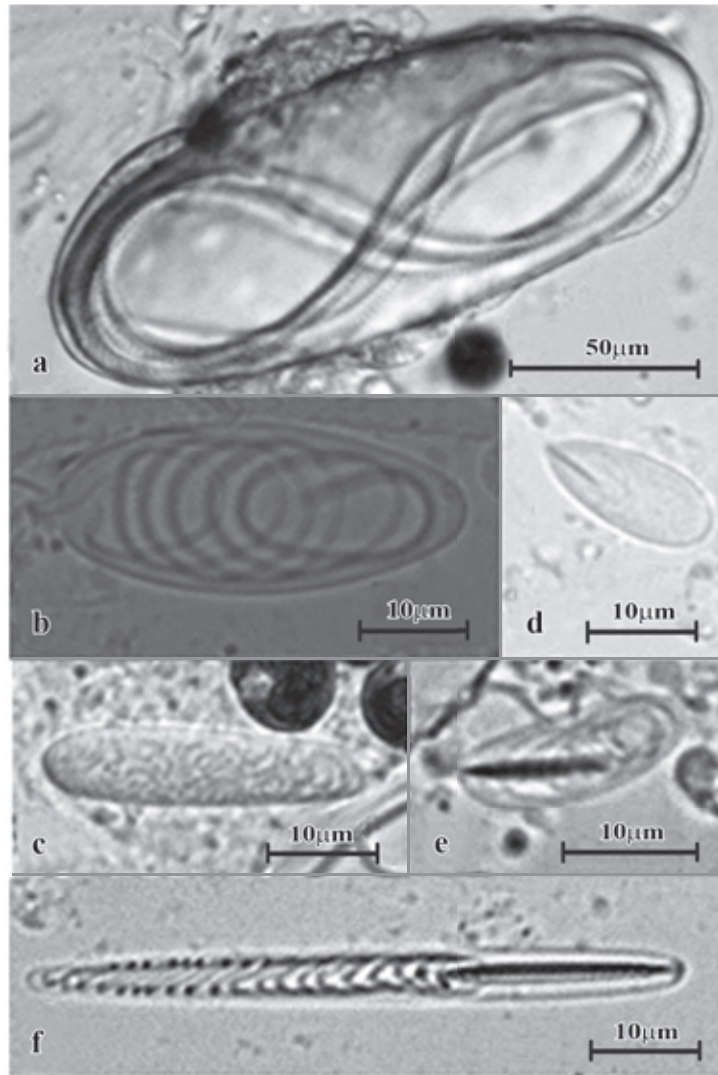


Figure 8. Cnidae of *Metarhodactis* aff. *Boninensis*: (a) Holotrichs I; (b) Holotrichs II; (c) Holotrichs III; (d) Microbasic *b*-mastigophore; (e) Microbasic *p*-mastigophore; and, (f) Hoplotelic microbasic *p*-mastigophore.

Holotrichs II is a long tubular-shaped structure featured with barbs. The tubules are coiled into three to ten loops (Figure 8b). The capsule length and width are 28.20–37.50  $\mu\text{m}$  and 9.87–14.58  $\mu\text{m}$ , respectively. This cnidae type is found in all parts of the body.

Holotrichs III is a long tubule furnished with barbs. The tubules are excessively coiled into

many loops in the capsule (Figure 8c). The capsule length and width are 22.98–33.53  $\mu\text{m}$  and 5.21–8.32  $\mu\text{m}$ , respectively. This cnidae type is found in all parts of the body.

Microbasic *b*-mastigophore is characterized by the tubule-shaped structure with a barbed (Figure 8d). The shaft is less than 3 times in capsule length. There is no V-shaped notch found at the shaft base

Table 1. Size ( $\mu\text{m}$ ) of cnidae found in different body parts of *Metarhodactis* aff. *Boninensis*

Cnidae type	Body part				
	Discal tentacle	Marginal disc	Mesenterial filaments	Column	Pedal disc
Holotrichs I (length)	-	-	121.58-146.73	-	-
(width)	-	-	43.41-72.78	-	-
Holotrichs II (length)	28.20-37.50	28.39-34.57	30.17-35.65	29.25-35.32	29.81-35.15
(width)	10.38-13.16	10.25-14.18	10.27-14.58	10.84-12.15	9.87-12.53
Holotrichs III (length)	27.37-33.35	28.73-32.65	28.46-33.53	27.36-31.64	22.98-33.41
(width)	5.86-6.67	5.64-7.63	5.84-8.32	5.82-7.74	5.21-7.42
Microbasic <i>b</i> -mastigophores (length)	11.61-15.14	10.35-14.36	13.10-15.80	13.54-15.32	12.56-16.21
(width)	2.41-3.58	2.31-4.23	4.86-5.23	4.14-5.27	4.85-6.23
Microbasic <i>p</i> -mastigophores (length)	11.61-15.14	13.74-15.91	13.51-16.58	-	13.25-18.16
(width)	2.41-3.58	4.30-5.48	4.82-6.44	-	4.61-6.11
Hoplotelic microbasic <i>p</i> -mastigophores (length)	-	-	49.89-56.71	-	-
(width)	-	-	4.63-5.22	-	-

in the unreleased state. The shaft became tapered immediately after release. The length and width of the capsule are 10.35–16.21  $\mu\text{m}$  and 2.31–6.23  $\mu\text{m}$ , respectively. This cnidae type is distributed in all parts of the body.

Microbasic *p*-mastigophore is featured by the tubule-shaped structure with a barb (Figure 8e). The tubule possesses the spine. The shaft is less than 3 times in capsule length. There is a V-shaped notch found at the shaft in unreleased state and the shaft became tapered immediately after release. The length and width of the capsule are 11.61–18.16  $\mu\text{m}$  and 2.41–6.44  $\mu\text{m}$ , respectively. This cnidae type is nearly found in all parts of the body, except in the column.

Hoplotelic microbasic *p*-mastigophore is characterized by a tube with a barb (Figure 8f). The tubule is simple and smooth. The shaft is less than 3 times in capsule length. There is a V-shaped notch found at the shaft in unreleased state and the shaft became tapered immediately after release. The length and width of capsules are 49.89–56.71  $\mu\text{m}$  and 4.63–5.22  $\mu\text{m}$ , respectively. This cnidae type is found only in the mesenterial filament.

## DISCUSSION

Our study provides insight into the morphological characteristics and anatomical features of *M. aff. boninensis* from the coast of Prachuap Khiri Khan Province, Thailand. Our study is also the first to report the reproduction and sex organs in this species. *M. aff. boninensis* has male and female organs in separate individuals. Reproductive organs are present within mesoglea of mesenteries filament. Dioecious reproduction is also reported in other corallimorpharian, for example, *Rhodactis indosinensis* (Chen *et al.*, 1995; Chadwick *et al.*, 2000) and *Corynactis californica* (Holts and Beauchamp, 1993).

In corallimorpharian systematics, tentacle shape and its arrangement are diagnostic characters at the family and genus levels (Cha, 2007). *M. aff. boninensis* possesses only one tentacle shape, the papilliform unbranched tentacles, which are distributed throughout the endocoel and exocoel rows of the oral disc. In contrast, *M. boninensis*, originally described from Japan by Carlgren (1943), exhibits two tentacle shapes: papilliform branched tentacles in endocoelic rows, and papilliform unbranched tentacles in exocoelic rows (Table 2).

Table 2. Characteristic comparison of *Metarhodactis* aff. *boninensis* and *M. boninensis*.

Characteristics	Species	
	<i>Metarhodactis</i> aff. <i>boninensis</i>	<i>M. boninensis</i>
Oral disc		
Diameter	25-130 mm	25 mm
Color	orange to red	yellow in formalin
Discal tentacle	papilliform	papilliform, branch
Discal tentacle cnidae	II, III, MB, MP	II, MB, MP
Marginal tentacle /disc	no	no
Marginal tentacle cnidae	II, III, MB, MP	no recorded
Tentacle-free zone	no	no
Column		
Length	5-15 mm	4-12 mm
Color	brown	yellow in formalin
Cnidae	II, III, MB	II, MB, MP
Pedal disc		
Diameter	13-50 mm	15 mm
Color	lighter than column	yellow in formalin
Cnidae	II, III, MB, MP	
Mesenterial cnidae	I, II, III, MB, MP, HMP	I, II, HMP
Geographic distribution	Thailand	Bonin islands Mariana islands
Reference	This study	Cha (2007)

Remarks: I = Holotrichs I; II = Holotrichs II; III = Holotrichs III; MB = Microbasic *b*-mastigophore; MP = Microbasic *p*-mastigophore; HMP = Haptotrich microbasic *p*-mastigophore.

Papilliform tentacle is somewhat short, less than 2 mm, consisting of 2 tissue layers, epidermis and gastrodermis. Zooxanthellae densely reside within tentacle gastrodermal tissue. In Anthozoa, tentacle has many important functions such as prey capture, defense from predators, natation, zooxanthellae residence, and agonistic interaction (Fautin and Mariscal, 1991). For the agonistic interaction, anthozoans have variously modified tentacles. For examples, sea anemone has specialized tentacle called acrorhagi occurring on the margin of an oral disc (Honma *et al.*, 2005), and many corals possess elongated sweeper tentacle (Williams, 1991).

In corallimorpharians, *Corynactis carnea* has capitate tentacle with acrosphere, a tissue densely packed with nematocysts (Acuña *et al.*, 2007). *Discosoma sanctithomae* has marginal tentacle with acontia filament (Miles, 1991). Additionally, *Rhodactis rhodostoma* possesses specialized bulbous marginal tentacle and this species is commonly known as killer anemone (Langmead and Chadwick-Furman, 1999). In this

present study, *M. aff. boninensis* bears only papilliform tentacles, and has no marginal tentacle for agonistic interaction. However, we found that the marginal disc of *M. aff. boninensis* contains four cnidae types, and it is possible that *M. aff. boninensis* has a marginal disc, instead of marginal tentacle, to contact with cnidarian competitor. Considering the anatomical findings, we postulate that papilliform tentacles of *M. aff. boninensis* play a vital role in providing living place for zooxanthellae and defending territory.

A cnidocyte (cnidae) is a unique character of cnidarians, which is one of the important features in corallimorpharian taxonomy (Cha, 2007; Fautin *et al.*, 2009). Cnidocytes can be categorized into three types: nematocyst, spirocysts, and ptychocysts (Watson and Wood, 1988; Fautin *et al.*, 2009). Among them, nematocyst is the most diverse group of cnidocyte and has a function to capture or immobilize the prey. Our study reveals that *M. aff. boninensis* possesses the only nematocyst, and six types of cnidae were observed. Among the six

cnidae types, holotrichs I is the largest cnidae, up to approximately 146  $\mu\text{m}$  long and 72  $\mu\text{m}$  wide. *M. aff. boninensis* produces holotrichs I only in mesenterial filaments, while other corallimorpharian species, for example, *Amplexidiscus fenestrafer*, *Rhodactis rhodostoma* and *Discosoma sanctithomae*, bear holotrichs I over all of their body parts, except the column (Langmead and Chadwick-Furman, 1999). Additionally, *R. rhodostoma* and *D. sanctithomae* showed aggressive behavior by attacking adjacent organisms in competition for space (Langmead and Chadwick-Furman, 1999). Many previous studies suggested that holotrichs cnidocytes could have a function in offending and defending other cnidarian competitors (Lang and Chornesky, 1990; Miles, 1991; Langmead and Chadwick-Furman, 1999). It is likely that corallimorpharian species bearing holotrichs cnidae are able to display a defensive and/or an aggressive response to sessile neighbors. Although there are no reports regarding the offensive response of *Metarhodactis* species, the presence of holotrichs cnidae in this study implies the possibility of *M. aff. boninensis* displaying an aggressive interaction with other organisms. We suggest that further study of behavioral ecology is needed to clarify the interaction and social behavior between *Metarhodactis* and other marine species.

The presence of hoplotelic microbasic *p*-mastigophores in the mesenterial filament is an important taxonomic criterion to distinguish *Metarhodactis* species from *Discosoma* (Cha, 2007). Our anatomical study found the hoplotelic microbasic *p*-mastigophores in mesenteries of *M. aff. boninensis*, and this clearly supports its taxonomic position within the genus *Metarhodactis*. Our *M. aff. boninensis* specimens produced holotrichs III in all body parts and did not bear microbasic *p*-mastigophores. In contrast, *M. boninensis* from Japan had no holotrichs III, but had an abundance of microbasic *p*-mastigophores in the column (Cha, 2007). Taking into account the tentacle shape and cnidae type and distribution, we presume that our *M. aff. boninensis* is clearly distinct morphologically from *M. boninensis*, and accordingly the taxonomic status of *M. aff. boninensis* is ambiguous. Recently the advent of the molecular technique is used to shed light on the taxonomy and evolution of

corallimorpharian (Cha, 2007; Nilkerd *et al.*, 2015). It is obvious that further studies on taxonomic position and phylogenetic relationship of *M. aff. boninensis* and other *Metarhodactis* species in Thailand require the combined data of morpho-anatomical characteristics and genetic markers.

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## LITERATURE CITED

- Acuña, F.H., A.C. Excoffon and L. Ricci. 2007. Composition, biometry and statistical relationships between the cnidom and body size in the sea anemone *Oulactis muscosa* (Cnidaria: Actiniaria). **Journal of the Marine Biological Association of the United Kingdom** 87: 415–419.
- Carlgren, O. 1943. East-Asiatic Corallimorpharia and Actiniaria. **Kungliga Svenska Vetenskapsakademiens Handlingar** 20 (6): 1–43
- Cha, H. 2007. **Systematics of the order Corallimorpharia (Cnidaria: Anthozoa)**. Ph.D. Thesis, University of Kansas. 217 p.
- Chadwick, N. E., M. Spiegel, and I. Nir. 2000. Sexual reproduction in the tropical corallimorpharian *Rhodactis rhodostoma*. **Invertebrate Biology** 119 (4): 361–369.
- Chen, C.A., C.P. Chen and I.M. Chen. 1995. Sexual and asexual reproduction of the tropical corallimorpharian *Rhodactis* (= *Discosoma*) *indosinensis* (Cnidaria: Corallimorpharia) in Taiwan. **Zoological Studies** 34 (1): 29–40.



- Daly, M., M.R. Brugler, P. Cartwright, A.G. Collins, M.N. Dawson, D.G. Fautin, S.C. France, C. S. Mcfadden, D.M. Opresko, E. Rodriguez, S. Romano and J.L. Stake. 2007. The phylum Cnidaria: A review of phylogenetic patterns and diversity 300 years after Linnaeus. **Zootaxa** 1668: 127–182.
- Fautin, D.G. 1992. Phylogenetic relationships among Scleractinians, Actinarians, and Corallimorpharians (Coelenterata: Anthozoa). pp. 665–670. *In* **Proceeding of the Seventh International Coral Reef Symposium**. University of Guam Marine Laboratory. Guam.
- Fautin, D.G. 2011. *Corallimorphus niwa* new species (Cnidaria: Anthozoa), New Zealand members of Corallimorphus, and redefinition of Corallimorphidae and its members. **Zootaxa** 2775: 37–49.
- Fautin, D.G., J.M. Guinotte and J.C. Orr. 2009. Comparative depth distribution of corallimorpharian and scleractinians (Cnidaria: Anthozoa). **Marine Ecology Progress Series** 397: 63–70.
- Fautin, D.G. and R.M. Mariscal. 1991. Cnidaria: Anthozoa, pp. 267–358. *In* F.W. Harrison and J. A. Westfall, eds. **Microscopic Anatomy of Invertebrates**. New York.
- Fautin D.G., T.Zelenchuk and D.R. Veendran. 2007. Genera of orders Actiniaria and Corallimorpharia (Cnidaria, Anthozoa, Hexacorallia), and their type species. **Zootaxa** 1668: 183–244.
- Holts, L.A. and K.A. Beauchamp. 1993. Sexual reproduction in the corallimorpharian sea anemone *Corynactis californica* in a central California kelp forest. **Marine Biology** 116 (1): 129–136.
- Honma, K., H. Udono, T. Kohno, K. Yamamoto, A. Ogawa, T. Takemori, A. Kumatori, S. Suzuki, T. Matsuyama and K. Yui. 2005. Interferon regulatory factor 4 negatively regulates the production of proinflammatory cytokines by macrophages in response to LPS. **Proceedings of the National Academy of Sciences of the United States of America**. 102 (44): 16001–16006.
- Lang, J.C. and E.A. Chornesky. 1990. Competition between scleractinian reef corals -a review of mechanisms and effects, pp 209–252. *In* D. Dubinsky, eds. **Ecosystems of the world**. Elsevier, Amsterdam.
- Langmead, N. and N. Chadwick-Furman, 1999. Marginal tentacles of the corallimorpharian *Rhodactis rhodostoma*. 1. Role in competition for space. **Marine Biology** 134: 479–489.
- Luna, L.G. 1968. **Manual of histologic staining methods of the armed forces institute of pathology**, 3<sup>rd</sup> ed. Mc Graw Hill, New York, 258 p.
- Miles, J. 1991. Inducible agonistic structures in the tropical Corallimorpharian, *Discosoma sanctithomae*. **The Biological Bulletin** 180: 406–415.
- Nilkerd, B., A. Swatdipong, J. Salaenoi and S. Tunkijjanukij. 2015. Pilot survey of Corallimorpharian (Cnidaria) diversity in the gulf of Thailand. **Kasetsart University Fisheries Research Bulletin** 39 (2): 31–46.
- Östman, C. 1991. Scanning electron microscopic observations on Scandinavian scyphozoans with special references to their nematocysts. pp. 283–292. *In* **Proceedings of the II Workshop on Jellyfish in the Mediterranean Sea**, Map Technical Rep. UNEP. Athens.
- Östman, C. 2000. A guideline to nematocyst nomenclature and classification, and some notes on the systematic value of nematocysts. **Scientia Marina** 64 (1): 31–46.
- Watson, G.M. and R.L. Wood. 1988. Colloquium on Terminology, pp. 21–23. *In* D.A. Hessinger and H.M. Lenhoff, eds. **The Biology of Nematocysts**. Academic Press, San Diego.
- Williams, R.B. 1991. Acrorhagi, catch tentacles and sweeper tentacles: a synopsis of ‘aggression’ of actiniarian and scleractinian Cnidaria. **Hydrobiology** 216(1): 539–545.