Asia-Pacific Network for Global Change Research (APN)

Research Institute of Aquaculture No. 3 (RIA 3)

A.V. Zhirmunsky Institute of Marine Biology,
Far East Branch of the Russian Academy of Sciences

Proceedings of the Workshop

COASTAL MARINE BIODIVERSITY AND BIORESOURCES OF VIETNAM AND ADJACENT AREAS TO THE SOUTH CHINA SEA

Nha Trang, Vietnam, November 24–25, 2011

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Vladivostok–Nha Trang
Dalnauka

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STATUS OF MARINE TURTLE POPULATIONS IN QUANG NGAI, BINH DINH AND PHU YEN PROVINCES, VIETNAM

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Introduction

From 1990 until now there was some researches of international organizations (IUCN, WWF, CMS-UNEP etc.) and national institutes about the distribution and species composition of sea turtles in Vietnam. Especially, in the report “Status of sea turtles in Vietnam” (2001) and in the “National Action Plan for Conservation Marine Turtles until 2010”, conducted by IUCN and the Ministry of Fisheries, almost all areas of Vietnamese coastal areas has been surveyed. However, researches on sea turtle population in southern – central coastal provinces are insufficient, even though this area has many long beaches, coral reefs and seagrass beds, and is an ideal place for sea nesting and foraging sea turtles. Three provinces in southern – central Vietnam in this study are Quang Ngai, Binh Dinh and Phu Yen, and they have a total of 467 km of coastline with many long and beautiful beaches. Nevertheless, this area is also a logistical center of offshore and inshore fishery industry, with thousands of fishing vehicles. These fishing and related activities may directly or indirectly affect sea turtle population in this area. Therefore, the most important and urgent task is investigating the species composition, distribution and amount of sea turtles in the areas. From this result, the relevant authorities will be able to make the plan and procedures to protect sea turtles in the area.

In order to collect sufficient data on status of sea turtle populations in southern – central area, the International Union for Conservation of Nature (IUCN), in cooperation with the Institute of Marine Environment and Resources, has conducted a research on species composition, distribution and threats to sea turtle population in three southern – central provinces: Quang Ngai, Binh Dinh and Phu Yen. Besides survey activities, the training and raising awareness course were also conducted for local fishermen and local fishery officers.

Methodology

1. Time and locations:

The survey was taken in 10 districts of three provinces: Quang Ngai, Binh Dinh and Phu Yen, from 14/9/2010 to 27/9/2010. The total length of coast is 467 km, co-ordinates from 120º42′ N to 150º25′ N.

2. Interview method:

- In each location, the following stakeholders were interviewed: the fishery management authorities, the local fishery officers, fishermen, people living around nesting beaches etc., in order to collect historical information of sea turtles.

- The interview form designed and used in survey process including the following information: the number and species of nesting sea turtle, nesting locations, nesting season, the size of species, the foraging species and locations, catching frequency, major threats in areas, etc.
- Interview at the seminar on the threat from fishing operations for sea turtles: the type of fishing, fishing gear, fishing methods that most affected the sea turtles.
- Based on surveys and interviews, survey the field at the nesting locations of sea turtles will be conducted.

3. Field survey methods:

- At the nesting beaches, conduct patrols and collect data on area characteristics and located on the map with GPS devices.
- Collecting data of species, position of nests, nesting season.
- Surveying threat to sea turtles to reproduce: current use of the beach, environment, and awareness of local residents in protecting sea turtles.

Results

The status of sea turtles at the study sites

From the information obtained from sea turtle survey in September 2010 in three provinces Quang Ngai, Binh Dinh, Phu Yen, we can propose three stages as follows (Figs. 1, 2):

- Before 1980: the period of the sea turtle population is stable, not affected much from humans.

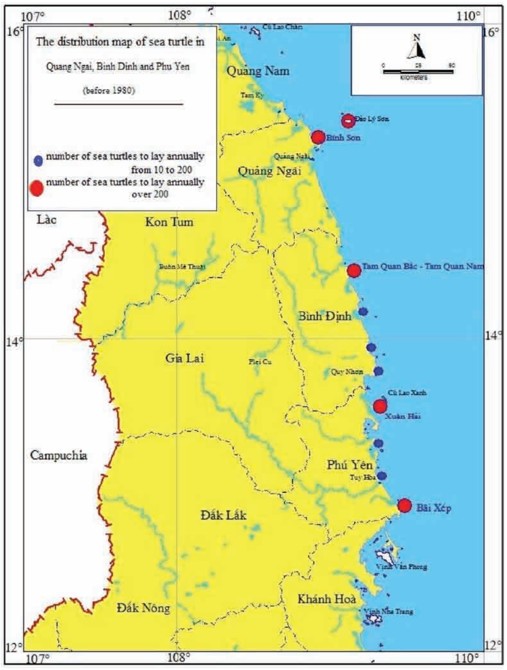

Fig. 1. The distribution map of sea turtles in Quang Ngai, Binh Dinh and Phu Yen (before 1980).

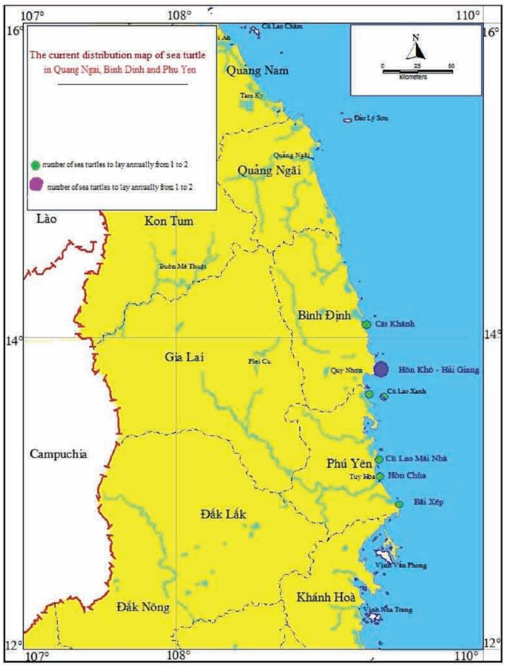

Fig. 2. The current distribution map of sea turtles in Quang Ngai, Binh Dinh and Phu Yen.

- From 1980 to 2000: stage of serious decline due to overexploitation, under a lot of negative impacts from humans.
- From 2000 to present: populations have been severely depleted.

Quang Ngai Province

In the 1980s, about 200 individuals of sea turtle laying eggs, mainly Green turtles species (*Chelonia mydas*) (local names: Vich, Trang Bong, Du) and Hawksbills sea turtle (*Eretmochelys imbricata*), and a small number of Leatherback sea turtle (*Dermochelys coriacea*) have layed eggs on the beaches of Quang Ngai Province. Among these beaches, Bai Dai (Long Beach) has a length of about 10 km, belonging to three communes: Binh Chau, Binh Phu, Binh Hai, and it was the biggest nesting beach. Around the Ly Son Island, sea turtles nestings were found in hundred times every year at the beaches of An Hai commune, Bai Dai of An Vinh Long commune.

Nevertheless, after a long period of exploitation, almost all the breeding sea turtles at these beaches have been eliminated. From the 2000s until now, it can hardly be seen a sea turtle go to nest at Quang Ngai's beaches, even in offshore beaches at Ly Son Island, according to the comments of local people.

Binh Dinh Province

In Binh Dinh Province, there are two species of sea turtles nesting: Green turtle and Hawksbill turtle. From 1980 and earlier, most of the beaches of Binh Dinh had nesting sea turtles. In some concentrated beaches, the number of breeding sea turtles was up to 40–90 nesting females each year, such as Hoai Nhon beach (10 km long), the beach at Cat Tien–Nhon Ly commune (7.5 km long), and the beach at Nhon Hai commune. From 1980 to 2000, while the number of nesting sea turtles has been seriously decreased, some turtles still appeared on beach of Tam Quan Bac – Tam Quan Nam. From 1990 to 1995, people still caught about 40 individuals a year in whole province. However, from 2000 until now, sea turtles are have hardly been seen nesting in the above beaches. The only two places where Green sea turtles still lay eggs are Hai Giang beach and Hon Kho – Nhon Hai commune – Quy Nhon City, with a small number of about 20 females totally. The beach at Hon Kho Island is about 100 m length but only 40 m is suitable for sea turtles to lay eggs.

Green turtles and Hawksbill turtles are still foraging in Binh Dinh's water area. In a rock reef at Nhon Ly commune, in 2010 the fishermen working on bottom trawl fishing boat have seen 5 individuals of Green turtles. In Hoai Nhon, Phu My and Phu Cat districts, within the years of 2008 and 2009, a fisherman reported seeing a 40 kg weight Hawksbill turtle and about 200 kg Leatherback turtle in the offshore water area.

Phu Yen Province

Based on information recorded in Phu Yen, only two breeding species of sea tutles are Green turtle and Hawksbill turtle and three foraging species are Green turtle, Hawksbill turtle and Leatherback turtles. In 1980s and earlier, coastal areas of Song Cau, Tuy An, Tuy Hoa City Dong Hoa district had a large number of sea turtles appeared on shore to lay eggs every year (the breeding period from May to late September), most of the coastal beaches, including Xuan Hoa beach (6 km), Xuan Thinh beach, An Hai beach (Tuy An district), beach in Tuy Hoa City, and Bai Xep (Hoa Tam commune, Dong Hoa district). Almost all of these beaches at that time had about 70–90 females of sea turtles came to lay eggs every year. Especially, in period from 1975 to 1980, the Bai Xep beach had up to 200 individual females per year.

Recently, most of these beaches are completely destitute or have very small number of nesting sea turtles. The Bai Xep beach, the bigest nesting site in the past, has only one or two clutches per year. Some other places such as Mai Nha island, An Hai beach, Hon Chua Island are supposed to be the places where sea turtles are still nesting, however, none of interviewees could confirm the number nesting and the species. According to participants, beside Green turtles, few years ago some place at Phu Yen Province also had Hawksbill turtle nesting such as Bai Tram (a small part of An Hai beach), but with a very small number (one or two individuals per year).

In Phu Yen, fishermen in gill-net boats usually catch by opportunity three species of sea turtle including Green turtles (the most numerous species), Hawksbills turtles and a small number of Leatherback turtles. The Green turtle is usually caught in water area from Xuan Hai to the mouth of Xuan Dai bays, 20 km coast length from the Cu Lao Xanh Island to the end of Tuy An, around Chua Island, Vung Ro water area, with some approximately 10 individuals per year. The Hawksbills turtle is caught infrequently in water area around Cu Lao Xanh Iland, Chua Iland with small number of 3 to 5 individuals per year. The number of Leatherback turtle is even lower, they are only caught or seen for two or three years.

Conclusion

Before 1980s, the study areas were a big nesting ground for sea turtles in Vietnam. Three species of sea turtle used to nest here including Green turtle (*Chelonia mydas*), Hawksbill turtle (*Eretmochelys imbricata*) and Leatherback turtle (*Dermochelys coriacea*). The most important beaches can be listed as follows: long beach at Binh Son, Long beach at Hoai Nhon, Cat Tien beach at Phu Cat, Xep beach at Dong Hoa. The foraging population of sea turtle concentrated from the rock reef of Nhon Ly (Binh Dinh) to Tuy An (Phu Yen). This area has a number of rock reefs and coral reefs, suitable for sea turtle living and foraging.

Recently, only Green turtle are still nesting at Hon Kho and Hai Giang beaches (Nhon Hai district, Binh Dinh) with a small number (about 5 to 10 individuals per year). The other beaches sometimes have sea turtles observed to nest but with very small number (1 or 2 individuals per year) and not frequently.

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OPHIUROIDS (ECHINODERMATA, OPHIUROIDEA) OF THE NHATRANG BAY (VIETNAM): FAUNA, SYMBIOTIC RELATIONSHIPS AND IMPORTANCE FOR SYSTEMATIC AND EVOLUTIONARY STUDIES

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Ophiuroids (brittle-stars) are important component of benthic communities and possess numerous unusual morphological and ecological features. Despite this, Vietnam ophiuroid fauna remains largely poorly investigated. In October and November, 2009, brittle-stars of Nhatrang Bay have been studied using various methods including scuba-diving, predominantly on coral reefs and rocky susbstrates up to 25 m. Additional materials from other expeditions were also considered.

About 20 species ophiuroids have been identified. Most of them have broad distribution in the Indo-West Pacific. Three species from two families (Ophiocomidae и Ophionereididae) – *Ophiocoma pusilla*, *O. occidentalis*, and *Ophionereis porrecta* are new records for the Vietnam fauna. Unique symbiosis case has been discovered for the Vietnam ophiuroids for the first time.

Importance of the present materials is not restricted only to a faunal study. Newly collected ophiuroid specimens are incorporated into broad project conducted by our group targeted for modern reassessment of the systematics and phylogeny of the echinoderm class Ophiuroidea.

OPISTHOBRANCH MOLLUSCS OF VIETNAM (GASTROPODA: OPISTHOBRANCHIA)

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Studies on opisthobranch molluscs (including well-known nudibranchs) currently among most dynamically developed fields of marine biodiversity research. Enormous diversity, intricate evolutionary history and numerous biological pecularities featured this remarkable group of the phylum Mollusca. Despite general advancements, currently there is a great deficiency of taxonomic works on opisthobranch regional faunas. In this respect, among the less studied world's faunas is Vietnam opisthobarnchs. Prior to present study was single review dealing only with the nudibranch molluscs (Risbec, 1956), and now is much outdated. Therefore, modern review of the Vietnam fauna of opisthobranch molluscs is presented for the first time. Present study is based on numerous specimens collected during last decade, predominantly in Nhatrang Bay. For each species short synonymy, diagnostic features, data on the biology and distribution are provided. Every species included into this review is illustrated by original colour photographs. In total, 150 species of opisthobranch molluscs is recorded including most orders, i.e. Cephalaspidea, Anaspidea, Umbraculida, Sacoglossa, Notaspidea, Doridacea and Nudibranchia. As one of the important results of the present study, 116 new for the Vietnamiese fauna species of the opisthobranch molluscs are recorded for the first time.

PHYSIOLOGICAL PERFORCE OF WHITE SHRIMP, *LITOPENAEUS VANNAMEI*, CULTURED IN FRESHWATER MEDIUM BY POTASSIUM APPLICATION

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Potassium is essential for shrimp especially that cultured in the freshwater medium. Potassium acts as electrolyte balance in the body cells, particularly in the delivery of nerve impulses and releasing energy from protein, fat and carbohydrate in the metabolism process. This study aims to determine the performance of white shrimp (*Litopenaeus vannamei*) cultured in freshwater with the addition of potassium. Research was conducted at the Wet Lab Institute for Coastal Aquaculture (RICA), Maros. The test animals that used were shrimp vaname having of age 62 days with initial weight average 5.80±0.02 g. The study used completely raandomized design (RAL) which consists of 4 treatments and 3 replications. The treatments tested were the application of KCl as source of potassium into freshwater medium having the salinity of 1-0 ppt with the concentration of 25, 50, 75 and 0 ppm (control) each for treatment A, B, C and D, respectively. Shrimp was adopted in brackishwater with the salinity of 25 ppt during 10 days before application. Dilution of salinity was done using fresh water during 3 days from 25 ppt down to 1 ppt, continued by the rearing in freshwater during 30 days. Variable measured were the level of osmotic activity, the rate of oxygen consumption, blood glucose levels, survival rate, and the weight growth and daily spesific lenght. The results showed that the application of potassium with the concentration of 25–75 ppm into rearing freshwater medium can enhance the ability of osmoregulation and reduce stress levels of the white shrimp on the freshwater medium which may increase the rate of growth and their survival rate. The statistic analysis showed that the osmotic activity, oxygen consumption rate and blood glucose levels exibited a significant difference ($P<0.05$) among the treatments. The best performance of white shrimp with osmoregulation level, oxygen consumption rate and the minimum blood glucose levels was found on the treatment with the application of 55.05–56.43 ppm potassium.

BIOLOGY OF THE CARIBBEAN BIVALVE *MYTILOPSIS SALLEI* (DREISSENIDAE) IN SOUTHEAST ASIA

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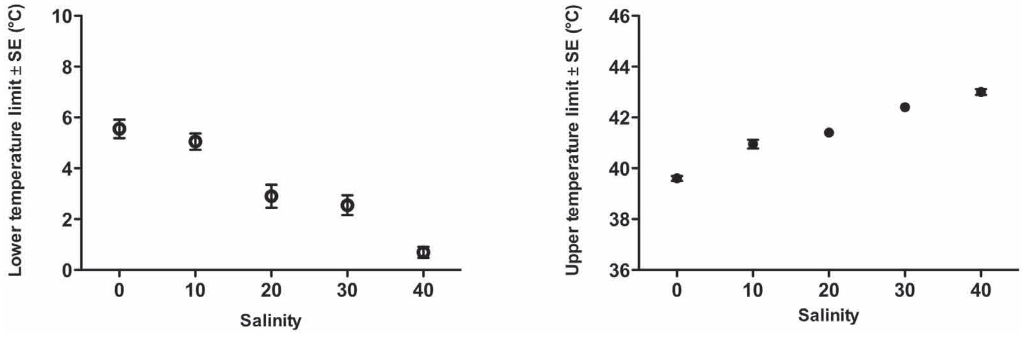
Amongst several recorded marine aliens in Southeast Asia*, Mytilopsis sallei* (Récluz) is one of a few Caribbean species that has established itself for the long-term outside its native range. Large, persistent populations occur in and around maritime ports from India through Thailand, Malaysia, Singapore, Hong Kong, Taiwan, China and Japan. Some of these populations have existed for more than 30 years, which may serve as sources for ensuing invasions elsewhere in the vicinity. *M. sallei* was most recently found along the Israeli coast on the Mediterranean Sea (Galil, Bogi, 2009). In Singapore, the first specimens of *M. sallei* were discovered in the early 1980s in the mangroves, and extensive populations were subsequently found in tidal canals (Tan, Morton, 2006). This was about the same time that similar populations were documented from other port cities in East Asia.

Vessels are likely vectors for their current distribution in Southeast Asia, although the exact routes and mode of transfer remain poorly documented. Pathways through the Panama Canal via the eastern Pacific, as well as across the Atlantic via tropical West Africa to India are equally likely, given the high volume of ship traffic plying these routes both in historical and modern times. Larval transport in ballast water and adults attached by byssus on ships' hulls are two major means by which this bivalve may have travelled across the world. High COI haplotype variability in South and East Asian populations (Wong et al., 2011) of *M. sallei* suggests that genetic transfer between individuals across existing populations is likely to be an ongoing process. *Mytilopsis* populations have also been observed in the eastern and central Pacific as well as tropical West Africa, but unresolved difficulties have precluded a more detailed analysis of their taxonomic status. Considerable intra-specific variation in shell morphology has resulted in a plethora of specific names that await taxonomic revision.

Although the bivalves appear to thrive best in the lower half of the intertidal zone in tropical estuaries, laboratory experiments suggest that they are able to tolerate a wide range of temperatures and salinities. Adult individuals initially acclimatized to different salinities at normal seawater temperature (29–30ºC) were able to tolerate salinities between 0 and 40, although at higher salinities, i.e. 45 and 50, 100% mortality was recorded after 48 hours. The upper and lower thermal limits of *M. sallei* were determined at different salinities (0, 10, 20, 30 and 40) under rapid temperature change, i.e. 1ºC hr^{-1}. Thermal tolerance increased significantly with salinity at both high and low temperatures, although cold tolerance was more affected at lower salinities (See Figure). Surprisingly, *M. sallei* showed the widest temperature range for survival (between 0.7 and 43.2ºC) in hypersaline conditions (salinity 40). At normal salinities within its natural range, this thermal tolerance window was slightly narrower, i.e., 2.9–41.4ºC (salinity 20) and 2.6–42.4ºC (salinity 30). In hyposaline conditions, both hot and cold survival limits



Effect of salinity on lower and upper thermal survival limits of *Mytilopsis sallei* (Dreissenidae), based on a rapid temperature change of 1ºC per hour (n=20 for each salinity observed) between salinities of 0 and 40; (Left) mean lower temperature survival limits; (right) mean upper temperature survival limits.

were compromised but greater reduction in its lower temperature limit was observed, i.e., 5.6ºC (salinity 0) and 5.1ºC (salinity 10). The ability to withstand cold and hot conditions at both high and low salinities could explain its wide latitudinal distribution, although such physiological capabilities remain to be elucidated for this species. High tolerance to extremes in salinity and temperature also explains its considerable biofouling persistence and survival in the face of rapid changes in the environment.

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THE INDO-PACIFIC PEN SHELL *ATRINA VEXILLUM* IN SOUTH-EASTERN SIAM BAY (SOUTH CHINA SEA)

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At the south-eastern coasts of Siam Bay (=the Gulf of Thailand) (southwest Vietnam), one species of the genus *Atrina* (Pinnidae) was found, namely, the flag pen shell *Atrina vexillum* (Born, 1778). It is characterized by elongated, wedge-shaped shell (Fig. 1). It attains big size and usually reaches 30 cm (maximum 48 cm) (Poutiers, 1998). Along with all species of the family Pinnidae, *A. vexillum* is a sessile semi-infaunal suspension-feeder and it lives vertically embedded in the bottom sediments, usually mud or muddy sand, anchoring by net of byssus threads. It is a tropical species distributed in the Indo-Pacific region from eastern Africa to eastern Polynesia, in western, south-western and central Pacific; north to Japan and Hawaii, and south to Australia. It is a commercial species.

The decline in *A. vexillum* populations threatened for the last decades due to various pollutants, sea resorts, anchoring of yachts, as well as unauthorized fishing. Despite the need for conservation, knowledge of the ecology and monitoring of the structure and condition of the main populations of *A. vexillum* inhabited Siam Bay are limited. The present study is a contribution to the knowledge of *A. vexillum* ecology, growth, and structures of the populations inhabiting the southwestern Vietnam coasts. The hypotheses of differences in growth and population structure of *A. vexillum* were investigated according to sub-areas in eastern Siam Bay characterized by different environmental factors.

Fig. 1. Flag pen shell *Atrina vexillum*. The outer shell surface with the elementary growth layers.

The study area of south-eastern Siam Bay was subdivided in three sub-areas. The first sub-area (S1) was identified near the coasts of Phu Quoc Island which is the nearest (among the studied sub-areas) to the top of the bay and nearby to the coastline of continent. The second sub-area (S2) was at the coasts of An Thoi Islands located at the south of Phu Quoc Island not far from the continent. The third sub-area (S3) was situated near Tho Tu Island, in the open part of the bay, nearby the boundary of the Siam Bay. Specimens of *A. vexillum* were sampled in 1986. In each sub-area, different aspects of population demography were studied (i.e., size structure, age structure and growth).

A comparison between the populations inhabiting these sub-areas was carried out and we found important differences between them. Population structure varied with sub-areas and habitat types. At open S3, maximum and mean (±SE) size was the highest (shell length (L), 320 mm, 272.7±13.2 mm, nonmetering the very large specimen that had shell 438 mm). At S2 situated between S1 and S3, mean L was 171.8±4.5 mm, and max L was equal 224 mm. At S3, the most deeply embedded within the bay, L did not exceed 182 mm, mean L=166.7±9.5 mm. The significant differences between all mean values of shell length were revealed by t-test ($P<0.01$). Length frequency of *A. vexillum* was wide-ranging; nevertheless, the specimens with a shell length of 170–190 and 290–310 mm were the most frequently found at S1 and S3 sub-areas, respectively. At S2, the size structure of *A. vexillum* population was bimodal with two peaks for 150–170 and 170–190 mm shell length.

At S2, the wet total weight varied from 82 to 500 g (236±18 g on average), the soft tissue wet weight was 15–105 g (41±3 g), and wet weight of the posterior adductor muscle was 5–30 g (13±1 g). The shell height varied from 85 to 140 mm (116±3 mm), and shell width was 27–49 mm (40±1 mm). Tissue wet weight was 10.7–25.0% of total wet weight, that is lower than for *Pinna nobilis* (Garcia-March et al., 2007), but higher than for *Pinna trigonium* (=*P. fumata*) (Silina, 2010) and the wet weight of the posterior adductor muscle was 3.1–7.7% of total wet weight and 19.0–37.0% of tissue wet weight, that is higher than for *P. nobilis* and *P. trigonium* (Garcia-March et al., 2007; Silina, 2010).

The growth of wet total weight (W_{total}, g), wet soft tissue ($W_{soft\ tissues}$, g), wet muscle (W_{muscle}, g) and shell weights (W_{shell}, g) was not isometric with shell length (L, cm) increase, as the exponents were many less than 3:

$W_{total} = 0.160L^{2.560}$, R = 0.820;
$W_{soft\ tissues} = 0.020L^{2.674}$, R = 0.852;
$W_{muscle} = 0.010L^{2.498}$, R = 0.794;
$W_{shell} = 0.130L^{2.466}$, R = 0.846.

Classical allometric model is not supported for other Pinnidae species too (Rabaoui et al., 2007; Silina, 2010). Besides, the linear parameters of the *A. vexillum* also did not change isometrically during their life, as the exponents were many less than 1:

$H = 1.582L^{0.704}$, R = 0.791, where H is shell height, cm;
$D = 0.698L^{0.614}$, R = 0.628, where D is shell width, cm.

It means that shell of the *A. vexillum* becomes more prolonged and less convex (gibbous) with increase of its age.

The outer shell layer of the individuals of family Pinnidae is calcitic prismatic that is contribute to appearance of elementary growth layers on the outer surface of the shell (Fig. 1). Previously, we have revealed that the width of the elementary growth layers of *P. trigonium* varied accordingly to seasons with periods of slow growth rates during late autumn – early spring and high growth rates during spring–autumn (Dorofeeva et al., 1987). It allows determining the age of each individual by counting the quantity of shell portions with thin elementary growth layers. Additionally, there were the annual rings on the muscle mark (track) on the inner shell surface of *A. vexillum*; they corresponded to the quantity of shell portions with thin elementary growth layers. For the individuals sampled in February, the muscle marks were ended with such ring; thus, the ring was formed during the winter (Fig. 2). Butler and Srewster (1979) also have used such rings on the muscle mark for age determination of *Pinna*

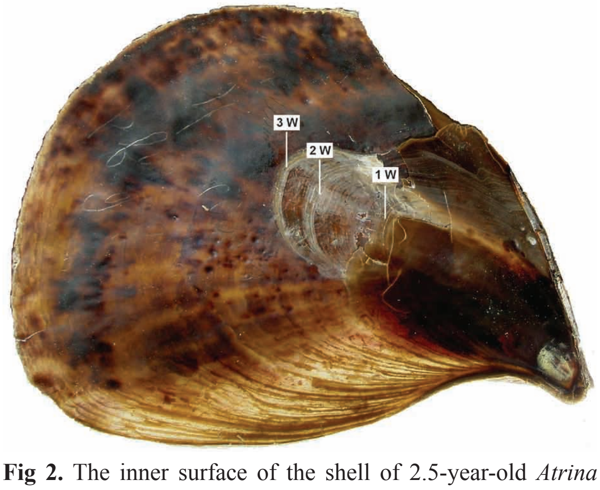

Fig 2. The inner surface of the shell of 2.5-year-old *Atrina vexillum* sampled in February. The muscle mark is ended with the winter annual ring. 1w, 2w and 3w are winter rings.

bicolor. It was revealed that growth rates varied with shell size, with a peak at the first year of *A. vexillum* life, followed by a sharp decline.

The *A. vexillum* individuals inhabited S1 had the slowest growth rates and up to an age of 3.5–5.5 years their shells were smaller than shells of specimens from other studied populations in Siam Bay. Growth rates were higher at S2 than at S1, but statistically significantly ($P<0.05–0.01$, for ages 4.5 and 5.5 years old, t-test) slower than at open S3 (see Table).

Growth rates of the flag pen shell *Atrina vexillum* at the different sub-areas of the south-eastern Siam Bay

Age, years	An Thoi Islands		Tho Tu Island	
	N, ind.	Length, mm	N, ind.	Length, mm
2.5	1	134	0	–
3.5	8	159.6±6.0	1	195
4.5	11	187.3±7.2	3	231.3±8.7
5.5	3	203.3±2.3	3	288.0±9.3
6.5	0	–	0	–
7.5	0	–	4	311.8±5.0

Thus, it was revealed that the farther to the Bay (to the direction of its top), the slower *A. vexillum* growth rates and the shorter life span. The explanations for this phenomenon may be as follows. It is known that the water salinity of Siam Bay <31.26‰ (30.80‰ on average), and the bay is under influence of runoff of rivers. Members of the family Pinnidae is intolerant to the water salinity differentials. However, at open S3, the water salinity is about 32.0‰ as well as in the open part of the South China Sea. Moreover, concentration of suspended particular matter (SPM) increases from open S3 to S2 and farther to S1 from 0.69 mg/l to 1.26 mg/l (Report, 1986). All species of suspension filter feeding pinnids are sensitive to high concentration of SPM that adversely affects filtering apparatus, feeding and respiration of mollusk. Besides, concentration of suspended organic carbon also increases from 17% (S3) to 31% (S1) of dry suspended matter. Concentration of chlorophyll "a" is 0.25–0.29 μg/l in the water at open S3, but it is 0.57 and 0.94–1.22 μg/l at S2 and S3, respectively. However, water temperature increases in this direction from 25.3–26.2ºC to 27.5–28.2ºC in February (Report, 1986). Therefore, oxygen concentration decreases in the direction of S3–S1 due to high level of organic matter decomposition, especially at high water temperature. Thus, it is 5.31 ml/l in the water of open S3 and 5.09 ml/l at S2 and S1 (Report, 1986). Low oxygen concentration also adversely influences the respiration and condition of the studied bivalve species and, in turn, its growth rates and life span. The results contribute to increase the knowledge of population ecology of *A. vexillum* and to provide useful information for implementing conservation policies.

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MOLECULAR DIVERSITY OF FATTY ACIDS IN MARINE OPISTHOBRANCHIA

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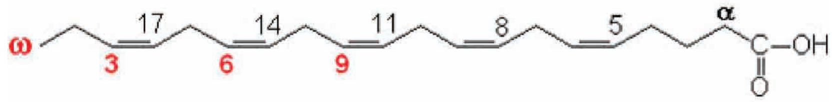
The enormous biodiversity of marine habitats is mirrored by the molecular diversity of chemical compounds found in marine animals, plants and microbes. The Opisthobranchia are known as beautiful sea slugs numbering 5000 to 6000 species, which live in all kind of habitats, from the intertidal to the deep sea, from the polar regions to the tropical reefs. Nevertheless they are more diverse and abundant in tropical waters. Many of them are specialists on certain food species. Some nudibranchs feed on sponges, others on bryozoans, polyps or jellyfish, corals, tunicates, barnacles or sea anemones; other groups have specialized on algae.

The Opisthobranchia mollusks are quite famous for many unique biological phenomena, as there are the defensive strategies by using highly toxic chemicals from their food or producing them by themselves (Wägele et al., 2006), or by incorporating the stinging cells of cnidarian prey and using them against possible predators. Another unique character for a certain group is the possibility to incorporate chloroplasts from their algal food and to use these as metabolite suppliers, therefore acting like plants.

Interesting is the symbiotic relationship of certain groups with unicellular algae zooxanthelles. The secondary metabolites that are chemical defenses provide the main focus for biochemists, whereas information about the lipids and fatty acids of Opisthobranchia is scrappy (Zhukova, 2007). Lipids play an important role in the biochemistry and physiology of the marine animals being major constituents of cell membranes, storage components and are used as hormones that play roles in regulating metabolism. The common building block for most of the different types of lipids is the fatty acid. The fatty acid hydrocarbon chains are usually between 12 and 30 carbon atoms long. Unsaturated fatty acids have double bonds in the chain. For example, eicosapentaenoic acid 20:5n-3 or 20:5Δ5,8,11,14,17:



Fatty acids of the mollusks are known to be influenced by taxonomic relations and environmental conditions, and also depend on physiological conditions and mainly nutrient habits and food availability (Joseph, 1982). High biological specificity of fatty acids, along with their structural diversity, has allowed fatty acids to be used as biochemical markers in marine ecology for the determination of trophic relations of the animals (e.g., Dalsgaad et al., 2003). Uncommon acids or group of fatty acids may serve as useful biochemical indicators (see Table). Usefulness of the fatty acid profiles for describing the nutrition of marine invertebrates and bacterial input (bacterial symbiosis) in total fatty acid pool of the animals are also known.

The 15 species of Opisthobranchia, collected in the Nha Trang Bay of the South China Sea, Vietnam, were analyzed for fatty acids. GLC analyses of fatty acids was performed with a Shimadzu GC-17A gas chromatograph (Kyoto, Japan) equipped with a flame ionization detector and SUPELCOWAX 10 (Supelco, Bellefonte, PA) capillary column (25 m × 0.25 mm i.d.), 210ºC and also SPB-5 (Supelco, Bellefonte, PA) capillary column (25 m × 0.25 mm i.d.), 230ºC was used. Helium was the carrier gas. Individual components of FAME were identified by comparing retention time data

Biomarker fatty acids (FA) of the potential food sources for the Opisthobranchia

Potential food sources	Specific fatty acids
Sponges	Very long chain FA with C24 – C30 chain length: Δ5,9-24:2; Δ5,9-25:2; Δ5,9-26:2; Δ5,9,19-26:3; Δ5,9-27:2 etc.
Symbiotic zooxanthellae	16:0, 18:4n-3, 18:5n-3, 22:6n-3
Heterotrophic bacteria	Branched-chain FA (*iso-, anteiso-*), Odd-chain FA: C15, C17, C19
Green algae	16:2n-6, 16:3n-3, 18:2n-6, 18:3n-6, 18:3n-3, 20:4n-6, 20:5n-3
Hydroids	18:1n-9, 22:6n-3

with those obtained for authentic standards, and using the ECL data and confirmed by GC-MS of MEFA and their N-acylpyrrolidide derivatives. GC-MS analyses of FAME were performed on a Shimadzu GCMS-QP5050A instrument (Kyoto, Japan) fitted with a MDN-5S (Supelco, Bellefonte, PA) capillary column (30 m × 0.25 mm i.d.).

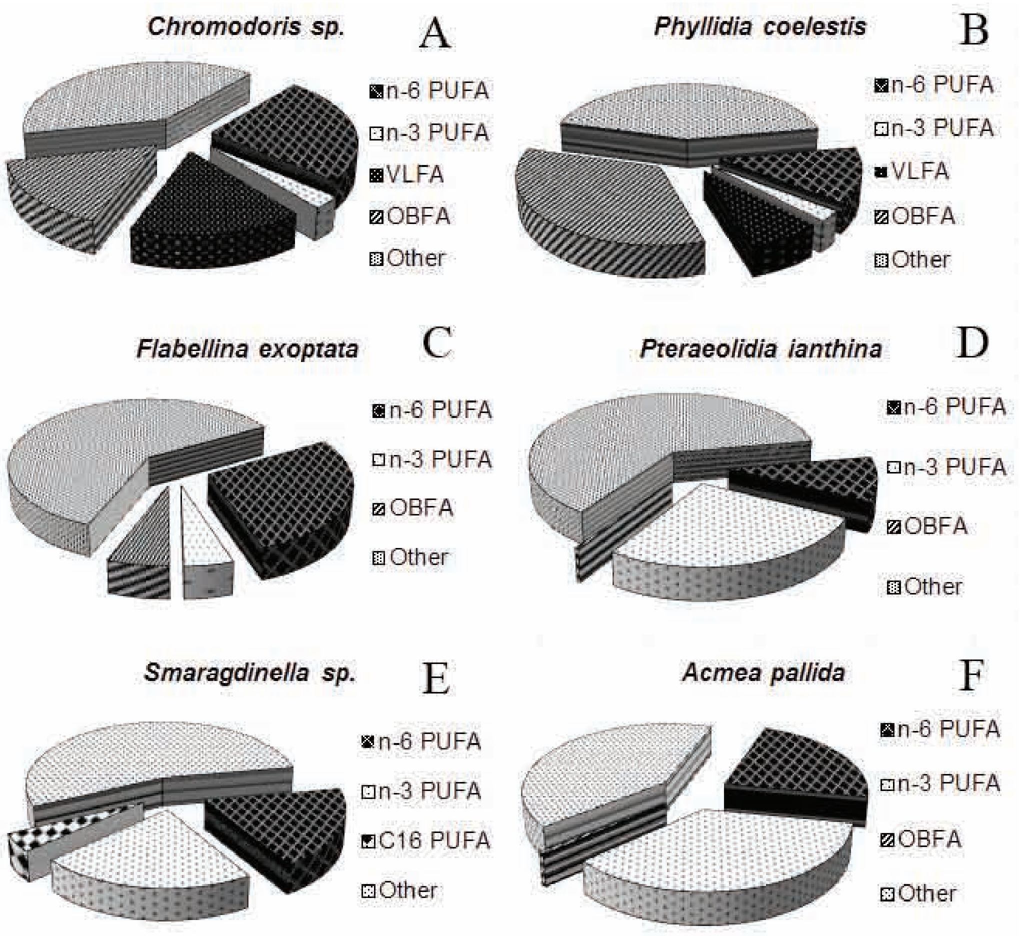
Fatty acid distribution in the species of order Nudibranchia, *Chromodoris* sp. and *Phyllidia coelestis* (Bergh, 1905), *Flabellina exoptata* (Gosliner et Willan, 1991) and *Pteraeolidia iantina* (Angas, 1864) and the species of order Cephalaspidea *Smaragdinella* sp., are shown in the Figure. For comparison, fatty acids of marine temperate limpet *Acmaea pallida* (Gould, 1859) are presented. The sea slugs exhibited a wide diversity of the fatty acids. Significant differences between species were revealed.

Nudibranch mollusks *Chromodoris* sp. and *P. coelestis* are common on tropical reefs. The fatty acids profiles of the nudibranchs differed from that of the other gastropods by presence of significant amounts of very long chain fatty acids (VLCFA) specific for sponges, so-called demospongic fatty acids (Fig., A, B). These doridins are carnivorous and specialized feeders on sponges. Utilization of this food probably is responsible for a high level of the demospongic acids in these species. Majority nudibranchs are suspected to feed on certain sponge species, which are known to distinguish in their fatty acid composition. Indeed, a series of VLCFA with double bonds at Δ5,9 found in high concentrations in the tropical nudibranchs distinguished between species *Chromodoris* sp. and *P. coelestis*. Among VLCFA in *Chromodoris* sp. Δ5,9-25:2 and Δ5,9-26:2 were the main (4.0 and 6.0%, respectively), whereas in *P. coelestis* they were minor components (1.7 and 0.9%). Moreover, only in the *P. coelestis* iso-Δ5,9-25:2 was identified, and concentration of Δ5,9-24:2 was twice as much as compared with *Chomodoris* sp. It confirms that these nudibranchs feed on different sponge species.

The fatty acid composition of marine mollusks is considered to be characterized by predominance of n-3polyunsaturated fatty acids (PUFA), 20:5n-3 and 22:6n-3 (Fig., F), which constitute usually near a half of total fatty acids. In contrast, the nudibranchs did not exhibit this feature; these two marine PUFA were minor components and in sum constituted 1.6 and 2.1% of the total fatty acids.

Another unique feature of the fatty acid composition of the nudibranchs relates to a high abundance of the odd-chain and branched (*iso-* and *anteiso-*) fatty acids, specific for bacteria and usually named bacterial fatty acids (Gillan, Johns, 1986) (Fig., A, B). They are normal as trace components in the most marine invertebrates. The presence of a large amount of bacterial acids was exceptional. More probable they are of dietary origin from sponge, which are known associated with bacteria, or may have come from bacteria living in symbiosis with the sea slugs. Indeed, symbiotic bacteria were recently found in the epithelium cells of the mantle and notum of the nudibranch *Dendrodoris nigra* (Zhukova, Eliseikina, in press). Some of the symbionts are sometimes the producers of the host's secondary metabolites that have defensive and protective functions for their hosts (Proksch et al., 2002).

As opposed to the doridins, VLCFA were not found in the aeolidins, *Flabellina exoptata* and *Pteraeolidia ianthina*, carnivorous nudibranch species feed on hydroids



Fatty acids of the tropical Opistobranchia *Phyllidia coelestis*, *Chromodoris* sp., *Pteraeolidia ianthina*, *Flabellina exoptata*, *Smaragdinella* sp. and temperate limpet *Acmea pallida*. Abbreviations: OBFA – odd-numbered carbon chain and branched fatty acids; VLFA – very long chain fatty acids; PUFA – polyunsaturated fatty acids.

(Fig. C, D). In the comparison with other species of nudibranchs (doridins) specialized on feeding on sponges the high concentrations of palmitic acid 16:0, oleic acid 18:1n-9 and arachidonic acid 20:4n-6 in *F. exoptata* were partly due to diet origin of these fatty acids from hydroids. *F. exoptata* is reported to feed on the hydroids *Halocordyle distincta* named feathered hydroid and *Eudendrium* sp.

Pteraeolidia ianthina was notable by significant amounts of specific fatty acids 16:0, 18:4n-3, 18:5n-3 and abundance of docosahexaenoic acid 22:6n-3 (up to 19% of the total fatty acids), which were absent or found in trace amounts in the other Opisthobranch species studied. These fatty acids are typical for dinoflagellates and serve as biochemical markers of these marine microalgae. Really, the aeolid nudibranch *P. ianthina* has evolved symbiotic partnerships with zooxanthelles. Zooxanthelles are photosynthetic microalgae (Dinoflagellata), which equally provide the slug with photosynthetic products. High amounts of the fatty acids specific for symbiotic dinoflagellates in *P. ianthina* indicate on significant uptake of organic matter produced by zooxanthelles in the total pool of fatty acids of this nudibranch. The nudibranch is known to farm these zooxanthellae within its own digestive diverticula. Partly, the nudibranch acquires the zooxanthelles probably from its food, hydroids, in which those zooxanthelles already live. It also obtains food by preying on several species of hydroids, including *Pennaria disticha*, a relatively large and conspicuous non-native species. During digestion, some of the hydroid's nematocysts are retained in the cerata of the nudibranch for use in defense.

The herbivorous *Smaragdinella* sp. similar marine temperate limpet *Acmaea pallida* possessed great amounts of algal derived PUFA such as eicosapentaenoic acid, 20:5n-3 and arachidonic acid, 20:4n-6 (Fig., E). The concentration of 20:5n-3 in the limpet *Acmaea pallida* reaches to 30% of the total fatty acids (Fig., F). *Smaragdinella* named as a "limpet" among the cephalaspid "bubble shells" is common in intertidal zone of Van Phong and Nha Trang Bays. It inhabits dead shell of the barnacles *Tetraclita squamosa*. It is nocturnal active, feeding on microalgae growing on the rocks while the tide is low. Considerable amount of 18:2n-6, 18:3n-4 and C16PUFA found in this representative of order Cephalaspidae is an indicator of feeding mainly on green microalgae.

Thereby, Opisthobranchia species exhibited a wide diversity of fatty acids. The fatty acid composition varied according to the species mainly due to different food supply, internal biosynthetic activity and symbiotic partnerships. Differences in diets of the mollusks are evident from their fatty acid composition.

Acknowledgments

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NEMERTEAN WORMS (NEMERTEA) OF THE VIETNAMESE COASTAL WATERS

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Nemertea is a phylum of vermiform invertebrates commonly known as ribbon worms, which includes about 1300 species (Kajihara et al., 2008). These worms inhabit different types of bottoms including silt, sand, algae, sea-grasses, and dead corrals, and are distributed vertically from the supralittoral to the abyssal zone. About 300 species of ribbon worms are now known from the seas of Northeast and Southeast Asia (Crandall et al., 2002; Chernyshev, 2008). However, the nemertean fauna of this region is investigated rather irregularly, with almost no information on the nemerteans of Vietnam coastal waters. Joubin (1903) described *Cerebratulus velatus* from the Gulf of Tonkin (North Vietnam), but the generic position of this species is still unclear. Dawydoff (1940) found 20 different nemertean larvae (pilidiae) in Nha Trang Bay (South Vietnam), and although adult worms were not identified, the variety of larvae definitely indicates high diversity of heteronemerteans and hubrechtellids. He also listed two heteronemertean species for Nha Trang Bay – *Beseodiscus unistriatus* and *B. hemprechi* (Dawydoff, 1952).

During the Russian-Vietnamese expeditions in 2005, 2007 and 2010 along the coast of Vietnam (from Co-To Island in the north to Ladd Reef in the south), I collected 257 specimens corresponding to 78 nemertean species (see Table). Many of these species appear to be undescribed, while only one species, *Ototyphlonemertes ani*, has been described before (Chernyshev, 2007).

The numbers of nemertean species found on the coast of Vietnam

Taxa	Species (genera)	Undescribed species	New for Vietnam
Order Tubulaniformes			
Tubulanidae	2 (1)	2	
Order Archinemertea			
Cephalotrichidae	2(1)	1	
Cephalotrichellidae	2(2)	1	1
Order Heteronemertea			
Valenciniidae	1(1)	1	
Baseodiscidae	2(1)		1
Lineidae	23(>9)	>15	4
Order Polystilifera			
Suborder Reptantia	7(>3)	?	
Order Monostilifera			
Suborder Cratenemertea	3(2)	3	
Suborder Eumonostilifera	36(>15)	>30	3
incl. Tetrastemmatidae	20(>3)	18	1
Total	**78 (>38)**	**>54**	**9**

Nine species are shown to be new for Vietnamese coastal waters: *Cephalotrichella alba*, *Baseodiscus delineatus* (Fig. 1C), *Notospermus tricuspidatus* (Fig. 1A), *N. geniculatus* (Fig. 1B), *Micrura callima* (Fig. 1D), *Lineus binigrilinearis* (Fig. 1E), *Plectonemertes sinensis* (Fig. 1F), *Diplomma serpentina* (Fig. 1H), and *Tetrastemma roseocephalum*. Although *Micrura callima* was described from Australia (Sundberg, Gibson, 1995), it appears to be also common for Vietnam. Another common species, *Diplomma serpentina*, was recently redescribed from Japan, Philippines and Indonesia (Kajihara et al., 2011). Hence, both species are probably widely distributed in the tropical and subtropical waters of the Pacific and Indian Oceans. In addition, three species (*C. alba*, *L. binigrilinearis*, *P. sinensis*) were previously only known from Hong Kong coastal waters, while *T. roseocephalum* is the only nemertean with a distribution ranging from boreal (Hokkaido) to tropical (Gulf of Tonkin) waters.

The large number of new species is probably due to the fact that tropical nemerteans have been hitherto only scarcely studied. The majority of the collected species belongs to the families Lineidae and Tetrastemmatidae, which is common for many regions of the northern hemisphere. Specific for the tropical waters is the occurrence of the polystiliferous nemerteans (Reptantia) that inhabit coral communities. Unfortunately, most reptantian species were described without any data on live specimens rendering the identification of these nemerteans difficult. However, the findings of two coloured archaeonemerteans appear considerably interesting. The first species, *Cephalothrix suni* in lit. (="*Cephalothrix* sp. VIE, Network 21" Chen et al., 2010), possesses a pigmented pattern on the ventral body side (Fig. 1G), while the second, *Balionemertes* sp. n., has pigmentation on both body sides and six pairs of eyes (two dorsal, two ventral, and two lateral). *Balionemertes* and *Cephalotrichella* are able to swim with eel-like movements.

Two new larvae of pilidiophoran nemerteans, *pilidium* ex gr. *gyrans* (Chernyshev, 2006) and *pilidium* ex gr. *auriculatum* (Chernyshev, 2011a) from Van Phong Bay (South Vietnam) have been previously described. The distinguishing feature of the latter form is the presence of short unpaired strand connecting the juvenile to the posterior wall of the pilidial epidermis.

Using phalloidin labeling in conjunction with confocal laser scanning microscopy (cLSM), I obtained new data on body wall and proboscis morphology of some nemertean species. Thereby, all archinemerteans investigated so far (including *Cephalothrix linearis* from the White Sea and *C. simula* from the Sea of Japan) exhibit diagonal muscles above the circular musculature (Fig. 2A, B, D). In *Balionemertes* sp. these muscle fibers are especially numerous (Fig. 2C) forming a thick layer of 7−10 μm. Thus, it appears likely that a body wall with a layer of outer diagonal muscles is an autapomorphy of Archinemertea. The presence or absence of the inner circular musculature is used in the systematics of Cephalotrichidae s.l. CLSM study demonstrates that all species of the archinemerteans possess this muscle layer (Fig. 2A, B), which can be easily overlooked in histological sections. The strands of outer longitudinal muscles were found in the proboscis of *Balionemertes* and *Cephalotrichella* (Fig. 2E). In both genera ganglionic cell tissue of lateral nerve cords encloses inner longitudinal muscle fascicle (Fig. 2 A – *ilf*).

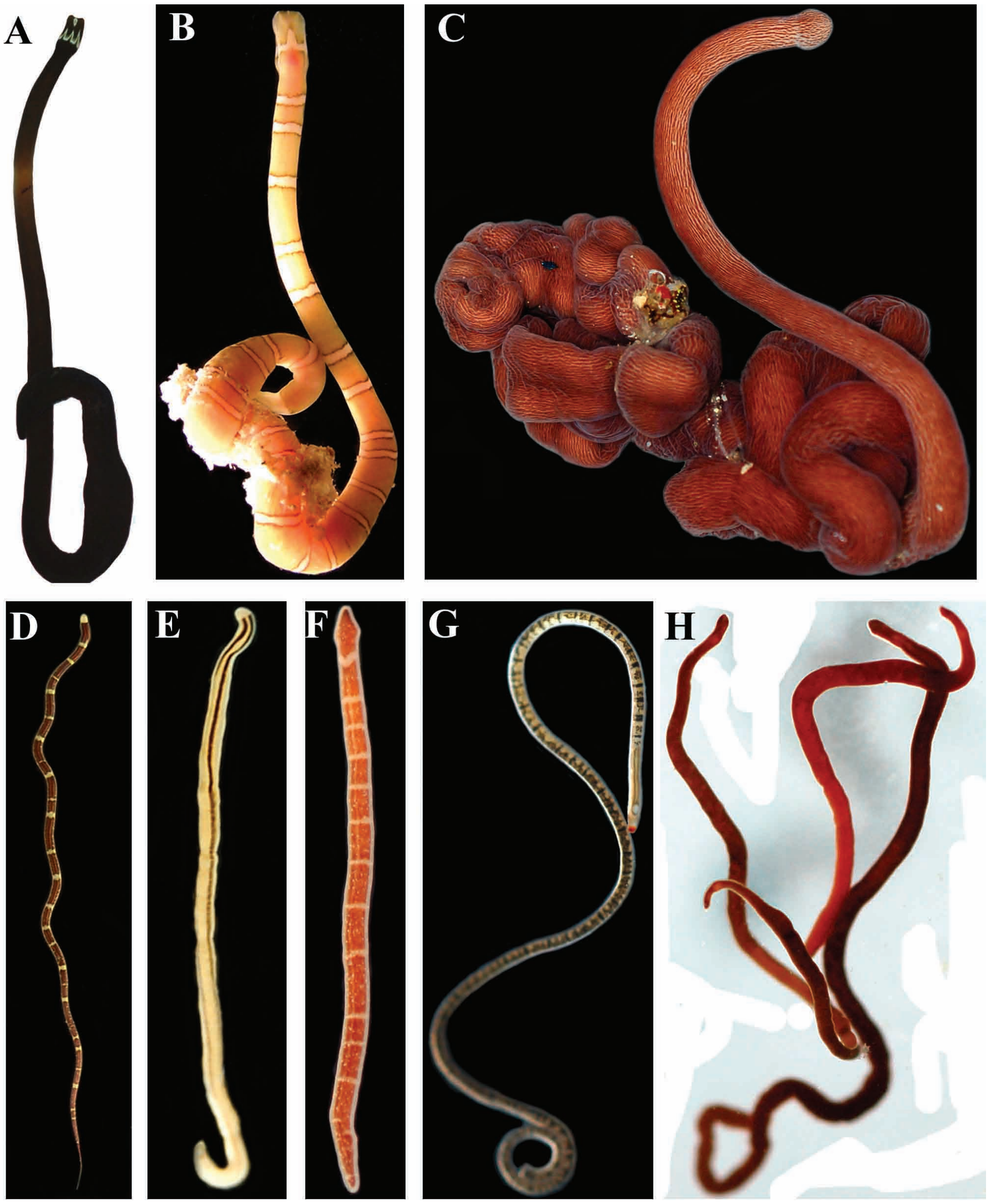

Fig. 1. Habitus of some species of nemerteans from the coast of Vietnam. A – *Notospermus tricuspidatus* (Cù Lao Thu Island); B – *Notospermus geniculatus* (MacHenry Reef); C – *Baseodiscus delineatus* (Cù Lao Thu Island); D – *Micrura callima* (Cù Lao Ré Island); E – *Lineus binigrilinearis* (Van Phong Bay); F – *Plectonemertes sinensis* (Cù Lao Chàm Island); G – *Cephalothrix suni* in lit. (Nam Du Islands); H – *Diplomma serpentina* (Nam Du Islands).

These unique states may be synapomorphies of the recently established family Cephalotrichellidae (Chernyshev, 2011b). Accordingly, as demonstrated in archinemerteans, the body wall musculature in nemerteans is of taxonomic relevance, and, therefore,

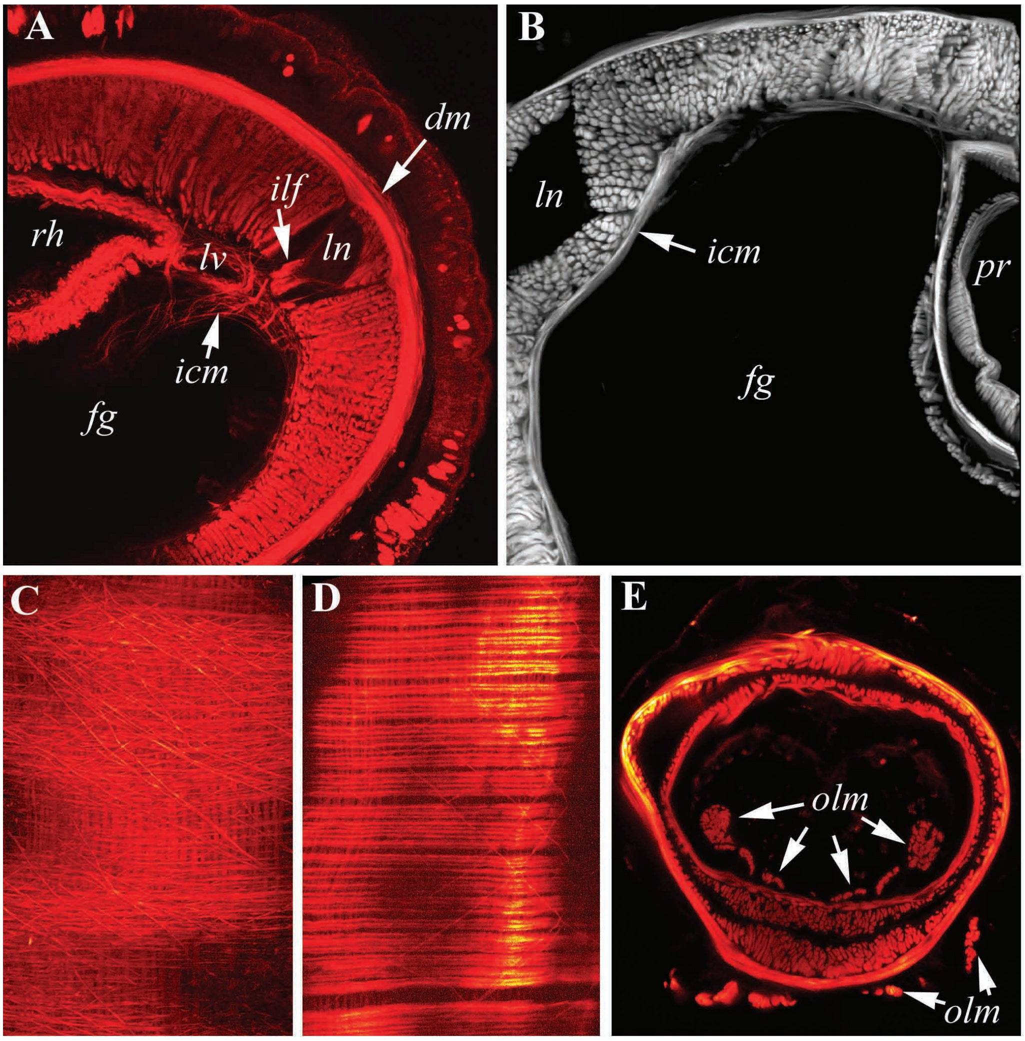

Fig. 2. Confocal laser scanning microscopy micrographs of the transversal (A, B, E) and longitudinal (C, D) sections of the archinemerteans. A – *Balionemertes* sp. (foregut region); B – *Cephalothrix linearis* (foregut region); C - *Balionemertes* sp. (outer diagonal and circular layers of body wall musculature); D – *Cephalothrix* cf. *simula* (outer diagonal and circular layers of body wall musculature); E – *Cephalotrichella alba* (partly everted proboscis). Abbreviations: dm – diagonal musculature, fg – foregut, icm – inner circular musculature, ilf – inner longitudinal muscle fascicle, ln – longitudinal nerve cord, lv – lateral blood vessel, olm – outer longitudinal musculature, pr – proboscis, rh – rhynchocoel.

renders phalloidin labeling and cLSM useful tools for future investigations on nemertean larval musculature that can be easily overlooked in histological sections.

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THE COMPOSITION OF PLANT SPECIES IN SONG NGU ISLAND, CUA LO TOWN, NGHE AN PROVINCE, VIETNAM

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Introduction

Ngu Island lies at a distance over 4 km from the coast. The island consists of two small islets. The bigger one is 133 m above the sea level, and the smaller one is 88 m. Twin Banks Mountain look like two fishes. So, it's called Song Ngu (Twin Fishes). There are pagoda, many wild plants and also wildlife. The island has actually become an attractive tourism site for visitors coming to Cua Lo. The flora in Ngu Island is rich and almost not experiences human interaction.

Methods

We used common method, which was introduced by Klein (1978) and Nguyen Nghia Thin (1997) for collected samples. The collection of samples was carried out by expanding of radius to area where is no presence of new species. For the species identification we used the method of morphological comparison based on "Plants of Vietnam" which was introduced by Pham Hoang Ho (1999–2000). We checked scientific names by using Brummit (1992).

Results

Based on our survey, we have identified 262 species, 124 genera, 61 families belonging to five divisions of higher plants – Psilotophyta, Lycopodiophyta, Polypodiophyta, Pinophyta, Magnoliophyta. However, their distribution was inequal. The division of Magnoliophyta predominated with 91.22%, others have ratio about 10% (Table 1).

Table 1. Distribution of studied orders

Phylum	Family		Genus		Species	
	Quantity	Percentage (%)	Quantity	Percentage (%)	Quantity	Percentage (%)
Psilotophyta	1	1.64	1	0.81	2	0.76
Lycopodiophyta	1	1.64	1	0.81	2	0.76
Polypodiophyta	4	6.56	9	7.26	18	6.87
Pinophyta	1	1.64	1	0.81	1	0.38
Magnoliophyta	54	88.52	112	90.32	239	91.22
Total	**61**	**100.00**	**124**	**100.00**	**262**	**100.00**

Most Species-Rich Genera

Based on collected data, there are 6 genera which have more than three species such as *Ficus* (12 species), *Ardisia* (6 species), *Croton* (5 species), *Lithocarpus* (5 species), *Litsea* (4 species), *Grewia* (3 species). Total number of species of these genera were 35 (13.36% of the species in studied area). Results are shown in Table 2.

Using Value of Plants

Comparing with previous literature, we identified 158 species (60.31%), which have various utilities as medicinal herbs, to produce oil or sap, decorative plants, plants having nutrition value or used as timber. The noticeable of them are medicinal herbs with 50 species (19.08%). The decorative plants belong to 35 species (13.36 %), timbered plants are 34 species (12.98%) (Table 3).

Rare Species

Based on data of the Vietnamese Red Book (Ministry…, 2007), we identified 11 rare species with different levels. There are three species at dangerous level (could be threatened to extinct in level V), three species at rare level (could become level R), four species at T level, one species could be at rare level.

Conclusions

We identified 262 species, 124 genera in 61 families belonging to 5 higher botanic orders such as Psilotophyta, Lycopodiophyta, Polypodiophyta, Pinophyta, Magnoliophyta at Ngu Island, Cua Lo Town, Nghe An Province.

There are six most abundant genera containing 3 to 12 species. They are *Ficus* (12 species), *Ardisia* (6 species), *Croton* (5 species), *Lithocarpus* (5 species), *Litsea* (4 species), *Grewia* (3 species). There were identified 158 species (60.31%) of various use including medicinal plants, oil, resin, ornamental plants, building, handcrafts, food and plants for timber. In that group, medicinal plants have dominated with 50 species (19.08%). There are 11 species in the study area which were assigned to the list of endangered species in Vietnam Red Book with many different levels.

Table 2. Most species-rich genera

No.	Genus	Number of species	Percentage (%)
1	*Ficus*	12	4.58
2	*Ardisia*	6	2.29
3	*Croton*	5	1.91
4	*Lithocarpus*	5	1.91
5	*Litsea*	4	1.53
6	*Grewia*	3	1.15
	Total	**35**	**13.36**

Table 3. Utility of studied species

No.	The used value of plant species	Number of species	Percentage (%)
1	Herbs	50	19.08
2	Oils, resin	9	3.44
3	Ornamental plants	35	13.36
4	Building, handcrafts	10	3.82
5	Food	20	7.63
6	Timber	34	12.98
	Total	**158**	**60.31**

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BIODIVERSITY OF SOFT CORALS ALCYONIIDAE (CNIDARIA: OCTOCORALLIA) AND THEIR TAXONOMY PROBLEM

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One challenge for taxonomists and for reef ecologists as well is to assess whether species that are broadly distributed, and for which widespread variation and/or distinct morphotypes are known, represent complexes of sibling species. The solution of these problems is very important for any biodiversity studies in coral reefs and for the marine ecosystems conservation as well. Although morphological features are the primary means of species identification among colonial stony corals and octocorals, for close to a century, workers have commented on the extraordinary morphological variability exhibited by these animals in response to environmental factors such as light, water flow, and depth. Octocorals, like many other colonial benthic invertebrates, exhibit remarkable levels of morphological variability. The basis for this variability has been largely unexplored.

Alcyoniidae is the most important family of the Octocorallia being the world-wide distributed in the ocean. However, the identification of Alcyoniidae to species level is often extremely difficult, and in the diverse central Indo-Pacific fauna it is quite often impossible. There is a large number of very similar species, many of which are still undescribed, but the majority of the species known and named at present time need revisions. However, taxonomic revisions of known species, necessary for adequate identifications, often cannot be made due to the poor state of old literature (Fabricius, Alderslade, 2001). Quite fairly revisions only exist for the few main genera, including *Sinularia*, *Sarcophyton* and *Lobophytum* (Verseveldt, 1980, 1982, 1983). However, as was outlined by McFadden at al. (2006), Verseveldt's taxonomic revisions are based on the type material only; most of his species descriptions regarded a single specimen. The number of morphological characters that he used for taxonomic classification was also small, including only (1) colony growth form based on preserved specimens (often on a colony fragment), (2) spacing of autozooids and siphonozooids on the colony surface (in the case of *Lobophytum* and *Sarcophyton*), and (3) the size and shape (judged subjectively) of sclerites from the (a) polypary surface, (b) interior of the polypary, (c) surface of the stalk or base, and (d) interior of the stalk or base. For each species, his description includes only a photograph of the preserved type specimen(s) and hand-drawn illustrations of only few sclerites from each anatomical region of the colony. Identifying a colony to species therefore requires matching sclerite samples to the limited selection illustrated for the type specimen. An exact match is rarely possible; most often, positive identification to species is simply not possible using this limited typological approach. Comparison of the original type material with Verseveldt's (1982, 1983) species descriptions and of Verseveldt's sclerite slides with slides which have been prepared subsequently from the same type material has revealed that his methods of slide preparation inadvertently resulted in the loss of many of the smaller sclerite forms. Moreover, comparison of his original slides with the illustrations he made from them indicates that he did not always illustrate the full range of sclerite types found on a slide, and occasionally omitted forms that could be species diagnostic. Especially it could be important to know the sclerites' armature of the polyp to separate the species in these genera as was shown by recent molecular-phylogenetic studies (McFadden et al., 2006, 2009).

Among the Alcyonidae, the soft corals of the *Sinularia*, *Sarcophyton* and *Lobophytum* are conspicuous, ecologically important members of shallow reef communities throughout the Indo-West Pacific. They are particularly common and conspicuous in shallow nearshore reef flat habitats where they often form large monospecific aggregations (Fig. 1). Study of their diversity and ecology is, however, hindered by incomplete knowledge of their taxonomy and the two last genera even cannot always be distinguished reliably (McFadden et al., 2006). Species recognition is hampered by the occasions in which the variability within a species appears high, e.g. the highly variable colony growth morphology. The bright and single known example is the case with uniting two known species of *Sinularia* which species were historically distinguished by very different colony shape (*S. brassica* and *S. dura*) into one under the older name basing on the matching of the spiculation and occurrence of the discontinuous range of the intermediate colony' shapes. The authors have been able to examine a considerable number of specimens covering a large variety of growth forms; the variation within a

Fig. 1. The monospecies settlement of *Sinularia flexibilis*, Vung Island, Nha Trang Bay. The depth is 7 m.

single resulting species was from a stalked colony with a convoluted capitulum consisting of closely appressed marginal folds to a stalked colony with a capitulum consisting of short lobes. A possible explanation for the variation in colony morphology could be that colonies adopt lobe production and a faster growth rate in response to an increase of available light (Benayahu et al., 1998).

Therefore, it is indeed possible that environment-correlated variation in growth in *Sinularia* has led to eco-morphs which are linked by a continuum of growth forms similar to what has been suggested for some scleractinian corals. Awareness of such a variation requires examination of series of specimens from the entire distributional range of a given taxon. Not less intriguing is the possibility that part or a whole range of such variations could be the result of cross-fertilization within sibling species or hybridization. It looks like the establishment of interspecies boundaries requires a molecular/genetic approach along with the examination of a comprehensive specimen collection, which might reveal other possible boundaries than those based on morphological characters only.

At present, such a wide variation in colonial form among soft corals is best recognized in the genus *Sinularia*; perhaps it is indicative of further species-concept problems to be encountered in this group (Benayahu et al., 1998). The species of *Sinularia* traditionally have been classified into five intrageneric groups – known as five Verseveldt's (1980) identification keys, – based on variation of a single microscopical character, the shape of the clubs from the polypary' surface. Inside of each key, the species should be recognized using shape and size of preserved specimens along with the very few characters of the other sclerites. However, this latest revision of *Sinularia* has incomplete data about the colony body sclerites; moreover, information about polyp sclerites is absent. It can obscure the identity of samples. For example, *Sinularia manaarensis* was described for a piece of colony from the Gulf of Manaar, Ceylon. Verseveldt firstly recognized the specimen as distinct from *S. gardineri* (Pratt, 1903), presented sclerites drawings (1980) and placed the species into his *Sinularia* group 4 as having clubs without a central wart. However, his drawings were not a comprehensive representation of all sclerite types in *S. manaarensis*; the information of polyp sclerites was not presented. The second finding of *S. manaarensis* (in Nha Trang Bay, South China Sea), shows much more about the sclerites set – the sclerites occurrence in the polyp, more large sclerites in the colony surface layer and some clubs with well distinct central wart (Dautova, Savinkin, 2009). The only difference with Verseveldt's description for surface sclerites is the maximal size of the clubs with thickened blunt-ended shafts – 0.4 mm *vs* 0.7 mm long (with wider handles) in the Nha Trang's material. The incompleteness of the single colony described as holotype, which circumstance was noted by Verseveldt, may be a reason, but the geographical or ecological variability should not be excluded as a reason for this difference.

A molecular phylogeny (constructed using mitochondrial gene msh1 sequences) recovered five well-supported clades, which were not congruent with the traditional intrageneric taxonomic groups (McFadden et al., 2009). The morphological characters attributed to each clade include the detailed data on the polyp' armature in addition to the shape of the clubs in the surface tissues. The overall growth form of the colony also distinguishes some clades. Polyp sclerites have for the most part been overlooked taxonomically in *Sinularia* (Verseveldt, 1980). As a result information on these characters is lacking in many species descriptions, published earlier, but construction of a molecular phylogeny has led us to recognize the phylogenetic and taxonomic importance of previously overlooked morphological characters (McFadden et al., 2009). The overwhelming majority of publications from the last decades of the 20 century are unusable because of inaccurate hand-made sclerite drawings. Apart from the fact that these drawings very often were made using low microscope magnifications, the opinion that the full sclerite set per specimen/species is necessary for the sample identity was established just towards the end of the 20th century.

Although *Sarcophyton* and *Lobophytum* are quite easily distinguished from *Sinularia* by the presence of siphonozooids, the morphological distinctions between *Sarcophyton* and *Lobophytum* are less obvious. In keys to the octocoral family Alcyoniidae, these two genera are distinguished from one another primarily by colony growth morphology (Verseveldt, 1982). *Sarcophyton* typically forms colonies in which the polyp-bearing region (disc or polyparium, typically wider than the stalk) is raised on a prominent stalk, often resembling a mushroom (Fig. 2A). The disk may have a highly folded margin but remains smooth. In contrast, in species of *Lobophytum* the polyp bearing area is commonly not much wider than the stalk and usually has prominent lobes or ridges on its upper surface; colonies are often thick and plate-like (Fig. 2B). The two genera can be distinguished further by the form of the sclerites that are found in the interior coenenchymal tissue of the colony stalk or base. In *Sarcophyton*, these sclerites are usually large (to 2.0 mm) and spindle shaped and commonly have irregularly distributed tubercular ornamentation, while the interior sclerites of *Lobophytum* tend to be smaller (less than 0.5 mm) and oval, with the tubercles arranged in regular, transverse

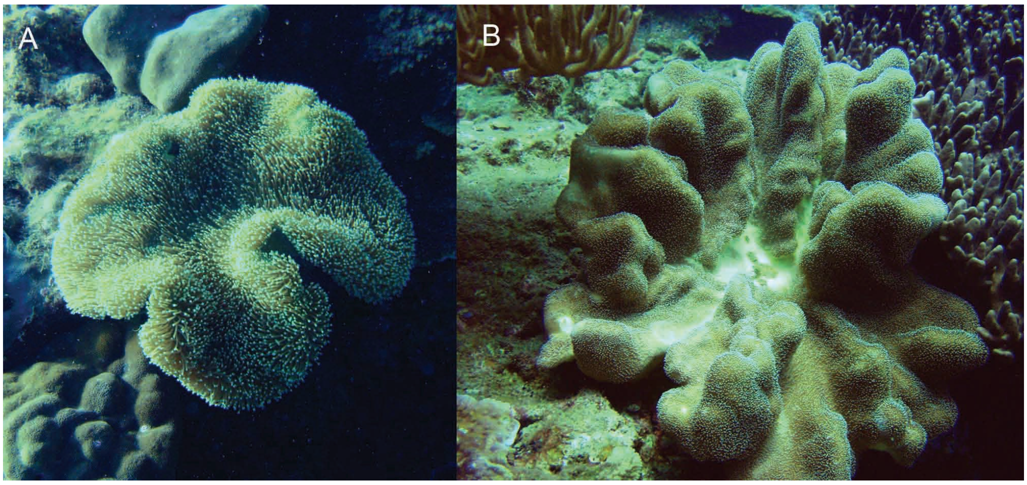

Fig. 2. A – *Sarcophyton serenei*, the typical mush-room colony shape with wide polyparium, Tre Island, Nha Trang Bay; B – *Lobophytum ransoni*, the polyparium is wide and low, Mun Island, Nha Trang Bay.

girdles. The clubs from the *Lobophytum* colony surface tend to have less defined heads than those of *Sarcophyton* (Verseveldt, 1983). However, these distinctions between the genera blur in cases where colony growth form is intermediate (Fig. 2A, B), or in which species with the growth form typical of one genus have sclerites more typical of the other (McFadden et al., 2006).

The examination of the phylogenetic relationships among species in the two genera using DNA sequence data (mitochondrial gene msh1) suggests that the conventional taxonomic division between *Lobophytum* and *Sarcophyton* does not reflect the evolutionary relationships among species assigned to these two genera. Three very well-supported clades are evident in the analysis: one encompassing species with typical *Sarcophyton* morphological characters, one comprising morphologically typical *Lobophytum* species, and a third distinct clade that includes a mix of nominal species from each genus. This mixed clade includes a number of taxa that have traditionally been assigned to *Lobophytum* on the basis of colony growth morphology but whose interior sclerites bear closer resemblance to those of *Sarcophyton*. Within each clade are multiple examples of specimens identified to different species that nonetheless share identical msh1 haplotypes. Conversely, some specimens identified as belonging to the same species fall into different genetic sub-clades. The lack of clear genetic distinctions among species in each clade is likely the combined result of two factors, the most obvious of which is that the msh1 gene simply may not be sufficiently variable to distinguish closely related species within these genera (McFadden et al., 2006). The second important factor was the above mentioned poor state of old literature.

It can be concluded that the confused state of the taxonomic status of many Alcyoniidae taxa (including the species boundaries verification) is a combined product of our present lack of understanding of intraspecific variation and a historical lack of taxonomic and ecological work on these corals in general. Ecological, reproductive, and behavioral differences in conjunction with the study of morphological and genetic variation are suggested to provide important taxonomic characters that may help to distinguish morphologically cryptic yet genetically distinct forms.

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CHARACTERIZATION OF BIOSURFACTANT PRODUCING BACTERIA *PSEUDOMONAS* SP. HP55, *ACINETOBACTER HAEMOLYTICUS* QN15 AND *RHODOCOCCUS RUBER* TD2 ISOLATED FROM VIETNAM COASTAL ZONES

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Introduction

Biosurfactants are surface-active compounds with both hydrophilic and hydrophobic regions in their structures and mainly produced by bacteria and yeast. The biosurfactants can reduce the interfacial tension at oil/water interfaces. These compounds can be roughly divided as lipopeptides, glycolipids, proteins and high-molecular-weight polymers of polysaccharides, lipopolysaccharides proteins or lipoproteins. The best-studied microbial surfactants are glycolipids. Among these, the best-known compounds are rhamnolipids, trehalolipids, sophorolipids and mannosylerythritol lipids (MELs). Biosurfactants are attracting much interest in many fields such as environmental application (oil bioremediation), industrial application (oil recovery and processing), biomedical application (antimicrobial activity, anti-adhesive), food processing. Biosurfactants have gained importance in these fields due to their unique properties such as higher biodegradability and lower toxicity (Banat et al., 2010). With these potential advantages, the study of biosurfactants has been interested in many countries. In Vietnam, few papers about marine yeast or bacteria isolated from Jet A1 were recently published (Pham et al., 2010). Here, in this paper, we reported some results of characterization of some marine biosurfactant producing bacteria in order to apply to oil polluted treatment.

Material and Methods

- HP55, QN15 and TD2 strains were isolated from Haiphong, Quangnam and Vungtau coastal zones of Vietnam.
- Medium for isolation of marine biosurfactant producing bacteria is API RP38 1% NaCl. Medium for marine bacterial biosurfactant production is Gost 1% NaCl supplemented with 1% diesel.
- Cellular morphology was observed by scanning electron microcospy S4800.
- E24 index was determined according to Purthi and Cameptra (1995).
- Biosurfactant compounds were extracted from marine bacterial strains according to Sivapathasekaran et al. (2009), then characterized by FT-IR analysis.
- Testing growth inhibition of pathogen by biosurfactant compounds by using agar-diffusion method.
- Toxic testing of biosurfactant compound to *Chaetoceross muelleri* and larva of *Diadema setosum* was performed by EPAM-94-EPA Marine.

Results and Discussion

1. Morphology characterization

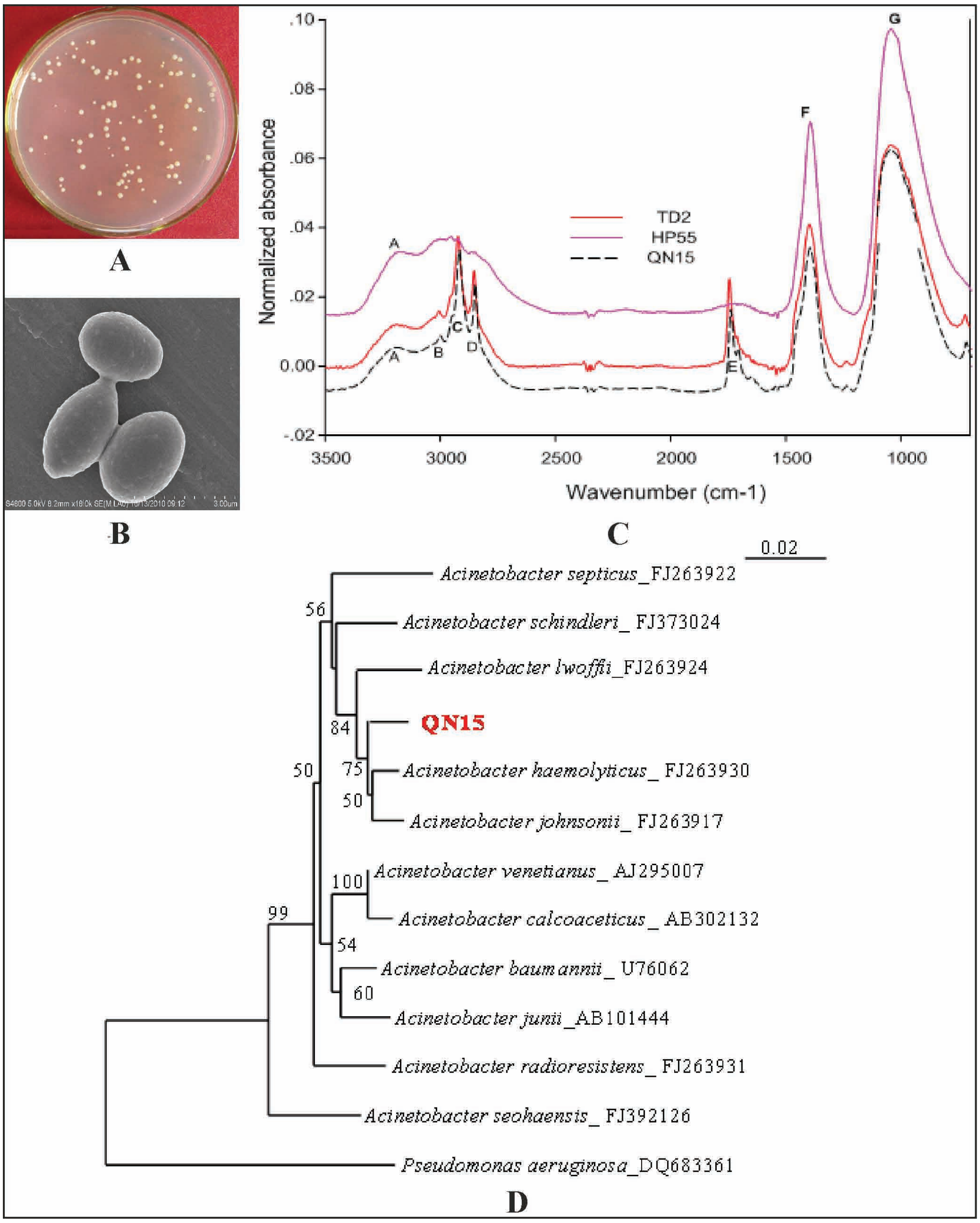
Three marine biosurfatant-producing strains HP55, QN15 and TD2 were isolated from coastal zones of Haiphong, Quangnam and Vungtau (see Figure). Their colony and cellular morphology was different, the HP55 cells were rod shape with single polar flagellum, TD2 cells also rod shape but without flagella and QN15 were oval shape. Gram stainning results showed that HP55 and QN15 strains were Gram negative whereas the other was Gram positive. Emulsion index of them was very high. E24 index of HP55, QN15 and TD2 was 85%, 69% and 65%, respectively. The E24 index indicated that these strains were high biosurfactant producing bacteria.

2. Phylogenetic characterization

Data of 16S rRNA analyses showed that HP55, QN15 and TD2 strains were defined as *Pseudomonas* sp. (100% identity), *Acinetobacter haemolyticus* (99% identity) and *Rhodococcus ruber (*100% identity), respectively. These bacteria were known as biosurfactant producers in agreement with Banat et al. (2010).

3. Biosurfactant characterization

Infrared spectrum analysis (FT-IR) showed that all three kinds of biosurfactant have hydrophilic hydroxyl (-OH) groups (band A). Bands B, C, D appeared CH^2 or CH^3 groups in 3000-2800 cm^{-1} region. These results demonstrated that there are straight functional H-C groups or branch functional HC groups in these biosurfactants. Result of analysis of 1453, 1398 cm^{-1} (band F) confirmed further that these compounds contain alkyl groups in the molecule. Carbonyl (C=O) groups also found in 1743 cm^{-1} and 1709 cm^{-1}(brand D) and these were esters groups (brand E). However, biosurfactants which were separated from HP55 strain did not contain esters group (not had E band as result of analysis of TD2 and QN15). Results of infrared spectrum analysis at G band, 1084–1152 cm^{-1}, confirmed again that biosurfactant producing TD2 and QN15 had esters carbonyl group. Altogether, biosurfactants produced by TD2 and QN15 strains are



Colony morphology of QN15 (A), cellular morphology of QN15 under SEM (B), analysis of biosurfactant compounds extracted from HP55, QN15, TD2 strains (C), phylogenetic trees of QN15 strain by 16S rRNA analysis (D).

esters, they contained carbonyl and alkyl group (branch B). Biosurfactant was produced by HP5.5 did not contain esters group but had straight or branch hydrocarbon, these were quite different from the two remaining substances.

4. Inhibition of biosurfactant to growth of pathogen

Biosurfactant extracted from HP55 strain can inhibit the growth of some pathogenic bacteria such as *Escheria coli* ATCC 35128, *Sarcina lutea* ATCC 9341, *Staphylococcus aureus* ATCC 6538 and *Vibrio paraheamolyticus* HH1 (data are not shown). Our results were suitable with the antimicrobial activity of biosurfatant against pathogenic grampositive and gramnegative bacteria reported by Das et al. (2008).

5. Toxic testing of biosurfactant compound to *Chaetoceross muelleri* and larva of *Diadema setosum*

Using CHEPHAM10 method, toxicity of biosurfactant producing by TD2 and QN15 were further tested with *Chaetoceross muelleri* and larva of *Diadema setosum*. With tested species *Chaetoceross muelleri*, results showed that QN15 and TD2 had no toxic with the development of *Chaetoceross muelleri*. In control experiment, without QN15 and TD2, average density of *Chaetoceross muelleri* was 1.8 x 10^6 cell/ml. At present QN15 and TD2 in testing experiment, average density of *Chaetoceross muelleri* was the same, 1.4 x 10^6 and 1.2 x 10^6 cell/ml. With tested species larva of *Diadema setosum,* in control experiment using fresh sea water and in testing experiment addition of QN15 and TD2 product in order to compare the growth of *Diadema setosum* in two conditions. In testing experiment, ratio of larva of *Diadema setosum* was 70% in comparison with control experiment. These results indicated QN15 and TD2 product inhibited the growth of larva of *Diadema setosum* but in permit level. Altogether, results implied that both compounds are non-toxic to algae *Chaetoceross muelleri* and larva of *Diadema setosum*.

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PHILINORBIS TERAMACHII HABE, 1950 (GASTROPODA: OPISTHOBRANCHIA: CEPHALASPIDEA) FROM COASTAL WATERS OF VIETNAM

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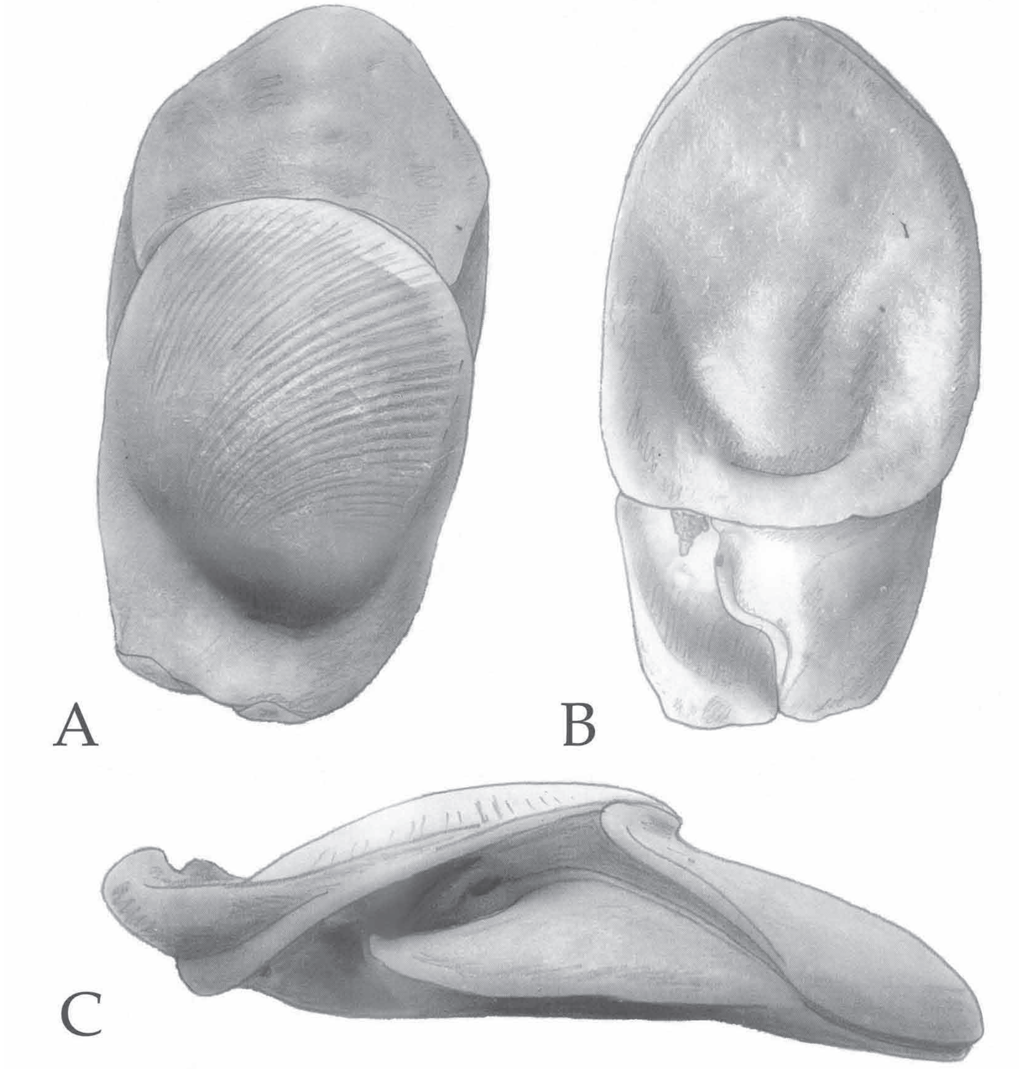
Philinorbis teramachii Habe, 1950 was previously known in several specimens from the Tosa Bay, southern Shikoku, Japan, 80–120 m, in sandy mud (Habe, 1950; Higo et al, 1999) and recorded for Taiwan at a depth of 120 m (The Taiwan Malacofauna Database, 2011). The description of the species consists of the description and drawing of only the shell (Habe, 1950, p. 52, pl. 9, figs. 3, 4).

One specimen of this rare species was found in the South China Sea near Vietnam (R/V "Odissey", 25.09.1984, 11º01′ N, 109º55′ E, depth 460 m, mud, coll. by Dr. B.I. Sirenko). The body of preserved specimen (28 mm length and 15 mm width) has no traces of any pigmentation and it is significantly flattened. The cephalic shield (see Fig., A – dorsal view) is 14 mm in length and 13 mm width and it has oval-pentagonal form. The line of posterior end of the cephalic shield is slightly concave, without lobes. Posterior shield is 8 mm in length and is only partly covered with the big flat internal shell. The shell (15 mm height and 13 mm width) is with nearly circuit counter represented by the large flattened body whorl, the upper edge has small triangular process arising under the small spire. Spiral sculpture consists of numerous well-expressed straight grooves. Foot (see Fig., B – ventral view) is 19 mm in length and it does not extend for the full length of the body. It is wide and without parapodia. There is a large genital opening on the base of right hand side of posterior foot corner (see Fig., C – lateral view) from which open seminal groove runs anteriorly along the side of the body to open into eversible head penis at the right anterior corner of the head. Gill lies behind of genital opening. Posterior end of the posterior shield is developed into wide flat "tail" 7 mm length with small triangular appendages on the end. This "tail" bearing mantle groove on the inner side is unusual for both Philinidae and Aglajidae. Buccal bulb (7 mm in length and 5 mm breadth) is large and muscular. There is radula with a pair of large lateral teeth in a row in the lower part of posterior end of the buccal bulb. Thin-walled crop was filled with gray mud and some foraminifera.

Habe (1976) noted that *Ph. teramachii* resembles *Pseudophiline hayashii* Habe, 1976 and both are more similar to *Philine* Ascanius, 1772 than to any of the aglajid genera. But as Habe believed, the last species has no radula and no gizzard plates, and he placed the both genera to Aglajidae (Habe, 1976). Gosliner (1980, p. 357) did not agree with Habe and has cited *Pseudophiline hayashii* as *Philine hayashii*. Later Habe described radula for *P. hayashii* and he believed that this species belong to Philinidae too (Habe, 1982). According to our data on morphology of *Ph. teramachii* and to description of *P. hayashii* by Habe (1976), the both species have similarity in body shape and in morphology of the shell and digestive system; they belong to *Philinorbis* Habe, 1950. *Pseudophiline* Habe, 1976 is the junior synonym of *Philinorbis. Philinorbis* includes two species: *Philinorbis teramachii* Habe, 1950 and *Philinorbis hayashii* (Habe, 1976) – comb. nov.



Morphology of the *Philinorbis teramachii* Habe, 1950 from Vietnam. A – dorsal view, B – ventral view, C – lateral view.

Ph. teramachii, the type species of *Philinorbis*, has differences from *Philine aperta* (Linnaeus, 1767) in absence of derived parapodia, more open mantle cavity and presence of the "tail". The caudal lobes in all philinids and *Melanochlamys* Cheeseman, 1881*,* most *Philinopsis* Pease, 1860 and some *Aglaja* Renier, 1807 are short and blunt while in other aglajids, as in *Chelidonura* A. Adams, 1850*,* they are variously elaborated (Gosliner, 1980), asymmetric, with one or two lobes. These outgrowths extend posteriorly from the left side or from the left and right sides of the posterior shield. The long symmetric process in *Philinorbis* extends posteriorly from the entire back surface of posterior shield, it is not divided on left and right lobes. *Philinorbis* is the valid genus of Philinidae.

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MOLECULAR PHYLOGENETIC RELATIONSHIP OF VIETNAMESE GROUPERS (*EPINEPHELUS* AND *PLECTROPOMUS*) BASED ON SEQUENCES OF 16S mtDNA

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Introduction

Groupers (Serranidae, Epinephelinae) are coral reef fish, distributed throughout tropical and warm-temperate seas around the world, and are currently represented by 15 genera and 159 species. The genus *Epinephelus* comprises approximately 100 species of perciform fishes worldwide (Heemstra, Randall, 1993).

In Vietnam, there has been considerable growth in grouper and other finfish grow-out aquaculture in net cages throughout the 1990s. Around 10 species were reported both in wild conditions and culture distributed primarily in Quang Ninh and Hia Phong in the north and Khanh Hoa, Phu Yen in the south (Le, 2004; Pierre et al., 2008). The morphological characteristics such as color, body and pattern (typically used to identify individual species in the field) have obvious limits which lead to confusion in many species within groupers (Chen et al., 2008; Zue, Yue, 2008).

The 16S gene of mtDNA (16S mtDNA), a useful molecular marker for biological evolution researches, has been widely used for the research of fishery phylogenetic relationships between genera and species which has solved some classification and phylogenetic problems (Craig et al., 2001; Zhu et al., 2008). The aim of this study is to construct the phylogenetic relationships of Vietnamese groupers of genera *Epinephelus* and *Plectropomu*s to create the input data for the conservation of fisheires resources.

Material and Methods

Sampling

Wild groupers (*Epinephelus fasciatu*s, *E. bruneus*, *E. coioides*, *E. bleekeri* and *E. merra*) were collected by hook-and-line and *Epinephelus* spp. and *Plectropomus leopardus* were purchased from sea cage culture in Nha Trang Bay (12°15′ N, 109°21′ E) in 2007 and 2008. Samples were transferred to the laboratory in cold condition, and then stored at -70^0C for further analysis.

DNA extraction, PCR amplification and sequencing

DNA extraction was performed using WIZARD SV genomic DNA purification kit (Promega) following manufacturer's instructions. Five μl of lysate was used as template in PCR reactions to amplify the 16S of mitochondrial DNA (16S mtDNA) using the primers 16SF 5′-CCGGTCTGAACTCAGATCACGT-3′ and 16SR 5′-GTTTACCAAAAACATGGCTTC-3′ (Espiritu et al., 2001). PCR reactions were performed in the total of 50μl including 2μ DNA template, 5μl 10X Taq buffer, 0.25mM each dNTP, 2μM mỗi primer (10mM), 1 unit of Taq polymerase (5U/1μl) and distilled water to a final volume. Biorad thermocyclers (Icycler) were used at the following temperature program: initial denaturation at 95°C for 5 min, followed by 35 cycles at 94°C for 40s, 47°C for 30s and 72°C for 1 min, and a final extension at 72°C for 5 min.

PCR products were separated on 1.5% agarose gel stained with ethidium bromide, and then analyzed by the GelDoc using the software Quantity One (Bio-Rad). PCR products were purified using an E.Z.N.A. Cycle-Pure Kit (Omega Bio-tek, Doraville, USA) as the manufacturer's instructions. Cycle sequencing was performed using a dye-labelled dideoxy terminator (Big Dye Terminator v. 3.1, Applied Biosystems) with the same primer as PCR reaction following the temperature program: 96°C for 20s, 50°C for 20s, and 60°C for 4 min. Products were analyzed using an ABI Prism 3700 DNA Analyzer (Applied Biosystems). Sequence contigs were assembled using Contig Express in the software package Vector NTI v. 11.

The Basic Logical Alignment Search Tool (BLAST, http://www.ncbi.nlm.nih.gov/BLAST) was used to identify the resulting sequences. Sequences were initially aligned by eye using the sequence editor BioEdit 7.0 (Hall, 1999), followed by a computer multiple sequence alignment in Clustal X Ver. 1.8 (Thompson et al., 1997) using default parameters and visual verification.

Phylogenetic Analysis

Phylogenetic analyses were conducted based on the sequences of 16S mtDNA of 7 species of *Epinephelus* (*Epinephelus bleekeri*, *E. brunneus*, *E. coioides*, *E. fasciatus*, *E. malabaricus*, *E. merra* and *E. fuscogutatus*) and 1 *Plectropomus* species (*Plectropomus leopardus*). Six other *Epinephelus* and 2 *Plectroponus* species whose 16S mtDNA sequences were downloaded from the GenBank. *Variola louti* was used as outgroup**.**

Data were analyzed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). Standard unweighted MP and ML were performed in PAUP 4.0 (Swofford, 2001). Prior to ML and BI analyses, best-fit models of nucleotide substitution were selected by the Akaike Information Criterion as implemented by Modeltest 3.7 (Posada, Crandall 1998) and Mrmodeltest 2.2 (Nylander, 2004).

In the Bayesian analysis, substitution model base frequency was estimated, four chains were used and the analysis was run for 1 million generation with the sampling frequency of 100. Trees from the “burn-in” generation were discarded and a final 50% majority-rule consensus tree constructed from remaining trees. Numbers at the interior braches of the majority-rule consensus tree represent posterior probability (PP) (Huelsenbeck, Ronquist 2001).

Bootstrap (BT) support values were used to assess the robustness of the findings. Bootstrap values were computed from 100 replicates randomized 10 times with TBR addition sequence. Due to computational time, calculations of bootstrap values under the MP criterion were conducted with heuristic searches replicated 1000 times and 100 times for ML analysis. Tree display and editing was performed with TreeView 1.6.6 (Page, 1996).

Results

Sequence characters

The length of 16S gene segments of grouper species and *Variola louti* which was designed as outgroup were 550 bp amplified by PCR (Fig. 1). Considering a total of 550 bp for the analysis, 29 were variable sites, and 102 bp were parsimony-informative sites, 419 bp were constant site. Except the outgroup, the mean percentage divergence was 8.5% and 3% from genus *Epinephelus* and *Plectropomus*, respectively. The percentage divergence between *E. bleekeri* and *E. akaara* was only 1%, which was the lowest. However, the percentage divergence between *E. tauvi*na and *E. fuscogutatus* was 7.1%, which was the highest. The percentage sequence divergences ranged from 2.3% to 3.7% among other species of *Plectropomus*.

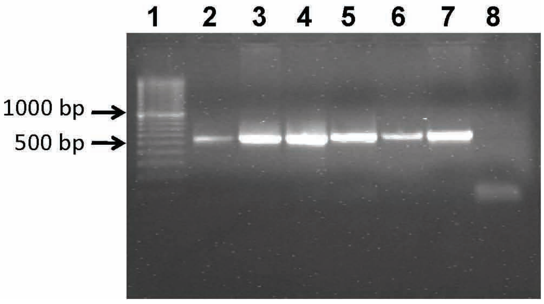

Fig. 1. PCR products of 16S mtDNA gene of grouper species**.** Lane 1: DNA marker 100 bp. Lane 2–7, PCR products of grouper species. Lane 8: negative control.

Phylogenetic Tree

Unweighted MP analysis produced a single tree with the tree length 237, onsistency index (CI) 0.7131, retention index (RI) 0.7799. Based on the partial 16S sequences of *Epinephelus* fishes and using *Variola louti* as outgroup, a molecular phylogenetic tree was constructed by MP, ML and BI methods. The values of bootstrap (BT) and posterior probability (PP) of nodes were indicated above the branch (Fig. 2). The figure showed that all species of the genus *Epinephelus* in this study produced one monophyletic tribe with distinct subgroup were clustered to sisters as follow. The first group include *E. fasciatus*, *E. merra* and *E. tauvina*; the second *E. bleeleri*, *E. akaara* and *E. sexfasciatus*; the third *E. coioides* and *E. malabaricus*, and the fourth *E. latifasciatus* and *E. fuscogutatus*. Three species *E. aeeoatus*, *E. howlandi* and *E. bruneus* have undetermined taxonomic positions. The relationship was highly supported by BT and PP value (more than 70%).

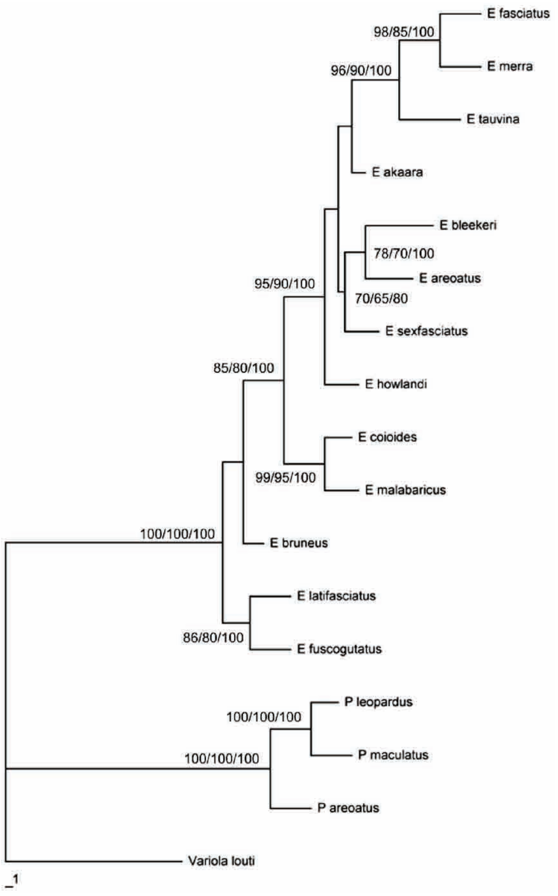

Fig. 2. Phylogram obtained from the 16S mtDNA analysis of grouper species; maximum parsimony tree with bootstrap support values (1000 and 100 replicates for MP and Mlanalysis, respectively) and posterior probability (BI analysis) along the branch. *Variola louti* were used as outgroups

Three *Plectropomus* species were also clustered in mormophletic group as *P. leopardus* was clustered to *P. maculatus*, and together they are sister clade to *P. aerolatus*.

Discussion

The groupers belong to fish of protogynous hermaphrodite and undergo sex reversal from female to male, which can be different from other genera of family Serranidae. At the same time, the interspecific fishes of grouper had difficulty in morphologically distinguishing, especially at the larval period, which was more difficult. Thus, synonymic phenomena often occur (Koedprang et al., 2007).

Zhu et al. (2008) examined the phylogenetic relationships of seven endemic species of groupers − *Epinephelus awoara*, *E. brunneus*, *E. coioides*, *E. longispinis*, *E. sexfasciatus*, *E. spilotoceps* and *E. tauvina* in China using the sequences of the mtDNA Cyt b gene. The result show that cluster relationships between *E. awoara* and *E. sexfasciatus*; *E. coioides* and *E. tauvina*; *E. longispinis* and *E. spilotoceps* were consistent with phenotypes in taxonomy. In addition, grouper species in the Atlantic Ocean were associated closely with those in the Pacific Ocean.

In this study, *Epinephelus* and *Plectropomus* species, all the species of assembled together, forming a monophyletic group, respectively. Using 16S mtDNA, *E. coioides* and *E. malabaricus* were clustered together, while *E. tauvina* was sister to *E. fasciatus*, *E. merra*. *E. sexfasciatus* was in the same clade with *E. bleeleri* and *E. akaara*, *E*, *coioides* and *E. tauvina* display close relationship (sequence difference is 1%) with Cyt b marker, while they are distribute in distinct clade and sequence difference is 5%. With 16S mtDNA.

However, our results were somehow consistent to those obtained on the phylogenetic tree of the subfamily Epinephelinae (Seranidae) carried out by Craig and Hasting (2007) based on molecular marker 12S and 16S of mtDNA.

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THE STUDY ON THE CHANGE OF SPECIES COMPOSITION AND DISTRIBUTION OF MARINE ALGAE FROM CAT BA AND HA LONG BAY

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Cat Ba – Ha Long are located in the west of the Tonkin Gulf and they are belonging to Cat Ba Biosphere Reserves area and Ha Long World Heritage. Here, a lot of research on seaweeds were conducted and the results showed that in recent years, the composition and distribution of seaweeds have been changed significantly. Some species of seaweeds previously distributed in Cat Ba and Ha Long disappeared. This article deals with variation of species composition and distribution of seaweeds in the study area.

RESOURCES OF MARINE MACROPHYTES AND THEIR USE IN VIETNAM

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Total number of marine plants dwelling along the Vietnamese coast with numerous islands and banks amounts to about 1000 species. 600 species of them are macrophytes including 300 species of Rhodophyta, 130 species of Heterokontophyta and more than 120 species of Chlorophyta (Phang-Hoang Ho, 1967). 200 species of these species are potentially useful and about 60 species are used in Vietnam in diet, medicine and for getting phycocolloides (see Table).

Useful seaweeds and their use in Vietnam

Species	Use for food	Medical use and food additions	Getting of polysaccharides	Export
Rhodophyta				
Acanthophora spicifera (M. Vahl) Børgesen			+	
Ahnfeltiopsis spp.		+		
Betaphycus gelatinus (Esper) Doty ex P.C. Silva	+	+	+	+
Caloglossa leprieurii (Montagne) J. Agardh		+		
Catenella nipae Zanardini	+			
Chondracanthus intermedius (Suringar) Hommersand			+	
Dermonema virens (J. Agardh) Pedroche & Ávila Ortíz [=*D. dichotomum* J. Agardh]	+	+		
Eucheuma arnoldii Weber-van Bosse	+			
E. denticulatum (N.L. Burman) F.S. Collins & Hervey	+	+	+	+
Gelidiella acerosa (Forsskål) Feldmann & G. Hamel	+		+	
Gelidium crinale (Hare ex Turner) Gaillon	+		+	

Table (continued)

Species	Use for food	Medical use and food additions	Getting of polysaccharides	Export
G. pulchellum (Turner) Kützing	+		+	
G. pusillum (Stackhouse) Le Jolis	+		+	
Gloiopeltis furcata (Postels & Ruprecht) J. Agardh	+			
G. tenax (Turner) Decaisne	+			
Gracilaria spp.	+	+	+	+
G. arcuata Zanardini	+			
G. blodgettii Harvey	+			
G. canaliculata Sonder [=*Gracilaria crassa* Harvey ex J. Agardh]	+			
G. coronopifolia J. Agardh	+			
G. firma Chang et Xia	+			+
G. foliifera (Forsskål) Børgesen	+			
G. gigas Harvey	+			
G. heteroclada Zhang et Xia	+		+	+
G. salicornia (C. Agardh) E.Y. Dawson	+			
G. tenuistipitata C.F. Chang & B.M. Xia	+		+	+
G. textorii (Suringar) De Toni	+			
G. vermiculophylla (Ohmi) Papenfuss [=*G. asiatica* Zhang & Xia]	+		+	+
Grateloupia lithophila Børgesen	+			
G. ramosissima Okamura	+			
Hydropunria eucheumatoides (Harvey) Gurgel & Fredericq [=*Gracilaria eucheumatoides* Harvey]	+			
Hypnea boergesenii T. Tanaka	+	+		
H. japonica Tanaka	+	+		
H. musciformis (Wulfen) J.V. Lamouroux	+	+	+	
H. valentiae (Turner) Montagne	+	+	+	
Kappaphycus alvarezii (Doty) Doty	+	+		+
K. cottonii (Weber-van Bosse) Doty ex P.C. Silva	+	+	+	+
K. inermis (F. Schmitz) Doty ex H.D. Nguyen & Q.N. Huynh	+	+		

Table (continued)

Species	Use for food	Medical use and food additions	Getting of polysaccharides	Export
K. striatus (F. Schmitz) Doty ex P.C. Silva	+	+	+	+
Eucheuma denticulatum (N.L. Burman) F.S. Collins & Hervey	+	+	+	+
P. suborbiculata Kjellman	+			
P. vietnamensis T. Tanaka & Pham-Hoàng Ho	+			
Heterokontophyta				
Sargassum spp.	+	+	+	+
S. carpophyllum J. Agardh	+		+	+
S. aquifolium (Turner) C. Agardh [=*S. crassifolium* J. Agardh; *S. binderi* Sonder ex C. Agardh]	+		+	+
S. glaucescens J. Agardh			+	+
S. graminifolium C. Agardh			+	+
S. hemiphyllum (Turner) C. Agardh	+	+		+
S. henslowianum C. Agardh			+	+
S. ilicifolium [= *S. cristaefolium* C. Agardh; *S. duplicatum* Bory de Saint Vincent; *S. duplicatum* (J. Agardh) J. Agardh; *S. sandei* Reinbold]	+		+	+
S. mcclurei Setchell			+	+
S. oligocystum Montagne	+		+	+
S. polycystum C. Agardh	+		+	+
S. siliquosum J. Agardh			+	+
S. swartzii C. Agardh	+			+
S. vachellianum Greville			+	+
Turbinaria spp.		+		
Chlorophyta				
Caulerpa racemosa (Forsskål) J. Agardh	+			
C. lentillifera J. Agardh	+			
Codium spp.	+	+		
Gayralia oxysperma (Kützing) K.L. Vinogradova ex Scagel et al. [=*Monostroma oxyspermum* (Kützing) Doty]	+			
Ulva lactuca Linnaeus	+	+		
U. prolifera O.F. Muller		+		
U. reticulata Forsskål	+	+		

Sargassaceae

In total, about 70 species of the genus *Sargassum* were found in Vietnam (Nhuyen Huu Dai, 1997), from which the greatest stocks had *S. carpophyllum*, *S. aquifolium*, *S. ilicifolium*, *S. glaucescens*, *S. graminifolium*, *S. henslowianum*, *S. mcclurei*, *S. oligocystum*, *S. polycystum* and *S. vachellianum* (Huynh, Nguyen, 1998; Nguyen, Huynh, 2007).

The greatest stocks were concentrated in the north (Tonkin Bay), in central Vietnam and in the south (the Gulf of Thailand). Recently, all biomass of the *Sargassum* spp. is collected from natural stocks in connection with great demand on the sargassaceae seaweeds (obtaining of fucoidan, fertilizer production and the increase in sale price). Such irrational use of natural resources led to catastrophic decline in total annual production of sargassaceae fields.

***Gracilaria, Hydropuntia* and other agarophytes**

There were found more than 20 species of Gracilariaceae: four species of *Hypnea* (*H. japonica*, *H. boergesenii*, *H. valentiae*, *H. musciformis*) and one species of *Gelidiella* (*Gelidiella acerosa*) (see Table).

Some species of *Gracilaria*, such as *G. vermiculophylla*, *G. tenuistipitata*, *G. firma*, *G. foliifera*, *G. gigas*, *G. textorii* are growing in the north Vietnam, *G. coronopifolia*, *G. heteroclada*, *Hydropuntia eucheumatoides* – in the south Vietnam, *G. blodgettii*, *G. salicornia*, *G. crassa* and *G. tenuistipitata* distributed along the whole coast.

During the last decades, yearly, 1500–2000 ton of dried mass of agarophytes are collected in the Quang Ninh, Hai Phong, Nam Dinh, Thai Binh, Nam Dinh, Thanh Hoa, Thua Thien Hue, Quang Nam, Da Nang, Binh Dinh, Phu Yen, Khanh Hoa, Ninh Thuan, Ba Ria-Vung Tau provinces.

Kappaphycus, Eucheuma and Betaphycus

Kappaphycus cottonii, *K. striatus*, *K. enermis*, *Eucheuma arnoldii*, *E. denticulatum* and *Betaphycus gelatinus* are growing along the shore of central Vietnam. *K. arnoldii* dwells in Ninh Thuan Province (Ninh Hai District). *K. cottonii*, *K. striatum* and *E. arnoldii* were also found on Truong Sa, Nam Yet, Son Ca and Song Tu Tay (Spratly Islands). Annual production of *K. cottonii*, *K. striatum* and *E. arnoldii* amounted to about 3000 ton dry weight.

Of the greatest economical importance are numerous species of brown algae of the genera *Sargassum* and red algae of the genus *Gracilaria* and *Hudropuntia. Sargassum* spp. are used as human food, as fertilizer for agricultural fields, and also for alginate and fucoidan production. Agar is produced from *Gracilaria* spp. and used in food industry and as human food. In the last years, *Kappaphycus alvarezii*, introduced from Japan, as a source of carrageenan, is widely cultivated in central and south Vietnam. Various dishes and beverages are cooked from the seaweed. The export of *K. alvarezii* is of great economical significance.

The most species used as human food are *Porphyra crispata*, *P. suborbiculata*, *P. vietnamensis*, *Gelidiella acerosa*, *Gracilaria vermiculophylla*, *G. coronopifolia*, *G. firma*, *G. heteroclada*, *G. salicornia*, *G. tenuistipitata*, *Hydropuntia eucheumatoides*, *Kappaphycus cottonii*, *Betaphycus gelatinus*, *Hypnea muscoides*, *H. valentiae, Dermonema virens*, *Gloiopeltis* spp. (Rhodophyta), *Ulva lactuca*, *U. reticulata*, *Caulerpa racemosa*, *C. lentillifera* (Chlorophyta) and *Sargassum* spp. (Heterokontophyta).

Obtaining of fucocolloids from seaweeds in Vietnam is insignificant in comparison with the countries of South-East Asia (China, Japan, Korea, Philippines and Indonesia) that is connected with relatively insignificant stocks of useful seaweeds, poor development of cultivation and the absence of high technology and methods on processing of marine plants.

There are some cultivated species of *Gracilaria*: *G. tenuistipitata*, *G. vermiculophylla*, *G. heteroclada*, *G. firma* and *Gracilariopsis bailiniae*, two species of *Kappaphycus* (*K. alvarezii* and *K. striatus*), *Eucheuma denticulatum* and small quantity of the green alga *Caulerpa lentillifera*.

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MORPHOLOGY OF THE PROBOSCIS OF SOME NEMERTEANS FROM VIETNAMESE AND RUSSIAN COASTAL WATERS

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All nemertean worms (except *Arhynchonemertes axi*) possess a proboscis – a tubular organ which lies in a coelomic cavity called the rhynchocoel. This organ is used principally in the capture of prey, but may also be used as a defensive weapon or as a locomotory organ. The proboscis of 22 nemertean species from Vietnam and Russia were examined with confocal laser-scanning (cLSM) and transmission electron microscopy (TEM).

According to TEM data, the endothelium of the proboscis in all studied nemerteans is organized as a pseudostratified myoepithelium. It consists of two cell types resting on the basal extracellular matrix (ECM): apically situated supportive cells and subapical myocytes covered by the cytoplasmic sheets of the supportive cells. Myoepithelial cells form the inner circular musculature of the proboscis, and it appears that this muscle layer in all nemerteans is endothelial. We distinguished three types of the proboscis endothelium. The first type consists of one row of muscle cells (4 to 6 µm thick) (Fig. 1A). A single myocyte or a small group of myocytes (2–4 cells in each) is covered by the sheets of one supportive cell. The endothelium of this type was described in *Riseriellus occultus* (Montalvo et al., 1998a), *Malacobdella grossa* (Magarlamov, Chernyshev, 2010), and was found in *Tubulanus punctatus* and *Hubrechtella juliae* (present investigation). The endothelium of the second type, like the first one, consists of one row of muscle cells, but is only 1 µm thick (Fig. 1B). Each contractile process contains about 5–9 myofibrillae (from 0.3 to 0.8 µm in diameter). One myofilament-containing cytoplasmic process protrudes basally from the myocyte soma and then is divided into smaller protrusions. These protrusions are separated from each other by the basal cytoplasmic sheets of the supportive cells. This type of endothelium was found in the proboscis of *Carinina* sp. and *Cephalothrix simula*. The endothelium of the third type can be up to 60 µm thick (*Baseodiscus delineatus*); it consists of 4 to 7 rows of muscle cells containing groups of 8–14 cells (Fig. 1C). The apical and lateral surfaces of each group are surrounded by the cytoplasmic sheets of one supportive cell. This endothelium was discovered in *Baseodiscus delineatus* (Magarlamov, Chernyshev, 2011) and some reptantian hoplonemerteans from Vietnam (present investigation).

Our TEM investigations have not revealed any nonspecialized ciliated cells in the proboscis epithelium, but there are relatively abundant sensory cells with a single central cilium enclosed by one or two rings of microvilli (Fig. 1D). Confocal laser-scanning microscopical observations of anti-α-tubulin immunolabelled proboscis enabled the finding of sparse rudimentary cilia in endothelial supportive cells (Fig. 2A). The glandular epithelium of the proboscis in many anoplan nemerteans contains cells forming unique nematocyst-like structures named pseudocnidae (Turbeville, 2006). According to our investigations, pseudocnidae are lacking in *Baseodiscus delineatus*. We studied the ultrastructure of the pseudocnidae of *Hubrechtella juliae* – they are rod-shaped and 15–25 µm in length, with long tubular core (filament) situated acentrically in the subcortical layer (Fig. 1E).

In the proboscis of *Riseriellus occultus* some types of gland cells appear in associated pairs forming two kinds (A and B types) of adhesive glandular systems (Montalvo et al., 1998b). In *Tubulanus punctatus* we have found the glandular system corresponding to type B, which consists of a pseudocnid forming-cell and a mucus-like cell (Fig. 1H). The supportive cells of the proboscidial epithelium are rather regularly arranged around each glandular system. The glandular proboscis cells of *Malacobdella grossa* and *Hubrechtella juliae* do not form any glandular systems.

The basal part of the glandular epithelium has a layer of glia-like cells which form the so-called basiepithelial nerve plexus (BNP), which shows α-tubulin immunoreactivity (Fig. 2B). The BNP has been described in the proboscis of all main groups of

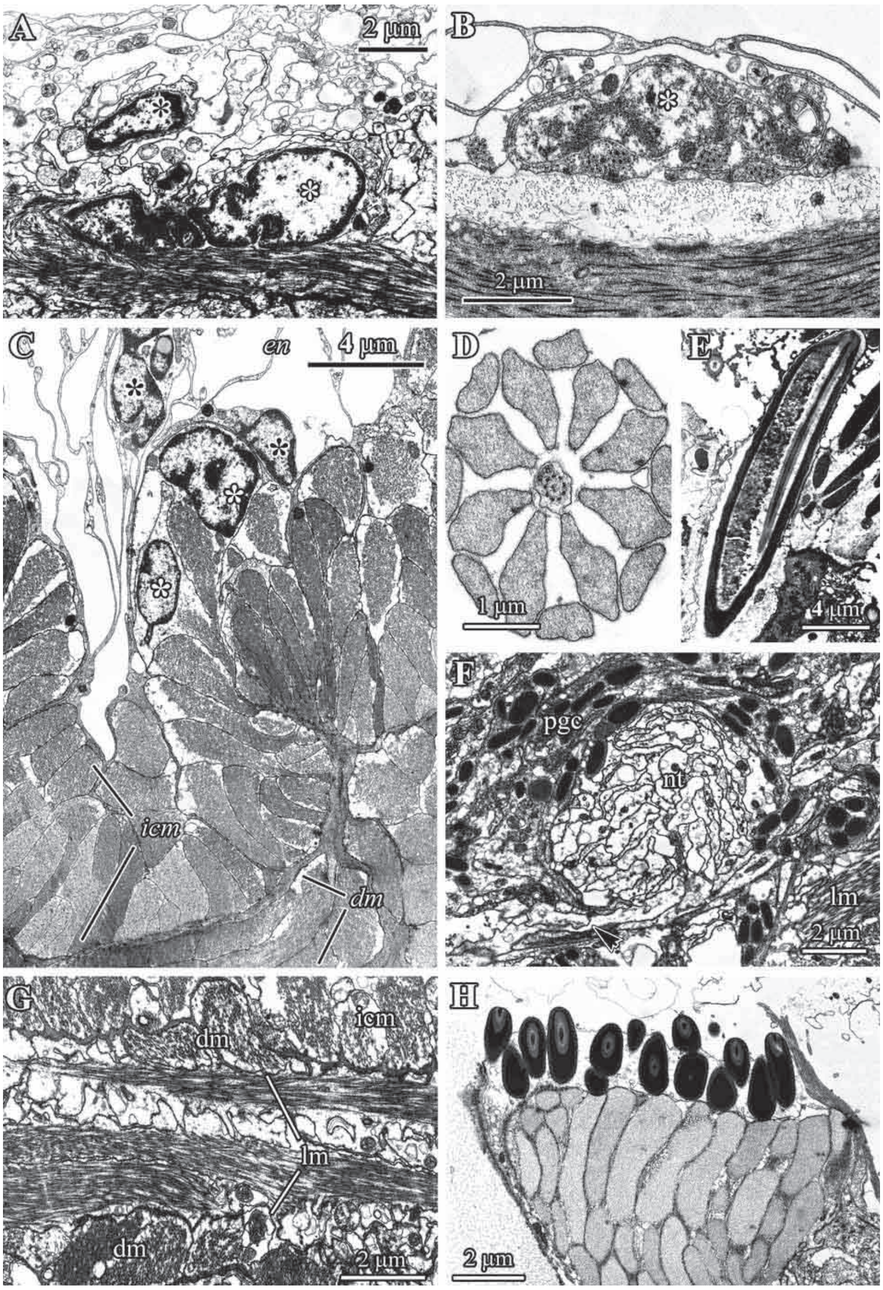

Fig. 1. Transmission electron micrographs of the longitudinal (B and F) and transverse (A, C–E, G, H) sections of the proboscis. (A) endothelial subapical myocytes of *Carinina* sp.; (B) supportive cells and myocytes of the endothelium of *Hubrechtella juliae*; (C) low-power micrograph of the proboscis of the *Baseodiscus delineates*; (D) apical part of the sensory cells of *H. juliae*; (E) pseudocnidae of *H. juliae*; (F) basiepithelial nerve plexus of *H. juliae*; (G) musculature layers of *H. juliae*; (H) *Tubulanus punctatus*, glandular system of type B. Black asterisk – supportive cells, white asterisk – myocytes. Abbreviations: en – endothelium, dm – diagonal musculature, icm – inner circular musculature, lm – longitudinal musculature, nt – nerve trunk, pgs – process of glia-like cells.

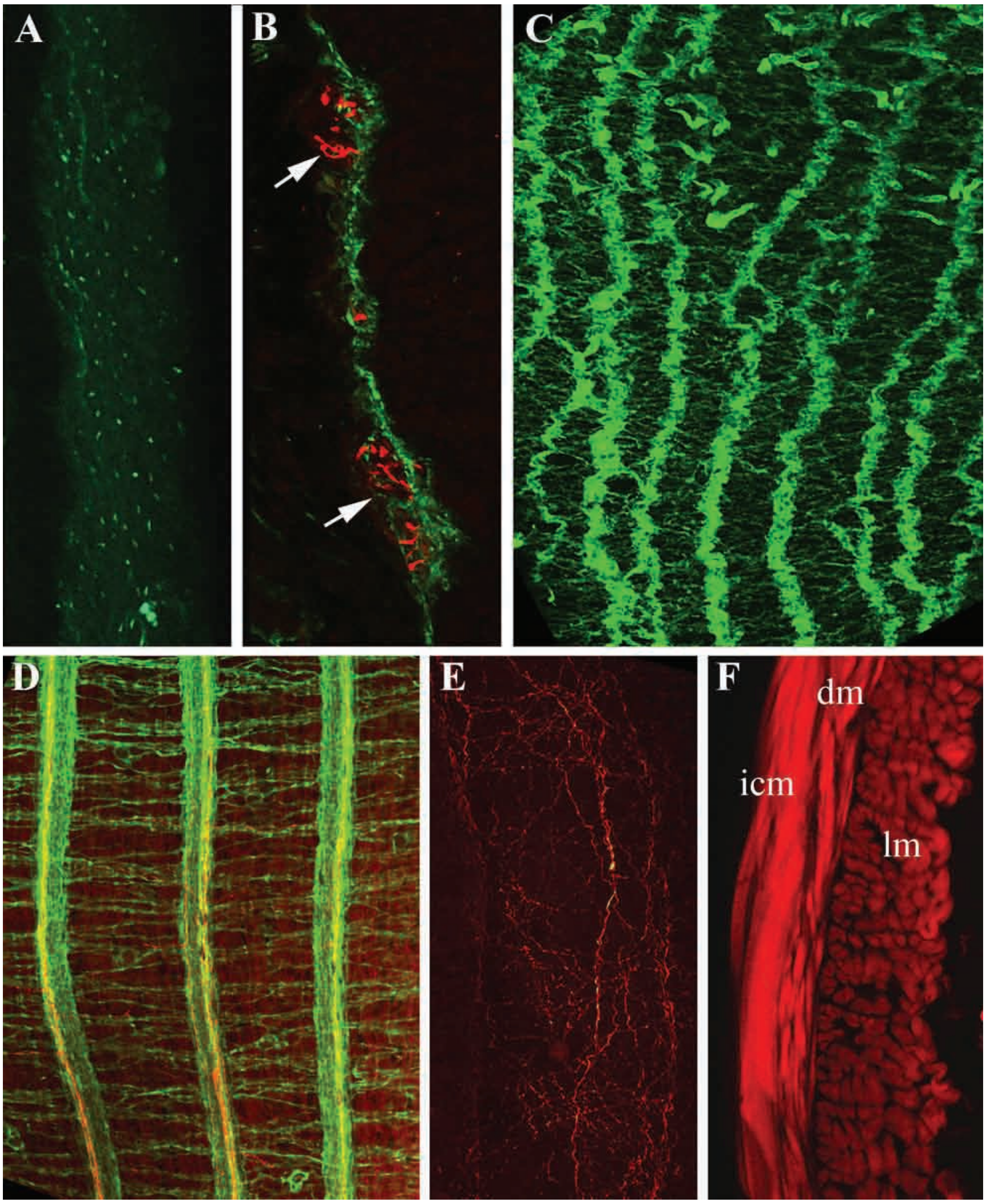

Fig. 2. Confocal laser scanning microscopy micrographs of the α-tubulin- and serotonin- immunoreactivity (A–E) as well as musculature (F) in the proboscis. (A) *Baseodiscus delineatus*, endothelial cilia (α-tubulin immunoreactivity); (B) intraepithelial nervous plexus of *B. delineatus* (transversal section, green – α-tubulin staining, red – serotonin staining), arrows – nerve trunks; (C) nervous plexus of *B. delineatus*, overlay of maximum projection of α-tubulin staining; (D) intramuscular nervous plexus of *Quasitetrastemma stimpsoni*, overlay of maximum projection of α-tubulin (green) and serotonin (red) staining; (E) serotonergic nervous plexus of *Micrura callima*, overlay of maximum projection; (F) musculature of *B. delineatus* (transversal section, phalloidin staining). Abbreviation: dm – diagonal musculature, icm – inner circular musculature, lm – longitudinal musculature.

the nemerteans. In paleo-, archaeo- and many heteronemerteans, the longitudinal nerve trunks (from two in Tubulanidae and Cephalotrichidae to 30 and more in *Baseodiscus*) pass inside the BNP (Fig. 1F). The nerve trunks are surrounded by glia-like cells and not separated from the glandular epithelium by the ECM, i.e., they have an intraepithelial position. In hoplo- and some heteronemertean, nerve trunks are separated from the BNP and are situated inside the proboscis musculature; in these nemerteans the BNP consists of glia-like cell processes only.

Using cLSM microscopy and antibody labeling we obtained new data on the proboscis nervous system morphology. In all studied hoplonemerteans, 10–16 longitudinal nerve trunks are linked by connective nerves forming grid-like nervous plexus (Fig. 2D). In *Baseodiscus,* 30–35 longitudinal nerve trunks have irregular connective nerves and anastomoses (Fig. 2C). In *Micrura callima* and *M. kulikovae* nervous plexus consists of numerous branched nerves (Fig. 2F).

Until recently, the diagonal musculature of the proboscis was known only in a few heteronemerteans, but, according to cLSM data, this layer is found in all anoplan nemerteans (Chernyshev, 2010). Typically the diagonal musculature is situated between the outer circular and longitudinal muscle layers, but *Baseodiscus delineatus*, *B.* cf. *princeps* and *Hubrechtella juliae* possess an unusual arrangement of the muscle layers. In *Baseodiscus* species, a single diagonal layer is situated between the inner circular and longitudinal muscle layers (Fig. 1C); *H. juliae* has two diagonal layers – the fist has a typical position, and the second lies as in *Baseodiscus* (Fig. 1G). In two archaeonemerteans from Vietnam, *Cephalotrichella alba* and *Balionemertes* sp., the proboscis possesses additional (outer) longitudinal musculature.

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EFFECTS OF GROW OUT TRANSPLANTATION TIMING ON GROWTH AND REPRODUCTION OF JUVENILE OYSTER, *CRASSOSTREA GIGAS* IN GAMAKMAN BAY, OFF THE SOUTHERN COAST OF KOREA

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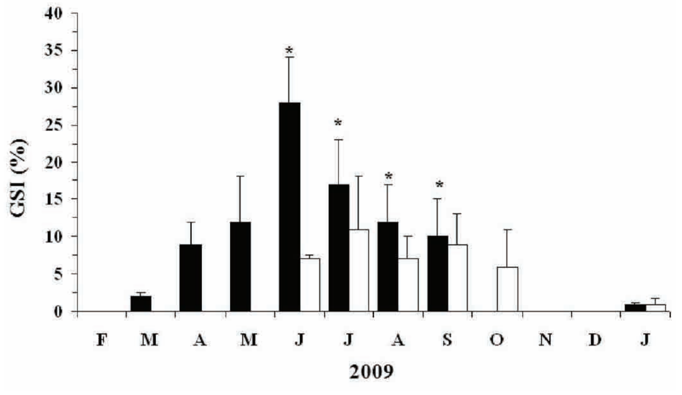
Pacific oyster, *Crassostrea gigas*, is the most important shellfish resources in Korea and has been widely cultured in numerous semi-enclosed bays along the south coast. In Korean oyster farming industries, the seed oysters are supplied from the wild, naturally occurring in water. The oyster spats are collected from the natural habitat during late June to August using 5–6 m long oyster culture string tied with numerous oyster shells and hardened for 7–9 months period in the intertidal zone where they periodically exposed to atmosphere daily with tidal fluctuation (Choi, 2008). Pacific oyster spat hardening is a common practice in Korea as well as in Japan and the hardened oyster showed higher survival rate, improved meat quality and greater adaptability to varying environmental condition than non-hardened oyster in the grow out field (Ventilla, 1984; Arakawa, 1990). Traditionally in Korea the hardened spat grow out transplantation is performed in May or June. To understand the effect of grow out transplantation timing on the growth and reproduction two populations of hardened oyster spats were differently transplanted into the Gamakman bay in January and May 2009, and monitored over a 12 months period.

The oysters grown rapidly after the grow out transplantation into the bay and the January transplanted oyster (JTO) reached 3 months earlier marketable size than that of May transplanted oyster (MTO). During the monitoring the shell length reached to 98±14 mm (for JTO) and 81±12 mm (for MTO) in November when the oysters were 1.5 years old. The condition index (CI) of JTO showed a seasonal fluctuation with gametogenesis, increased with the advance of gonad maturation and highest value was recorded in June, when most of the oysters were in ripe condition. CI suddenly declined in July and continued up to October, indicates the synchronous spawning activity of the oysters. But in MTO, CI did not clearly correspond with gametogenesis, increased throughout the study period with significantly lower values than that of JTO ($P<0.05$).

Histology revealed that sex was determinable during February–March with a male preponderance sex ratio when the oysters were earlier gonad maturation stage. With the advance of gonad maturation the females outnumbered the males, indicating the protandric nature of the oysters. The overall female to male sex ratio of JTO was 1:0.74 and MTO was 1:1.01. Microscopic observation confirmed 1.7–2.7% hermaphroditic population from the mature and spawning stage oysters. During the monitoring the gonad



Monthly variations of GSI (%) of January (solid bar) and May (open bar) transplanted oysters during the study period from Gamakman Bay, Korea. The values are mean ±SD. The asterisk (*) indicates significant variation for the *t*-test at *P*<0.05.

maturation and spawning of JTO was faster than that of MTO and the rapid gonad was associated with the utilization of spring season storage energy and increasing water temperature of the bay. Spawning occurred during June to October at 20–25ºC and most of the oysters became reproductively inactive from October to onward.

The reproductive effort estimated by indirect enzyme-linked immunosorbant assay (ELISA) was increased with the advance of gonad maturation and the highest mean gonado-somatic index (GSI) 28±6% of JTO was recorded in June when most of the oysters were ready for spawning (see Figure). The GSI sharply decreased in July and continued up to October, indicates a massive spawning activity of the oyster. But the mature individual of MTO represented only 11±7% GSI with a lees quantity of gamete release during the spawning period (see Figure). Fecundity (calculated by dividing the total amount of eggs estimated using ELISA by the weight of a single egg, 13 ng, Kang et al., 2003) of JTO was ranged 2.7 to 51.7 million eggs/oyster and the highest monthly mean fecundity 28.0±11.2 million was estimated in June. But in MTO highest mean fecundity 2.3±1.1 million was estimated in September. Results indicate that the spawning process of JTO was faster than that of MTO with significantly higher number egg produced and possibly released to the environment.

The proximate composition of the oyster tissue showed a seasonal cycle in relation to gametogenic development and differed between the groups. The carbohydrate content increased in spring and dramatically decreased in summer in accordance with high energy catabolism for spawning. The higher carbohydrate reserve at the earlier gonad maturation was coincided with the higher reproductive effort of JTO than that of MTO. Protein level was higher during the spawning and post spawning period when the carbohydrate level was lower, indicates that the oysters used the protein as an alternative energy source during that period.

The faster growth and spawning with significantly higher reproductive effort indicates the aquaculture potentiality of JTO and can significantly contribute to the spat recruitment in the bay. This study suggest that the traditional grow out transplantation time needs to be re-evaluated for the sustainable oyster aquaculture in the Gamakman Bay where the oyster industry is facing a shortage of natural spat supply.

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SPRATLY ARCHIPELAGO AS A POTENTIAL RECOVERY RESERVE OF BIODIVERSITY OF COASTAL AND ISLAND REEFS OF VIETNAM

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In the Spratly Archipelago, there are approximately 600 coral reefs and atolls, scattered in the South China Sea north of the Philippine Islands in Sabah to southern Palawan and over distances of more than 500 km. Reefs, raised above the sea surface in the maximum tide, include, at least 26 islands and rising rocks (McManus, 1994). Itu Aba and Taiping Islands are one of the largest islands of the archipelago.

Indo-Pacific Province, which includes the Spratly Archipelago, is characterized by high diversity of marine life. Here, at least 70 genera of hermatypic corals are found (Veron, 1995; Latypov, 2005). In its coral reef ecosystems, over 400 species of corals (Veron, Hodgson, 1989), 1500 species of reef fish and 200 species of marine algae (McManus, 1994) are found. The exact number of all marine animals and plants in the South China Sea is difficult to assess because of inadequate state of taxonomic data, but the total number of species that are found at all depths from Spratly reefs likely approaches to bumber of species in the Indo-Pacific and reaches several thousand species. Coral reefs are common in the shallow waters of the South China Sea. High spatial diversity and productivity of coral reefs provide not only a variety of habitats for marine organisms, but also support the existence of juvenile fish larvae for fisheries, shellfish, crustaceans, cephalopods and other animals. Because corals play a key role in marine ecosystems of the South China Sea, they are in need of better understanding of the role of the coral fauna in the region for marine resource conservation and management in the future.

The species diversity on Spratly reefs as in most reefs of the Indo-Pacific (Veron, 1995; Latypov, 2007) is represented by five genera: *Acropora* (52 species), *Montipora* (20), *Porites* (15), *Favia* (14), *Fungia* (10), constituting 40% of the total hermatypic scleractinian identified in the archipelago, four morphological types of reefs are formed: those typical for most reefs of the Indo-Pacific; reefs with short and long reef flat zones; reefs with steep reef slope (Fig. 1) as well as typical atolls sized up to 34 km long and 5 km wide. In various zones of coral reefs, species richness varies from 102 to 179 species. The largest number of species are observed on the reef slope and in the lagoons of atolls (214 and 190 species, respectively). In these zones, the highest degree of substrate covered by corals and other related animals are observed (see Table).

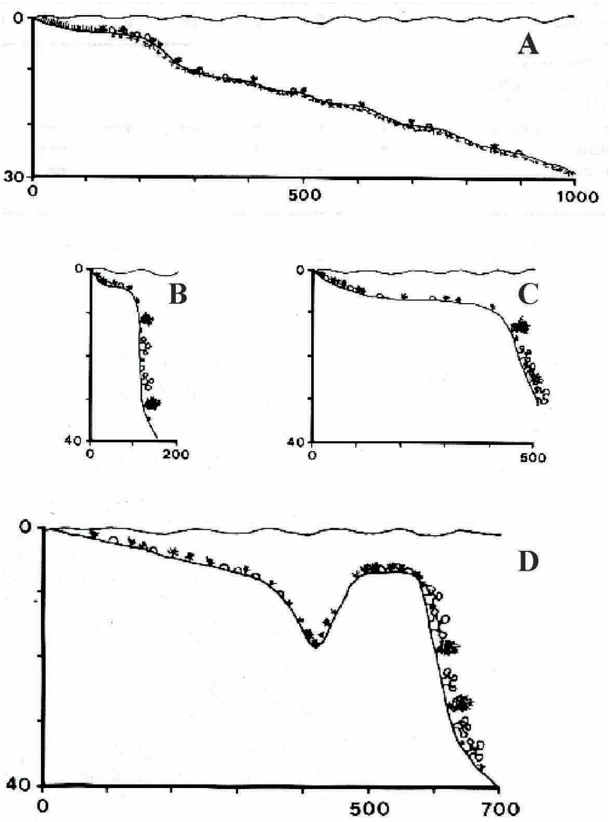

Fig. 1. Various profiles and lengths of reef: A – normal reef zone, B – short, C – long steep reef slope, D – large reef flat with a steep reef slope with of platform in its upper part. On the abscissa axis, depth, on the ordinate axis, length, m.

The most common species belong to genera *Acropora*, *Montipora*, *Pocillopora*, *Porites*. Continuous coral thickets of *Acropora cytherea*, *A. digitifera*, *A. cuneata*, *Pocillopora verrucosa*, *Montipora speciosa*, *Porites nigrescens*, *P. cylindrica* can form mono-settlements in an area covering tens of square meters. Numerous species of the reef slope often produce a variety of massive and sub-massive colonies (genera *Porites*, *Pocillopora*, *Goniastrea*, *Favia*, *Favites*) and solitary colonies of alcyonarian *Sinularia*, *Sarcophyton* and hydroid corals of *Millepora* (Fig. 2).

Branching colonies of *Pocillopora damicornis*, *P. verrucosa*, *P. eydouxi*, *Acropora monticulosa* and *A. gemmifera* are commonly found at almost all reef flat zones in most areas. Corals *A. digitifera*, *A. palmerae*, *Favia speciosa*, *Leptoria phrygya*, *Platygyra lamellina* and a hydroid *Millepora platyphylla* tend to form large colonies reaching in diameter more than 1 m, providing up to 50% of the substrate surface. The sand glades of reef flat and reef slope are populated by numerous solitary Fungiidae (up to 45 specimens/m^2) *Cycloseris cyclolites*, *C. costulata*, *Fungia fungites*, *F. scutaria*, *Sandolitha robusta* and *Herpolitha limax* constituting 30–40% of the substrate surface.

The vertical sections of the reef slope are generally dominated by colonies of soft corals genera *Sarcophyton*, *Lobophytum*, *Dendronephthya* and a gorgonacean *Junceella*, some of them can reach 0.5 m in diameter.

Degree of substrate covered by different organisms and free of macrobenthos sites, %

Components	Depth, m			
	10	15	20	30
Scleractinians	75.38	41.90	17.54	4.57
Acropora	40.00	33.72	11.01	5.34
Dead corals	6.12	3.45	21.33	12.24
Sea grass	48.81	20.03	6.66	5.33
Calcareous algae	12.67	17.90	3.73	6.19
Soft corals	15.50	20.52	10.58	12.05
Sponges	10.14	8.66	4.70	1.08
Sand	11.86	2.33	15.66	10.60
Corallogenous limestone	4.70	22.06	23.54	52.65
Gravel	0.00	11.99	3.61	0.92

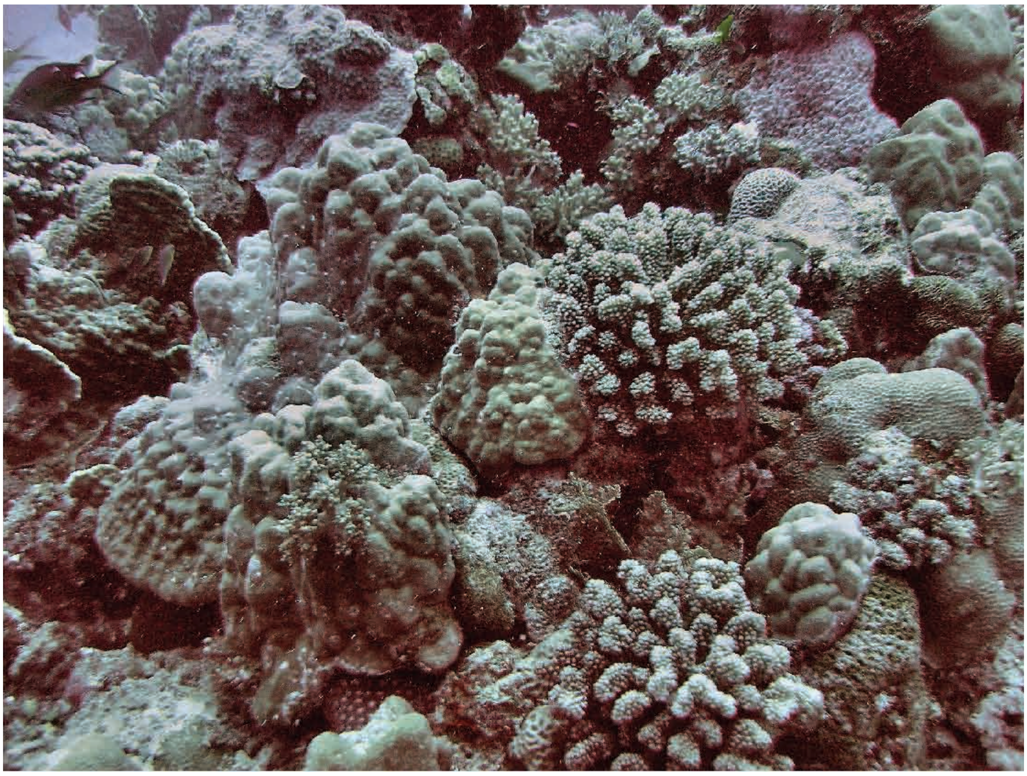

Fig. 2. Multi-species settlement on the reef slope of the Lodd Atoll, depth 18 m.

Usually, the structural form of the atolls observed at lowest tide. In the middle of atolls, in the lagoon corallogenous lifting reef flat can be found which is covered with corals at a degree of 10–30%, mainly by branchy *Pocillopora* and encrusting *Montipora.* A similar situation is observed with respect to morphology of Thun Tay (Tuan et al., 1997). Significant areas of lagoons are often occupied by algae spreading in intertidal areas. Scleractinian are usually limited in number here. They are mostly small isolated colonies of *Stylophora*, *Seriatopora*, *Favia*, *Goniastrea*, *Montipora*, *Porites*, *Goniopora*. The area of the coral projective cover on the bottom did not exceed 5–7%. In the inner reef slope of the atolls (Thun Tai, Lodd, Sinton, etc.), there is a well developed coral community with a high share of the scleractinian genera *Pocillopora*, *Acropora*, *Montipora* and *Porites* (substrate covering percentage – 30–50%). Here, 3–5 species of branchy scleractinians *Montipora digitata*, *P. verrucosa*, *P. nigrescens*, *Hydnophora exesa* usually dominate sometimes reaching up to 100% of substrate cover. The reef slope complex in the upper divisions are inhabited by blue coral *Heliopora coerulea*, hydroids *Millepora platyphylla*, *M. dichotoma*, massive, plate and encrusting colonies genera *Porites*, *Pachyseris*, *Echinopora*, *Acropora*. The area of the projective cover of substrate in the zone of multi-species settlement may reach about 100%, deeper – 20–40%.

On reefs of Australia, Vietnam, Indonesia and the Philippines, 360–370 species of scleractinian belonging to 70 genera are found (Veron, 1995; Latypov, 2007).

Fig. 3. Fish community on the reef slope of the Lodd Atoll, depth 25 m.

On reefs of Spratly Archipelago, 304 species of corals belonging to 66 genera are identified which makes up 76% of the total species of scleractinian of the the entire coral fauna of the Pacific and Indian oceans. Of course, further explorations of Spratly Archipelago corals would increase species richness by no less than 350 species. Coral reef fauna of Spratly Archipelago represents well the tropical fauna of the Indo-Pacific.

Stability of coral ecosystems of islands of the archipelago and their exceptional biodiversity on most reefs are explained by the fact that the islands are uninhabited, i.e., by the lack of human pressure and by their great distance from the mainland. Coastal continental ecosystems of the South China Sea can be dependent on the ecosystems of Spratly Archipelago with respect to resources of larvae. Due to the predominance of monsoon currents (McManus, 1994), Spratly Islands reefs are a source of larvae that may replenish disturbed coral reefs in Vietnam. It is likely that the islands of the archipelago and similar groups of uninhabited reefs provide a mechanism to stabilize the supply of small invertebrates and fish of coastal areas of the South China Sea. This is becoming more and more important because in the coastal areas of Vietnam, Indonesia, Philippines, strong overfishing is clearly recorded (McManus, 1994). The Spratly reefs and lagoons have noticeable concentrations of various fish ranging from 37 to 54 specimens/m^3 (Fig. 3). Dispersal of larvae of the Spratly reef ecosystems may contribute to the production of fish, other seafood and coral reefs in nearby regions. Thus, coral reefs in the archipelago can be seen as a “savings bank” where the commercially important invertebrate and fish are preserved from overfishing, and this is a constant flow of maggots to areas with depleted marine resources.

In broad terms, the Spratly reefs are important ecologically, with abundant and relatively undeveloped resources. Currently, there is a complex political dispute over the Spratly Islands involving Vietnam, China, Malaysia, Taiwan and the Philippines. Brunei established an exclusive fishing zone encompassing Louisa Reef in the southern part of the archipelago in 1984 without public ownership in their islands. In the 2000s, China joined the ASEAN in discussions to establish a “code of conduct” for the South China Sea. There is a proposal (McManus, 1994) of conservation of genetic diversity of reef ecosystems and the region. It is proposed to establish a transboundary international maritime reserve zone (park, reserve) in Spratly Archipelago under possible protectorate of Vietnam, China and the Philippines.

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FINDING OF *CLADOLABES SCHMELTZII* LUDWIG, 1875, A NEW SPECIES OF HOLOTHURIANS (ECHINODERMATA: HOLOTHUROIDEA) FOR THE NHA TRANG BAY, VIETNAM

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Being exclusively marine animals, echinoderms and especially holothurians are common component of benthic communities. Many species of holothurians are edible and therefore they have significant ecomonic value. Thus, investigation of the biodiversity of the holothurians is essential for the faunistics and zoogeography. Proximity of Nha Trang Bay to the Indo-Malasian Centre of the marine fauna biodiversity which borders with South China Sea suggests existence of rich echinoderm fauna and especially sea cucumbers. Identification of holothurians is difficult sometimes because of high variation of color and exterior characters. Sometimes one can find the cryptic species therefore small sea cucumbers can be misidentified as juveniles of other species.

During our research jointly with the Institute of Oceanography VAST (Nha Trang, Vietnam), we have found some new for the South China Sea echinoderm species including a holothurian *Cladolabes schmeltzii* Ludwig, 1875. We provide here information about sites where the species was found in Nha Trang Bay, new information about external morphology, geographical range and taxonomical significance of some morphological characters.

The materials were collected by the joint expeditions of the A.V. Zhirmunsky Institute of Marine Biology FEB RAS and the Institute of Oceanography VAST during 2009−2010. Samples were collected by SCUBA diving, photographs of alive holothurians were made using the Nicon D50 camera. For preparation of plates with the sclerites, the water solution of $NaClO_2$ was used. Photograpgs of the sclerites were obtained by the digital camera at optimal magnification and with scanning electron microscope (SEM). We follow Miller and Pawson (1984) and Pawson (1970; sticks) in terminology used for various sclerites (table, perforated plate, sometimes disk) and calcareous ring (ten pieces, five radial and five interradial).

Order **Dendrochirotida** Grube, 1840
Family **Sclerodactylidae** Panning, 1949
Subfamily **Cladolabinae** Heding et Panning, 1954
Genus *Cladolabes* Brandt, 1835
Cladolabes schmeltzii (Ludwig, 1875)

Thyonidium schmeltzii Ludwig, 1875: 94, figs. 20a, b; Massin, 1999: 96–98, figs. 80a–e, 81, 113d (synonymy).

Description. Body length of the alive specimens up to 7 cm and up to 1.8 cm in width (Fig. 1A, B, C). Body length of fixed specimens from 1.5 to 4.6 cm (Fig. 1D, E), weight from 0.18 to 3.9 g. Five double series of ambulacral arms. Body pentagonal in cross section, tips become narrow (except for one specimen). All specimens have ambulacral arms with widened tips, arms in two series at dorsum and in four series at ventrum. Mouth and anal openings at apposite tips of body. Calcareous ring as a tube with

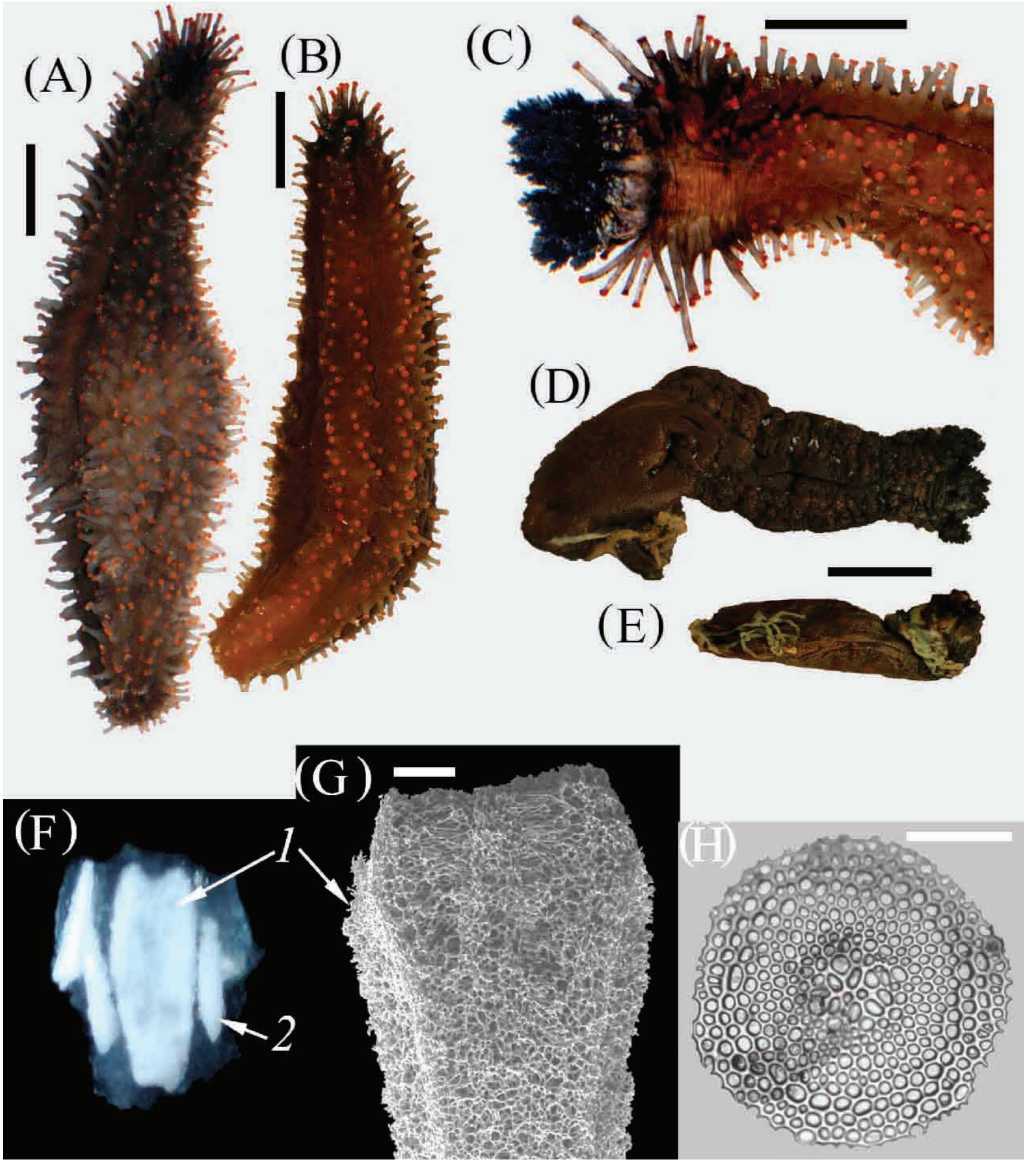

Fig. 1. *Cladolabes schmeltzii* (Ludwig, 1875), Nha Trang Bay, South China Sea. A – alive specimen, view of bivium; B – alive specimen, view of trivium; C – alive specimen, anterior body tip, view from ventral side; D – MIMB 25754; E – MIMB 25755; F – calcareous ring (1 – interradial, 2 – radial). Scale bar: A–E – 10 mm; G – 0.2 mm; H – 0.1 mm.

a 1/5 of body length. Radial calcareous ossicles (Fig. 1F) about two times wider than the interradial ones. Calcareous ossicles (Fig. 1G) have loose microstructure. Eight large and numerous small Polian vesicles; numerous (up to 80) short stone canals. Retractor muscles of pharynx attached at half-way of body length.

Sclerites. There are many club-shaped sticks performed from spicules with one tip ornamented with spikes and others formed as delicate plates perforated by several holes (Fig. 2D). Calcareous ring has loose ossicles (Fig. 1F, G). Ambulacral arm tips armed by calcareous heavily perforated disc (Fig. 1H) with diameter of 0.25–0.30 mm. Shortened sticks with one tip crowned by spikes and another tip widened and it forms perforated basis. The sticks with double lengthwise rods were found (Fig. 2B). Some sticks have short rod and wide basis (Fig. 2A), some spicules have no rods and look like wide basis which has a crown of spikes (Fig. 2B). There are additional oblong rosettes (Fig. 2E).

Colour. Alive holothurians black but when we took photos with flash they become reddish (Fig. 1A, B), arm tips red. Fixed specimens have brown color (Fig. 1D, E).

Geographic distribution. Australia (Queensland, GBR – type locality), Philippines, Palau Islands, Indonesia (North Sumbawa, East of Flores, East Kalimantan, Sulawesi), China; 0–10 m.

The main character of the species is shape of the sticks from body wall (Liao, Clark, 1995). Moreover, Liao and Clark (1995) and Massin (1999) noted calcareous rings and additionally Massin (1999) presented drawings of the sclerites in form of perforated plates from ambulacral arms, rosettes and sticks from tentacles. The specimens which were found in Nha Trang Bay obviously belong to *C. schmeltzii,* because the sticks from body wall and elements of calcareous ring were found.

Specimens of Massin (1999) are larger than Vietnamese ones, but holothurians found from China seashore by Liao and Clark (1995) are smaller than our specimens. Sizes of our specimens correspond to size range noted in this species by previous authors.

Liao and Clark (1995) provided iformation only about sticks, and Massin (1999) added the drawings of sclerites of various parts of body. The perforated plates from Massin (l.c.) are undoubtedly parts of the disks which arm the tube-feet tip. Our oblong rosettes are similar to tentacle rosettes from Massin (1999). Massin (1999) noted that *C. schmeltzii* has 7 long and numerous Polien vesicles, and many small stone canals. We found 8 large Polien vesicles and about 80 stone canals. The short sticks with broadened basis and ossicles with the broadened basis and the spikes found only (Fig. 2A, C) in the anterior part of body (we assume in tentacles wall and/or pharynx) are completely absent in Liao and Clark (1995) and Massin (1999) specimens. Our illustrations of calcareous ring are more similar with those from Liao and Clark (1995) and differ from Massin (1999). A lack of the ossicles from ambulacral podia in our specimens may be explained by the long period of storage in ethanol. It is uncertainly how long time specimens were stored and fixed by formalin in Massin (1999). Storing of specimens in formaldehyde is detrimental for calcareous elements.

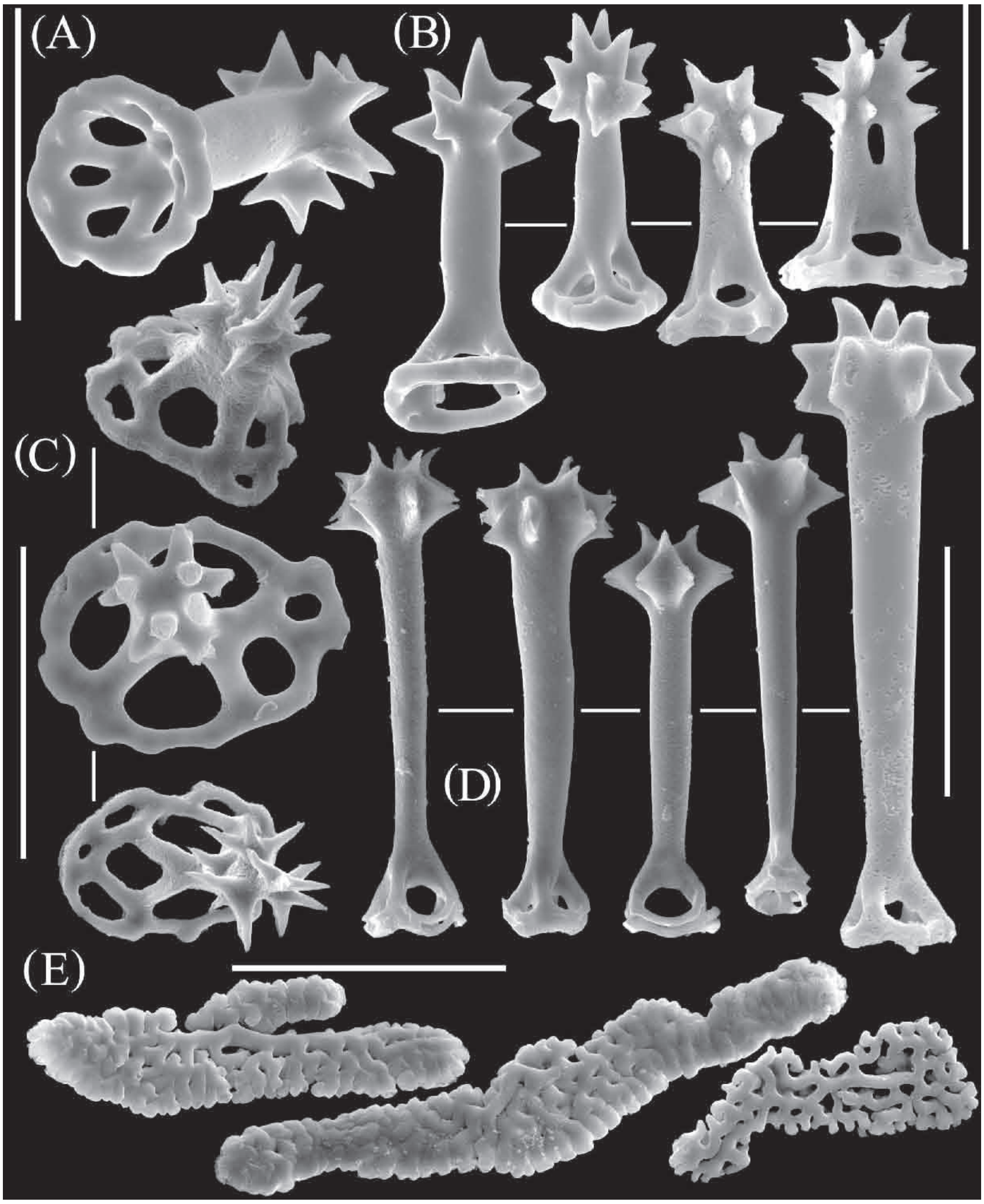

Fig. 2. *Cladolabes schmeltzii* (Ludwig, 1875). Sclerites. A – rods from tentacles, slanted; B – rods from tentacles; C – modified rods from tentacles; D – rods from the body wall; E – rosettes from tentacles. Scale bar: 0.05 mm.

Formalin fixation, perhaps, saved the color of body in specimens of Massin (1999); our specimens from Nha Trang Bay have another intravital body color being black in the sea and at laboratory, and having reddish shades in color when photographed with flash-light (Fig. 1A, B). Color of the tube-feet tips is similar to the illustrations of Massin (1999).

The morphological differences between the sea cucumbers from Nha Trang Bay and from China and Indonesia in sizes and details of the skeletal elements can be considered as intraspecific variation. Indeed, they do not have taxonomic value. Massin (l.c.) noted that larger specimens have larger sticks, but we did not observe the differences in sizes of the sticks in our specimens. Descendants from asexual reproduction can be smaller than younger specimens. In such cases, the age can be determined using the ossicle sizes. So, we noted the following differencies: specimens of Massin (1999) have more light color, the specimens from Hainan Island (Liao, Clark, 1995) and Nha Trang Bay have more dark color. The calcareous ring ossicles in specimens of Massin (1999) have long thin offshoots. New record of *C. schmeltzii* makes the geographical range of this species wider. Finding of this species in Vietnam is supported by system of currents in Centre of Biodiversity (Hoeksema, 2007). However, new location is far from Chinese habitats (Liao, Clark, 1995) as well as from type locality.

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THE STUDY OF BIOCHEMICAL RESISTANCE FACTORS OF THE BLEACHING OF CORAL REEFS OF VIETNAM

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Introduction

With approximately 3260 km of coastline (excluding islands), marine resources constitute an important natural asset for Vietnam. The diversity of marine natural resources makes an important contribution to the national economy, through the provision of marine products (fish, invertebrates, algae, etc.), energy (crude oil and gas), raw materials (mineral resources), storm protection and recreation (Pham Quoc Long et al., 2008).

While information on Vietnam's marine biodiversity is not complete, 11000 species have so far been recorded in Vietnam's marine and coastal waters including

2458 species of fish, 653 species of sponges, 653 seaweed and so on (Nguyen Chu Hoi et al., 1998). Species diversity is known to increase from north to south, and fish abundance is higher in offshore than in inshore coral reefs. Vietnam's known diversity of corals is comparable with that of Indonesia or the Philippines.

Coral reefs provide home to over 25% of all marine life and are extremely important for biodiversity. It is considered as national treasure of Vietnam, forms unique marine communities with huge productivity and gives an opportunity to quickly develop several branches of Vietnam economy (Vu Trung Tang, 1997). However, they are very fragile sensitive ecosystems that can only stand a narrow tempurature range. Climate change poses threat in the world's reef systems and the most visually dramatic impact is seen on coral discoloration (*bleaching,* the loss of symbiotic zooxanthellae as a result of overheating of sea water in combine with other stress factors), which leads to irreversible loss of corals. In recent decades, as a result of bleaching, more than 30% of all coral reefs of the oceans have been killed.

In recent years, there have been some investigations on the biodiversity of corals in some areas of Vietnam carried out by scientists from Vietnam and Russia. From obtained results, the data on coral bleaching and the reasons for the process is being clarified gradually.

Scientific Overview and Goals

a. Overview

It is reported that zooxanthellae belong to one genus of microalgae, dinoflagellate, a group of *Symbiodinium*. According to the analysis of ribosomal DNA, zooxanthellae are divided into several phyllotypes. It has been shown that different zooxanthellae have different resistance to the increase of water temperature. Corals that contain zooxanthellae sensitive to higher temperature discolor and die when lose them while in the same conditions, corals containing thermally stable zooxanthellae still survive. It is anticipated that the loss of zooxanthellae has the link to changes in the functioning of cell walls of these micro-algae and coral cells in which they are located. Many functions of cells depend on the fluid lipid bilayer that forms the basis of bio-membranes. There is evidence of significant changes in the content of some fatty acids (FAs) of discolored corals, which may be part of zooxanthellae specific membrane lipids.

b. Goals and Objectives

The objectives of the present project is to obtain new fundamental information on the composition of lipids and FAs of cleaner zooxanthellae and host cells in healthy stony and soft corals of Vietnam, as well as colonies of different degrees of bleaching. Other task of the project is to determine the relationship between heat-resistant individual zooxanthellae corals and their lipid composition, examine the sustainability of individual species of corals to bleaching, the dynamics of lipids, the role of individual lipid classes and individual FAs in the process. These studies also provide new data for the works in the field of FA biosynthesis, symbiotic lipid markers and taxonomy of corals. Offer a method for monitoring the ecological conditions of coral reefs using lipid markers, offers advice on choosing thermally stable species for artificial coral reef restoration, as well as on using lipids to prevent coral bleaching.

Methods

We used the methods of GC and GC-MS to establish the composition and structure of fatty acids, in particular, the unusual FAs, with the help of mass spectra. We used also methodology using gradient centrifugation in combination with mild ultrasonic treatment to separate the host organism and symbionts of corals. A new approach will be used to compare the composition of lipids in healthy symbionts natural colonies of corals, which lost zooxanthellae, and corals, which are cultivated in artificial conditions.

In order to determine the sustainability of certain species of Vietnamese corals with the raise of water temperature, analysis of ribosomal DNA to identify coral's zooxanthellae, isolated from colonies with different degrees of thermal stability was implemented.

We identified the dynamics of the lipids from corals and zooxanthellae in the bleaching of the colonies, and determined the relationships between heat-resistant individual zooxanthellae in corals and composition of lipids of biomembranes. The role of individual lipid classes and individual FAs in the process of coral bleaching were defined, and a method of monitoring of ecological conditions of coral reefs using the analysis of lipid markers, to advise on the choice of heat-resistant species of corals for the recovery of coral reefs as well as on the use of lipids to prevent discoloration was presented.

Results

The project has been carried out by scientists from both sides, Vietnam and Russia, and three expeditions in 2005, 2007 and 2010 were successfully conducted. During these cruises, we had collected thousands of marine samples, the biodiversity and characteristics of reef communities were also investigated.

a. Research works of Vietnam scientists since 2005

Main works planned were to:

- Organize light diving work to collect mass species of both stony and soft corals containing and not containing zooxanthellae, keep a record of the materials. Collect corals at different stages of bleaching. Determine the total lipids of the collected biological specimens.
- Define qualitative and quantitative composition of fatty acids and the major classes of lipids, identify major classes of lipids, establish the chemical structure of unusual fatty acids, which are formed when corals lose zooxanthellae. Perform a preliminary fractionation of lipids on the degree of unsaturation and the number of carbon atoms, synthesize derivatives of these lipids for GC and GC-MS analyses to establish the chemical structure of unusual fatty acids.
- Develop a methodology of obtaining pure fractions of zooxanthellae and host cells. Get the total fractions of zooxanthellae and host cells. Carry out the methodology for long-term investigation of corals in artificial conditions. Obtain preliminary data on the thermal stability of individual species of corals.
- Compare the composition and content of lipids between corals with zooxanthellae, corals without zooxanthellae and corals having lost zooxanthellae. Test the use of lipids in preventing coral bleaching in artificial and natural conditions. Submit research results in the form of articles in scientific journals.

b. Research works of Russian scientists since 2005

Main works planned to:

- Obtain fractions of zooxanthellae and the host cells of mass species of stony and soft corals of Vietnam.
- Define qualitative and quantitative composition of fatty acids and major classes of lipids of zooxanthellae and host cells, preliminarily fractionate lipids on the degree of unsaturation and the number of carbon atoms, transform these FAs to their derivatives for GC analyses, establish the chemical structure of unusual fatty acids .
- Make a comparison of the lipids of zooxanthellae and host cells between groups of corals, determine zooxanthellae lipid markers. Develop an analytic methodology of zooxanthellae ribosomal DNA. Obtain data on the composition of zooxanthellae corals of Vietnam with different thermal stability.
- Determine the relationship between heat-resistant individual zooxanthellae corals and composition of lipids of biomembranes. Define the changes in the composition of lipids in corals and zooxanthellae in the discoloring. Define the role of individual lipid classes and individual FAs in the process of coral bleaching.
- Suggest a method for monitoring the ecological conditions of coral reefs using lipid markers. Give advice on the choice of heat-resistant species of coral reefs for reconstruction of Vietnamese reefs.
- Conduct training of Vietnamese experts. Submit research results in the form of articles in scientific journals.

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ESTIMATING ECONOMIC DAMAGE TO CORAL REEFS AND SEAGRASS IN VUNG TAU PROVINCE CAUSED BY THE IMPACT OF OIL SPILLS

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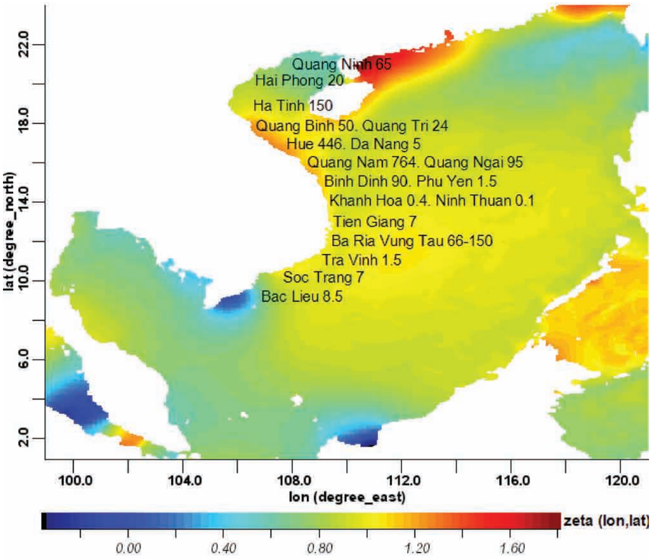
Introduction

Oil spill is always one of the most serious environmental problems in coastal areas. Oil spill could pollute large regions, causing enormous losses to both economy, society and environment. Thus, the problem of calculating the economic damage to marine ecosystems is important when oil spills occurs in order to devise plans for prevention and appropriate compensation.

Material and Methods

1.1. Material

We used data sets of the questionnaires through direct interviewing people in the study area when the spill occurred and the statistical data of oil spills along the coast of Vietnam (see Figure).



Distribution of collected oil spill along the coast of Vietnam (tons).

1.2. Methods

We used habitat equivalency analysis method to measure compensation for the losses of ecosystem services. This method requires that compensative project must provide replacement services so that the total economic value at least equal to the total economic value of services lost. This means that the size of the compensative project must be sufficient to compensate the value of services lost. It was combined with some statistical calculations on SPSS and IDL.

Results

With calculating for 150 tons of oil spilled in Vung Tau Province, one-year project started is 2011 with value ratio injured/restored: 1:00, discount rate per unit time (%): 3.00, of which 1) number of affected area units (hectare) = a, pre-injury service level (%) = b, pre-restoration service level (%) = c (Tables 1–5).

Table 1. Estimating the oil spills occurred and the the area of ecosystems affected

Hard corals			Soft corals			Seagrass		
Primary project: 15 years		Compensatory project: 12 years	Primary project: 10 years		Compensatory project: 15 years	Primary project: 3 years		Compensatory project: 5 years
a	b	c	a	b	c	a	b	c
24.5	17.11	17.9	7.3	95.3	25.7	250	65.59	0

Seagrass Analysis

Table 2. Service loss at injury area

SERVICE LOSS AT INJURY AREA					
Year	Services Lost			Raw SAYs lost	Discounted SAYs lost
	Beginning	End	Mean		
2011	34.41%	22.94%	28.67%	71.687	71.687
2012	22.94%	11.47%	17.20%	43.012	41.760
2013	11.47%	0.00%	5.73%	14.337	13.514
2014	0.00%	0.00%	0.00%	0.000	0.000
Total discounted SAYs lost: Lpp_cb					126.961

Table 3. Service gain at the compensatory area

SERVICE GAIN AT THE COMPENSATORY AREA					
Year	Services Gained			Raw SAYs gained	Discounted SAYs gained
	Beginning	End	Mean		
2011	0.00%	20.00%	10.00%	0.100	0.100
2012	20.00%	40.00%	30.00%	0.300	0.291
2013	40.00%	60.00%	50.00%	0.500	0.471
2014	60.00%	80.00%	70.00%	0.700	0.641
2015	80.00%	100.00%	90.00%	0.900	0.800
2016	100.00%	100.00%	100.00%	1.000	0.863
2017	–	–	–	–	28.754
Discounted SAYs gained per unit area: Gcp_cb					31.92
Replacement habitat size (hectare): 1.00*126.962/31.919: Rlg_cb					3.978

Hard Corals Analysis

Table 4. Service loss at injury area and service gain at the compensatory area

SERVICE LOSS AT INJURY AREA					
Year	Services Lost			Raw SAYs lost	Discounted SAYs lost
	Beginning	End	Mean		
2011	82.89%	77.36%	80.13%	19.631	19.631
2012	77.36%	71.84%	74.60%	18.277	17.745
2013	71.84%	66.31%	69.07%	16.923	15.952
2014	66.31%	60.79%	63.55%	15.570	14.248

Table 4 (continued)

SERVICE LOSS AT INJURY AREA

Year	Services Lost			Raw SAYs lost	Discounted SAYs lost
	Beginning	End	Mean		
2015	60.79%	55.26%	58.02%	14.216	12.630
2016	55.26%	49.73%	52.50%	12.862	11.095
2017	49.73%	44.21%	46.97%	11.508	9.638
2018	44.21%	38.68%	41.44%	10.154	8.256
2019	38.68%	33.16%	35.92%	8.800	6.947
2020	33.16%	27.63%	30.39%	7.446	5.707
2021	27.63%	22.10%	24.87%	6.092	4.533
2022	22.10%	16.58%	19.34%	4.739	3.423
2023	16.58%	11.05%	13.81%	3.385	2.374
2024	11.05%	5.53%	8.29%	2.031	1.383
2025	5.53%	0.00%	2.76%	0.677	0.448
2026	0.00%	0.00%	0.00%	0.000	0.000
Total discounted SAYs lost: Lpp_shc					134.010

SERVICE GAIN AT THE COMPENSATORY AREA

Year	Services Gained			Raw SAYs gained	Discounted SAYs gained
	Beginning	End	Mean		
2011	17.90%	24.74%	3.42%	0.034	0.034
2012	24.74%	31.58%	10.26%	0.103	0.100
2013	31.58%	38.43%	17.10%	0.171	0.161
2014	38.43%	45.27%	23.95%	0.239	0.219
2015	45.27%	52.11%	30.79%	0.308	0.274
2016	52.11%	58.95%	37.63%	0.376	0.325
2017	58.95%	65.79%	44.47%	0.445	0.372
2018	65.79%	72.63%	51.31%	0.513	0.417
2019	72.63%	79.48%	58.15%	0.582	0.459
2020	79.48%	86.32%	65.00%	0.650	0.498
2021	86.32%	93.16%	71.84%	0.718	0.535
2022	93.16%	100.00%	78.68%	0.787	0.568
2023	100.00%	100.00%	82.10%	0.821	0.576
2024	–	–	–	–	19.194
Discounted SAYs gained per unit area: Gcp_shc					23.732
Replacement habitat size (hectare): 1.00*134.01/23.732: Rlg_shc					5.647

Soft Corals Analysis

Table 5. Service loss at injury area and service gain at the compensatory area

SERVICE LOSS AT INJURY AREA					
Year	Services Lost			Raw SAYs lost	DiscountedSAYs lost
	Beginning	End	Mean		
2011	4.70%	4.23%	4.46%	0.326	0.326
2012	4.23%	3.76%	3.99%	0.292	0.283
2013	3.76%	3.29%	3.52%	0.257	0.243
2014	3.29%	2.82%	3.05%	0.223	0.204
2015	2.82%	2.35%	2.58%	0.189	0.168
2016	2.35%	1.88%	2.11%	0.154	0.133
2017	1.88%	1.41%	1.64%	0.120	0.101
2018	1.41%	0.94%	1.17%	0.086	0.070
2019	0.94%	0.47%	0.70%	0.051	0.041
2020	0.47%	0.00%	0.23%	0.017	0.013
2021	0.00%	0.00%	0.00%	0.000	0.000
Total discounted SAYs lost: Lpp_shm					1.582

SERVICE GAIN AT THE COMPENSATORY AREA: Gcp_shm					
Year	Services Gained			Raw SAYs gained	Discounted SAYs gained
	Beginning	End	Mean		
2011	25.70%	30.65%	2.48%	0.025	0.025
2012	30.65%	35.61%	7.43%	0.074	0.072
2013	35.61%	40.56%	12.38%	0.124	0.117
2014	40.56%	45.51%	17.34%	0.173	0.159
2015	45.51%	50.47%	22.29%	0.223	0.198
2016	50.47%	55.42%	27.24%	0.272	0.235
2017	55.42%	60.37%	32.20%	0.322	0.270
2018	60.37%	65.33%	37.15%	0.372	0.302
2019	65.33%	70.28%	42.10%	0.421	0.332
2020	70.28%	75.23%	47.06%	0.471	0.361
2021	75.23%	80.19%	52.01%	0.520	0.387
2022	80.19%	85.14%	56.96%	0.570	0.412
2023	85.14%	90.09%	61.92%	0.619	0.434
2024	90.09%	95.05%	66.87%	0.669	0.455
2025	95.05%	100.00%	71.82%	0.718	0.475
2026	100.00%	100.00%	74.30%	0.743	0.477
2027	–	–	–	–	15.897
Discounted SAYs gained per unit area: Gcp_shm					20.607
Replacement habitat size (hectare): 1.00*1.581/20.607: Rlg_shm					0.077

Conclusion

The final results of the total cost of each project in Vung Tau region when 150 tonnes of oil spills occurred.

Hard corals			**Soft corals**			**Seagrass**		
Primary project: 15 years (USD)		Compensatory project: 12 years (USD)	Primary project: 10 years (USD)		Compensatory project: 15 years (USD)	Primary project: 3 years (USD)		Compensatory project: 5 years (USD)
α	β	ξ	α	β	ξ	α	β	ξ
365544.9	241539.2	101646	6175.8	2584	1386	2150625	2083547.3	99450
708730.1			**10145.8**			**4333622.3**		

α = the cost of buying, growing coral reefs; β = the annual management costs;
ξ = the compensative cost chi phí đền bù

REPRODUCTIVE STRATEGIES IN SOFT CORALS (CNIDARIA: OCTOCORALLIA) AND THEIR BIODIVERSITY CONSERVATION

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Recently, populations of tropical shallow-water stony and soft corals have dramatically decreased because of anthropogenic pollution and other factors which have direct impacts on their habitats – coral reefs. Continuing reductions in abundance and distribution caused by increases in the magnitude and frequency of detrimental factors indicate that future impacts on coral reef community structures may be severe and the human induced threats have been suggested to interact with natural threats (e.g., El Nino Southern Oscillation, ENSO, and storms). The analysis of the different reproductive strategies of the soft corals can be suggested as extremely important for understanding of the mechanisms of the biodiversity conservation in coral reefs ecosystems. An investigation of the reproduction modes of soft corals and ecological factors affecting it is essential in order to implement successful resource management programs. However, only a few studies were conducted on the effects of environmental parameters, such as sedimentation and turbidity (Dai, 1991; Schleyer, Celliers, 2003), depth, current, and flow (Fabricius et al., 1995). Biological factors such as predation and competition (Sammarco et al., 1983) are deemed unlikely to influence the abundance and fauna richness of soft corals.

Soft corals belong to the very few organisms on the planet which are capable of reproducing both sexually and asexually. The nature of sexual reproduction among corals varies by species. About three-quarters of all stony corals form hermaphroditic colonies. These colonies have the ability to produce both male and female gametes. In most octocorals, male and female reproductive organs (gonads) are in separate male and female colonies. Separation of the sexes in different coral colonies is called gonochorism. The sexuality of corals – whether hermaphroditic or gonochoric – tends to be consistent within species and genera, although there are exceptions. Regardless of whether corals are gonochoric or hermaphroditic, there are two modes of fertilization that lead to the formation of larvae: broadcast spawning and brooding. In the Pacific, and especially on the Great Barrier Reef of Australia, most species are hermaphroditic spawners and release their gametes into the water simultaneously. Because colonies may be separated by wide distances, this release must be both precisely and broadly synchronized, and is usually done in response to multiple environmental cues.

Alcyonaceans exhibit internal brooding of larvae, surface brooding, and broadcast of gametes with external fertilization (Benayahu, 1991). The majority of tropical Alcyoniidae studied to date are gonochoric broadcast spawners with external fertilization and larval development. They have one annual spermatogenic cycle while their oogenesis is completed over a longer period and achieved through overlapping oogenic cycles. Broadcast spawning soft corals have been found to have short, seasonal, and synchronized spawning episodes. On the Great Barrier Reef, Australia, the soft corals spawn during the multispecies mass spawning event (Aliño, Coll, 1989; Benayahu, 1991). The seasonality of reproduction may depend on the geographical location of the soft coral population. Thus, the reproductive season of soft corals in the Red Sea occurs over extended periods and this may lead to temporal reproductive isolation among alcyonaceans (Benayahu, 1997). However, in contrast, alcyonaceans in the Great Barrier Reef spawn in multispecific spawning episodes (Aliño, Coll, 1989). The mode of reproduction is almost identical within a given genus, except for the genus *Alcyonium* in which there are both brooding and broadcasting species (Benayahu et al., 1990; McFadden, Hochberg, 2003). However, the geographic variation in reproductive timing even can be expected in the same species as it was shown for *Lobophytum pauciflorum* from southern Taiwan and the Great Barrier Reef (Fun et al., 2005).

At this time it is unclear to what degree reproductive strategies in octocorals are influenced by the surrounding environment or is dictated by genetic constraints; however, it is likely that both brooding and broadcast spawning may each confer certain reproductive advantages, especially in different environments. The participation of broadcasting spawning octocorals in synchronized mass spawning events may yield ecological benefits by reducing predation pressure on newly-spawned gametes in reef environments. Such highly synchronized spawning events may be critical to reproductive success in broadcast spawners by enhancing fertilization rates. Among brooders, spawning synchronicity may not be as crucial, because there is retention of eggs, either inside the polyps or on the colony surface until fertilization occurs (Dahan, Benayahu, 1997b). Brooding may be a strategy which compensates for low fecundity by enhancing survival of larvae by providing a refuge from predation.

The amount of time between fertilization and larvae achieving competency remains unknown for most species of octocorals. Once larvae become competent to metamorphose they must locate a suitable environment in which to settle. Successful recruitment depends upon encounter and selection of appropriate habitat, thus an ability to delay metamorphosis in the absence of a suitable environment may enhance the probability of survival by increasing the time available to locate more favorable conditions.

Richmond (1989) proposed that planula with zooxanthellae may benefit from nutritive algal metabolites, allowing them to extend their competency period. Contrary to this prediction, Ben-David-Zaslow and Benayahu (1998) found no significant difference in competency period or larval longevity between several species of zooxanthellate and azooxanthellate soft corals from the Red Sea. This suggests that for lecithotrophic octocoral larvae, nutrient sources within the oocyte are a key important factor controlling larval competence and longevity, and ultimately dispersal capabilities.

Numerous environmental features including substrate, light/dark cues, and water motion may influence coral settlement and successful recruitment. Many species of shallow-water octocoral larvae preferentially settle in shaded microhabitats, such as the underside of settlement plates, which behavior may be an avoidance response to high light intensity, low tides, competition from filamentous algae, grazing pressure, and sedimentation (Benayahu, Loya, 1987; Aliño, Coll, 1989; Dahan, Benayahu, 1997b and others). Once an octocoral planula locates an appropriate site to settle it undergoes metamorphosis to a feeding polyp (see Fabricius, Alderslade, 2001: Fig. 12). However, a limited number of studies indicate that successful settlement and recruitment into a population occurs at a low rate, at least among shallow-water alcyonacean octocorals (Farrant, 1987; Lasker et al., 1998). Extremely high post-settlement mortality of new recruits implies that successful settlement may be more closely tied to water column and post-settlement survival than to gamete production and fertilization rates (Lasker et al., 1998).

Cloning by somatic division, i.e., budding, fission or fragmentation, occurs among a wide variety of anthozoans, including octocorals (Fig. 1). Studies to date indicate that asexual reproduction in octocorals is most common among tropical soft

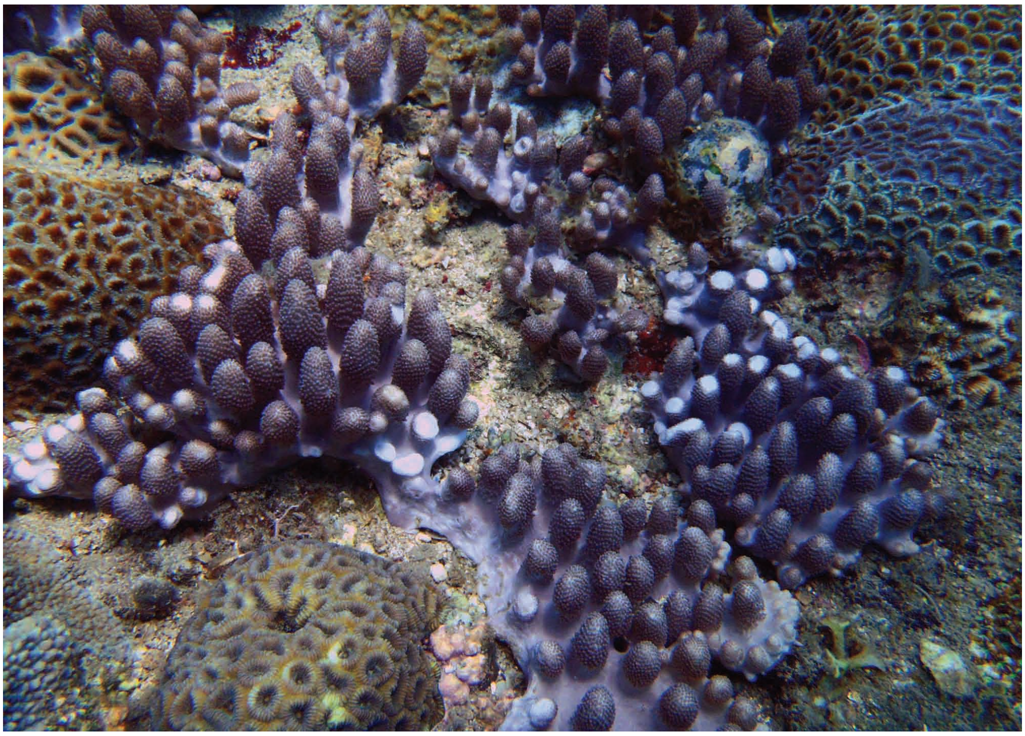

Fig. 1. Cloning by somatic division, i.e., fission or fragmentation, in soft coral *Paralemnalia*. Nha Trang Bay, Vietnam, 2011.

corals, especially in the families Alcyoniidae, Clavulariidae, Nephtheidae, and Xeniidae. Octocorals exhibit a particularly large range of mechanisms of clonal propagation. Among the described mechanisms, for example, budding of daughter colonies is found in *Sarcophyton gemmatum* (Verseveldt, Benayahu, 1978), colony fission in *Xenia macrospiculata* (Benayahu, Loya, 1985), autotomy of small-sized fragments in *Dendronephthya hemprichi* (Dahan, Benayahu, 1997a), and the generation of new colonies by stolons in *Efflatounaria* sp. (Karlson et al., 1996). There is evidence that asexual propagation, via vegetative growth, allows for high population growth rates due to the rapid colonization of substrate (Benayahu, Loya, 1987). Thus, species capable of this reproduction strategy often exhibit high local abundances and an ability to rapidly recover following disturbance (Highsmith, 1982; Dahan, Benayahu, 1997b). Soft corals (Octocorallia, Alcyonacea) tend to form large monospecific aggregations on coral reefs, composed of numerous colonies (Fig. 2), and most probably derived by asexual processes (Benayahu, Loya, 1977, 1981). The benefits of producing disjuncted modules by soft corals are discussed in terms of spreading the risk of mortality and exploitation of patchily distributed resources (McFadden, 1986). Typically fission occurs by producing two (occasionally three) daughter colonies of approximately equal size. Fission occurs throughout the year, but its highest frequency in most populations is surveyed during several months (McFadden, 1991).

Fig. 2. Large monospecific aggregation of numerous colonies *Sinularia manaarensis*. Nha Trang Bay, Vietnam, 2005.

Located in the tropical monsoon area of South East Asia, marine waters of Vietnam are characterized by high biodiversity of coral fauna and abundance of coral reefs. However, the coral reef ecosystems are threatened by human impacts and global climate changes that tend to reduce the species diversity and bring more rare and endangered species to extinction. The monitoring of the balance between the sexual reproduction and asexual fission in the soft and stony corals populations is urgently needed to predict and explain the future shift in the biodiversity in the region. This work was supported by the grant FEB RAS 09-III-A-06-195 (Dr. T.N. Dautova, Leader of the project).

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MANAGEMENT OF WILD HARD CLAM (*MERETRIX LYRATA*) RESOURCES IN THE SOUTHERN COAST OF VIETNAM

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Introduction

The hard clam industry has been given consideration for development 5–7 years ago in the southern coast of Vietnam where it has a very good potential for this high economic value species in association with the MSC certification provided to hard clam in

Bentre Province from the end of 2009. However, the dependency on unstable occurrence of wild seed, decrease of cultured areas, and increase in costs for grow-out activities have strong effects on the supply of raw clams for processing and export. It was roughly estimated in 2009 that about 30 thousand ha were good for hard clam culture while the supply of clam seed was enough for stocking of approximately 8 thousand ha only (Ministry of Agriculture and Rural Development – MARD, 2010). The lack of related information is also a difficulty for management of wild hard clam resources. This study was aimed to provide a better understanding on all of the related groups of stakeholders and a set of solutions for appropriate management of wild hard clam resources in the southern coast of Vietnam.

Research Methodology

This study was carried out from January 2010 to March 2011. Data were collected from major groups of stakeholders relating to hard clam resources: (1) 8 free collectors of wild seed; (2) 4 artificial reproduction hatcheries; (3) 20 nursery sites; (4) 8 clam seed traders; (5) 25 grow-out farms; (6) 26 raw clam traders; (7) 4 processors/exporters of hard clams; and (8) 16 local managers/officers and associations.

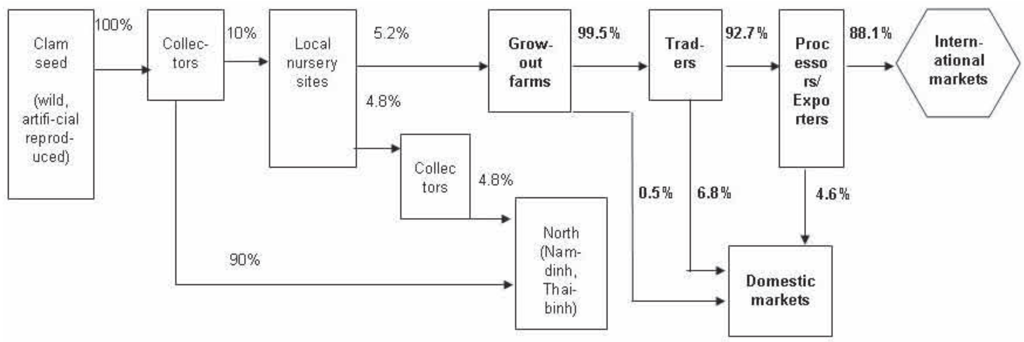
Descriptive analysis and cross-tabulations were used for making a clear picture on the technical-economic characteristics of each stakeholder group as well as their problems and suggestions for further development.

Results and Discussions

The hard clam industry in the south of Vietnam can be presented as in the following figure (see Figure).

Exploitation of wild seed of hard clam

The occurring frequency of wild clam seed in the southern coast was about 0.5–1.0 time/year at the density of 2164 (±1792) ind./m^2. Main season for collection of wild clam seed was from April to June (Lunar calendar) at the size of 288 thousand ind./kg (75% of respondents). The yield of captured wild seed was 107 (±126) kg/ha/year. The collectors received a total gross income of 365.8 (±327.3) million VND/ha/year (the exchange rate was USD 1=VND 18000 at the end of 2009 and the beginning of 2010) and the ratio of net income to total costs was 9.7 times (±11.9). There were many factors making the difficulties for estimation of the stock and captured production of wild clam spats. It was roughly estimated in 2009 that about 30 thousand ha were good for hard clam culture while the supply of clam seed was enough for stocking of 7300 ha in 2008 and 8 thousand ha in 2009, approximately. But how to manage the locations where wild seed of hard clam occurred was the most important issue which was associated with the lack of labors for spats collection of wild seed.



Marketing channels of hard clam industry in the south of Vietnam (2009–2010). Note: bold numbers are percentage of total raw clam production.

Artificial reproduction of hard clam

In order to contribute to a better supply of hard clam seed, the first reproduction hatchery of hard clam in the south of Vietnam was established in 2006 in Tiengiang Province. After 2 years of operation with some successes, the reproduction technique of hard clam had been transferred to some other coastal provinces in the south. In 2009 and 2010, the numbers of hard clam hatcheries in this region were 7 and 11, respectively. These hatcheries were established for reproducing the spats stage I (0.5 to 1 million ind./kg) and spats stage II (50 thousand to 200 thousand ind./kg). On average, each clam hatchery was designed with a total volume of spat nursing tanks of 1750 m^3 (about 6 m^3/nursing tank) and a productivity of 52.8 thousand spats stage II per cycle (from 8–10 cycles/year). However, the real capacity was only about 26.1% of the designed production. The average total variable cost for operating hatchery was VND 14.7 million/m^3/cycle with the survival rate to spats stage II was 5.5% (±4.0). The hatchery owners received a total income of 98.7 (±71.9) million VND/m^3/cycle and the ratio of net income to total costs of 2.7 (±1.7) times. The most important obstacles for hatchery operation were: insufficient supply of good broodstocks and lack of original source of qualified feed. Some large-scale hatcheries in the north of Vietnam has hired technicians from China for operation of their hatcheries, however, the “know how” of better results from these technicians in reproduction of hard clam is still a secret.

Nursery of hard clam seed

Nursery of clam spats has been practiced at small-scale from 2006 and there were 53 nursery sites in the region in 2009–2010. Wild seeds were nursed from stage I to stage II on nylon earthen pond with the areas of 1065 (±530) m^2/site. The nursery sites were operated about 1–2 cycles/year with the density of 136000 ind./m^2 (±58000) at the size of 508 thousand spats/kg (±334). The survival ratio after nursing of 81 days (±22) was 67% (±19.5) and productivity was 74 thousand spats/m^2/cycle. Total variable costs were 9261.6 million VND/ha/cycle, of which 95.6% was spent for buying clam spats. And then the ratio of net income to total costs was 0.7 time/cycle (±0.5). Uncertain quality of all types of clam spats were due to the complexity in collection and trading was major issue.

Traders of hard clam seed

The traders of hard clam seed bought 100% of their purchased clam spats from the collectors/exploiters with the size of wild seed of 91 thousand ind./kg (±112). They resold 10% of seed at local areas and 90% to the traders came from northern provinces, especially Namdinh and Thaibinh provinces. Each clam seed trader bought an average amount of 0.75- 69.0 tons/year with an additional cost of 48.7 thousand VND/kg which brought about a profit of 3 million VND/kg and the ratio of net income to total costs of 0.2 time. The seed traders had problems with poor quality of wild seed caused by the complicated process from exploitation to selection and preservation. In addition, there was a high competition among the traders (both local ones and those from the north).

Growing-out farms of hard clam

Two most common types of growing-out of hard clams were private farms and cooperatives. The average grow-out area was 17.6 ha for private farms and 551.7 ha for co-operative farms. Clam seed with small size (649±990 ind./kg) were stocked from February to April (Lunar month), while the seed with medium size (313±259 thousand ind./kg) were stocked from June to August (Lunar month). The respective stocking densities were 312.5 thousand ind./m^2 (±2,594) and 64 ind./m^2 (±51). In order to get the average harvest size of 46 (±7) ind./kg, the cultured duration was 22 months/cycle or /crop for small size seed and 12 months/cycle for medium ones. The total income was 211.2 million VND/ha/crop (±254.1) with the ratio of net income to total costs of 0.13 time/crop (±0.29). The farms with more availability and better management of their own wild seed had lower production cost but higher yield, total income and profit. The most serious problems for grow-out farms were the lack of seed, capital, and poor quality of water and man-power while the mass mortality become very common.

Traders of raw hard clam

All of raw hard clam production harvested by grow-out farmers was sold to traders who mainly came from Namdinh and Thaibinh provinces in the north and Bentre and Tiengiang provinces in the south. Average production of raw clam per trader was about 28.8–921.7 tones/year. And then they resold 93.2% of total purchased clam production to processors and the remaining was redistributed at the local markets (6.8%). Added costs of trading raw clam was 0.8 thousand VND/kg with a profit of 3.5 thousand VND/kg and the ratio of net income to total costs was 0.2 time. Insufficient supply of seed made the trader to follow the auction of grow-out farms/sites from different places while the size of hard clams depended on the orders of processors.

Processors/exporters of hard clam

The export of hard clam products has been started since the beginning of 1990s and exported production was 25747 tons in 2007. Unfortunately, Vietnamese Association of Seafood Processors and Exporters (VASEP) had not given enough consideration on this industry until 2010. About 2/3 of total production of live hard clams were processed and exported, especially to Spain, Italy and Portugal. Domestic markets consumed about 1/3 of total production of live hard clams and 5% of processed products only (Le Xuan

Sinh, 2009). MSC certification provided to hard clam of Bentre province at the end of 2009 is a good opportunity for hard clam industry in Vietnam. Three typical processed products were whole clam, coverless, and haft shell. The export price in the past several years show an average profit of 0.05–0.1 USD/kg of raw materials. However, decreasing cultured area and increasing production costs of grow-out farms/sites have strong effects on the supply of raw materials for processing and export. Increase of added value and diversification of products are important. In 2010, VASEP and MARD identified that hard clam ranked the third in the priority for export of Vietnam seafood, after black tiger shrimp and Pangasius catfish. The considerations also need to be given to the competition from other species of bivalves in the domestic markets and that from China.

Sector managers/local officers

About 87.5% of sector managers/local officers and associations though that hard clam was one of the key species for coastal aquaculture but not well managed. In order to have a sustainable development of hard clam industry in the study area and Vietnam, an improvement should be given to: (1) better planning of the areas for broodstocks and seed protection and exploitation, nursery, and growing-out activities; (2) encouragement of the reproduction and nursery models; (3) more appropriate policies/regulations on allocation of lands, taxes and capital/credits; and (4) suitable scheme for integrated coastal management. In addition, it is impressive when the impacts of climate change on livelihoods were clearly recognized by the southern coastal community, in which, hard clam industry would be firstly and strongly affected (Le Xuan Sinh, 2011).

Conclusions

Hard clam is a new industry in Vietnam but has a good potential for development, especially in the southern coastal provinces. However, there is a serious lack of related information while these provinces have to face a various number of difficulties for further development of hard clam. This study helps to clarify the whole picture of hard clam industry, to provide the related and updated information in a systematic manner. A better management of the industry can not be achieved without the regards given to all groups of stakeholders participating in the industry. Planning, natural resourse management, seed reproduction, organization of growing-out activities, processing, and marketing of products, as well as adaptation to the impacts of climate change and improvement of linkages among stakeholdersalso are all important.

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HABITATION AND PRODUCTIONAL CHARACTERISTICS OF PERIPHYTONIC COMMUNITIES OF THE SOUTH VIETNAM REEFS

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Periphyton fouling communities are an important component of coral reef ecosystems. Autotrophic and heterotrophic organisms of these communities form a dense 3–5 mm thick cover on the surface of dead coral colonies and coral fragments and constitute peculiar microbiocenoses. Periphyton communities are dominated by tiny thalloid macrophytes, red calciferous coralline and filamentous algae (Sorokin, 1990).

Daily production of periphyton community down to 20–30 m deep reaches 2–3 g C m^{-2} day^{-1}, depending on the degree of substrate coverage (Cherbadgy, Propp, 1981; Littler et al., 1991). Taking into account an extremely intensive development of perihyton on reefs, these communities are assigned, by their significance, to key functional components of reefs, along with coral communities *per se* (Sorokin, 1990). However, we know presently very little about the effect of environmental factors on productional characteristics of such a community.

The aim of our study was to estimate *in situ* the productional characteristics of periphytonic community inhabiting waters of different hydrological and hydrochemical characteristics.

Materials and Methods

Complex studies of the effects of environmental factors – photosynthetically active radiation (PAR), oxygen concentration in surrounding waters (O_2), ammonium (NH_4), nitrate (NO_3) and orthophosphate (PO_4) in seawaters, as well as the contents in algal tissues of carbon (C), nitrogen (N), phosphorus (P), chlorophyll *a* (Ch *a*) and phycoerythrin (PE) – on the rate of oxygen exchange (M_{O2}) of periphyton were conducted under *in situ* conditions in the South China Sea in May 2007.

Debris of dead corals overgrown by algae were sampled by divers from depths of 5 m at Bath Long Island (20º08′N and 107º44′83 E) on May 20 and at the north-eastern part of Re Island (15º24′N and 109º08′ E) from depth of 10 m on May 24.

The daily rate of oxygen exchange (M_{O2}) of periphyton was measured aboard a research vessel, using a particular flow-through system which imitates the conditions that are characteristic of the habitats of the alga (Cherbadgy et al., 2010). In the evening, periphyton-overgrown coral fragments with an area of 3–5 cm^2 (2D) in 4–8 replicates (Table 1) were taken and placed into 800-ml transparent experimental vessels. Water samples were taken from aboard every 3 hours through 24 h period from the near-bottom layer and the flow, running through the experimental and control vessels, to determine the concentration of O_2; we collected additional samples at the midday and at the

Table 1. Values of environmental factors, contents of nutrients and photosynthetic pigments in algal tissues and the rates of oxygen exchange in periphyton during 24 h (M_{O2}), the rate of photosynthesis in the day time (P_{max}) and dark respiration (R_d) in the areas of Bath Long Island (depth of 5 m) and Re Island (depth of 10 m)

Region	Bath Long Island					Re Island				
Variable	Valid N	Means	Min	Max	St. Dev	Valid N	Means	Min	Max	St. Dev
PAR, µmol m^{-2} s^{-1}	108	95	0	336	110	72	36	0	114	45
O_2, mg l^{-1}	96	6.82	5.43	8.86	0.82	72	6.89	5.15	10.07	1.27
PO_4, µM	36	0.11	0.03	0.9	0.13	24	0.07	0	0.13	0.05
NH_4, µM	36	0.29	0.12	0.42	0,09	24	0.29	0	0.67	0.14
NO_3, µM	36	0.04	0.01	0.18	0.04	16	0.6	0.24	1.3	0.41
C, mg g^{-1}	8	43.4	29.5	57.4	14.4	4	78.1	57.2	108	20.7
N, mg g^{-1}	8	7.24	2.18	12.3	5.22	4	16.1	7.66	21.6	5.24
P, mg g^{-1}	8	0.41	0.01	0.82	0.42	4	1.25	0.51	2.24	0.65
Ch *a*, mg g^{-1}	8	287	56,3	542	154	4	168	100	205	41
PE, mg g^{-1}	8	39.1	0.55	121	41.5	4	139	16.5	307	107
M_{O2}, mg O_2 m^{-2} h^{-1}	64	97.7	−238	499	200	32	79.7	−93.4	345	160
P_{max}, mg O_2 m^{-2} h^{-1}	64	427	378	476	24.3	32	377	326	428	24.9
R_d, mg O_2 m^{-2} h^{-1}	64	−102	−70.1	−134	16	32	−69.3	−49.4	−89	9.61

midnight to determine NH_4, NO_3 and PO_4 levels. O_2 concentration in water samples was determined using Winkler test with some modifications; concentrations of NH_4, NO_3 and PO_4 were measured with a spectrophotometer. Contents of C, N and P in algae tissues were estimated with the method of wet burning; chlorophyll *a* concentration was determined spectrophotometrically in 90%-acetone extracts of algae. All indices were calculated per sm^2 of the substrate. To calculate the daily rate of oxygen exchange (M_{O2}), we used the following modified equation for an unstable regime in a flow-through system.

To describe the dependence of net production (P_n) on PAR intensity we used the hyperbolic equation tangent (Henley, 1993):

$$P_n = (P_{max} \times \tanh (\alpha \times PAR\ P_{max})) + R_d,$$

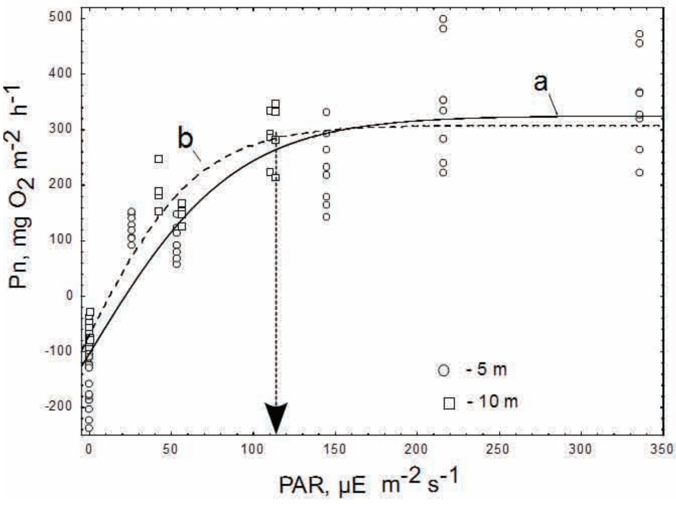
where P_n and R_d are the rates of net photosynthesis production and dark respiration, correspondingly, mg O_2 m^{-2} h^{-1}; α is the tangent of the angle of the slope of the initial part of the light curve; P_{max}, the maximum rate of photosynthesis; and PAR, photosynthetically active radiation, µE m^{-2} s^{-1}.

Results and Discussion

Bath Long Island is fringed with a coral reef that projects 300–400 m of the shore. The bottom in the area of study was 80% covered with reef-building corals. The remaining part of the bottom was occupied by fragments of dead corals and separate fractions of a calciferous porous mass covered with periphytonic foulings that were constituted of red coralline (*Lithothamnion* sp.), lamellar (*Rhodymenia* sp.) and filamentous (*Hypoglossum* sp., *Ostreobium* sp.) algae. Green algae were represented by thalloid (*Halimeda* sp.) and lamellar (*Anadyomene* sp.) forms. In addition to these algae, beds of the red agar-containing alga *Hydropuntia eucheumatoides*. Biomass of red algae in the periphyton of Re Island exceeded that of Bath Long Island.

Environmental conditions in the area of Bath Long Island and Re Island changed during the day, causing corresponding changes of M_{O2} in periphyton communities (Table 1). Concentration of PO_4 in the environment, both at Bath Long Island and Re Island had low values of 0.11 and 0.07 µM, respectively. Concentration of NH_4 in both areas had the same average values of 0.29 µM and distinctly varied during the day. At the same time, concentration of NO_3 in the environment significantly differed.

The contents of C, N and P in the periphyton tissues at Bath Long Island was significantly lower than in the area of Re Island (Table 1). The content of Ch *a* in shallow waters (5 m deep) of the Bath Long Island area was much higher than in the deep-water area of Re Island (10 m), while the content of PE had a reverse dependence. It might be the result of the fact that the periphyton of Bath Long Island was dominated in biomass by green algae, unlike the periphyton of Re Island dominated by red algae. Besides, with an increase of the depth, the PE content grows because of chromatic adaptation of these algae to light deficit.



Dependence of net photosynthesis rate (P_n) of periphyton on PAR intensity, simulated by hyperbolic tangent on the basis of experimentally obtained parameters of light curves. The experiments were performed in periphyton habitats under natural illumination, for 24 h. a – Bath Long Island, depth 5 m, b – Re Island, 10 m.

Dependence of the net photosynthesis rate (P_n) of periphyton on the intensity of PAR in these areas is perfectly described (r=0.92, p<0.000) by the hyperbolic equation tangent (see Figure).

In spite of a significant difference of habitation conditions in these areas (Table 1), P_n of the periphyton have there close values. So, at PAR intensity of 330 µE m^{-2} s^{-1}, penetrating in the midday to a depth of 5 m in the area of Bath Long Island, P_n of the periphyton reaches 320 mg

O_2 m^{-2} h^{-1} (Fig., a). At the same time only 118 µE m^{-2} s^{-1} PAR reach 10 m in the area of Re Island, the P_n value however reaches 300 mg O_2 m^{-2} h^{-1} (Fig., b). Further extrapolation of this curve does not lead to P_n increase.

At greater depths of habitation, the angle of the curve slope (α) grows, this shows a higher efficiency of utilization of weak light intensity by the periphyton community (Table 2). With this, the I_c value at which photosynthesis compensates the expenditures for community respiration reduces to 12.1 µE m^{-2} s^{-1}.

Table 2. Photosynthesis parameters of light curve (P-I) of (the mean ±SE, n=8). P_{max} – the greatest level of photosynthesis, mgO_2 m^{-1} h^{-1}; R_d – dark respiration, mgO_2 m^{-2} h^{-1}; α – the slope of the initial part of light curve; $I_c = R_d/\alpha$ – compensation intensity of PAR, µE m^{-2} s^{-1}; $I_k = P_{max}/\alpha$ – saturation level of PAR, µE m^{-2} s^{-1}; r – correlation coefficient calculated using the Levenberg–Marquardt algorithm for P-I curves; p – probability level

Region; depth, m	P_{max}±SE	R_d±SE	α±SE	I_c	I_k	r	p
Bath Long Island; 5 m	427±24.4	−102±16	4.83±0.74	21.0	88.4	0.92	<0.000
Re Island; 10 m	377±24.9	−69.3±9.6	5.71±0.62	12.1	66.1	0.89	<0.000

Thus, adaptation of periphyton community to light deficiency at depths occurs evidently through a change of species composition of algae and their chromatic adaptation to these conditions. Periphyton in these areas, like a majority of benthic macrophytes of tropical zone, has a high level of productivity in a wide light range of habitation, which is supported by the complex of adaptive reactions (Littler et al., 1991).

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ON THE SEA ANEMONE FAUNA OF THE COASTAL WATERS OF VIETNAM

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The present work is based on the samples taken in the coastal areas of Vietnam during the Russian–Vietnamese expeditions in 2007 and 2010 and literature data. There are many publications concerning the sea anemone species composition and distribution in South China Sea and adjacent waters (Carlgren, 1943; Dunn, 1978, 1981; England, 1987, 1992; Hartog, 1997; Astakhov, 2002; Ardelean, Fautin, 2004; Astakhov et al., 2008; Fautin et al., 2009) where about 40 species of sea anemones are recorded. However, information on the sea anemone fauna with regard to Vietnam coastal waters is lacking. In a majority of publications, reference to material from Vietnam is absent. In this work, the preliminary list of the sea anemone fauna is presented for this region of the Indo-Pacific for the first time (see Table). The species-level taxonomy of Actiniaria is given according to Carlgren (1949). Taxonomic categories such as Infraorder and Superfamily as defined by the International Code of Zoological Nomenclature (ICZN) correspond to Tribe and Subtribe of Carlgren (1949). In contradiction to the ICZN, the names of infraorders and superfamilies in Actiniaria have not yet been typified.

Preliminary list of the sea anemone species of Vietnam

Species	Areas	Habitat (depth; bottom deposits; actinian partners)	References
Order **Actiniaria** Suborder **Nynantheae** Infraorder **Athenaria** Fam. **Edwardsiidae** *Edwardsia annamensis* Carlgren, 1943	Lien Chien, Tourane	–	Carlgren, 1943
Fam. **Haloclavidae** *Peachia mira* Carlgren, 1943	Lien Chien, Tourane	–	Carlgren, 1943
Infraorder **Thenaria** Superfamily **Endomyaria** Fam. **Actiniidae** *Paracondylactis sinensis* Carlgren, 1934	Con Son Islands	–	Carlgren, 1943
Entacmaea quadricolor (Leuckart in Rüppell et Leuckart, 1828)	Van Phong Bay (12°39.9 N, 109°19.8 E); Cat Ba Island (20°44.6 N, 107°07.9 E); Cham Island (15°55.4 N, 108°32.6 E); Khanhhoa Province (12°03′–12°50′ N); Con Son Islands (8°38′24.9″–8°44′57.7″ N, 106°37′09.9″–106°40′08.5″ E)	0–25 m; splits and cavities of remnants and corals, branches of corals, block, the basalt slope; anemonefishes *Amphiprion clarkii*, *A. frenatus*, *A. polymnus*	Present data; Astakhov, 2002; Astakhov et al., 2008
Macrodactyla doreensis (Quoy et Gaimard, 1833)	Tho Chu Island (9°19.3 N, 103°29.6 E); Khanhhoa Province (12°03′–12°50′ N)	0.5–12 m; silt, sand, gravel, stones, corals; anemonefishes *Amphiprion clarkii*, *A. polymnus*	Present data; Astakhov, 2002
Fam. **Thalassianthidae** *Cryptodendrum adhaesivum* Klunzinger, 1877	Khanhhoa Province (12°03′–12°50′ N)	1–1.5 m; splits and cavities of corals; anemonefishes *Amphiprion clarkii*	Astakhov, 2002
Fam. **Stichodactylidae** *Stichodactyla haddoni* (Saville-Kent, 1893)	Khanhhoa Province (12°03′–12°50′ N); Con Son Islands (8°40′36.5″ N, 106°36′26.7″ E)	1–18 m; silt, sand, gravel; anemonefishes *Amphiprion polymnus*, *A. clarkii*	Astakhov, 2002; Astakhov et al., 2008

Table (continued)

Species	Areas	Habitat (depth; bottom deposits; actinian partners)	References
Stichodactyla mertensii Brandt, 1835	Thu Island (10°33.2 N, 108°55.1 E); Khanhhoa Province (12°03′–12°50′ N); Con Son Islands (8°38′24.9″ N, 106°37′09.9″ E)	2–18 m; the coral plate, splits and cavities of remnants and corals, the basalt slope, sand; anemonefish *Amphiprion clarkii*, *A. sandaracions*	Present data; Astakhov, 2002; Astakhov et al., 2008
Heteractis aurora (Quoy et Gaimard, 1833)	Khanhhoa Province (12°03′–12°50′ N)	0–16 m; silt, sand, gravel, the coral plate, remnants, the basalt slope; anemonefish *Amphiprion clarkii*	Astakhov, 2002
Heteractis malu (Haddon et Shackleton, 1893)	Khanhhoa Province (12°03′–12°50′ N)	0–7 m; silt, sand, gravel; anemonefish *Amphiprion clarkii*	Astakhov, 2002
Heteractis crispa (Hemprich et Ehrenberg in Ehrenberg, 1834)	Khanhhoa Province (12°03′–12°50′ N)	0.5–21 m; branches of corals, remnants, silt, sand, gravel, the basalt slope; anemonefish *Amphiprion clarkii*, *A. polymnus*	Astakhov, 2002
Heteractis magnifica (Quoy et Gaimard, 1833)	Tho Chu Island (9°19.3 N, 103°29.6 E); Khanhhoa Province (12°03′–12°50′ N); Con Son Islands (8°41′36.6′ N, 106°44′04.8″ E)	2–27 m; the coral plate, remnants, splits and cavities, the basalt slope; anemonefish *Amphiprion perideration*, *A. clarkii*	Present data; Astakhov, 2002; Astakhov et al., 2008
Fam. **Phymanthidae** *Heteranthus insignis* Carlgren, 1943	Con Son Islands	–	Carlgren, 1943
Superfamily **Acontiaria** Fam. **Hormathiidae** *Hormathianthus tuberculatus* Carlgren, 1943	Nha Trang Bay; Con Son Islands	–	Carlgren, 1943
Fam. **Sagartiidae** *Sagartianthus indosinensis* Carlgren, 1943	Con Son Islands	0 m	Carlgren, 1943
Fam. **Nemanthidae** *Nemanthus annamensis* Carlgren, 1943	Bach Long Vi Island (20°06.071 N, 107°43.596 E); Nha Trang Bay	5–22 m, Gorgonacea	Present data; Carlgren, 1943

Note. "–" – data are not available.

Thus, at least 16 species of Actiniaria belonging to 12 genera and 9 families were found on the coast of Vietnam. Partners of the sea anemone *N. annamensis* are species of Gorgonacea. Sea anemone *E. quadricolor*, *M. doreensis*, *C. adhaesivum*, *S. haddoni*, *S. mertensii*, *H. aurora*, *H. crispa*, *H. magnifica*, *H. malu* are hosts of anemonefishes, and some the clownfish sea anemones live in aquaria for decades.

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PATTERNS OF PALLIAL GONODUCT STRUCTURE OF MUD SNAILS IN THE GENUS *BATILLARIA* (CAENOGASTROPODA, CERITHIOIDEA, BATILLARIIDAE)

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Gastropods of the genus *Batillaria* Thiele, 1931 are widely distributed in intertidal zones from tropical to warm-temperate regions. Inner morphology of the genus is rather well studied at present. First, some general anatomical characters of *Batillaria* s.str.

were listed by R. Houbrick (1988). Recently, results of comprehensive morphological study of *Batillaria attramentaria* (G.B. Sowerby, 1855) and *B. zonalis* (Bruguiere, 1792) were published (Prozorova et al., 2010; Strong et al., 2011). Nevertheless, such taxonomically important character as structure of pallial gonoduct well deserves more ample treatment. To facilitate study of the structure and function of the *Batillaria* reproductive system, histology of pallial oviduct of *B. attramentaria* from the southern Russian Far East was examined. Female specimens fixed with 70% ethanol were dissected to extract portions of foot with pallial oviduct organs. Histological sections were prepared at 5–7 μ and stain with hematoxylin and eosin to examine under light microscope in detail. Results of the study are presented below in comparison with Pleuroceridae.

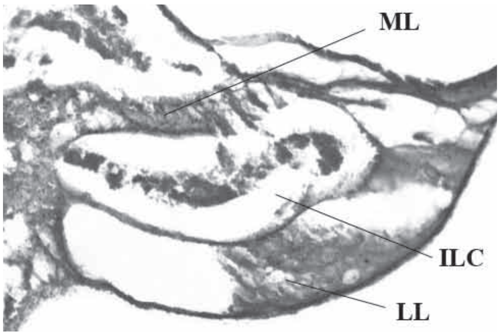

Fig.1. Transverse histological section of *Batillaria attramentaria* through proximal part of pallial oviduct showing fused part of interlamellar cavity.

Abbreviations used in figures: AG – albumen gland, DS – disoriented spermatozoa, ILC – fused part of interlamellar cavity, LL – lateral lamina, ML – medial lamina, OPP – opening of spermatofore bursa, OS – oriented spermatozoa, OSR – opening of seminal receptacle, R – rectum, SG – double sperm gutter, SR – seminal receptacle, WPP – muscle walls of spermatofore bursa.

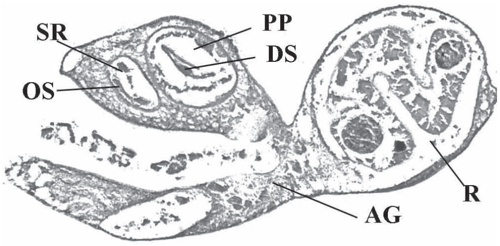

Fig. 2. Transverse histological section of *Batillaria attramentaria* through pallial oviduct showing distinguished chambers of seminal receptacle and spermatofore bursa.

Like other cerithioideans, batillariids are characterized by open pallial gonoduct in both sexes (Houbrich, 1988). Pallial oviduct, corresponding to almost open tube, consists of two laminae – medial and lateral (Strong, 2003; Prozorova et al., 2010; Strong et al., 2011). These laminae are fused in short posterior portion of pallial oviduct only (Fig. 1). Medial lamina covered by interstitial tissue comprises a semen-accepting pallial pocket (here spermatofore bursa) and seminal receptacle (Fig. 2). Both organs connect up mantle cavity separately. Length of closed part of spermatofore bursa is nearly equal to that of seminal receptacle.

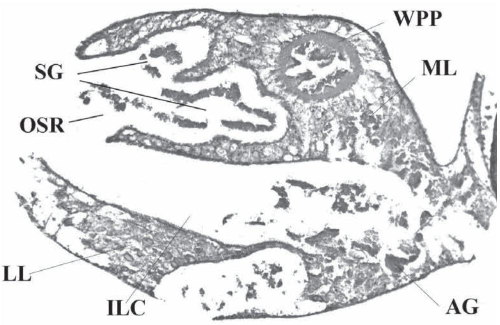

Fig. 3. Transverse histological section of *Batillaria attramentaria* through pallial oviduct showing double sperm gutter along with opening of seminal receptacle.

Spermatofore bursa walls have muscle structure along its whole proximal half and a little more (Fig. 3). In contrast to that, species of freshwater cerithioidean family Pleuroceridae are characterized by pallial pocket having muscle walls in proximal one third parts only. Opening of spermatofore bursa is less that one third of its length. Inner surface of spermatofore bursa is epithelium-lined. Spermatofores and disoriented spermatozoa fill the organ (Fig. 2).

Seminal receptacle located laterally to the bursa contains oriented spermatozoa (Fig. 2). Both organs spermatofore bursa and seminal receptacle are widely open to mantle cavity. Chambers of these organs are connected by wide double sperm gutter (Fig. 3). Septa dividing sperm gutter in two parts is visible in outer half of seminal receptacle as well.

Lateral lamina comprises two glands – distal albumen and proximal capsulate ones. Mucous capsulate gland is well developed in reproductive period. In other time, a short albumen gland is visible only. Cells of albumen gland have granular secret without mucus. There are no hematoxylin inclusions in their cytoplasm (Figs. 2, 3).

A new anatomical survey demonstrates some unique characters of the *Batillaria* oviduct in the comparison with that in other cerithioideans. Obtained results may be used to discriminate genera in the Batillariidae as well.

Acknowledgements

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THE SPECIES COMPOSITION OF THE CROAKER FISH (FAMILY SCIAENIDAE) IN THE COASTAL ZONE OF QUANG NINH AND HAI PHONG PROVINCES

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Introduction

Sciaenid fishes, croakers and drums, are important fisheries resources in the shallow warm seas and estuaries of the world. The Sciaenidae is the seventh largest family among the 150 families of Perciformes (perches), and includes about 80 genera and

300 species. Many croakers use river mouth and estuarine environments seasonally as nursery grounds for juveniles and as seasonal feeding grounds for adults. Others are year-round inhabitants of estuaries and coastal lagoons and a few are found in the reef habitats. In 2007, IUCN-Species Survival Commission identified this family as a conservation priority by establishing a Sciaenidae Red List Authority (RLA) to assess the risk of extinction of every croaker species and to recommend actions needed in the next decade to ensure their survival. A workshop on Global Sciaenidae Red List Assessment and Brazilian Regional RLA has been held in Manaus, Brazil from 9–14 November 2009 with more than 50 experts from all over the world to be presented for this valuation. Vietnam also sent a representative to take part in this meeting and contributed with information for 35 species of the croaker found in Vietnamese waters. However, most species were listed as the D rank (data deficient). This problem may relate to poor understanding of this fish family in Vietnam in generally and no information has been updated recently.

To solve this problem, the Institute of Marine Environment and Resources (IMER) has cooperated with the National University of Taiwan and the Amazon University in Brazil to carry out an expedition sampling. The survey was implemented in 2010 in the coastal areas of Quang Ninh and Hai Phong provinces, field survey were done both in dry and rainy seasons. The results of this study play as the crucial breaking ground step for a comprehensive study in term of biological features of this family and contribute greatly to the conservation of coastal fishery resources in Vietnam.

Materials and Methods

1. Time and locations:

Specimens were collected two times. First sampling was done in March 2010 during dry season, and the second in October 2010 during rainy season. Study area is located the coastal estuary on the coastline extedning more than 200 km from Hai Ha, Quang Ninh Province to Tien Lang, Hai Phong Province (see Figure).

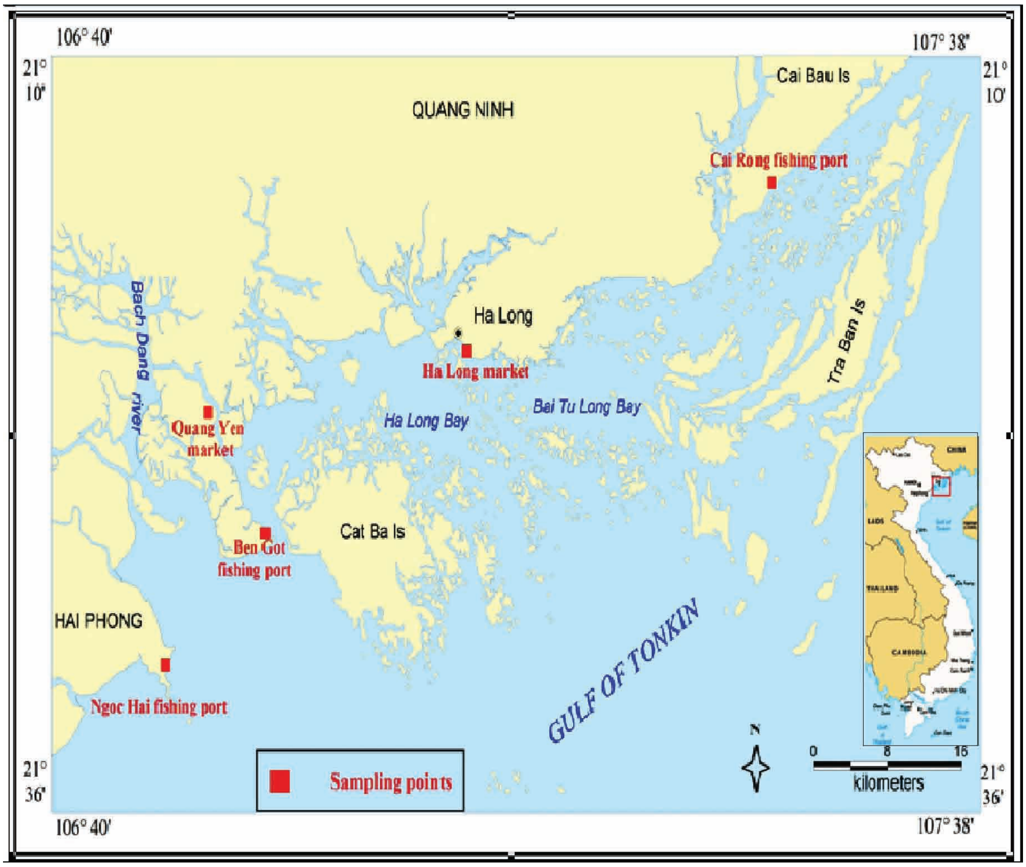
2. Sampling design:

- Specimens were collected from five markets and fish landing sites of the two provinces. The fish landing sites and fish markets are the focal points where the fishing boats land their catch and trade the fish. Therefore, fish collected here would represent for the natural distribution of fish in surrounding areas. The replicate speciments were collected with the variation of fishing areas, fishing water depths, substrate, and seasonal factors.

Sampling areas:

(1) The Cai Rong fishing port, Van Don District, Quang Ninh (coordinates: 21º03′37″ N, 107º25′40″ E). Fishes are catched from coastal waters in Tien Yen, Ha Coi, Ba Che.

(2) The Ha Long market, Ha Long City, Quang Ninh (coordinates: 20º07′51″ N, 107º44′55″ E). This is the common procurement of fishing vessels in the exploitation of Bach Dang estuary, Ha Long area.



A map of coastal areas of Quang Ninh – Hai Phong.

(3) The Quang Yen market, Quang Yen Town, Quang Ninh (coordinates: 20º56′19″ N, 106º47′56″ E). This is where the joint procurement of fishing at the Bach Dang estuary area, Quang Yen.

(4) The Ngoc Hai fishing port, Do Son Town, Hai Phong (coordinates: 20º43′10″ N, 106º47′40″ E). This is where the joint procurement of fishing in the area of Bach Dang, Cua Van Uc, Cat Hai, Son.

(5) The Ben Got fishing port, Cat Hai District, Hai Phong (coordinates: 20º48′05″ N, 106º34′12″ E). This is where the joint procurement of fishing at the Bach Dang estuary, Cat Hai, Do Son.

- Specimens are collected based on the boat caught fish near the shore (gill net and trawling sectors), in the markets and fishing ports at the selected study area.
- Selection of fresh specimens is decided by the good in shape of fins, scales and natural color. Then specimens were photographed before bringing them to the lab and stored in preserved sodium of formalin 20%.
- In the laboratory, the specimens were fixed in 70% ethanol solution for long-term preservation.

3. Species identification:

The method in this study for identifying fish species was based on external morphology, swimbladder and otoliths. The framework for this method was developed by FAO (2001) where they used phenotypes for determing the species. In this study, more specified identification of species were used based on fin rays of the spine, hole beard, the number of whiskers, lips, teeth and lateral line scales.

Results

1. Species composition of sciaenid fishes found in the coastal areas of Hai Phong and Quang Ninh provinces

Based on the analysis of specimens collected through the survey, 10 species of seven genera were found including: *Chrysochir*, *Collichthys*, *Dendrophysa*, *Johnius*, *Nibea*, *Otolithes*, *Pennahia*. Each genus containes only 1–2 species. Among 10 species found in the study area, 7 new records have been added to list of marine fish in the western side of Tonkin Gulf composed by Nguyen Nhat Thi (2008).

List of species in family Sciaenidae distributed in the coastal areas
of Quang Ninh and Hai Phong provinces in this study

1. *Chrysochir aureus* (Richardson, 1846)
2. *Collichthys lucidus* (Richardson,1844)
3. *Dendrophysa russelli* (Cuvier, 1830)
4. *Johnius borneensis* (Bleeker, 1850)
5. *Johnius carouna* (Cuvier, 1830)
6. *Johnius hypostoma* (Bleeker, 1853)
7. *Nibea soldado* (Lacepede, 1802)
8. *Otolithes ruber* (Bloch et Schneider, 1801)
9. *Pennahia anea* (Bloch, 1793)
10. *Pennahia pawak* (Lin, 1940)

List of species in family Sciaenidae distributed in the the western side
of Tonkin Gulf (Nguyen Nhat Thi, 2008)

1. *Argyrosomus nibe* (J. et Th., 1911)
2. *A. pawak* Lin, 1940
3. *A. amoyensis* (Bleeker, 1863)
4. *A. macrocephalus* (Tang, 1937)
5. *Otolithes argenteus* Cuv. et Val., 1830
6. *Bahaba taipingensis* (Herre, 1932)
7. *Johnius belengeri* (Cuv. et Val., 1830)
8. *J. cujus* (Ham et Buch, 1822)
9. *J. aneus* Bloch, 1793
10. *J. dussumieri* (Cuv.et Val., 1830)
11. *J. carutta* Bloch, 1793
12. *J. argentatus* (Houttuyn, 1782)
13. *Collichthys lucidus* (Rich., 1844)
14. *Miichthys miiuy* (Basilewsky, 1855)
15. *Chrysochir aureus* (Rich., 1846)
16. *Nibea albiflora* (Richardson, 1846)
17. *N. diacanthus* (Lac., 1802)
18. *Pseudosciaena crocea* (Rich.,1846)
19. *Bola coibor* (Ham., 1822)
20. *Dendrophysa ruselli* (Cuv. Et Val., 1830)
21. *Macrospinosa suja* (Ham., 1822)

Different results in this study compared with that of Nguyen N.T. (2008) may be related to the technical identifications in former studies that mainly used the outer morphology for identification purposes without using of the swimbladder and otolith. Moreover, Nguyen N.T. (2008) focused more on collection of the offshore specimens. Thus, the results of this study are significant contribution to a comprehensive list of species in the Tonkin Gulf.

2. Seasonal fluctuation in species composition

According to analysis of samples collected in two seasons: in the collection in March, we found eight species, in the first collection in November, we found 10 species. The two species not identified during the collection in March were *Johnius hypostoma* (Bleeker, 1853) and species *Nibea soldado* (Lacepede, 1802).

The species composition of sciaenid fishes for the two surveys

March 2010	September 2010
Chrysochir aureus (Richardson, 1846)	*Chrysochir aureus* (Richardson, 1846)
Collichthys lucidus (Richardson,1844)	*Collichthys lucidus* (Richardson,1844)
Dendrophysa russelli (Cuvier, 1830)	*Dendrophysa russelli* (Cuvier, 1830)
Johnius borneensis (Bleeker, 1850)	*Johnius borneensis* (Bleeker, 1850)
Johnius carouna (Cuvier, 1830)	*Johnius carouna* (Cuvier, 1830)
Otolithes ruber (Bloch et Sch., 1801)	*Johnius hypostoma* (Bleeker, 1853)
Pennahia anea (Bloch, 1793)	*Nibea soldado* (Lacepede, 1802)
Pennahia pawak (Lin, 1940)	*Otolithes ruber* (Bloch et Sch., 1801)
	Pennahia anea (Bloch, 1793)
	Pennahia pawak (Lin, 1940)

Conclusion

The sciaenid fishes in coastal areas of Quang Ninh and Hai Phong had 10 species in seven genera, including *Chrysochir*, *Collichthys*, *Dendrophysa*, *Johnius*, *Nibea*, *Otolithes*, *Pennahia*. Seasonal factors affected the fluctuation in the number of species. The survey in September brought two more species, *J. hypostoma* and *N. soldado* while they have not been in the survey of March.

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STATUS OF THE KNOWLEDGE OF THE INDO-PACIFIC ANADARINAE (MOLLUSCA: BIVALVIA)

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Introduction

Bivalve mollusks of the subfamily Anadarinae Reinhart, 1935 are members of the family Arcidae Lamarck, 1818 and they are widely distributed in tropical and subtropical zones of the World Ocean, although some species penetrate to temperate latitudes; the highest species diversity occurs in the Indian and Pacific oceans. Lim (1968) suggested that the sibfamily is comprised of about 60 valid Recent species.

Up to present, taxonomic and faunal accounts of the anadarines of Japan (Habe, 1965; Noda, 1966), China (Li, 1983), Russian Far East (Lutaenko, 1993), South Africa and Mozambique (Kilburn, 1983), southern India (Lutaenko, 2006), western Africa (Oliver, Cosel, 1992), Thailand (Vongpanich, 1996) and Vietnam (Evseev, Lutaenko, 1998) were published; there are modern data on the anadarines of Australia (Lamprell, Healy, 1998) and Red Sea (Oliver, 1992). Despite the abundant literature, taxonomy of the subfamily remains complicated and unclear. The reasons for that are ignorance of type material study, presence of many nominal species names introduced in the 19th century, high variability of shell morphology, and unstable generic subdivisions.

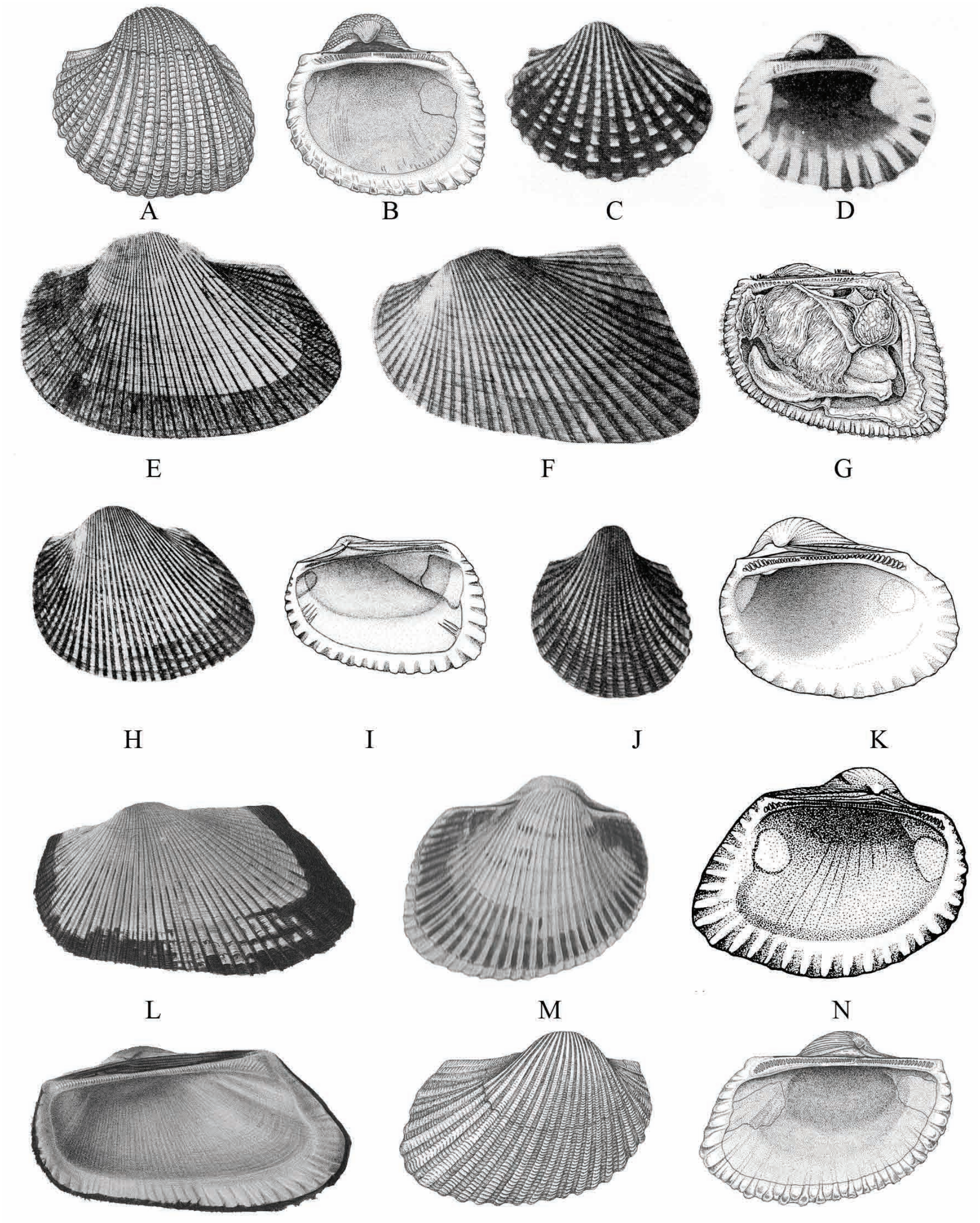
How Many Species of the Anadarines Exist in the Indo-Pacific?

The shelf waters of the Indo-West Pacific are spread out over an enormous area with incredibly rich biota; for instance, the area has more than 6000 species of mollusks (Briggs, 1995). Based on literature and original data, we estimated anadarine species richness and compiled a list of species living in the Indo-Pacific. In total, 38 species are known up to date from the area: *Anadara antiquata* (L.), *A. uropigimelana* (Bory), *A. crebricostata* (Reeve), *A. auriculata* (Lamarck), *A. craticulata* (Nyst), *A. ehrenbergi* (Dunker), ?*A. ambigua* (Reeve), *A. ferruginea* (Reeve), *A. dautzenbergi* (Lamy), *A. africana* (Sowerby), *A. pygmaea* (H. Adams), *A. consociata* (Smith), *A. vellicata* (Reeve), *A. pilula* (Reeve), *A. binakayanensis* (Faustino), *A. satowi* (Dunker), *A. cornea* (Reeve), *A. kagoshimensis* (Tokunaga), *A. natalensis* (Krauss), *A. troscheli* (Dunker), *A. kafanovi* Lutaenko, *A. gubernaculum* (Reeve), *A. guangdongensis* (Bernard, Cai et Morton), *A. broughtonii* (Schrenck), *A. inaequivalvis* (Bruguiere), ?*A. rufescens* (Reeve), *A. granosa* (L.), *A. rhombea* (Born), *A. nodifera* (Martens), *A. indica* (Gmelin), *A. erythraeonensis* (Jonas in Philippi), *A. mosambicana* (Bianconi), *A. kikaizimana* (Nomura et Zinbo), *A. deyrollei* (Jousseaume), *A. trapezia* (Deshayes), ?*A. addita* Iredale, ?*A. secticostata* (Reeve), *A. jurata* Iredale. Status of some species are much in need of further study with examination of types and molecular approach. The species richness of the anadarines is highest in the western portion of the Indo-Pacific (up to 22 in Chinese waters) and decreases to 7–8 in Red Sea and Southern Africa.

Status of the Subfamily Anadarinae

The subfamily Anadarinae was proposed by the Reinhart (1935) in the Arcidae along with nominative Arcinae Lamarck, 1809, and Noetiinae Stewart, 1930, with the following diagnosis: "Ventral margin closed, byssal gape lacking; sculpture consisting of large, regular radial ribs, sculptured with grooves or nodes in most species; surface of shell regularly rounded; ligamental area narrow to moderately wide, flat to widely V-shaped when viewed in cross section (with both valves joined); hinge straight or gently arched; teeth regularly diminishing in size from exremities to center, but usually not completely lacking in the center. Inner margin of shell crenulated; beaks never opisthogyrate, pointing either inward or forward. Type genus: *Anadara* Gray, based upon *Arca antiquata* Linné. Geologic range: Cretaceous to Recent." This diagnosis can be supplemented with: teeth are vertical or slightly oblique, byssus is absent at adult stage or very week, periostracum is thick and easily fallen away. It was probably Kilburn (1983) who first suggested that Anadarinae can be synonymized with Arcinae because of presence of species with some intermediate features such as *Anadara mosambicana* (Bianconi, 1856) and *A. erythraeonensis* (Jones in Philippi, 1851): these two species possess a narrow byssal gape and a rather flattened shell reminding some *Barbatia* (Arcinae). We proposed a new genus *Mosambicarca* Lutaenko, 1994 for this group (Lutaenko, 1994) and suggested that *Mosambicarca* represents a "primitive" anadarine with ancestral features (another transitional group is Hawaiarcinae Noda, 1986 (Noda, 1986). Newly described *Paranadara* Francisco, Barros et Lima, 2011 (Francisco et al., 2011) may belong to Hawaiarcinae if it is not a synonym of another arcid genus. Coan and Valentich-Scott (2012; in press) in their comprehensive review of the tropical bivalves of western America prefer to keep Anadarinae as a subfamily of Arcidae. We believe now that hawaiarcines can be regarded as a tribe Hawaiarcini in the Arcinae while *Mosambicarca* deserves status of tribe in the Anadarinae with amendment of diagnosis of the Anadarinae.

Anadarinae is a young group of arcoids appeared only at the boundary of the Paleogene and Neogene and widely evolved in the Miocene (Noda, 1966) and they clearly represent a single clade. Within the Arcidae, the subfamily split of the Arcinae and Anadarinae is based on the strength of the byssus corresponding to the attached or free-living modes of life, and this split corresponds to separate adaptive radiations, one epibyssate and one endobyssate (Oliver, Holmes, 2006). Molecular data evidence that *Anadara* forms a clade with good support (Matsumoto, Hayami, 2001; Marco, 2002). Morphologically, anadarines are not very diverse (see Figure). Inclusion of *Bathyarca* Kobelt, 1891, *Bentharca* Verrill et Bush, 1898, and *Samacar* Iredale, 1936 into the Anadarinae (Newell, 1969) is not substantiated and we support viewpoint of Oliver and Holmes (2006) that Bathyarcidae Scarlato et Starobogatov, 1979 may be recognized with more molecular studies (perhaps, as a subfamily). Status of species assigned to *Samacar* and a new subgenus *Samacar* (*Pseudoporterius*) Kamenev, 2007 (Kamenev, 2007) is confused; the latter is compared to *Porterius* Clark, 1925 which belongs to another family, Parallelodontidae Dall, 1898 with only one living species; parallelodontids are extinct and they are believed to be ancestors of Arcidae.



Morphological diversity of Anadarinae: A, B – *Anadara rhombea* (Born) (after Lutaenko, 2006); C, D – *A. granosa* (L.); E – *A. crebricostata* (Reeve); F – *A. vellicata* (Reeve); G – *A. vellicata* (Reeve), lateral view with left valve and mantle removed (after Biggs, 1973); H – *A. broughtonii* (Schrenck); I – *A. gubernaculum* (Reeve) (after Evseev, Lutaenko, 1998); J – *A. pilula* (Reeve); K – *A. africana* (Sowerby) (after Kilburn, 1983); L, O – *A. erythraeonensis* (Jonas in Philippi) (after Dunker, 1869 (in Dunker, 1858–1878)); M – *A.* cf. *inaequivalvis* (Bruguiere) (after Reeve, 1843–1844); N – *A.* cf. *kagoshimensis* (Tokunaga) (after Evseev, Lutaenko, 1998); P, Q – *A. ehrenbergi* (Dunker) (after Lutaenko, 2006); figs. C, D, E, F, H, J after Li (1983).

Generic Taxonomy of Anadarinae

In the 18th–19th centuries, a majority of the arcoidean bivalves including anadarines were regarded in the single genus *Arca* L., 1758 (Born, 1780; Bruguière, 1789; Lamarck, 1819; Reeve, 1843–1844; Nyst, 1848; and others), however, at the end of the 19th century a tendency to subdivide *Arca* into subgenera appeared (e.g., Smith, 1885) or even some anadarines were included into different genera (Dunker, 1858–1878). Lamy (1907) in his land-mark revision of the Arcoidea included all anadarines in *Arca* but he enumerated "sous-genre" in the beginning of *Arca* sections such as *Anadara*, *Senilia*, *Cunearca*, *Argina*. Number of genera and subgenera increased, and generic taxonomy of Anadarinae can be represented as follows:

System of Reinhart (1935)

Anadara (*Anadara*) Gray, 1847
Anadara (*Larkinia*) Reinhart, 1935
Anadara (*Senilia*) Gray, 1842
Anadara (*Argina*) Gray, 1842
Anadara (*Cunearca*) Dall, 1898
Anadara (*Scapharca*) Gray, 1847
Nemoarca Conrad, 1869

System of Iredale (1939)

[subfamilies in Arcidae were not discerned;
only *Anadara*-like genera are presented below]

Anadara Gray, 1847
Tegillarca Iredale, 1939
Scapharca Gray, 1847
Imparilarca Iredale, 1929
Potiarca Iredale, 1939
Mabellarca Iredale, 1939

System of Rost (1955)

Anadara (*Anadara*) Gray, 1847
Anadara (*Larkinia*) Reihart, 1935
Anadara (*Scapharca*) Gray, 1847
Anadara (*Cunearca*) Dall, 1898
Lunarca Gray, 1842

System of Olsson (1961)

Anadara (*Anadara*) Gray, 1847
Anadara (*Diluvarca*) Woodring, 1925
Anadara (*Rasia*) Gray, 1857
Anadara (*Senilia*) Gray, 1842
Anadara (*Larkinia*) Reinhart, 1935
Anadara (*Grandiarca*) Olsson, 1961
Anadara (*Cunearca*) Dall, 1898
Anadara (*Scapharca*) Gray, 1847
Anadara (*Sectiarca*) Olsson, 1961
Anadara (*Caloosarca*) Olsson, 1961
Anadara (*Esmerarca*) Olsson, 1961

System of Habe (1965, 1977)

Tegillarca Iredale, 1939
Potiarca Iredale, 1939
Scapharca Gray, 1847
Anadara Gray, 1847
Mabellarca Iredale, 1939
Diluvarca Woodring, 1925
Tosarca Noda, 1966
Imparilarca Iredale, 1929

System of Noda (1966)

Anadara (*Anadara*) Gray, 1847
Anadara (*Scapharca*) Gray, 1847
Anadara (*Hataiarca*) Noda, 1966
Anadara (*Tosarca*) Noda, 1965
Anadara (*Tegillarca*) Iredale, 1939
Anadara (*Kikaiarca*) Noda, 1966
Anadara (*Cunearca*) Dall, 1898
Anadara (*Potiarca*) Iredale, 1939

System of Lim (1968)

Anadara (*Anadara*) Gray, 1847
Anadara (*Scapharca*) Gray, 1847
Anadara (*Tegillarca*) Iredale, 1939
Anadara (*Larkinia*) Reinhart, 1935
Anadara (*Senilia*) Gray, 1842

System of Newell (1969)

Anadara (*Anadara*) *Gray*, 1847
Anadara (*Lunarca*) Gray, 1857
Larkinia Reinhart, 1935
Samacar Iredale, 1936
Scapharca (*Scapharca*) Gray, 1847
Scapharca (*Cunearca*) Dall, 1898
?*Scaphula* Benson, 1834
Senilia Gray, 1842
Bathyarca Kobelt, 1891
Bentharca Verrill et Bush, 1898

System of Keen (1971)

Anadara (*Anadara*) Gray, 1847
Anadara (*Cunearca*) Dall, 1898
Anadara (*Esmerarca*) Olsson, 1961
Anadara (*Grandiarca*) Olsson, 1961
Anadara (*Larkinia*) Reinhart, 1935
Anadara (*Rasia*) Gray, 1857
Anadara (*Scapharca*) Gray, 1847
Bathyarca Kobelt, 1891
*Lunarc*a Gray, 1842

System of Kilburn (1983)

[Anadarinae is not recognized;
only *Anadara* with subgenera are listed]

Anadara (*Anadara*) Gray, 1847
Anadara (*Scapharca*) Gray, 1847
Anadara (*Mabellarca*) Iredale, 1939
Anadara (*Tegillarca*) Iredale, 1939

System of Li (1983)

Anadara Gray, 1847
Scapharca Gray, 1847
Potiarca Iredale, 1939
Tegillarca Iredale, 1939
Mabellarca Iredale, 1939

System of Evseev and Lutaenko (1998)

Anadara (*Anadara*) Gray, 1847
Anadara (*Imparilarca*) Iredale, 1929
Anadara (*Diluvarca*) Woodring, 1925
Anadara (*Tosarca*) Noda, 1965
Anadara (*Potiarca*) Iredale, 1939
Anadara (*Tegillarca*) Iredale, 1939
Anadara (*Mabellarca*) Iredale, 1939
Anadara (*Scapharca*) Gray, 1847

Molecular analysis seems to support subdivisions of *Anadara* into several genera (*Anadara*, *Scapharca*, *Tegillarca*, *Diluvarca*) showing that such morphological characters as inaequivalvness (*Scapharca*) likely related to pleurothetic life and sculpture of radial ribs are useful in current classification (Matsumoto, Hayami, 2001). Coan and Valentich-Scott (2012) stated that the subgenera of *Anadara* are far poorly differentiated, with non-covarying characters, such as size, shell thickness, degree of valve overlap posteroventrally, and they abandoned their use stating that not all the genus-level taxa are necessarily synonyms of *Anadara*. However, they listed as synonyms *Scapharca*, *Larkinia*, *Senilia*, *Diluvarca*, *Cunearca*, *Potiarca*, *Caloosarca*, *Esmerarca*, *Grandiarca*, *Sectiarca*, *Tosarca*.

Re-consideration of the subgenera of *Anadara* is much in need using molecular approach but this should be done in combination with traditional phenetic-based analysis and especially taking into account rich paleontological record. We believe that at least *Anadara* s.s., *Scapharca*, *Tegillarca*, *Mabellarca* can be included in the genus *Anadara* as subgenera. *Bathyarca*, *Samacar* and *Bentharca* are obviously not members of Anadarinae.

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DEBROMOAPLYSIATOXIN, A CYTOTOXIC METABOLITE FROM THE MARINE CYANOBACTERIUM *LYNGBYA MAJUSCULA* FROM VIETNAM

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Introduction

Marine cyanobacteria would probably ranked along side the actinomycetes and myxobacteria as a prolific producer of unique natural products. Over the past 30 years, the research for bioactive secondary metabolites (natural products) from marine organisms has yielded a wealth of new molecules (estimated at approximately 17.000) with many fundamentally new chemotypes and extraordinary potential for biomedical research and applications (Blunt et al., 2008). Marine cyanobacteria belong to the most fruitful sources of marine natural products, with nearly 700 compounds described (Jones et al., 2009).

Majority of the papers is dominated by cyanobacterial collections from reef systems in Hawaii, the Caribbean, Madagascar, and Papua New Guinea. However, a few is known on the chemistry of marine cyanobacteria from other parts of the world, such as South East Asia where biodiversity is high (Tan, 2006). At present, a few is known on biological activity and chemistry of marine cyanobacteria from Vietnam. Here is the opportunity to search for novel cyanobacterial biomolecules from these marine areas which have not been studied intensively so far.

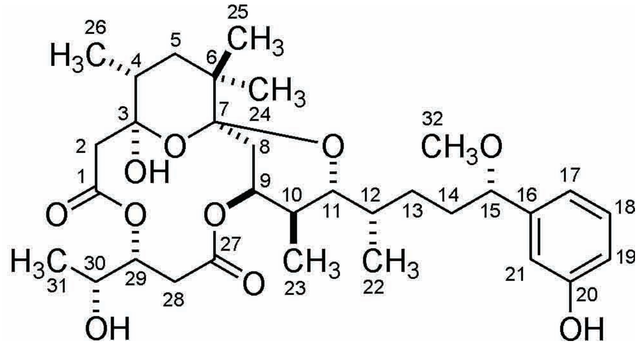
The filamentous marine cyanobacterium *Lyngbya majuscula* (Gomont) is of particular importance, as approximately 35% of all cyanobacterial bioactive compounds identified so far have been isolated from the genus *Lyngbya*, with 76% of these coming from *L. majuscula* (Jones et al., 2009). The compounds isolated from *L. majuscula* exhibit a variety of biological activities including antimicrobial, antiproliferative, immunosuppressant activities. Thus, it is accepted that the marine cyanobacterium *L. majuscula* is an exceptional source of novel potential pharmaceuticals. Although a lot of studies have been carried out and are still going on in the research for novel bioactive compounds from *L. majuscula*, there is little report on bioactive compounds isolated from this marine cyanobacterium growing near the coasts of Vietnam.

In our ongoing efforts toward finding novel and pharmacologically active marine cyanobacterial metabolites, the chemical and biological investigation of a *L. majuscula* strain collected in Vietnam at Hon Khoi locality in Khanh Hoa Province was undertaken. This chemical investigation of this strain resulted in the isolation of debromoaplysiatoxin. Debrommoaplysiatoxin was first isolated from the digestive gland of the sea hare *Stylocheilus longicauda* (Kato, Scheuer, 1974). This compound has been showed to be an activator of protein kinase C and to be a potent tumor promoter (Nagai et al., 1997) and displayed some antineoplastic activity (Mynderse et al., 1977). Herein, we wish to report debrommoaplysiatoxin isolated from *Lyngbya majuscula* strain collected in Vietnam exhibited cytotoxic activity against bladder cancer cell line 5637 with IC_{50} of 86 ng/ml.

Results and Discussion

Samples of the marine cyanobacterium *Lyngbya majuscula* were collected at Hon Khoi locality in Khanh Hoa Province, Vietnam on August 20, 2007. The methanol extract prepared from the biomass of *L. majuscula* showed strong cytotoxic activity against showed activity against bladder cancer cell line 5637. Bioassay-guided separation of this methanol extract on silica gel chromatography followed by reversed-phase HPLC afforded debromoaplysiatoxin (see Figure).

The identification of debromoaplysiatoxin was established by direct comparison of our spectroscopic data, including ^{1}H NMR, ^{13}C NMR, and HRMS in negative mode



Debromoaplysiatoxin, molecular formula $C_{32}H_{48}O_{10}$.

with those reported in the literature. ^{1}H NMR, ^{13}C NMR data of the isolated debromoaplysiatoxin, together with literature data for debromoaplysiatoxin reported by Nagai et al. (1997) are shown in Table. In addition, the HRMS in negative mode of isolated debromoaplysiatoxin, showed a molecular ion peak at *m/z* $[M\text{-}H\text{+}HCl]^-$ 627.2942 and the molecular weight was deduced to be 592 Dalton compatible with the molecular formula $C_{32}H_{48}O_{10}$. In our work, debromoaplysiatoxin displayed moderate cytotoxicity against bladder cancer cell line 5637 with IC_{50} of 86 ng/ml.

Experimental Section

Instruments

UV spectra were recorded in MeOH (Uvasol, Merk, Germany) with a UV-2102 PC UV-vis scanning spectrophotomater (Shimadzu). One-dimensional (^{1}H and ^{13}C-NMR spectra were recorded at 300K on a Bruker AVANCE DMX600 NMR spectrometer (^{1}H-NMR: 600 MHz; ^{13}C-NMR: 150MHz) locked to the deuterium resonance of the solvent, $CDCl_3$. Chemical shifts are reference to the residual proton signal of the solvent as an internal standard. Mass spectrometry: HR-ES-IMS (positive ion mode) were recorded on a high resolution Bruker MaXis mass spectrometer. Analytical and preparative HPLC were performed on a component system (Kontron Instruments, Italy).

Marine cyanobacterial samples

Samples of the marine cyanobacterium *Lyngbya majuscula* Harvey ex Gomont (Oscillatoriaceae), growing on rocks, dead corals, and gravel in the lower intertidal to subtidal zone of shores and exposed to calm to moderate wave action were collected by hand from a water depth of 0.1–1 m, placed into sample containers and shipped in the laboratory within the day. In the laboratory, the samples were air-dried in low natural light, lyophilized later, and stored at −20ºC until use. A voucher specimen is available in the Ernst-Moritz-Arndt – University Greifswald, Germany under the strain number LMVN.

Extraction and isolation

Lyophilized biomass (15 g) were extracted with 3 portions of 250 ml of n-hexane, ethyl acetate, methanol respectively. The extracts obtained were evaporated under vacuum to give the crude dried extracts. A portion of the methanol extract (240 mg) was applied onto a silica gel column and eluted with a stepwise gradient starting with 75% n-hexane to 100% EtOAc to 100% MeOH to yield twenty major fractions (F_1 to F_{22}). Among of them, the fraction F_{10} eluting with 50% n-hexane/EtOAc which exhibited strong cytotoxic activity. Further separation of F_{10} was done by semi preparative HPLC

^{13}C NMR and ^{1}H NMR data of isolated debromoaplysiatoxin with literature data of debromoaplysiatoxin reported by Nagai et al. (1997)

Position	Debromo-aplysiatoxin	Lit. values	Debromo-aplysiatoxin	Lit. values
	$^{1}\delta_C$	$^{2}\delta_C$	$^{3}\delta_H$	$^{4}\delta_H$ (J in Hz)
1	169.19	169.2 (0)	–	–
2	46.91	46.9 (2)	2.52 (β, d) 2.76 (α, d)	2.52 (β, d, 12.7) 2.76 (α, d, 12.5)
3	98.81	98.8 (0)	–	–
4	35.68	35.7 (1)	1.86 (m)	1.86 (m)
5	41.16	41.1 (2)	1.62 (ax, t) 1.05 (eq, dd)	1.62 (ax, t, 13.1 and 13.1) 1.05 (eq, dd, 3.6 and 13.4)
6	38.98	39.0 (0)	–	–
7	100.85	100.8 (0)	–	–
8	33.66	33.6 (2)	1.71 (ax, dd) 2.68 (eq, dd)	1.71 (ax, dd, 3.6 and 14.8) 2.68 (eq, dd, 3.0 and 14.7)
9	73.21	73.2 (1)	5.23 (m)	–
10	35.42	35.4 (1)	1.71 (m)	1.71 (m)
11	69.88	69.8 (1)	3.93 (dd)	3.93 (dd, 2.1 and 10.9)
12	34.24	34.2 (1)	1.52 (m)	1.52 (m)
13	31.24	31.2 (2)	1.31 (m) 1.39 (m)	1.31 (m) 1.39 (m)
14	36.12	36.1 (2)	1.64 (m) 1.97 (m)	1.63 (m) 1.97 (m)
15	85.83	85.8 (1)	4.0 (t)	4.0 (t, 6.5 and 6.5)
16	145.93	145.9 (0)	–	–
17	119.34	119.3 (1)	6.84 (dt)	6.84 (dt, 1,1 and 7.9)
18	129.79	129.8 (1)	7.13 (t)	7.13 (t,7.8 and 7.8)
19	114.98	115.0 (1)	6.71 (ddd)	6.71 (dt, 1,1 and 7.9)
20	158.34	158.3 (0)	–	–
21	114.67	114.6 (1)	6.92 (m)	6.92 (t,1 and 1)
22	13.52	13.6 (3)	0.79 (d)	0.79 (d,6.8)
23	13.04	13.0 (3)	0.71 (d)	0.71 (d,6.9)
24	26.79	26.8 (3)	0.83 (s)	0.83 (s)
25	23.61	23.6 (3)	0.8 (s)	0.8 (s)
26	16.48	16.5 (3)	0.86 (d)	0.86 (d,6.8)
27	170.36	170.4 (0)	–	–
28	34.69	34.6 (2)	2.92 (α, dd) 2.88 (β, m)	2.92 (α, dd, 11.1 and 18.2) 2.87 (β, dd, 2.8 and 18.1)
29	74.30	74.3 (1)	2.23 (m)	5.23 (m)
30	67.08	67.0 (1)	4.03 (m)	4.03 (m)
31	17.72	17.7 (3)	1.12 (d)	1.12 (d, 6.4)
32	56.60	56.6 (3)	–	3.17 (s)

(Synergi-RP 80A column, 250×10 mm, 4 micron, flow rate 3 ml/min, a solvent gradient from 20–100% acetonitrile/water in 52 min, detected at 210 nm) to afford debromoaplysiatoxin (5.3 mg, t_R=19.69).

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REVIEW OF THE MORPHOLOGY AND DISTRIBUTION OF MICROGASTROPODS OF THE GENUS *DIFFALABA* (CAENOGASTROPODA: CERITHIOIDEA: LITIOPIDAE)

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Representatives of the genus *Diffalaba* Iredale, 1936 are small intertidal gastropods (shell length is less than 2 cm), living on seaweeds and widely distributed in the Pacific Ocean from Australia to Japan and in the southern Russian Far East. The taxonomy of *Diffalaba* is not still clear because of scanty data on animal and larval shell morphology of the type species *Diffalaba opiniosa* Iredale, 1936 described from Australia

(Iredale, 1936). The distribution of that species known as Australian endemic is not well-known yet. There has been no revision of the Australian Litiopidae. As a consequence, there is no comprehensive data on the family composition in Australia and distribution of litiopid species (Beechey, 2010).

Besides *D. opiniosa*, four other species are referred to *Diffalaba* by different authors. Three species of the genus are known from Japan: *D. picta* (A. Adams, 1861), *D. hungerfordi* (Sowerby III, 1894) (Higo et al., 1999) and *D. vitrea* (Sowerby III, 1915) (=*Litiopa simplex* Yokoyama, 1927). First two species are recorded in Korea (Kwon et al., 2001; Lee, Min, 2002; Min et al., 2004). Beside that, *D. vitrea* is recorded in the southern Russian Far East in Vostok Bay and Peter the Great Bay, Sea of Japan (original data). Chinese *Diffalaba* species are usually regarded as *D. picta* (Seashells of China, 2004) because the species type originates from Shandong Province of China. The second species, *D. hungerfordi,* is probably occurs on China coasts everywhere because both these litiopids are known to inhabit Taiwan (The Taiwan Malacofauna Database, 2011). In Thailand, *D. hungerfordi* is recorded (Robba et al., 2003). In Vietnam, mollusks of the genus are rather rare and usually identified as *D. picta* (Thach, 2007) in spite of more oblong shell than in Japanese and Chinese specimens.

D. vladivostokensis (Bartsch, 1929) from the southern Primorye was the only species of the genus known in Russia (Bartsch, 1929; Golikov, Scarlato, 1967; Golikov, Kussakin, 1978; Volova et al., 1979). Sometimes, the species is cited as belonging to the *Alaba* H. et A. Adams, 1860 (Golikov, Kussakin, 1978; Volova et al., 1979; Kulikova et al., 2000; Golikov et al., 2001). More often *D. vladivostokensis* is considered as a junior synonym of *D. picta* (A. Adams, 1861) (Kuroda et al., 1971; Golikov, Scarlato, 1985; Higo et al., 1999; Gulbin, 2004, 2006; Kantor, Sysoev, 2006; Thach, 2007) inhabiting the coasts of Australia, Japan, Korea, China and the southern Russian Far East (Habe, 1968). Based on comparison of specimens from Japan, Korea and the southern Russian Far East we should agree with opinion that *D. vladivostokensis* is a synonym of *D. picta.* In Russia, that species inhabits northern part of the Japan Sea from Possjet Bay to Vostok Bay and probably southern Sakhalin and southern Nevelskoy Strait (Golikov et al., 2001).

Thus, the genus *Diffalaba* is represented at least by four species three of which inhabit Indo-Pacific region (OBIS Indo-Pacific Molluscan Database, 2006): tropical-subtropical to boreal Indo-West Pacific *D. picta*; tropical-subtropical *D. hungerfordi* and subtropical *D. vitrea.*

Two morphologically studied species of the genus, *D. picta* and *D. vitrea,* are characterized by smooth protoconch (Kulikova et al., 2000; Prozorova, Sitnikova, 2010; Prozorova et al., 2010; original data). In Litiopidae, not only *Diffalaba,* but two other genera – recent *Styliferina* A. Adams, 1860 (Sasaki et al., 2008) and fossil *Litiopella* Bandel et Kiel, 2000 lack the distinct spirals on the protoconch (Bandel, Kiel, 2000). In opposite with that, representatives of *Alaba* and *Litiopa* Rang, 1829 − *Alaba incerta* (Orbigny, 1842) and *Litiopa melanostoma* Rang, 1829 are characterized by protoconchs sculptured with numerous axial riblets and subsutural plaits (Houbrick, 1987). Furthermore, the protoconch microsculpture is a not structurally consistent feature in Litiopidae. A further comprehensive investigation of the morphology of Litiopidae and *Diffalaba* is in progress.

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TAXONOMIC IDENTIFICATION AND PHYLOGENETIC RELATIONSHIPS OF THE GROUPERS (*EPINEPHELUS* SPP.) COLLECTED ALONG THE COASTAL ZONE OF VIETNAM

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Introduction

Grouper is a common name for fish of any member in the subfamily Epinephelinae, family Serranidae, order Perciformes, class Actinopterygii, phylum Chordata. The largest genus *Epinephelus* in the family Serranidae comprises hundreds of species distributed in the sea over the world most of which are of considerable economic value (Heemstra, Randall, 1993). Groupers are residents widely distributed in the tropical and subtropical seas and usually migrants for long distance. In some regions, groupers are sea bottom residents living mainly in reefs. They are morphologically identified based on colour patterns and body shape which are in some species normally changed from juveniles to adults. That is the reason why nomenclature of groupers comprises of a variety of synonyms regarding to description of a particular species (Ding et al., 2006). Due to their homogeneous morphology, identification and reconstruction of the evolutionary/phylogenetic relationships among grouper species are mostly difficult. This gives rise to confuse some species with each others. Little is known about molecular identification and phylogenetic analysis in terms of revealing the difference within *Epinephelus* (Maggio et al., 2005). However, in recent years, mitochondrial genetic markers, i.e., cytochrome b (*cob*) and 16S rDNA sequences, have been used for molecular analysis and phylogenetic relationships of groupers of *Epinephelus* in eastern Atlantic and China seas (Maggio et al., 2005; Craig et al., 2007). In Vietnam, groupers ("*ca mu*" or "*ca song*") have contributed to the national economic development in the regards of the products of this valuable fish available from sea fishing and mariculture industry. Up to date, none of any analysis of molecular identification and phylogenetic relationships has been done for taxonomic clarification of groupers and *Epinephelus* spp. populations in Vietnam.

In this paper, we present the work using a portion of mitochondrial cytochrome b as molecular markers to identify species and to investigate the molecular relationships among some *Epinephelus* species collected along the geographic-coastal regions of Vietnam. The construction of overall phylogenetic tree of 58 samples for 12 *Epinephelus* of Vietnam and other epinepheline species with different biogeographical distributions over the world was included in the study.

Materials and Methods

Samples and genomic DNA extraction

Grouper species were collected in the coastal sea of Hai Phong, Quang Ninh, Nghe An, Phu Yen, Khanh Hoa, Ba Ria – Vung Tau Provinces of Vietnam (Fig. 1). Fresh muscle tissue was taken and preserved in 70% ethanol, stored in −20^{o}C until DNA extraction. Genomic DNA was extracted from specimens using the commercial QiaAmp DNA extraction kit (Qiagen Inc.) according to the manufacturer's instructions. The genomic DNA was eluted in 100 µl elution buffer, estimated using spectrophotometry (GBC UV/visible 911A spectrophotometer), stored at −20ºC until use and diluted to a working concentration of 100 ng/µl. The amount of 1 µl of this was used as template in the PCR of 50 µl volume.

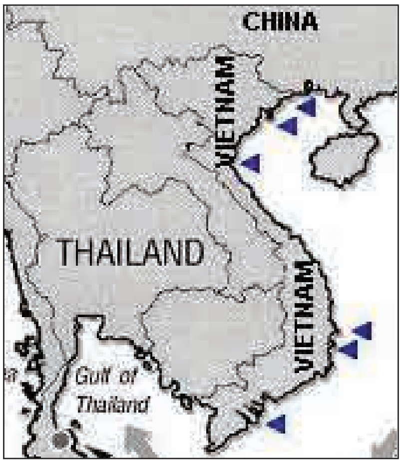

Fig. 1. Vietnam map and geographical locations of coastal area where samples were collected (arrows). A part of map was extracted from Fig. 1 in Antoro et al. (2006).

Primers, PCR amplification and sequencing

Forward primer EcobF: 5'ATGGCHAGCCTTCGHAAA 3' and reverse primer EcobR: 5'GATATTTGTCCTCAGGGGAG 3' were designed based on the *cob* sequences available in GenBank and used for PCR to amplify a 368 bp fragment. The reaction was carried out in a final volume of 50 µl, including 100 ng template and 20 pmol of each primer and a mix of remaining PCR components (PCR Master Mix from Promega). The PCR was performed in a MJ thermal cycler TC-100 (MJ Research Inc., USA) with an initiation at 94^{o}C for 5 min, then 40 cycles including denaturation at 94^{o}C for 1 min, annealing at 54^{o}C for 1 min, extension at 68^{o}C for 1 min and a final cycle of 10 min at 68^{o}C to complete the amplification. PCR products were detected on 1% agarose gels, stained with ethidium bromide in a Wealtech apparatus (Wealtech, USA) and photographs were recorded digitally. PCR products were purified and cloned using a TA cloning kit (Invitrogen Inc.). DNAs of recombinant plasmids were sequenced in the double direction with an automated DNA-sequencer ABI-3100 Avant Genetic Analyzer (Applied Biosystems).

Sequence analysis and phylogenetic construction

Partial cytochrome b sequences of the Vietnamese groupers were edited by SeqEd1.03; then aligned by MacVector8.2 package (Accelrys Inc.) with those of 40 *Epinephelus* species dowloaded from the GenBank. The fasta set of the alignment was imported into MEGA4.0 (Tamura et al., 2007) and molecular phylogenetic trees were constructed by Neighbor-joining method (NJ) with bootstrap of 1000 replicates.

Results and Discussion

Taxonomic identification based on sequence comparison

Nucleotide sequence of *cob* for each Vietnamese grouper specimen was used as an entry to search in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Based on the pair-wise identities of blast hits on query sequence, it revealed that among twelve Vietnamese grouper specimens, seven (EpHP; EpS1, EpS2; EpS5; EpS9; EpS10; EpS11) is highly identical to *Epinephelus coioides*; two to *E. lanceolatus* (EpS3 and EpS8); and one each to *E. coeruleopunctatus* (EpS14), *E. fario* (EpS16), *E. sexfasciatus* (EpS13) species, respectively. Alignment of nucleotide sequence of fifty eight *cob* sequences (including 12 Vietnamese and those of 40 *Epinephelus* species extracted from GenBank) confirmed that the taxonomic identity of the above listed species is well clarified. This indicated that there are, at least to date, five *Epinephelus* species exist along the sea coastal region of Vietnam such as *E. coioides*, *E. lanceolatus*, *E. coeruleopunctatus*, *E. fario*, *E. sexfasciatus* among which *E. coioides* is commonly found in China Sea (Ding et al., 2006).

Phylogenetic analysis for identification and taxonomic relationships

Fifty eight *cob* nucleotide sequences of 40 *Epinephelus* species (including 12 Vietnamese) were used for phylogenetic tree construction by Neighbor-joining (NJ) method (Tamura et al., 2007) (Fig. 2). The topology of the phylogenetic tree generated showed that the Vietnamese groupers were closely clustered as sisters: i) EpHP, EpS1, EpS2, EpS5, EpS9, EpS10, EpS11 with *E. coioides* group; ii) EpS14 with *E. coeruleopunctatus*; iii) EpS16 with *E. fario*; iv) EpS13 with *E. sexfasciatus*; and v) EpS3, EpS8 with *E. lanceolatus* (Fig. 2). The close relationships were supported by high bootstrap value with 1000 replicates (93–100%).

This study used 12 specimens of the Vietnamese groupers for molecular identification and phylogenetic analysis. The NJ topological structure tested with *cob* sequences confirmed that the *Epinephelus* population in Vietnam sea is of more than one commonly found *E. coioides*. Two species i.e., *E. coeruleopunctatus* and *E. lanceolatus* seem to be the first record and identification in the South China Sea along the Vietnam coastal region.

Wide distribution of certain *Epinephelus* species in seas may be reflected to its long distance migration between tropical and subtropical sea regions. The mitochondrial *cob* sequence used in this study has been proved to be as a reliable genetic marker for species identification according to data for sequence comparision and phylogenetic topology. However, the question of introgression of geographic inter-groups of some species and gene flow of the species distributing in the Pacific Ocean and the Atlantic Ocean may be raised for coevolution investigation (Craig et al., 2007; Ding et al., 2006). Importing breeds of groupers are also an important genetic issue in Vietnam. To make clear to these issues, more and diverse specimens would be collected and the nuclear markers (i.e, internal transcribed spacers, ITS-2) also studied for introgressive hybridization elucidation.

Conclusion

Molecular study of twelve grouper specimens collected along the coastal sea region of Vietnam revealed that there are, up to date, five species *E. coioides*, *E. lanceolatus*, *E. coeruleopunctatus*, *E. fario*, *E. sexfasciatus* exist, among which some seem to be the first record and first identification in the China sea along Vietnam. The phylogenetic topology confirmed the clades of those identified *Epinephelus* species of the Vietnam sea with high bootstrap value. Suggestion is that both mitochondrial and nuclear markers would be used in further studies to clarify taxonomic/phylogenetic analysis and issues of gene flow/introgressive hybridization among the *Epinephelus* population in Vietnam and South China Sea.

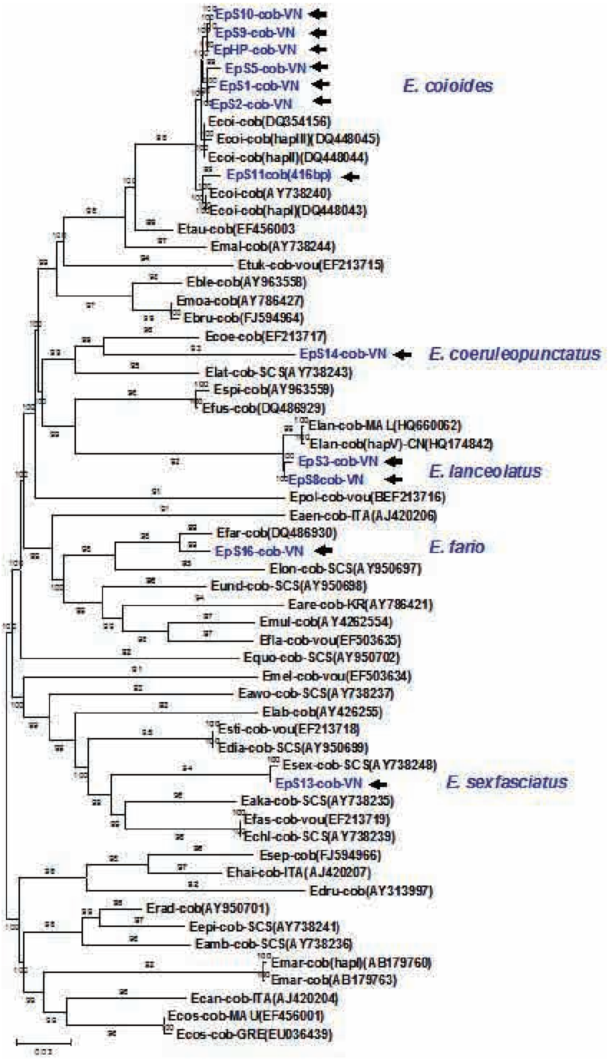

Fig. 2. Phylogenic tree of genus of *Epinephelus* species (including 12 *Epinephelus* Vietnam specimens) constructed based *cob* sequence, using Neighbor-joining method (NJ). Numbers at nodes represent bootstrap values (%) with 1000 replicates. The scale bar represents an interval of Tamura–Nei genetic distance. The Vietnamese specimens are designated as EpS and EpHP (arrows) and nomenclature of the *Epinephelus* species identified for them were marked at each group. The *cob* sequences of 40 *Epinephelus* species were downloaded from GenBank. GenBank numbers and origin of specimens are given when applicable. Abbreviations: Eaen: *Epinephelus aeneus*; Eaka: *E. akaara*; Eamb: *E. amblycephalus*; Eare: *E. areolatus*; Eawo: *E. awoara*; Eble: *E. bleekeri*; Ebru: *E. bruneus*; Ecan: *E. caninus*; Echl: *E. chlorostigma*; Ecoe: *E. coeruleopunctatus*; Ecoi: *E. coioides*; Ecos: *E. costae*; Edia: *E. diacanthus*; Edru: *E. drummondhayi*; Eepi: *E. epistictus*; Efar: *E. fario*; Efas: *E. fasciatomaculosus*; Efla: *E. flavocaeruleus*; Efus: *E. fuscoguttatus*; Ehai: *E. haifensis*; Elab: *E. labriformis*; Elan: *E. lanceolatus*; Elat: *E. latifasciatus*; Elon: *E. longispinis*; Emal: *E. malabaricus*; Emar: *E. marginatus*; Emel: *E. melanostigma*; Emer: *E. merra*; Emoa: *E. moara*; Emul: *E. multinotatus*; Epol: *E. polyphekadion*; Equo: *E. quoyanus*; Erad: *E. radiatus*; Esep: *E. septemfasciatus*; Esex: *E. sexfasciatus*; Espi: *E. spilotoceps*; Esti: *E. stictus*; Etau: *E. tauvina*; Etuk: *E. tukula*; Eund: *E. undulosus.*

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PALLIAL GONODUCT HISTOLOGY OF TWO SPECIES OF MEDICALLY IMPORTANT GENUS *PARAFOSSARULUS* (CAENOGASTROPODA, RISSOOIDEA, BYTHINIIDAE) FROM VIETNAM AND THE SOUTHERN RUSSIAN FAR EAST

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Freshwater snails of the genus *Parafossarulus* Annandale are widely distributed in East Asia from the southern Russian Far East on the north to northern Vietnam in the south. These mollusks are known as first intermediate hosts of 13 trematode species in 12 genera, including *Clonorchis* and *Opistorchis* as the most important for human (Besprozvannykh, Ermolenko, 2007). *Clonorchis sinensis*, the Chinese liver fluke, is believed to be the third most prevalent worm parasite in the world. That causes Clonorchiasis in humans, endemic disease to Southeast Asia. In Vietnam, 10% of people on average are infected with the Chinese liver fluke (Ngo, Ermolenko, in press). These facts explain importance of study of the *Parafossarulus* snails, especially their reproductive biology.

Scheme of reproductive system of the genus is generally known (Kim, 2005; Starobogatov, 1970). To facilitate study of the structure and function of its different parts, histology of pallial and renal portions of gonoduct of two *Parafosarulus* species was examined. Specimens of *Parafossarulus striatulus* Benson and *P. manchouricus* (Bourguignat) were collected by V. Besprozvannykh (IBSS FEB RAS) in rice paddies in Nam Dinh Province (northern Vietnam) and in Bolshaya Ussurka River drainage of Primorskiy Krai (southern Russian Far East). Snails fixed with 70% ethanol were dissected to extract portions of foot with pallial gonoduct organs. Histological sections were prepared at 5–7 μ and stain with hematoxylin and eosin to examine under light microscope in detail. Results of the study are presented below in comparison with other Bithyniidae.

Abbreviations used in figures: AG – albumen gland, BC – bursa copulatrix, DG – digestive gland, F – foot, K – kidney, PD – prostate diverticula, PO – pallial oviduct, SV– seminal vesicles.

The female reproductive system of *Parafossarulus* similar to other bithyniids comprised ovary, oviduct, bursa copulatrix and glandular part (Lilly, 1953; Kim, 2005; Starobogatov, 1970). Thickened tube of renal gonoduct is coiled (Starobogatov, 1970). Slit-like channel of pallial oviduct is glandular from ventral side only (Fig. 1). That comprises two glands (Starobogatov, 1970) – albumen and capsulate ones. Bursa copulatrix is smaller than that in other bithyniids and jointed to oviduct not distally, but in mid-posterior portion (Fig. 1).

The male reproductive system of the *Parafossarulus* species like that of other bithyniids comprised testis, seminal vesicles, prostate gland, vas deferens, penial duct and penis (Lilly, 1953; Kim, 2005; Starobogatov, 1970). Pallial part of the male reproductive system includes prostate gland and vas deferens. Males of *P. striatulus* from Vietnam were studied. In transverse histological sections, numerous seminal vesicles filled by spermatozoa with haploid nucleus inside are well visible (Fig. 2). Seminal vesicles, running from testis to the prostate gland are embedded in digestive gland. The cells of digestive gland have eosinophillic cytoplasm with granular secret (Fig. 2). Thicker portion of the seminal vesicles of Vietnamese *P. striatulus* (Fig. 2) are nearly 0.5–0.55 mm in diameter like that in the species from Nepal cited as *Bithynia striatula* (Kim, 2005). Pallial part of the male reproductive system includes prostate gland and vas deferens. Prostate (Fig. 3) comprises diverticula arranged in flat belt (Starobogatov, 1970).

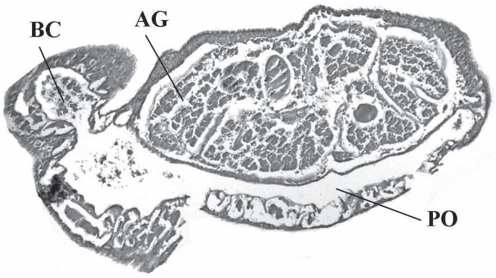

Fig. 1. Transverse histological section of *Parafossarulus manchouricus* female through pallial oviduct showing opening of bursa copulatrix.

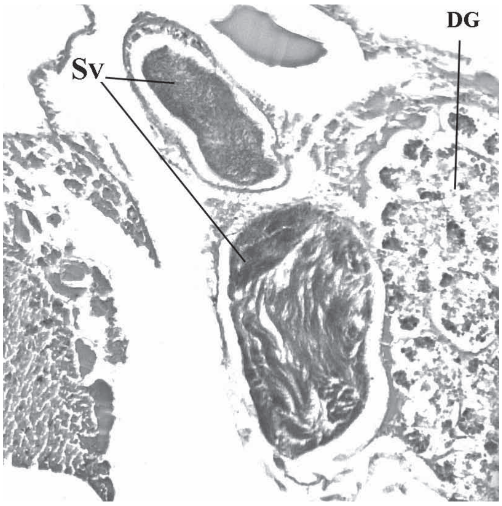

Fig. 2. Transverse histological section of *Parafossarulus striatulus* male through renal oviduct showing seminal vesicles.

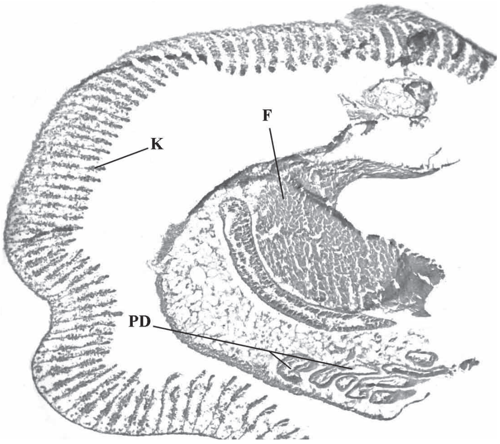

Fig. 3. Transverse histological section of *Parafossarulus striatulus* male through pallial oviduct showing diverticular structure of prostate.

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STUDY ON THE CONOPEPTIDES FROM CONE SNAILS COLLECTED FROM NHA TRANG, VIETNAM

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Introduction

Predatory marine snails of the genus *Conus* (family Conidae) with over 500 species may comprise the largest single genus of marine animals living today (Becker, Terlau, 2008). Their venoms are complex mixtures of small, disulfide-brigded polypeptide toxins (conotoxins) that inhibit the function of ion channels and neurotransmitter receptors. In recent years, there has been an increased use of mass spectrometric techniques to characterize crude *Conus* venoms, to identify specific toxins by their molecular mass, and to assist in the determination of conotoxin sequences including disulfide bond connectivities and post-translational modifications. It was showed that each *Conus* venom appears to contain a unique set of 50–200 small disulfide-bonded peptides (Lewis, 2009). It is estimated that there are up to 100,000 different peptides in the combined venoms from this remarkable genus. Thus, conus peptides have potentials to be developed as new research tools in neuroscience and as novel medications for treatment of pain, epilepsy and other neuropathic disorders. Several are being directly developed as diagnostic and therapeutic agents (Layer, McIntosh, 2006). We have investigated the venom of *Conus* species collected from Nha Trang coast. The venom polypeptides were isolated, purified and characterized. These toxins have been identified by mass directed approach based on LC-MS/MS analysis of crude venom. Beside, ω-conotoxins (ω-CTX) with MW of about 3 kDa was cloned, expressed in *E. coli*, purified and characterized.

Interesting that conotoxins from venom of *Conus* species found to produce analgesic activity in the formalin test on mice. The recombinant ω-conotoxin MVIIA also showed to have the same analgesic activity as compared tocommercial lidocain.

Materials and Methods

Cone snails such as *Conus textile*, *C. striatus*, *C. litteratus*, *C. leopadrus*, *C. quercinus*, *C. marmoreus*, *C. distans*, *C. stephano*, *C. betulinus*, *C. caracteristicus*, *C. vexillium* were collected from Nha Trang coast.

Extraction of peptides from venoms

Venoms were stripped from freshly dissected venom ducts according to the method of Cruz et al. (1976). Venom duct was homogenized using 50% acetonitrile/water as solvent and centrifuged at a speed of 10000 rpm and the supernatants retained. Crude venom extracts were lyophilized and stored at -20^{o}C before use.

Global reduction and alkylation

The purified HPLC fractions were reduced by DTT and alkylated by iodoacetamide (IAA). The resulting mixture was subsequently analyzed by ESI-MS/MS. The mass spectrum of the reduced/alkylated crude venom was compared with that of the crude venom. and the peaks shifting by 116, 232, and 348 Da were assigned as one, two, and three disulfide containing peptides (mass matches ±1 Da), respectively.

Proteolytic digestion

The samples after reduction and alkylation were digested with sequencing grade trypsin. The digested samples were directly analyzed by LC-MS and proteolytic fragments were further subjected to MS/MS.

Inflammatory pain assay

The analgesic effect of the conotoxins was determined by formalin test as described by Zhang et al. (2007). Swiss mice were used for the test.

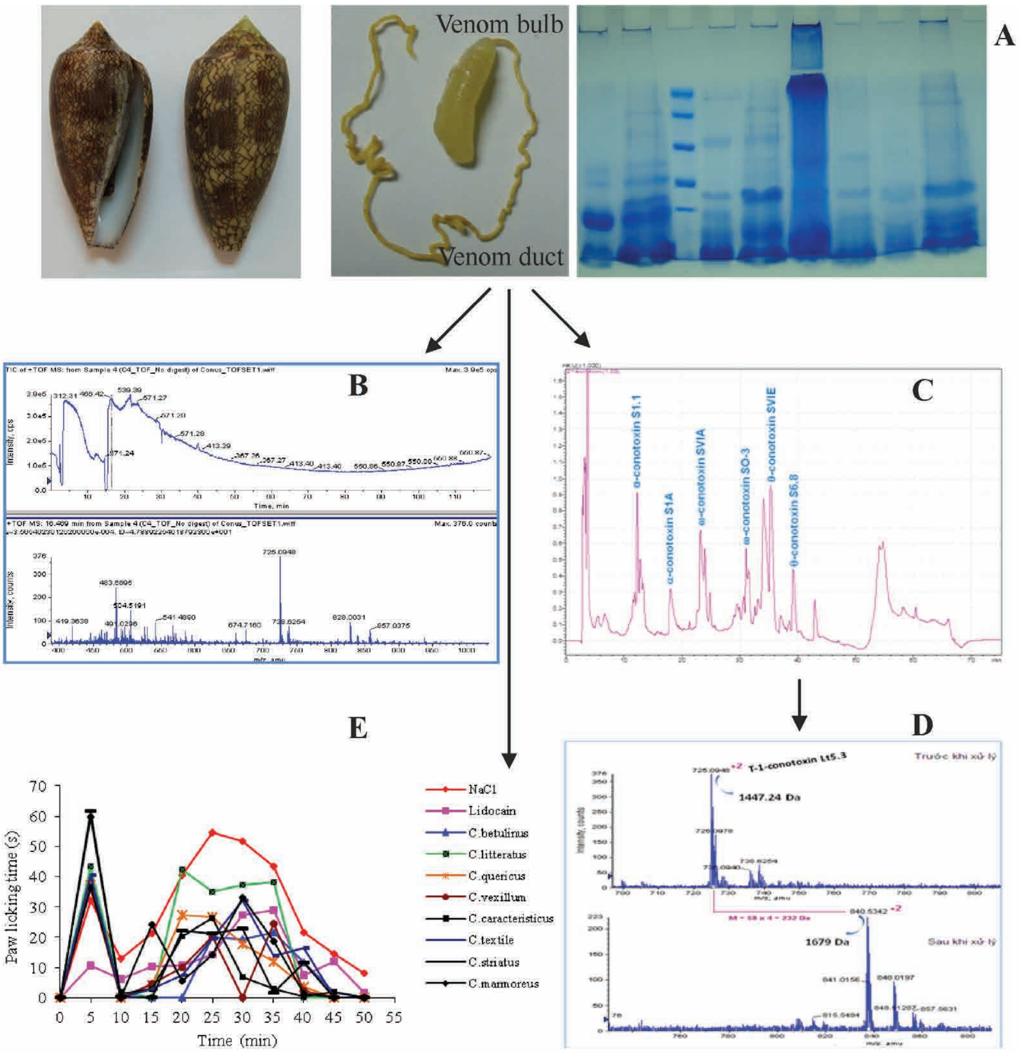
Construction and expression of recombinant conotoxin

The DNA sequence encoding omega-conotoxin (ω-CTX) was designed subcloned into pET-32a (+). The recombinant plasmid pET-32a (+)-CTX MVIIA was sequenced to confirm the desired construct with the gene sequence. *E. coli* BL21(DE3) was used as the host for the protein expression. The purified fusion protein was tested for the analgesic activity on mice.

Results

Purification and identification of *Conus* toxins

The Figure presents an example of our strategy for identification and characterization of the toxin components of crude venom samples that involves the following steps: (1) extraction of the crude venom (Fig., A); (2) on-line LC-ESI-MS profiling of the crude venom (Fig., B); (2) off-line separation of the crude venom (Fig., C) and MS profiling of the toxins before and after reduction/alkylation (Fig., D); (4) analgesic activity test of the conotoxin fractions on mice (Fig., E). It's interesting that after separation, ESI-TOF-MS analysis of each conotoxin fraction provided us with accurate molecular masses of the major toxin components. As it was shown in Fig., C, in one sample, six conotoxins were identified and named. The presence of disulfide bonds in each toxin was determined from the mass differences between the native and reduced toxin. In addition, the number of cysteine residues was calculated by examining the mass difference between the reduced and alkylated toxins (i.e., a difference of 58 Da/cysteine). Fig., C showed dominant mass observed in the untreated fraction at m/z 725.09. After reduction, this m/z is shifted to 840.53 indicating the presence of 2 disulfide bonds.



Overview of the isolation, purification and biochemical strategy of conotoxins from *Conus* venom. (A) A representative example of the shell of a *C. litteratus* specimen utilized in the present study, its venom apparatus and SDS-PAGE analysis of crude extract from its venom. (B) LC/MS chromatogram (m/z) of crude duct venom shows ES/MS (m/z). (C) RP-HPLC purification profile of *C. striatus* crude venom. (D) Comparison of fraction before and after reduction/alkylation. Top panel shows the ESI-MS of the crude venom and bottom panel shows the ESI-MS of reduced/alkylated crude venom. (E) Analgesic activity of crude extracts in the inflammatory pain assay in mice.

Analgesic activity

Analgesic activity of the studed conotoxins was examined using one hind paw injections into mice. The results showed that extracts of cude venom of ten *Conus* species were found to produce analgesic activity in the formalin test. The venom of *C. betulinus*, *C. vexillum*, *C. caracteristicus*, *C. textile*, *C. quericus* showed the better analgesic activity than that from others (Fig., E).

Recombinant ω-conotoxin MVIIA

Recombinant ω-conotoxin MVIIA was expressed in the soluble and fusion form with thioredoxin (Trx) with molecular weight of about 19.7 kDa. The construction of 6xHis tags facilitates the purification of the protein on Ni^{2+}-chelating sepharose column. The yield of the protein was about 30 mg/l. It was shown, recombinant ω-CTX MVIIA showed to have the same analgesic activity as lidocain.

Discussion

The complexity of cone snail venoms in terms of both the number and variety of their components. In recent years, mass spectrometric techniques have become more widely used in the study of complex mixtures, and in particular for venoms (Beatrix et al., 2009). The knowledge of molecular mass and number of disulfide bonds that can be readily obtained from MS and LC/MS serves as a useful indicator of both the structural and pharmacological type of toxins present in the venom. These techniques can be used to rapidly identify members of a particular structural class, or to search for novel or unique compounds. We have analyzed the venom proteome of ten species of *Conus* from Vietnam using an LC-ESI-MS/MS approach. The venoms were first fractionated using RP HPLC then individual fractions were analyzed using TOF-MS. The results revealed that more than 40 polypeptides were identified by this strategy and the venoms showed much more complex than previously realized. Rapid analysis of peptide toxins promises to massively expand the database of these fascinating bioactive molecules and facilitate many potentially informative of study. These include studies of the variation in toxin in individual cone snails from a given species and rapid screening of crude venom from different animal venoms against different ion channels. Recombinant technology represents an alternative approach of producing bioactive peptides. Conotoxin MVIIA is a highly potent and selective blocker of the N-type voltage sensitive calcium channels (VSCCs). A fusion protein Trx–CTX MVIIA expressed and purified from *E. coli* possesses significant analgesic activity. It provides a convenient way to produce large amount of Trx–CTX MVIIA and CTX MVIIA for neuroscience research and clinical trials.

Acknowledgments

This work was supported by grants from two projects of Vietnamese Academy of Science and Technology: (1) Screening and identification of pharmaco-bioactive peptides from some special marine organisms (*Conus*, ascidians) by proteomic techniques (2009–2010); (2) Study on the recombinant conotoxins and analgesic activity testing (2011–2012).

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POPULATION DYNAMICS OF *METAPENAEUS ENSIS* (DECAPODA: PENAEIDAE) IN GANH HAO RIVER, BAC LIEU PROVINCE, VIETNAM

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Introduction

Aquatic resources play an important role in developing countries, they not only contributed to livelihood but also provide significantly nutrition for local communities in Asian southeast countries (Smith, 2003). Greasy back shrimp (*Metapenaeus ensis*) is one of a commercial brackish-water crustacean species of penaeid family, and the species is a very important component of estuarine and marine ecosystems in tropics with its high fecundity and short lifespan (Garcia, 1998).

Postlarvae and adult stages of *M. ensis* are found on muddy bottoms in estuaries and coastal waters. In the Mekong River delta, *M. ensis* is being exploited in brackish waters in rivers and canals with artisanal fishing gears like bag-net, cast-net and trap-net, the bag-net is considered the largest scale in these artisanal fishing gears. Commercial value this species is not only high in Vietnam, but also in many countries in the world, and high demand in market because of the quality of its meat (King, 2001).The species was also studied with regard to various aspects in Australia, Hong Kong and Japan.

However, information about this species was very limited in Vietnam, many parameters were unknown such as growth coefficient, mortality coefficient and exploitation rate of *M. ensis,* and these aspects are very important in fisheries management to determine whether an area is overfished or under-fished to help better in fisheries managers to develop long-term strategies for a large proportion of the aquatic resources in the region.

Materials and Methods

The study was carried out in the Ganh Hao River, the largest river in Bac Lieu Province (9°15′ N, 105°45′ E), the coastal region of the Mekong delta of Vietnam; depth and width of this part river is 7–8 m and 120–400 m, respectively. The salinity is between 25–30 ppt.

The bag net is the fishing equipment used locally, it is designed to be fixed at the designated site. It is supported by two columns. Each bag net is designed with different mesh sizes in various parts of the body of the net; the mesh size is largest at mouth of the bag net (net 2a=3.5 cm) and gradually decreases to the end of the net (3.0 cm to 2.5 cm to 2.0 cm). The gradient in mesh size extends for 7–8 m of the net. At the end of the net, the mesh size is 1.5 cm. The total length of the bag net is 26.5–32.0 m, height and width of the net mouth are 5.5–6 m and 10–11 m, respectively. The net is always set in places with currents such as rivers and canals, it operates on the ebb when the water moves downstream. The principle of the bag net is that the net is set facing the tidal current, the shrimp and other aquatic animals are caught as they flow into it on the current.

Six stations were selected along the 30 km distance of Ganh Hao River for the sampling of *M. ensis*. These samples were collected directly from the bag-nets. The first station was in the estuary (0 km), the other sites extended upstream at locations of 6 km, 12 km, 18 km, 24 km, and 30 km along the river. The study was performed over 12 months at 6 sampling points at Feb., Apr., Jun., Aug., Oct. and Dec. Besides, 10% of largest shrimp individuals in each sampling time were also selected to check the mature stages; the fecundity of mature female was estimated from relationship between ovary weight and *CL* (Courtney et al., 1989).

The FAO–ICLARM Stock Assessment Tool (Gayanilo et al., 2005) was used to determine *K*, and *L* (L_∞) was estimated with the von Bertalanffy growth equation:

$$L_t = L_\infty(1 - \exp^{-[k(t-t_0)+St_s+St_0]})$$

Asymptotic length (L_∞) of *CL* (mm); *K* is the growth coefficient (year^{-1}). Total mortality coefficient (*Z*) was required to extrapolate the probability of capture from the length-converted catch curve (Pauly, 1984). The total mortality was based on *CL* composition calculated from the bag net samples. It was assumed that these bag nets are representative of all the bag nets used in the region. The instantaneous value of the fishing mortality coefficient (*F*), the natural mortality coefficient (*M*) was calculated from using Pauly's empirical estimate (1984), $Z = F + M$. The exploitation rate: $E = \frac{F}{Z}$.

However, biological character of *M. ensis* is to grow in the coastal region where shallow water and low salinity are observed, but they migrate to open sea for reproduction when reach pre-mature and mature stages (Garcia, 1998; Courtney et al., 1989), this migration depend on matured size of each species. I it assumed that *M. ensis* started migrating when *CL* attained 30 mm. So, migration is considered to exchange population size and effect to *F*, *E* and E_{max}. It supposes that α is migration index of *M. ensis* (that takes percentages of large individuals (30 mm *CL*), $0<\alpha<1$. If α is close to 0, it is less migration from the estuarine zone. If α is close to 1, it is high rate of migration from the estuarine zone.

- $\alpha=0$, when $0<CL$ mm<30; In this case: $Z_0=F_0+M$; $E_0=\frac{F_0}{Z_0}$ (population size is not affected by migration)
- $\alpha\neq0$, when CL mm>30; In this case: $Z_\alpha = F_\alpha+M$ + migration (population size is decreased)

$Z_\alpha=F_\alpha+M+(1-\alpha)$; $E_\alpha=\frac{F_\alpha}{Z_\alpha}$; ($\alpha$: 0.1÷0.9)

Migrated and observed assessment will be based on α values. M is assumed as constant for males and females and this value is not affected by α, E_{max} is computed respond with each value of α.

Results

Frequency of *CL* was identified from sampling in different months ranged from 14.5–33.5 mm for males and to 14.5–35.5 mm for females. These frequencies are shown in Fig. 1, the growth parameters L_∞=35.2 mm and 37.3 mm (*CL*) and K=1.6 year^{-1} and 1.5 year^{-1} for males and females, respectively.

The recruitment pattern of *M. ensis* was continuous throughout the year as shown in Fig. 2, with two peaks per year in April–May (16–24%) and July–August (8.4–10%). However, recruitment was continuous between these peaks, with the highest recruitment peak in April–May, the second highest peak in July–August, and the lowest values in December–February.

Besides, each value of α has corresponded with value of Z, F and E_{max}, the relationship of these values are shown in Fig. 3. It also indicated that negative correlation between α with E and E_{max}, but $E<E_{max}$ for males and females with any value of α, which means that *M. ensis* is under fishing pressure in the region, and mature individuals start migrating from August to December (Fig. 1).

Discussion

The *CL* (mm) has oscillated and not clear in peak among months, so breeding can occur many times per year. The season of reproduction of penaeid shrimp is affected by various environmental factors including rainfall, temperature and depth (Garcia, 1998). Its seasonal reproduction can be different from region to region. A study in Australia showed that seasonal spawning of *M. ensis* is around year, highest from September to November and lowest between January and February (Crocos et al., 2001).

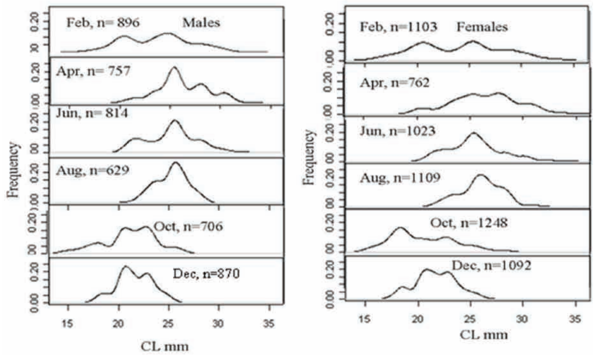

Fig. 1. Frequency *CL* (mm) of *M. ensis* males and females at the six sampling times.

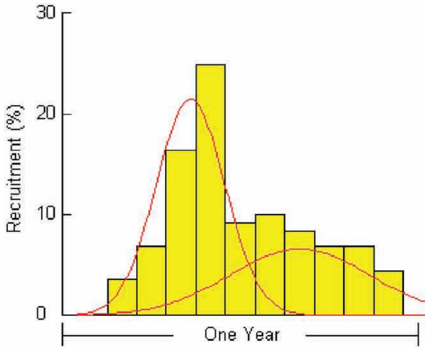

Fig. 2. Recruitment pattern of *M. ensis* per year.

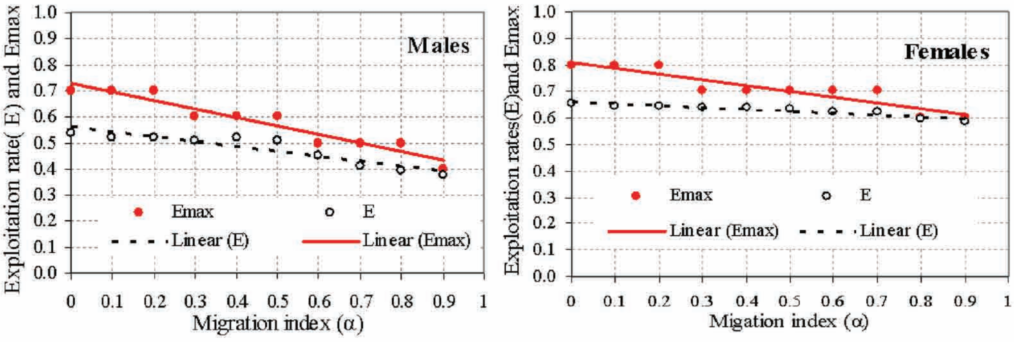

Fig. 3. Relative migration rates (α*)* and exploitation rates *E*, E_{max} for males and females.

The peak recruitment of *M. ensis* in Ganh Hao River was in April–May and in July–August, but it is continued chain and is not clear in those peaks shown in Fig. 1. The peak recruitment of *M. ensis* occurred in April–June and September–October in Hong Kong (Leung, 1997). Recruitment is affected by many factors including habitat, migration and environmental factors (King, 2001).

Results show that fishery is below the optimum level based on *E* ($E<E_{max}$). Therefore, the fishing pressure on the stock is not excessive when α: 0.1÷0.9, and greater yield could be attained with a reasonable increase in the fishing effort.

The bag net is a passive apparatus that obstructs the current and filters the shrimp from the water, so, shrimps are less vulnerable to it than to active equipment such as trawl nets which actually pursue the shrimp. Environmental conditions in the region can be not consistent for distribution of mature individuals. The same conslusion is drawn for Hong Kong, perhaps because of low salinity (Leung, 1997). Because mature females were found in 15–30 m deep and at salinity over 33 ppt (Crocos et al., 2001), individuals have larger 30 *CL* mm is considered to migrate to open sea from August to December, the migration of *M. ensis* has low proportion, and it had not caused effect to exploitation rates (Fig. 3).

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MACROBENTHOS OF THE INTERTIDAL ZONE WITH MANGROVE VEGETATION IN DAOCHAO AND ZANZOLA ISLANDS (BAI TY LONG ARCHIPELAGO)

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Basic publications concerning the intertidal zone with mangrove vegetation of Vietnam deal with the Gulf of Tonkin in the area of Cat Ba Island and Hai Phong (Gurjanova, Phuong Chang Hiu, 1972), and the south-eastern coast in the area of the Nha Trang (Gulbin et al., 1988; Nguen Van Chung et al., 1988). The present work is based on study of samples of macrobenthos taken in 1988 in the intertidal zone of the Bai Ty Long Archipelago, near Zanzola Island (20°40′ N, 107°20′ E) and Daochao Island (20°50′ N, 107°20′ E). For identification of intertidal horizons, we used Vaillant's principle of vertical stratification (Vaillant, 1891). The boundaries of the upper, middle, and lower horizons were determined as the highest tide mark; the mean high and mean low water stages, and the lowest possible tide mark, respectively. Regular diurnal tides are characteristic of the Daochao and Zanzola islands (highest tide – 3.9 m).

The intertidal zone with mangrove vegetation is located in inlets, bottom deposits consist of stony debris, dead coral fragments, shelly ground covered by 15–20 cm silt. A light wave action and minor water-freshening are typical here. Intertidal zone reaches 200 m wide. Fifty-three and 38 species of macrobenthos are found in Daochao and Zanzola islands, respectively. Brushwoods of the ground plant *Aegiceras* spp. and solitary brushes of mangroves, covering by sea water in the high water, can be observed in the upper horizon. Population of this inteitidal zone is impoverished and patchy pattern of the community' distribution is typical. A presence of the soft bottom provides conditions for development of various infaunal species (polychaetes *Bhawania cryptocephala*, *Leonnates persica*, *Onuphis eremite*, *Lambrineris shiihoi*, sipunculas *Antilleosoma antillarum*, *Golfingia elongate*, *Thysanocardia catherinae*). Epifaunal species inhabit the hard substrata (*Saccostrea echinata*, *Balanus retuculatus*, *Cthamalus malayensis*). Oysters *Ostrea foliolum* settle on the stems and branches of *Aegiceras*. Macrophytes algae are almost absent which is connected with faint transparence of the sea water.

In the upper intertidal horizon, only animal population is observed. In Daochao Island, community *O. foliolum* forms belt on the stems of *Aegiceras* spp. with total biomass of 2733.0 gWWm^2. Among *Aegiceras* branches, bivalve molluscs *Gafrarium pectinatum*, *Dicyanthifer manni*, *Kellia* sp. and gastropod *Nerita iusculpta* are found. The stones are covered by the oyster *S. echinata*, barnacles *B. reticulatus* and *Ch. malayensis*. There are abundant gastropods (*Nerita albicilla*, *N. signata*, *Lunella granulata*, *Cerithium morus*). Population of the upper horizon of Zanzola Island is more poor, but species composition in general is similar to those of Daochao Island. On the rocky coast, there is prevalent community of the bivalve mollusks *Isognomon ephippium+Hormomya mutabilis* with the total biomass of macrobenthos of 430.0 gWWm^2.

In the middle intertidal horizon of Daochao Island, sprouts of algae with total biomass of 56.4 gWWm^2 is observed. The total animal biomass is 897.4 gWWm^2. Belt-forming species *S. echinata* makes up 80% of the animal biomass. Numerous gastropods *Batillaria zonalis*, *C. morus*, bivalve molluscs *G. pectinatum*, *Anomalocardia squamosa*, sipunculid *Th. catherinae* and decapods *Mictyris longicarpus*, *Uca dussumieri*, *Thalamita crenata* are accompanying species. In Zanzola Island, community of bivalve molluscs *G. pectinatum+A. squamosa* is developed on the stones. Total animal biomass in this community is 1263.4 gWWm^2, dominant species makes up more than 80% total biomass. Bivalve mollusc *H. mutabilis* and polychaetes *Tharyx mulifilis* and *L. persica* frequently occur.

The lower horizon is silt-covered, there are rare shell and coral deposits. Macrobenthos is absent, only in Daochao Island sprouts of red algae *Peyssonnelia* sp., *Bastrychia binderi*, *Wurdemannia miniata* and green algae *Cladophora papenfussii* are found.

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