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TAXONOMIC REVISION OF THE FAMILY DOLIOLIDAE BRONN, 1862 (CHORDATA, TUNICATA, THALIACEA, DOLIOLIDA) FROM EAST COAST OF PENINSULAR MALAYSIA), WITH AN UPDATED WORLDWIDE DISTRIBUTION

NUR 'ALIAH BINTI ADAM[1] AND NURUL HUDA AHMAD ISHAK*[1, 2]

[1]*School of Marine and Environmental Sciences, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*
[2]*Institute of Oceanography and Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*

**Corresponding author: huda@umt.edu.my*

Abstract: The marine pelagic tunicate from the family of Doliolidae Bronn, 1862 in the coastal waters of Terengganu was studied for the first time, hereby presented in this paper. The distribution was analysed from 18 sampling stations alongside the Terengganu waters; including Pulau Bidong, Pulau Yu and Pulau Kapas. Samples were collected from April to July 2016 using 200µm Bongo net; towed vertically from a stationary vessel; and were preserved in a 5% buffered formaldehyde. Five species discovered in this family were identified as new records in Malaysian waters: *Doliolum denticulatum* Quoy and Gaimard, 1834, *Doliolum nationalis* Borgert, 1894, *Dolioletta gegenbauri* Uljanin, 1884, *Doliolina mulleri* Krohn, 1852 and *Dolioloides rarum* Grobben, 1882. A comprehensive review of the species description, diagnosis and a key to the phorozooid from the recorded species is herewith provided. We also deliver a detailed map of current and known worldwide occurrence of these five species, and thus consequently update the biodiversity of Malaysian fauna.

KEYWORDS: Doliolid, pelagic tunicates, South China Sea, Terengganu, taxonomy, biogeography

Introduction

Pelagic tunicates are large transparent animals that measure up to 25cm (Lavaniegos & Ohman, 2003). They are of significant elements of oceanic and coastal waters, which are widely distributed in the ocean (Soto *et al.,* 2001). They consist of two classes; the Thaliacea and the Appendicularia. Thaliacea comprises three orders: the Doliolidae and the Salpidae, which are filter-feeders with tubular bodies and alternating generations; and the colonial Pyrosomidae (Bone, 1998). Lately, these groups were placed in the limelight as they deserve due to their recognition as a substantial component of the pelagic food web (Kremer & Madin, 1992; Deibel, 1998, Madin & Deibel, 1998) and to the acknowledgement of their ecological, evolutionary and biogeochemical contribution (Andersen, 1998; Boero *et al.,* 2008).

Doliolids are relatively small, barrel-shaped animals that occur mostly in neritic regions and shelf break waters (Zeldis *et al.,* 1995; Van Soest, 1998). All doliolids are hermaphroditic and they have the most complex life cycle compared to any of the pelagic tunicates; consisting of no lesser than six different and successive morphological stages (Godeaux *et al.,* 1998; Paffenhöfer & Koster, 2011). They are of high nutritional value to predators, particularly fish due to the preference on their fatty acid composition (Pond & Sargent, 1998). Hydromedusae, pelagic polycheates, and sapphirinid copepods have been reported to prey on the doliolids (Harbison, 1998; Takahashi *et al.,* 2013; Takahashi *et al.,* 2015; Paffenhöfer, 2013). These doliolids are capable of feeding on particles ranging in the size of a bacterium to large diatoms, copepod eggs, and nauplii, thus causing their blooms to have a noteworthy effect on phytoplankton and mesozooplankton communities (Paffenhöfer *et al.,* 1995; Deibel, 1998; Paffenhöfer & Koster, 2005). Moreover, they are able to adapt and respond quickly to biological and physical changes in the environment (Capitanio *et al.,* 2005; Deibel & Paffenhöffer, 2009; Li *et al.,* 2011), which thus making them an excellent bio-indicator of any environmental changes.

The patchy distribution of doliolids in the water column (Alldredge & Madin, 1982; Voronina, 1998; Pakhomov & Hunt, 2017) combined with their delicate fragility have deterred many researcher from studying them in detail. They are particularly prone to damages both during the capture and subsequent preservation process (Foxton, 1965; Alldredge & Madin, 1982; Sameoto, 1984; Romeo *et al.,* 1992; Menard *et al.,* 1994; Nishikawa & Terazaki, 1996; Weikert & Godeaux, 2008; Liu *et al.,* 2012; Hereu *et al.,* 2014). At times when well-preserved specimens collection becomes almost impossible (Madin *et al.,* 1996), it may then cause significant difficulties in identifying the species (Diaz *et al.,* 2008).

The order of Doliolida Delage and Hérouard, 1898 contains two suborders: Doliolidina Godeaux, 1996 which consist of 2 families; the Doliolidae Bronn, 1862 with 4 genera and Doliopsoididae Godeaux, 1996 with 1 genera; and Doliopsidina Godeaux, 1996 which consist of 3 families; Doliolunidae Robison, Raskoff & Sherlock, 2005 with 1 genera, Doliopsidae Godeaux, 1996 with 2 genera and Paradoliopsidae Godeaux, 1996 with 1 genera. About 25 species are currently known worldwide (Purushothaman, *et al.,* 2017) and the discovery of new species is rare (Hereu *et al.,* 2014) with only two new species have been added in the 2000s (Robison *et al.,* 2005a; b). The Doliolidae is the largest family of this order with 17 described species in four genera (Chihara & Murano, 1997; Godeaux, 1998; Esnal & Daponte, 1999; Purushothaman *et al.,* 2017). The taxonomic identification for the species are mostly based on the gonozooid stage, which is a single and free-swimming, and are usually distinguished by their shape, and the numbers and arrangement of muscle bands which encircle their bodies (Borgelt, 1968; Esnal & Daponte, 1999).

Taxonomical studies of marine invertebrates in Malaysia are mainly focused on the groups of Crustacea (Copepoda), Cyanobacteria, eukaryotic algae, Protozoa, Platyhelminthes, Nematoda and Tunicata (Yasin *et al.,* 2015) while little is known on the Thaliacean's general biology, ecology and distribution, and this information is particularly scarce in this region. To date, there is no record on the presence of doliolids in Malaysian waters, least as it does on its taxonomical or biogeographical studies. Therefore, this study aims, to list the species of doliolid identified in Malaysian waters, to provide a comprehensive review of the descriptive and diagnostic characteristics, to aid species identification, and to illustrate the updated distribution map. Additionally, a key for the species identification of doliolid in Malaysian waters is also been included together.

Materials and Methods

Study Area

The surveyed region is located in the east coast of Malaysia (Fig. 1), specifically in Terengganu waters, which is located directly facing to the South China Sea (SCS). This includes Pulau Bidong, Pulau Kapas and Pulau Yu areas. The SCS lies in the tropical zone of the western Pacific Ocean, off the southeast corner of the Asian continent, and the region in general is primarily subjected to the monsoon season (Chu *et al.,* 1999). Asian tropical monsoons are generally divided into three parts, i.e. the Northeast Monsoon (NEM) from November to March, the Southwest Monsoon (SWM) from May to September, and Inter-Monsoon periods; April and October (Azanza *et al.,* 2008).

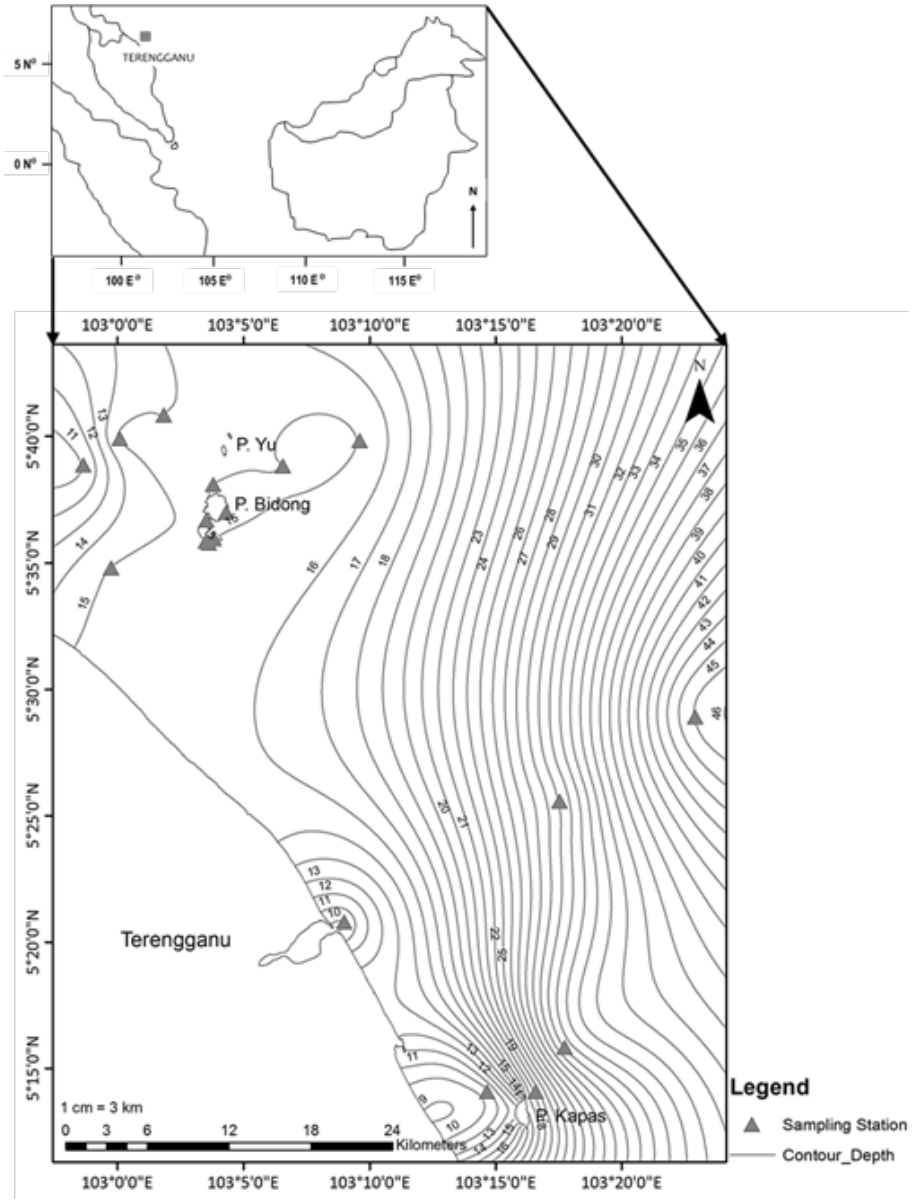

Figure 1: Top inset picture, location of Terengganu in Malaysia. Bottom magnified picture, Location of the 18 sampling stations (red triangle) along Terengganu waters.

Terengganu coastal waters' physical properties and dynamical movements are influenced by SCS condition. Its coastal area is a shallow shelf area with water depth of less than 80 m (Daud *et al.,* 2016). This region experiences a relatively dry season from April to July, while the heaviest precipitation received in November and December which sometimes reaches more than 1,000 mm of rainfall (Camerlengo & Somchit, 2000). It is reported that the surface waters during SWM averaged around 29 °C (Nicholson, 2011; Thompson & Tkalich, 2014) of which are relatively warmer as compared to during northeast monsoon, where the temperature and salinity recorded are lower (Saadon & Carmerlengom, 1996; Chua, 1984; Zainal, 1993).

Field Method

Four monthly samplings were conducted from April 2016 to July 2016 during Southwest Monsoon (SWM) (warmer water) period at 18 sampling stations (5°14'– 5°40'N, 102°58'–103°22'E) around Terengganu waters. Specimens were collected with a cylindrical-conical paired-Bongo net of mesh size 200 µm and mouth diameter of 60 cm, fitted with a calibrated flowmeter to determine the volume of filtered water during each tow (unit: m^3). The net was towed vertically from different depths (15 – 45 m), but always started at 1 m above the seafloor. The specimens collected were preserved immediately in a 5% buffered formaldehyde which had been diluted with seawater prior to the observation, identification and counting. Physico-chemical parameters of surface seawater such as salinity, temperature and pH were recorded in situ using a multiprobe (HydroLab Quanta Multiparameter Water Quality probe) at each station.

Laboratory Method

In the laboratory, the zooplankton samples were sorted and all doliolids were picked out from the samples. Phorozooids or gonozooids were submerged for 24 hours in a 2% Rose Bengal or Toluidine blue solution for a better observation of the taxonomic characters, and later were observed under a stereoscopic and a compound microscope. Thaliaceans were identified to their species level based on taxonomy works of Thompson's (1948), Godeaux's (1998) and Esnal and Daponte's (1999). The specimens were identified by the observation of their muscle bands, shape of stomach and intestine and the whole body view of the specimen. Identified specimens were photographed using a digital camera mounted onto the stereo microscope. All photographs are original, based on the collected material of this study. These identified specimens were deposited in the Repository and Research Center South China Sea Museum Specimens lot numbers: Dolioletta UMTTn0001, Dolioloides UMTTn0002, and Doliolum UMTTn0003 for reference collection in Universiti Malaysia Terengganu (UMT) in Kuala Nerus.

Results

List of Species

In the present study, a total of 5 species from the genus Doliolidae have been identified, described, drawn and illustrated taxonomically. These species were the first record in Malaysian waters. They were as the following:

Domain Eukarya
Supergroup Opisthokonta
Kingdom Animalia Linaeus, 1758
Phylum Chordata Bateson, 1885
Subphylum Tunicata Larmarck, 1816
Class Thaliacea Nielsen, 1995
Order Doliolida Delage & Hérouard, 1898
Suborder Doliolidina Godeaux, 1996
Family Doliolidae Bronn, 1862
Genus *Doliolum* Quoy and Gaimard, 1834
1. *Doliolum denticulatum* Quoy and Gaimard, 1834
2. *Doliolum nationalis* Borgert, 1894

Genus *Dolioletta* Borgert, 1895
3. *Dolioletta gegenbauri* Uljanin, 1884

Genus *Doliolina* Garstang, 1894
4. *Doliolina mulleri* Krohn, 1852

Genus *Dolioloides* Garstang, 1894
5. *Dolioloides rarum* Grobben, 1882

*Derived from World Register of Marine Species (WoRMS)

Description and Distribution of Doliolidae from the East Coast of the Malay Peninsular

1. Doliolum Denticulatum (Quoy & Gaimard, 1834)

Doliolum denticulatum Quoy & Gaimard, 1834 (cited from Kott, 2005, p. 204); Grobben, 1882; (Fraser, 1947b, pp. 1 - 4); Madin, 1991, p. 107; Godeaux, 1998, pp. 279 – 283; Boltovskoy, 1999, pp. 30 - 32; S. Kim, Lee, & Kim, 2010, pp. 11 – 12; Franco *et al.,* 2017, p. 13; Purushothaman *et al.,* 2017, p. 8

Specimens Examined

A1: 4 phorozooids and gonozooids, 5°38'53.08"N, 102°58'39.07"E, April 2016; A2: 3 phorozooids and gonozooids, 5°39'55.02"N, 103°0'6.03"E, April 2016; A3: 1 gonozooids, 5°40'51.01"N, 103° 1'51.06"E, April 2016; A4: 2 phorozooids and gonozooids, 5°38'6.00"N, 103° 3'48.06"E, April 2016; A6: 2 phorozooids and gonozooids, 5°39'50.59"N, 103° 9'36.64"E, April 2016; B4: 1 gonozooids, 5°37'0.62"N, 103° 4'19.04"E, May 2016; B6: 4 phorozooids and gonozooids, 5°34'48.33"N, 102°59'45.63"E, May 2016; C1: 6 phorozooids and gonozooids, 5°20'47.59"N, 103° 8'59.91"E, Jun 2016; C2: 33 phorozooids and gonozooids, 1 larvae, 5°25'33.93"N, 103°17'32.72"E, Jun 2016; C3: 74 phorozooids and gonozooids, 7 old nurses, 2 larvae, 5°28'54.80"N, 103°22'55.42"E, Jun 2016; D2: 5 phorozooids and gonozooids, 5°14'5.20"N, 103°16'36.04"E, July 2016; D3; 7 phorozooids and gonozooids, 5°15'50.06"N, 103°17'44.13"E, July 2016.

Description

Phorozooid (Fig. 2.1, Top): The body length reaches to 3.9 mm. The test is thin, but usually rigid. The body is barrel-shaped with eight continuous muscles and with no projection. The alimentary canal started at MV, forming a wide dextral arch at MVII and the end at MVI around the cloacal floor. Anus parietal is on the right side at MVI. Branchial septum strongly arched, extending dorsally to MII and ventrally to MIII, its posterior bend extending to MVI ¾. Short endostyle is from MII – MIV. Neural ganglion is at MIII ¾. Gonad is absent.

Gonozooid (Fig. 2.1, Bottom): The body length reaches to 3.7 mm. Additional of gonad. Ovary is at the intestinal coil at MVI ½ before the testis. Testis is club-shaped, elongate in the antero-posterior axis, dorsal to the digestive tract, opening at MIV ½ and extending forward sometimes beyond MII. Other morphological characters resemble the phorozooid stage.

Old nurse (Fig. 2.2, Top): Barrel-shaped without visceral mass, except for a heart. Muscular bands MII – MVIII are fused into a continuous sheath. Budding is located on the dorso-ventral projection.

Larvae (Fig. 2.2, Bottom): Larval stage tailed, with elongate envelope. Without any intermediate vesicle between the tail and cephalo-enteron.

Remarks

Gonozooid, phorozooids, old nurse and larvae of this species were found during the sampling of this study. Specimen characteristics are closely a resemblance of those of previous description of Thompson (1948), Godeaux (1998) and Esnal & Daponte (1999).

Distribution

Doliolum denticulatum is the most common and abundant species of Doliolidae which are widely distributed in the Atlantic, Indian and Pacific Ocean, and they are also the most abundant one (Esnal & Daponte, 1999; Jitlang *et al.,* 2007). It was first found by Quoy & Gaimard (1834) on Agulhas Bank in the southeast African waters of the Indian Ocean. It has also been found in the Red Sea and Gulf of Suez (Godeaux, 1974; Khalil & El Rahman, 1997), Gulf of Aden, Hanish Hill, N'Djebel Tair, Commission Plain, Atlantis (Godeaux, 1987) and in the Gulf of Aqaba (Godeaux, 1998). Records from the North Atlantic Ocean includes of Grobben's (1882) finding from Messina, occurrence in Mediterranean (Godeaux, 1974; Costello *et al.,* 2001), Bay of Naples (Uljanin, 1884; Lo Bianco, 1904), Alboran Sea and Western Mediterranean Basin (Madin, 1991), Bay of Villefranche (Bone & Trueman, 1984), Catalan Sea (Katechakis *et al.,* 2002; 2004) and the Levantine Sea (Weikert & Godeaux, 2008). Records from the South Atlantic Ocean stems from Esnal and Daponte's (1999) and Nogueira *et al.,* (2015) finding from Brazil. Herdman (1898), Thompson (1948) and Ahmad Ishak (2014) have recorded the presence of this species in the South Pacific Ocean. Meanwhile records from the North Pacific Ocean includes finding from San Diego

(Tokioka, 1937; 1960), Baja California (Berner & Reid, 1961; Berner, 1967; Lavaniegos & Ohman, 2003), Kaoping Trench, southwestern waters of Taiwan (Tew & Lo, 2005; Liao *et al.,* 2013; Franco *et al.,* 2017), Korean waters (Chae *et al.,* 2008; Kim *et al.,* 2010) Kuroshio Extension and the Oyashio–Kuroshio Mixed Water Region of Japan (Takahashi *et al.,* 2013; 2015). This species was also found in the Central Pacific Ocean at the northwest continental shelf of South China Sea (Li *et al.,* 2011). The compilation of previous literature and present data distribution of *Doliolum denticulatum* is shown in Figure 2.3.

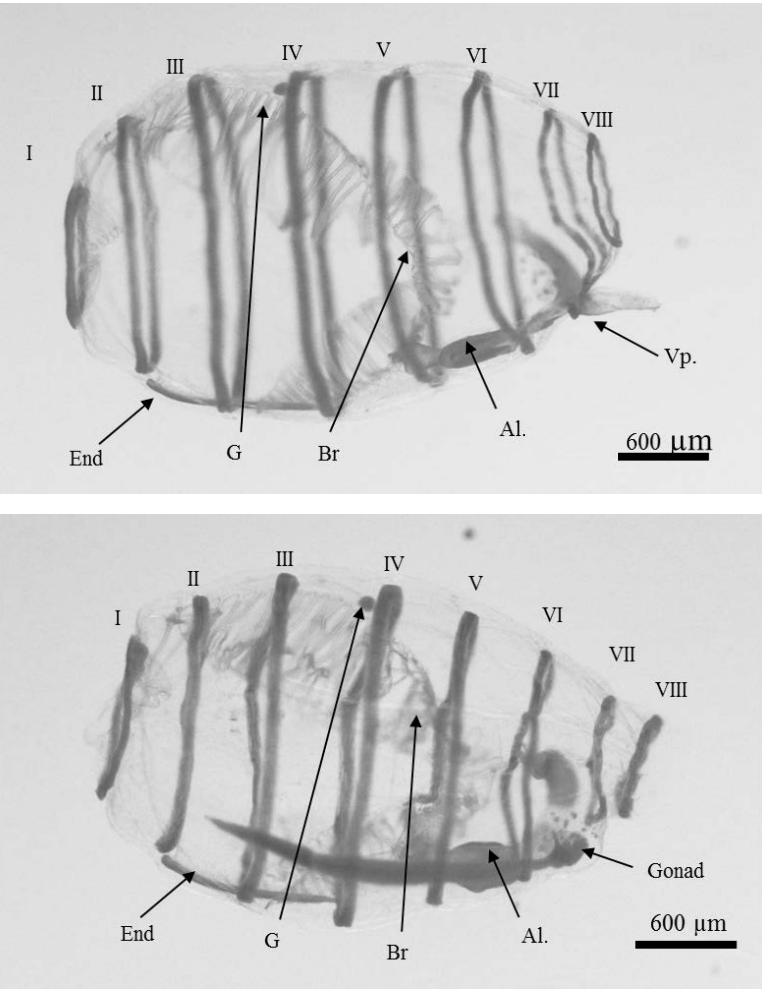

Figure 2.1: *Doliolum denticulatum* (Quoy & Gaimard, 1834). Top, Phorozooid stage, lateral view; Bottom, Gonozooid stage, lateral view. Al., alimentary canal; Br, branchial septum; Dt., dorsal tubercle; End, endostyle; G, ganglion; N, nucleus ; Vp., ventral peduncle.

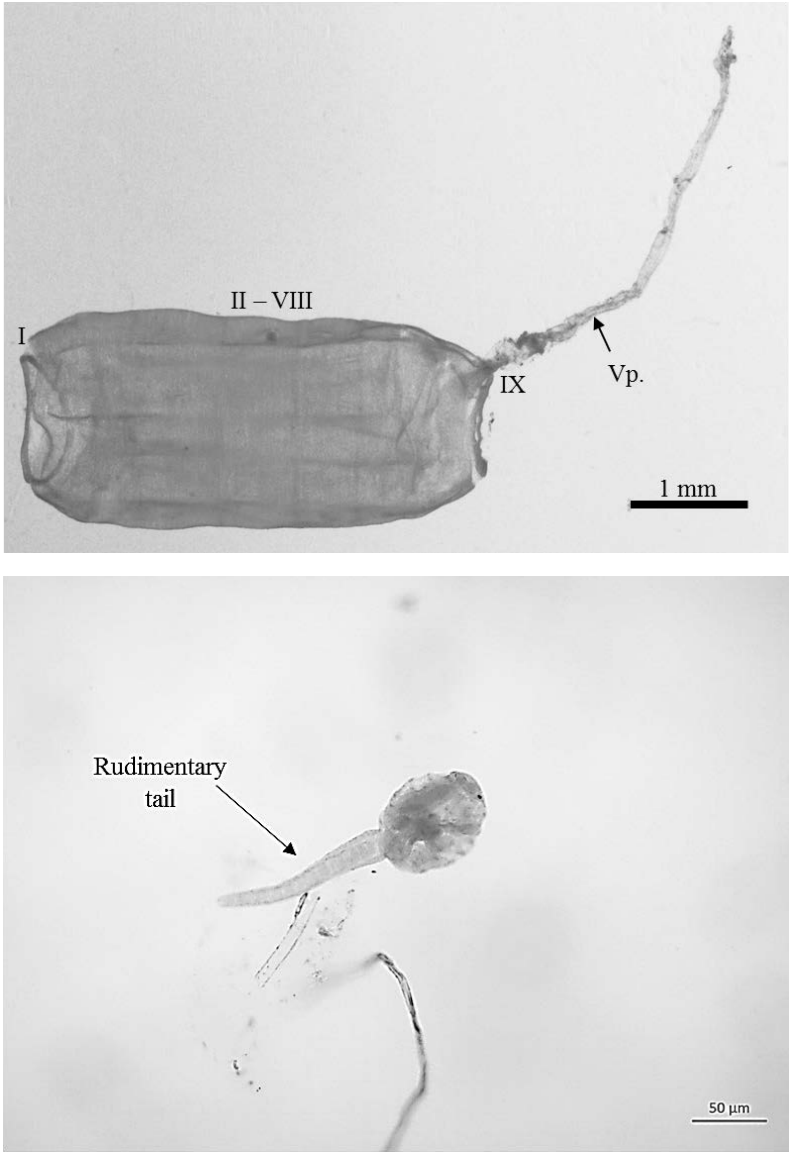

Figure 2..2: *Doliolum denticulatum* (Quoy & Gaimard, 1834). Top, Old Nurse stage, lateral view; Bottom, Larvae stage, lateral view; Vp. ventral peduncle.

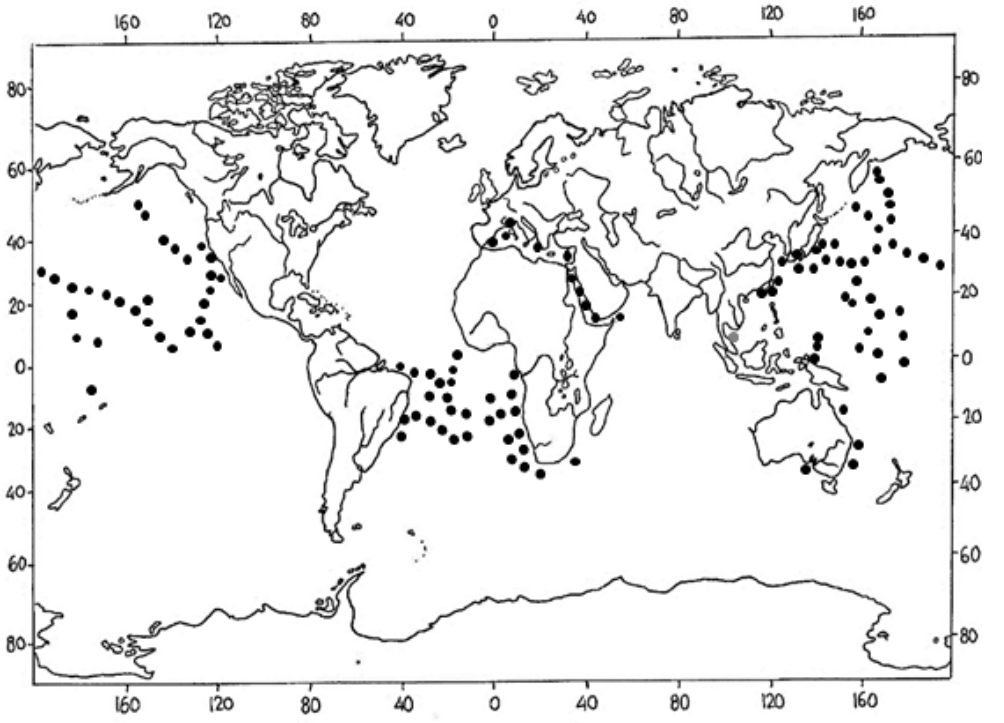

Figure 2.3: Distribution of *Doliolum denticulatum* (Quoy & Gaimard, 1834). Red dots: specimen examined by the author; black dots: records based on compilation of previous literature data.

2. Doliolum Nationalis Borgert, 1894

Doliolum nationalis Borgert, 1894 (cited from Berill, 1950, pp. 282 – 284); Fraser, 1947b, pp. 1 - 4; Fraser, 1962, p. 81, plate XX; Madin, 1991, p. 107; Godeaux, 1998, pp. 279 – 283; Boltovskoy, 1999, p. 34; S. Kim, Lee, & Kim, 2010, pp. 11 – 12; Purushothaman et al., 2017, p. 8

Specimens examined

C3: 1 phorozooid, 5°28'54.80"N, 103°22'55.42"E, Jun 2016.

Description

Phorozooid (Fig. 3.1): A single example of the phorozooid zooid of this species was collected, in a rather bad condition. The body length is 3.3 mm. The test is thin. The body is barrel-shaped with eight continuous muscles and with no projection. The alimentary canal started at MV, forming a wide dextral arch at MVII and the end at MVI around the cloacal floor. Anus parietal is on the right side at MVI. Branchial septum is strongly arched, extending dorsally to MII and ventrally to MIV ½ or MV, its posterior bend extending to MVI ½. Short endostyle is from MII – MIV. Neural ganglion is at MIII ¾. Gonad is absent.

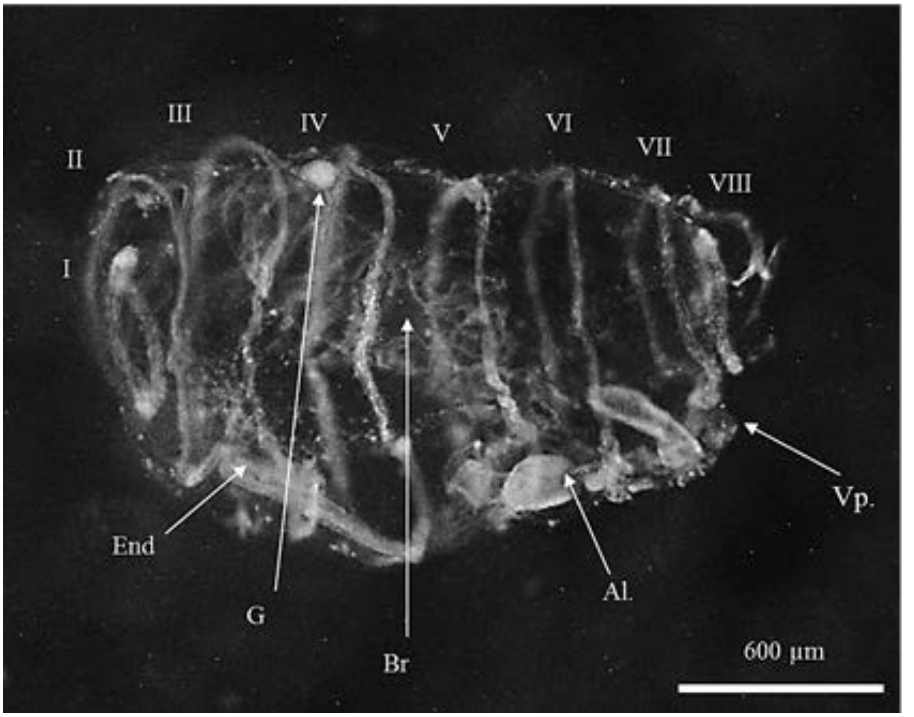

Figure 3.1: *Doliolum nationalis,* (Borgert, 1894). Phorozooid stage, lateral view. Al., alimentary canal; Br, branchial septum; Dt., dorsal tubercle; End, endostyle; G, ganglion; N, nucleus ; Vp., ventral peduncle.

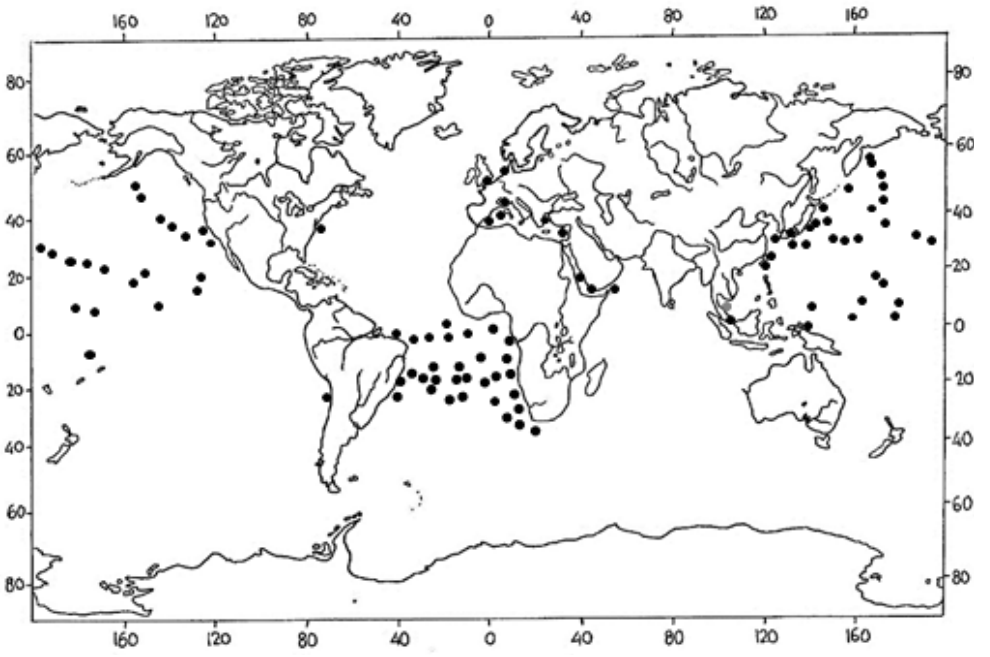

Figure 3.2: Distribution of *Doliolum nationalis* (Borgert, 1894). Red dots: specimen examined by the author; black dots: records based on compilation of previous literature data.

Remarks

Only phorozooid of this species were found during the sampling of this study. Judging from the condition of the specimen of this single specimen, death had occurred prior to their capture. Although I is able to figure out the basic morphological characters of this species such as the shape of its alimentary canal and the arrangement of its muscle bands, I however is unable to presume the minute details of its morphological characteristics such as the count of the gill slit of the specimen. Specimen characteristics are closely a resemblance of those of previous description of Thompson (1948), Godeaux (1998) and Esnal & Daponte (1999).

Distribution

Doliolum nationalis is less frequent and also less abundant species of Doliolidae Esnal & Daponter, 1999 distributed in the Atlantic, Indian and Pacific Ocean. It was first found by Borgert (1894). Records from the North Atlantic Ocean includes the occurrence in Bay of Naples (Uljanin, 1884; Lo Bianco, 1904), Plymouth (Russell & Hastings, 1931), Eastern Mediterranean (Godeaux, 1974; van der Land & van Soest, 2001), British Isles (Fraser, 1982), Bay of Villefranche (Holland, 1989; Godeaux, 1989; Bone *et al.,* 1996; Menard *et al.,* 1997), Alboran Sea and Western Mediterranean Basin (Madin, 1991), German largeht (Lindley *et al.,* 1990; Edwards *et al.,* 1999), northern part of the Levantine Sea (Weikert & Godeaux, 2008) and Greek waters (Koukouras, 2010). Records from the South Atlantic Ocean stems from Esnal and Daponte's (1999) and Nogueira *et al.* (2015) finding from Brazil. Neumann (1906) has found this species in the Indian Ocean and it has also been found in the Red Sea and Gulf of Suez (Godeaux, 1974), Gulf of Aden, Hanish Hill and N'Djebel Tair (Godeaux, 1987). Thompson (1948) has recorded the presence of this species in the South Pacific Ocean. Meanwhile records from the North Pacific Ocean includes finding from San Diego (Tokioka 1937; 1960), Tokyo (Hirose *et al.,* 1999), Korean waters (Chae *et al.,* 2008; Kim *et al.,* 2010), Mejillones Bay (Apablaza & Palma, 2005), Kuroshio Extension and the Oyashio–Kuroshio Mixed Water Region of Japan (Takahashi *et al.,* 2013; 2015) and waters of Taiwan (Liao *et al.,* 2013; Franco *et al.,* 2017). This species was also found in the Central Pacific Ocean at Singapore Straits (Tey, 1967) and the northwest continental shelf of South China Sea (Li *et al.,* 2011). The compilation of previous literature and present data distribution of *Doliolum nationalis* is shown in Figure 3.2.

3. Dolioletta Gegenbauri Uljanin, 1884

Doliolum gegenbauri Uljanin, 1884 (cited from Berill, 1950, pp. 286)
Dolioletta gegenbauri: Garstang, 1933 (cited from Godeaux, 1998, pp. 279 – 283); Fraser, 1947, pp. 1 - 4; Madin, 1991, p. 107; Boltovskoy, 1999, p. 15 – 18; (Kott 2005.) S. Kim, Lee, & Kim, 2010, pp. 12 – 13; Purushothaman *et al.,* 2017, p. 8; Franco *et al.,* 2017, p. 13

Specimens examined

A6: 1 old nurse, 5°39'50.59"N, 103° 9'36.64"E, April 2016; B1: 1 phorozooids, 5°35'51.35"N, 103° 3'31.21"E, May 2016; B2: 1 phorozooids, 5°35'57.49"N, 103° 3'49.21"E, May 2016; B4: 4 phorozooids and gonozooids, 5°37'0.62"N, 103° 4'19.04"E, May 2016; B5: 2 phorozooids and gonozooids, 1 old nurse, 5°35'47.15"N, 103° 3'37.61"E, May 2016; C1: 38 phorozooids and gonozooids, 20 old nurses, 5°20'47.59"N, 103° 8'59.91"E, Jun 2016; C2: 209 phorozooids and gonozooids, 69 old nurses, 2 larvae, 5°25'33.93"N, 103°17'32.72"E, Jun 2016; C3: 111 phorozooids and gonozooids, 63 old nurses, 2 larvae, 5°28'54.80"N, 103°22'55.42"E, Jun 2016; D1: 4 phorozooids and gonozooids, 5°14'5.31"N, 103°14'40.12"E, July 2016; D2: 3 old nurses, 5°14'5.20"N, 103°16'36.04"E, July 2016; D3; 3 phorozooids and gonozooids, 1 old nurse, 5°15'50.06"N, 103°17'44.13"E, July 2016.

Description

Phorozooid (Fig. 4.1, Top): The body length reaches up to 7.4 mm. Test is thin and soft, with some rigid. The body is barrel-shaped with eight continuous muscles and a ventral peduncle. The alimentary canal started at MV, dextrally coiled at MVII ¼ and the end at MVII around the cloacal floor. Anus parietal is on the right side at MVI. Branchial septum strongly arched, extending dorsally to MII and ventrally to MVII, its posterior bend extending to MVII ¾. Short endostyle is from MII – MIV. Neural ganglion is at MIII ¾. Gonad is absent.

Gonozooid (Fig. 4.1, Bottom; Fig 4.2): The body length reaches up to 8 mm. Additional of gonad. Ovary is at the intestinal coil at MVII ¾ before the testis. Testis is club-shaped, elongate in the antero-posterior axis, dorsal to the digestive tract, opening at MVII ¾ and extending forward to MII. Other morphological characters resemble the phorozooid stage.

Old nurse (Fig. 4.3): Robust in appearance. Muscle bands broader than ½ the interspaces. MIII and MIV are wider than the rest, MIII is slightly dominant.

Oozoid (Fig. 4.4, Top): In young oozoids, alimentary canal extending horizontally in the sagittal plane, endostyle extends from MII to MVI, stomach in front of MVII.

Larvae (Fig. 4.4, Bottom): Larvae is with a rudimentary tail encased in a spherical envelope.

Remarks

Oozoids, phorozooids, gonozooids, old nurse and larvae of this species were found during the sampling of this study. Specimen characteristics are closely a resemblance of those of previous description of Thompson (1948), Berill (1950), Godeaux (1998) and Esnal & Daponte (1999).

Distribution

Dolioletta gegenbauri is a common species of Doliolidae (Nishikawa & Terazaki, 1996) with widest distribution area scattered around the Atlantic, Indian and Pacific Ocean. It was first found by Uljanin (1884) in Bay of Naples (Uljanin, 1884; Lo Bianco, 1904). Distribution records from the North Atlantic Ocean is then followed by finding from the occurrence in Plymouth (Russell & Hastings, 1931), Eastern Mediterranean (Godeaux, 1974; Costello *et al.,* 2001), Bay of Biscay (Bone & Ryan, 1974), Western Atlantic Ocean (Pomeroy, 1980; Pomeroy & Deibel, 1980), Gulf of Stream (Deibel, 1985), Alboran Sea and Western Mediterranean Basin (Madin, 1991), Bay of Villefranche (Holland, 1989; Bone *et al.,* 1996; Menard *et al.,* 1997; Pond & Sargent, 1998) and the northern part of the Levantine Sea (Weikert & Godeaux, 2008). Records from the South Atlantic Ocean stems from Esnal and Daponte's (1999), Tavares (1967) and Nogueira et al. (2015) finding from Brazil and South Atlantic Bight continental (Frischer et al., 2014). This species has also been found in the Red Sea (Godeaux, 1960), Gulf of Aden, Hanish Hill and Commission Plain (Godeaux, 1987), Bay of Bengal (Madhupratap *et al.,* 1980) and Parangipettai India (Kannathasan, 2012). Records from the North Pacific Ocean includes finding from San Diego (Tokioka, 1937; 1960), Japan (Nishikawa, 1995; Nishikawa & Terazaki, 1996; Nakamura, 1998; Hirose *et al.,* 1999); Kuroshio Extension and the Oyashio–Kuroshio Mixed Water Region (Takahashi *et al.,* 2013); California (Berner, 1967; Lavaniegos & Ohman, 2003), Mejillones Bay (Apablaza & Palma, 2005), North Pacific Ocean's Subarctic Frontal Zone (Ignell, 2006), Korean waters (Kim *et al.,* 2010) and waters of Taiwan (Franco *et al.,* 2017). Meanwhile Thompson (1948) and Ahmad Ishak (2014) had recorded the presence of this species in the South Pacific Ocean. This species was also found in the Central Pacific Ocean at the northwest continental shelf of South China Sea (Li *et al.,* 2011). The compilation of previous literature and present data distribution of *Dolioletta gegenbauri* is shown in Figure 4.5.

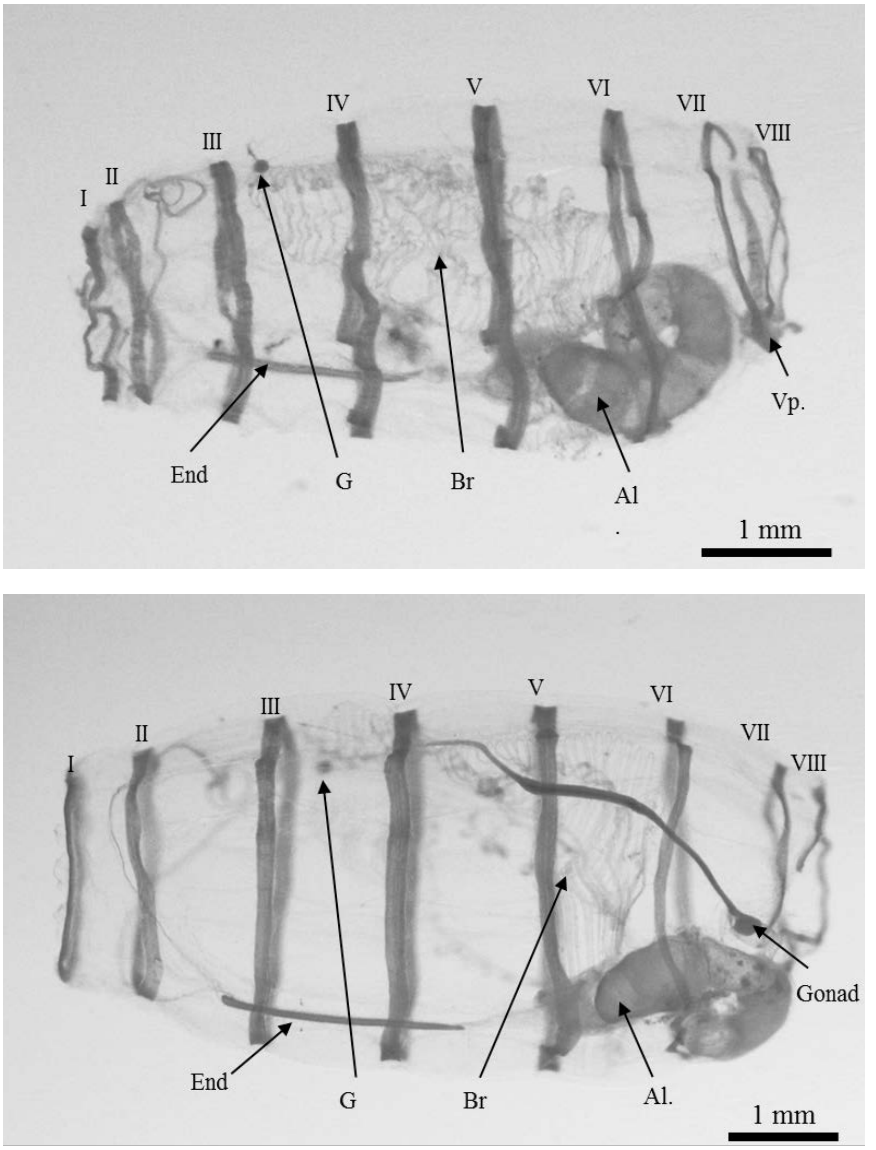

Figure 4.1: *Dolioletta gegenbauri* (Uljanin, 1884). Top, Phorozooid stage, lateral view; Bottom, Gonozooid stage, lateral view. Al., alimentary canal; Br, branchial septum; Dt., dorsal tubercle; End, endostyle; G, ganglion; N, nucleus ; Vp., ventral peduncle.

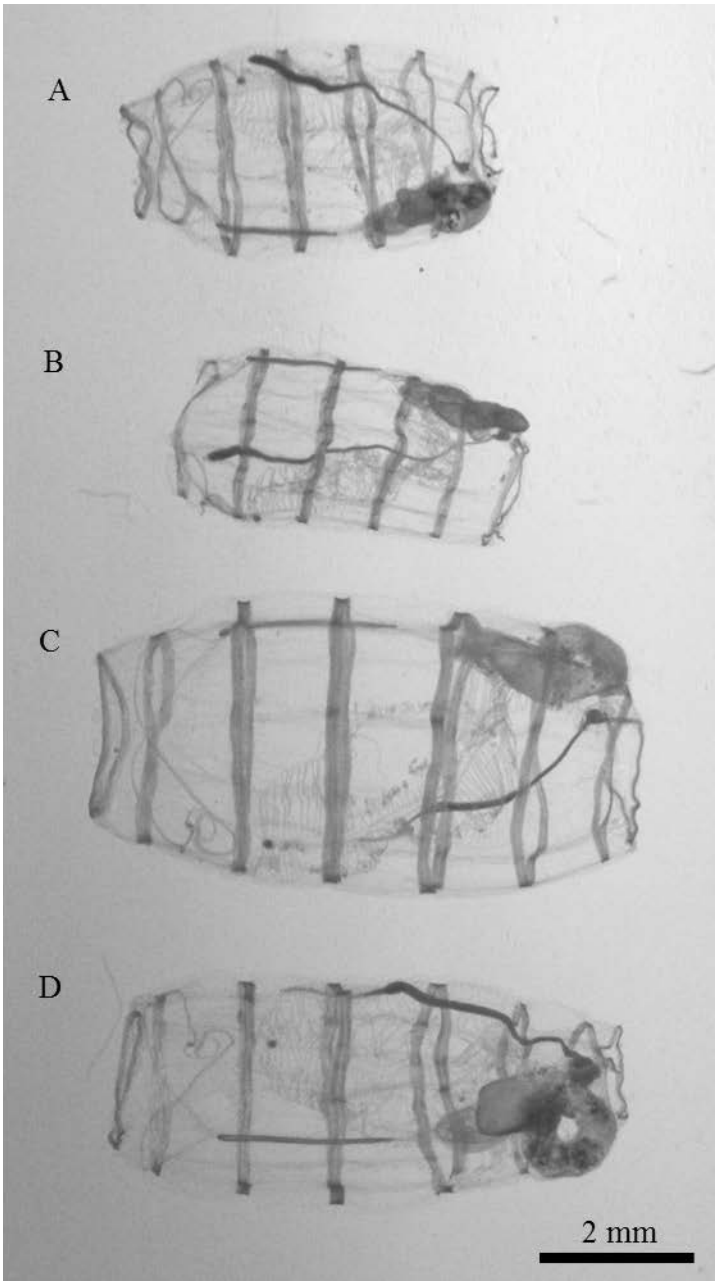

Figure 4.2: *Dolioletta gegenbauri* (Uljanin, 1884). A – D, Gonozooid stage, lateral view; showing the variation of testis elongation.

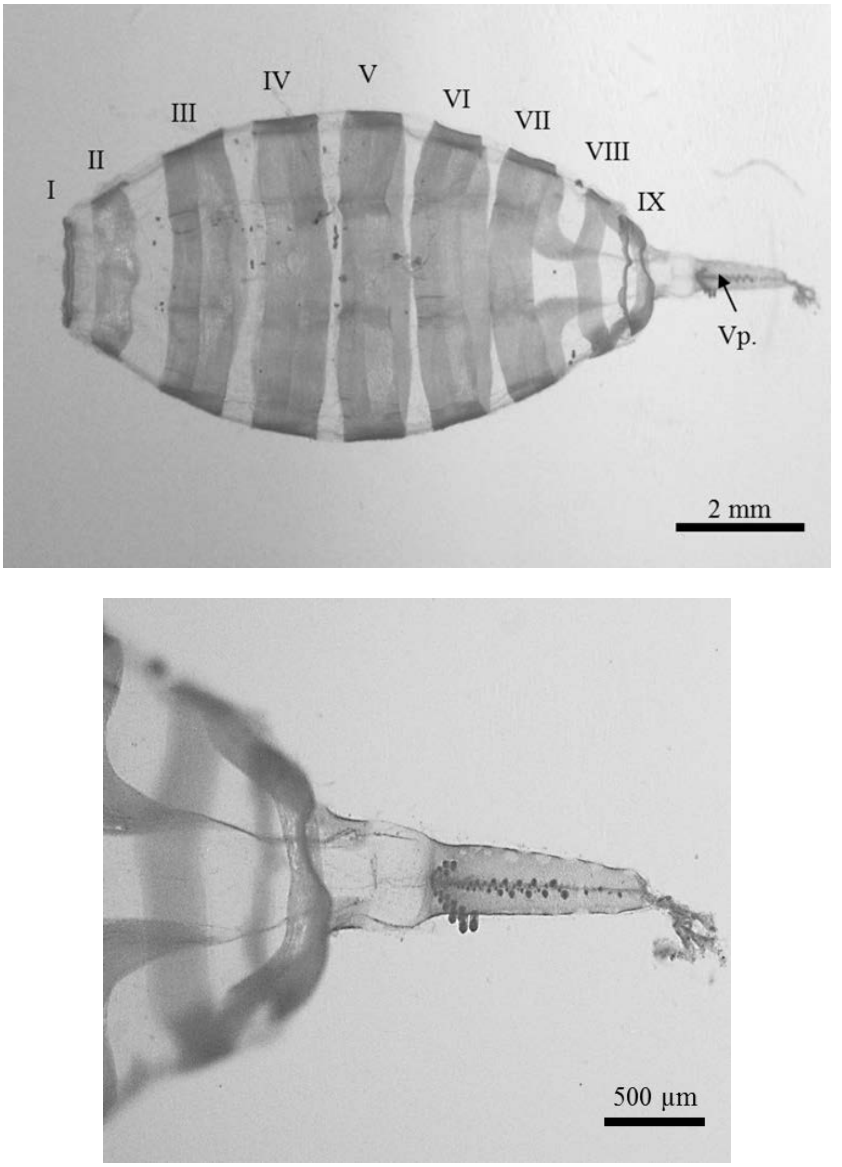

Figure 4.3: *Dolioletta gegenbauri* (Uljanin, 1884). Top, Old nurse stage, lateral view; Bottom, posterior part.

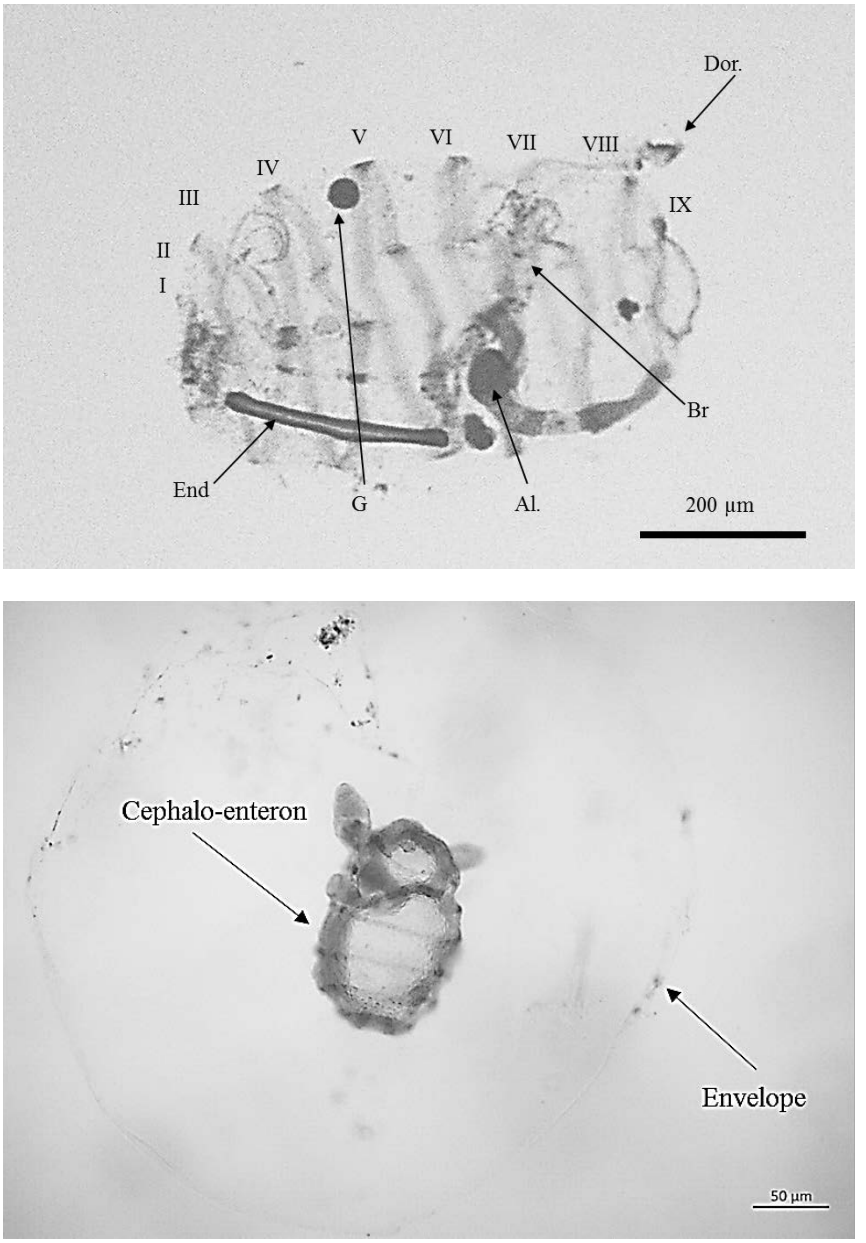

Figure 4.4: *Dolioletta gegenbauri* (Uljanin, 1884). Top, Young oozoid stage, lateral view; Bottom, Larvae stage, lateral view. Al., alimentary canal; Br, branchial septum; Dor., dorsal appendix; End, endostyle; G, ganglion; N, nucleus.

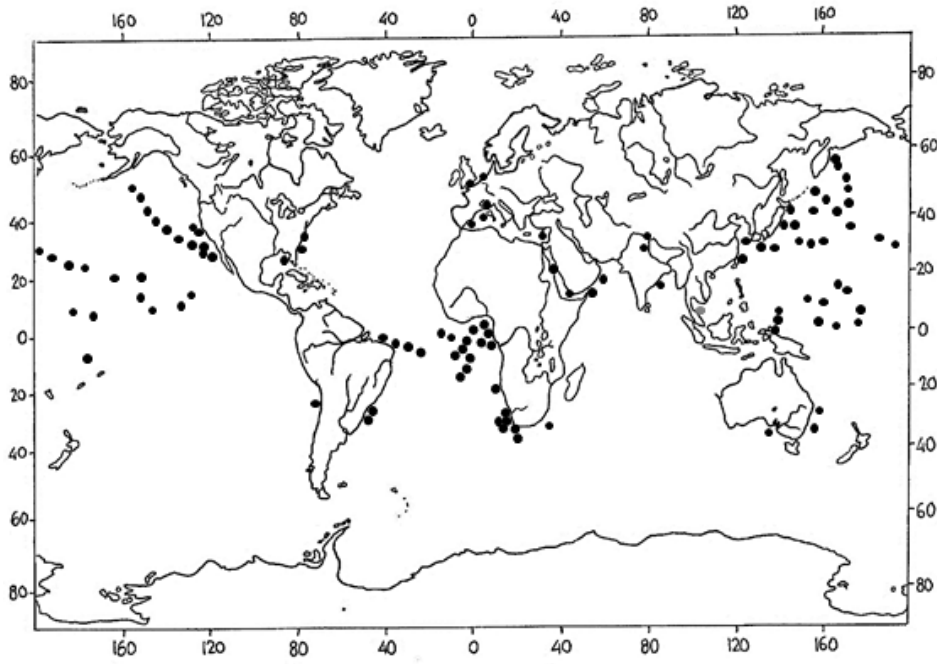

Figure 4.5: Distribution of *Dolioletta gegenbauri* (Uljanin, 1884). Red dots: specimen examined by the author; black dots: records based on compilation of previous literature data.

4. Doliolina mulleri Krohn, 1852

Doliolina muelleri; Godeaux, 1998, pp. 279 – 283; Fraser, 1947, pp. 1 - 4; Madin, 1991, p. 108; Boltovskoy, 1999, p. 22 – 23; Purushothaman *et al.,* 2017, p. 8; Franco *et al.,* 2017, pp. 13 – 14

Specimens examined

C1: 9 old nurses, 5°20’47.59”N, 103° 8’59.91”E, Jun 2016; C2: 2 phorozooids and gonozooids, 54 old nurses, 5°25’33.93”N, 103°17’32.72”E, Jun 2016; C3: 2 phorozooids and gonozooids, 51 old nurses, 1 larvae, 5°28’54.80”N, 103°22’55.42”E, Jun 2016; D2: 6 old nurses, 5°14’5.20”N, 103°16’36.04”E, July 2016; D3; 9 old nurses, 5°15’50.06”N, 103°17’44.13”E, July 2016.

Description

Oozoid (Fig. 5.1, Top): The body is tiny, length up to 0.7 mm. The test is thin. The body is barrel-shaped with nine continuous muscles and a dorsal appendix. The alimentary canal started at MVI, forming an upright U-shaped. The branchial septum bears four pairs of slits, extending upwards from MV to MVII. Short endostyle is from MII – MIV. Neural ganglion is at MIV ¾. Gonad is absent.

Old nurse (Fig. 5.1, Bottom): Eurymyonic; muscle bands broader than ½ interspaces. Amphiclinous; muscle bands 2-8 gradually becoming broader then narrower both anteriorly and posteriorly; myoplane at 3-4. Broad muscles (usually contracted and of dark appearance after preservation) separated by small gaps. MIV is slightly broader than MIII.8

Larvae (Fig. 5.2): Larval stage tailed, with elongate envelope and intermediate vesicle between tail and cephalo-enteron.

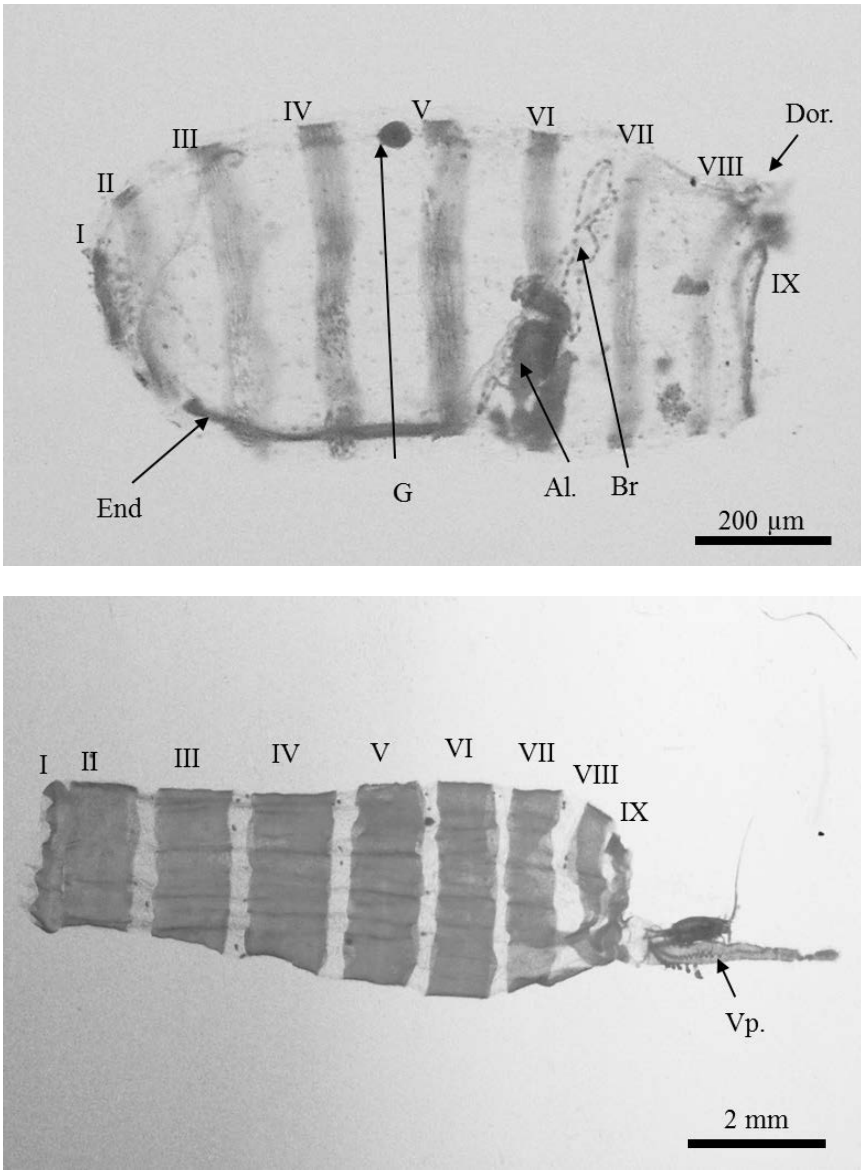

Figure 5.1: *Doliolina mulleri* (Krohn, 1852). Top, Young oozoid stage, lateral view; Bottom, Old nurse stage, lateral view. Al., alimentary canal; Br, branchial septum; Dor., dorsal appendix; End, endostyle; G, ganglion,; N, nucleus; Vp., ventral peduncle.

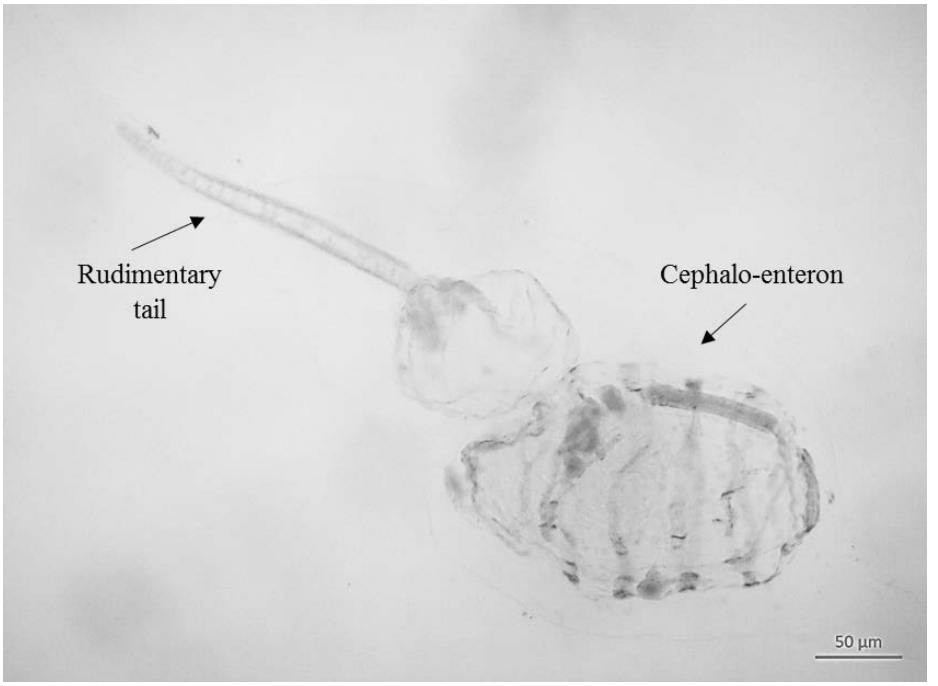

Figure 5.2: *Doliolina mulleri* (Krohn, 1852). Larvae stage, lateral view.

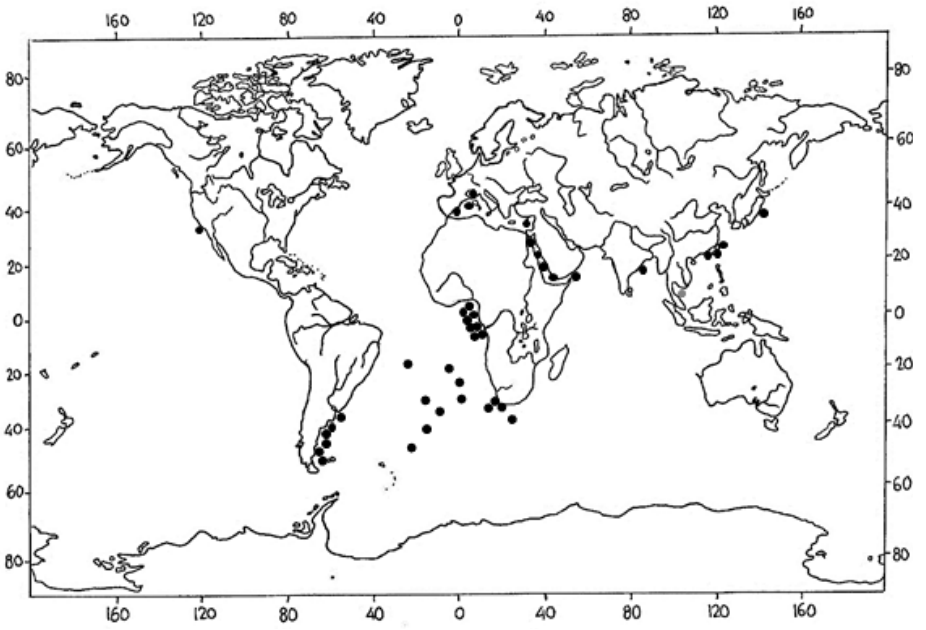

Figure 5.3: Distribution of *Doliolina muelleri* (Krohn, 1852). Red dots: specimen examined by the author; black dots: records based on compilation of previous literature data.

Remarks

Oozoids, old nurse and larvae of this species were found during the sampling of this study. Specimen characteristics are closely a resemblance of those of previous description of Thompson (1948), Godeaux (1998) and Esnal & Daponte (1999).

Distribution

Doliolina muelleri is an uncommon species of Doliolidae which scattered scarcely in the Atlantic, Indian and Pacific Ocean. It was first found by Krohn (1852). Records from the North Atlantic Ocean includes the occurrence in Bay of Naples (Uljanin, 1884; Lo Bianco, 1904), Eastern Mediterranean (Godeaux, 1974), Rade de Villefranche (Bone & Trueman, 1984; Bone *et al.,* 1997), Alboran Sea and Western Mediterranean Basin (Madin, 1991) and northern part of the Levantine Sea (Weikert & Godeaux, 2008). Records from the South Atlantic Ocean stems from Esnal and Daponte's (1999) and finding from Brazil by Tavares (1967) and Nogueira *et al.* (2015). This species has also been found in the Red Sea and Gulf of Suez (Godeaux, 1974), Gulf of Aden, Hanish Hill N'Djebel Tair; Commission Plain; Atlantis (Godeaux, 1987) and Parangipettai India (Kannathasan, 2012). Records from the North Pacific Ocean includes finding from San Diego (Tokioka, 1960), Kaoping Trench in southwestern waters of Taiwan (Tew & Lo, 2005; Liao *et al.,* 2013; Franco *et al.,* 2017) and Kuroshio Extension and the Oyashio–Kuroshio Mixed Water Region of Japan (Takahashi *et al.,* 2013). The compilation of previous literature and present data distribution of *Doliolina muelleri* is shown in Figure 5.3.

5. Dolioloides rarum Grobben, 1882

Dolioloides rarum Grobben, 1882, pp. 265 - 267; Godeaux, 1998, pp. 279 – 283; Fraser, 1947, pp. 1 - 4; Boltovskoy, 1999, p. 15 – 18; Purushothaman *et al.,* 2017, p. 8.

Specimens examined

A2: 2 old nurses, 5°39'55.02"N, 103°0'6.03"E, April 2016; A3: 1 old nurses, 5°40'51.01"N, 103° 1'51.06"E, April 2016; A4: 3 phorozooids and gonozooids, 2 old nurses, 5°38'6.00"N, 103° 3'48.06"E, April 2016; A6: 2 phorozooids and gonozooids, 5°39'50.59"N, 103° 9'36.64"E, April 2016; B1: 1 old nurses, 5°35'51.35"N, 103° 3'31.21"E, May 2016; B5: 4 phorozooids and gonozooids, 5°35'47.15"N, 103° 3'37.61"E, May 2016; B6: 1 phorozooids and gonozooids, 5°34'48.33"N, 102°59'45.63"E, May 2016; C1: 7 phorozooids and gonozooids, 5°20'47.59"N, 103° 8'59.91"E, Jun 2016; C2: 2 phorozooids and gonozooids, 23 old nurses, 5°25'33.93"N, 103°17'32.72"E, Jun 2016; C3: 1 gonozooids, 29 old nurses, 5°28'54.80"N, 103°22'55.42"E, Jun 2016; D1: 7 phorozooids and gonozooids, 2 old nurses, 5°14'5.31"N, 103°14'40.12"E, July 2016; D2: 3 phorozooids and gonozooids, 5°14'5.20"N, 103°16'36.04"E, July 2016; D3; 3 old nurses, 5°15'50.06"N, 103°17'44.13"E, July 2016.

Description

Oozoid (Fig. 6.1): The body length reaches 1.8 mm. The test is thin. The body is barrel-shaped with nine continuous muscles and a dorsal appendix. Alimentary canal extending horizontally in the sagittal plane; endostyle is from MII to MV; stomach is at MVI. Anus parietal is on the right side at MVIII. Branchial septum is extending upwards to MVI. The gill slits count is four or five. Neural ganglion is at MV ½. Gonad is absent.

Old nurse (Fig. 6.2): Muscle bands broader than ½ the interspaces. Muscle bands MII – MVIII not forming a gradually increasing and diminishing series.

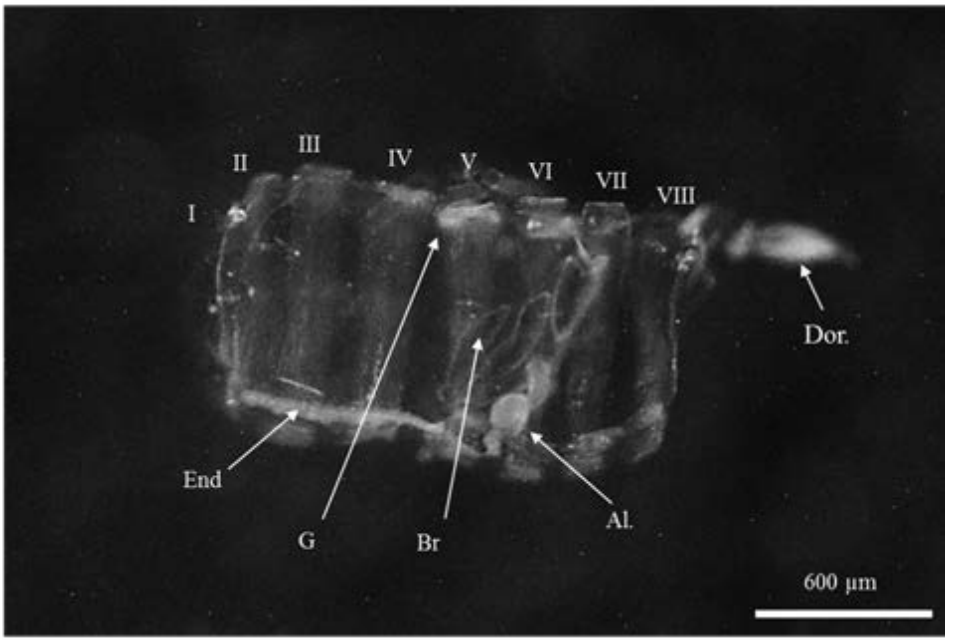

Figure 6.1: *Dolioloides rarum* (Grobben, 1882). Young oozoid stage, lateral view. Al., alimentary canal; Br, branchial septum; Dor., dorsal appendix; End, endostyle; G, ganglion; N, nucleus.

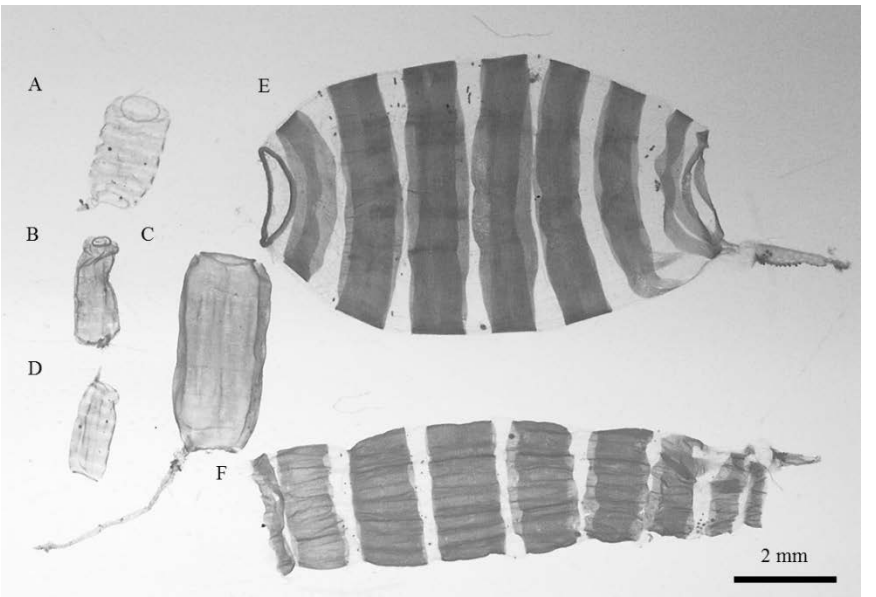

Figure 6.2: Showing variety of Doliolida old nurse stage.

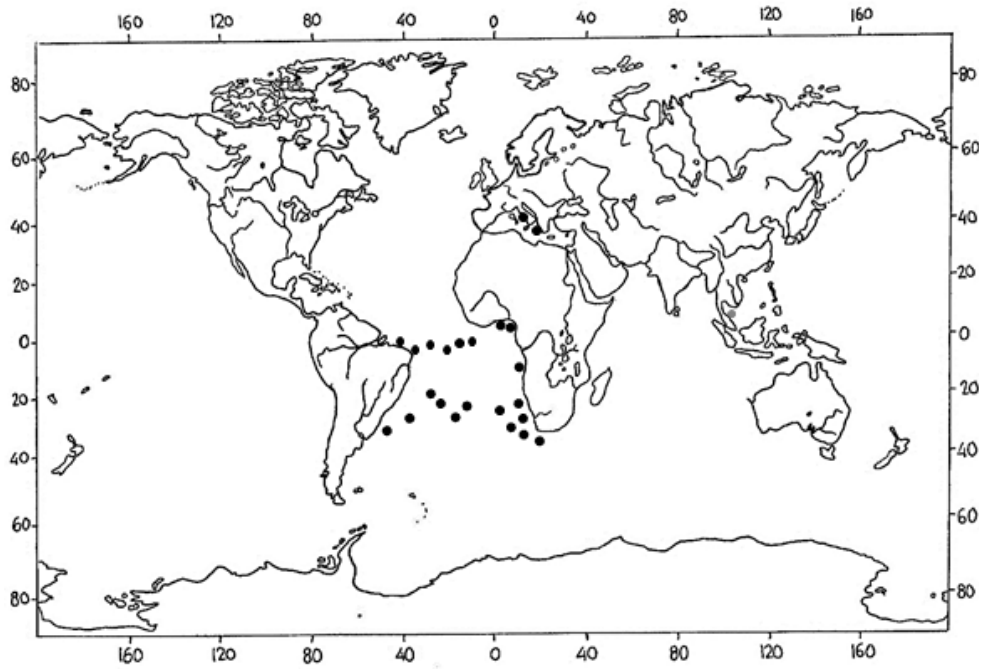

Figure 6.3: Distribution of *Dolioloides rarum* (Grobben, 1882). Red dots: specimen examined by the author; black dots: records based on compilation of previous literature data.

Remarks

Only oozoids and old nurse of this species were found during the sampling of this study. Specimen characteristics are closely a resemblance of Grobben (1882) and Esnal and Daponte in 1999.

Distribution

Dolioloides rarum is a rare species of Doliolidae with previous distribution records occurring only in the Atlantic Ocean. It was first found by Grobben (1882) in Messina, followed by the occurrence in Bay of Naples (Uljanin, 1884; Lo Bianco, 1904) and Mediterranean (Costello *et al.,* 2001). Records from the South Atlantic Ocean stems from Esnal and Daponte's (1999). This is the first time Dolioloides rarum has been found in South China Sea, outside from the Atlantic Ocean. The compilation of previous literature and present data distribution of Dolioloides rarum is shown in Figure 6.3.

Key to Doliolid Species (phorozooids) from East coast of Malay Peninsular

1. Alimentary canal extended horizontally ... *Dolioloides rarum*
   - Alimentary canal forming loop or arched dextrally ... 2
2. Alimentary canal forming loop ... 3
   - Alimentary canal forming a wide dextral arch ... 4
3. Alimentary canal forming an upright U or S shaped loop in the sagittal plane ... *Doliolina muelleri*
   - Alimentary canal forming a close loop coil in the middle of the cloacal floor ... *Dolioletta gegenbauri*
4. Branchial septum strongly arched from M II dorsally to M III ventrally ... *Doliolum denticulatum*
   - Branchial septum strongly arched from M II dorsally to just in front of M V ventrally ... *Doliolum nationalis*

Discussion

Taxonomy

In the present study, the focus was on the taxonomy, diversity and biogeography of Doliolidae in the east coast of Malaysia. Doliolid have been known to have the most complex life cycle, and at each stage there bring upon its own identification problems. Differentiating the sample of gonozooid and phorozooid stage from Terengganu waters for genus level *(Doliolum, Dolioletta, Doliolina* & *Dolioloides)* was straightforward as they have distinct morphological characteristics, especially in their shape of alimentary canal. However, the identification of the species within the genus *Doliolum*; for *D. denticulatum* and *D. nationalis* proves to be a bit difficult. Aside from having undistinguishable larvae and old nurse stage, there are not much features that differentiate the gonozooid and phorozooid stage of these two species (Esnal & Daponte, 1999). Moreover, the single phorozooid of *D. nationalis* seems to be unfit for critical taxonomy study due to its poor condition and shrinkage. The most distinct character between the gonozooid and phorozooid stage of these two species was the extension of their branchial septum which was luckily seen in the single *D. nationalis* sample, thus conforming its identity. The branchial septum of *D. denticulatum* is strongly arched from M II dorsally to M III ventrally while the branchial septum of *D. nationalis* is strongly arched from M II dorsally to just in front of M V ventrally.

Distribution and Biogeography

Lavaniegos and Ohman (2003) forwarded that interpreting the long-term changes discovered within the environment could be aided by monitoring the biogeographic distribution of the pelagic tunicates. This is due to the human activities which often affect the distribution patterns of marine fauna and the biology of the ocean (Kiørboe, 2011). Even though with their irregular distribution, doliolids are an important element of the ocean ecosystem due to its large distribution coverage (Godeaux, 1973; 1985; 1986; 1987; 1998b; Borgert, 1894; Garstang, 1933: Tokioka & Berner, 1958a; b; O'Sullivan, 1983). In the South China Sea, a total of 6 species in the family Doliolidae were observed (Zhang *et al.,* 2003a, b; Liao *et al.,* 2013; Franco *et al.,* 2017). Fraser (1962) stated that the biogeography of thaliaceans is vital to be investigated due to the potential role as indicators for fluctuations of ocean currents and temperature. This claim can be supported by the findings of Neumann (1913), Garstang (1933), Berner & Reid (1961), Blackburn (1979) and Lazarus & Dowler (1979) where species such as *Doliolum denticulatum* proved to be a good indicator of the warm waters and is strongly limited by low temperatures.

The results of our study showed a total of five species in the Terengganu waters: *Doliolum denticulatum, Doliolum nationalis, Dolioletta gegenbauri, Dolioloides rarum* and *Doliolina muelleri.* These five species occurring simultaneously in the same station only happened once, where the sampling site has a bottom depth that reaches over 45 m. We hypothesised that doliolid abundance could be higher in deeper and colder waters since doliolids seems to prefer waters of lower temperatures and salinities (Godeaux, 1973; 1987). *Dolioletta gegenbauri* showed the highest densities, totaling up to 107.87 ind. /m 3, being present at 11 sampling stations. It is not surprising as this species is considered as a very frequently encountered and abundant species which also showed a very wide distributional range (Esnal & Daponte, 1999). *Doliolum denticulatum* showed the widest distribution range, being present at 12 sampling stations. The closely related species Doliolum nationalis is less frequent and also less abundant, only 1 single specimen of *Doliolum nationalis* were able to be collected. *Doliolina muelleri* is uncommon in the sample even though it shows a very wide distributional range worldwide. This may be due to the preference of 15°C temperature (Neumann, 1913) and our study area is not ideal for this species to thrive. *Dolioloides rarum* is the rarest species identified in the sample. This is consistent with other finding that states its more

erratic occurrence (Esnal & Daponte, 1999) and it was last recorded in Mediterranean (Costello *et al.,* 2001).

Uncollected Doliolid Species

Analyzing and comparing the studies of thaliaceans distribution from all China Seas (Table 1), we observed that there is one species which were recorded in the China Seas that could not be identified from Terengganu waters as yet: *Dolioletta tritonis.*

Table 2: Doliolids encountered in the China Seas from previous literature.

	Taiwan waters (Zhang *et al.,* 2003a; b; Franco *et al.,* 2017)	Korea waters (Kim *et al.,* 2010)	Singapore Straits (Yap & Lee, 2016)	Terengganu waters (present study)
Family: Doliolidae				
Doliolum denticulatum	/	/		/
Doliolum nationalis	/	/	/	/
Dolioletta gegenbauri	/	/		/
Dolioletta tritonis		/		
Dolioloides rarum	/			/
Doliolina muelleri	/			/

The thaliaceans diversity result of this study might be undervalued due to a few limitations throughout the course of the study. In fact, our research was conducted within a span of four months within a year and not throughout a single year and that the area coverage for the sampling site is limited. This could cause some problems for the comparison with other kinds of work of the same nature. Next, deep-water or seasonally restricted occurrences may also influence the results of the study. The collection of organisms was carried out at restricted regions covering only the southwest monsoon season and at an average depth of 15 m. Through sampling in deeper waters and with a wider sampling location, we could expect to have found a higher diversity of doliolids species.

Conclusion

Despite bearing on several limitations, this study provides valuable baseline information regarding the distribution of salps in Malaysian waters, given that there are no historical records of *Doliolum denticulatum, Doliolum nationalis, Dolioletta gegenbauri, Dolioloides rarum* and *Doliolina muelleri* have been previously identified in the area, and consequently extends the discovery of latitudinal distribution of these species in the South China Sea. This study contributed to serve as a starting point for further research, especially in the area of ecology of these organisms. Supplementary studies on the abundance and distribution in different seasons and locality would be an accommodating effort in making future comparisons, and thus for the better understanding of the changes of the pelagic tunicates' community structure in the South China Sea experiences.

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Discovery, and also to Mr. Khyril, Mr. Hafiz and Ms. Muna for their help on board. Thanks are also due to reviewers whom will be commenting on the manuscript for further improvement.

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