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COMMUNICATION

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COMMUNICATION

Additional description of the Algae Hydroid *Thyroscyphus ramosus* (Hydrozoa: Leptothecata: Thyroscyphidae) from Palk Bay, India with insights into its ecology and genetic structure

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Abstract: The Algae hydroid *Thyroscyphus ramosus* of the Indian subcontinent is the most easily recognizable fleshy colonial hydroid playing a vital role in benthic communities. Though this fauna is abundant, it has remained unexplored for the past nine decades in India. This study provides a detailed report of the morphology, ecology and geographical locations of *T. ramosus*. Morphological traits such as maximum height, gonophore, and theca twist directions were studied in detail. The molecular biological data confirms the identity of *T. ramosus* and its abundance in Palk Bay, India. Important molecular markers such as 18S, 16S rRNA sequences of *T. ramosus* were analyzed and compared with similar species in NCBI. Using 18S sequence data, it is proven that *T. ramosus* is a distinct and valid species, however, interestingly the 16S rRNA forms clades with other species of the same genera (*T. fruticosus* and *T. bedoti*) rather than the same species. Moreover the *mt*COI forms a different clade with other genera. Furthermore, these data may enhance the advancement of identification in non-monophyletic conditions.

Keywords: Distribution, molecular, morphology, Palk Bay, *Thyroscyphus ramosus*.

இந்திய துணைக் கண்டத்தில் உள்ள ஆல்கா ஹைட்ராய்டாகிய தைரோசிபஸ் ரமோசஸ், மிகவும் எளிதில் அடையாளம் காணக்கூடிய சதைப்பற்றுள்ள காலனித்துவ ஹைட்ராய்டு ஆகும். இவை கடலின் அடிப்பரப்பிலுள்ள உயிரனங்களிடையே மிக முக்கிய பங்கு வகிக்கிறது. இவை இந்தியாவில் ஏராளமாக இருந்தபோதிலும், கடந்த தொண்ணூறு ஆண்டுகளுக்கு மேலாக இந்த உயிரனத்தை பற்றி அறியப்படாமலேயே இருந்தது. தற்போதைய ஆய்வு தைரோசிபஸ் ரமோசஸின் உருவவியல், சூழலியல் மற்றும் புவியியல் இடங்கள் போன்ற விரிவான அறிக்கையை வழங்குகிறது. உருவவியல் பண்புகளான அதிகபட்ச உயரம், இனப்பெருக்கம் மற்றும் உடல்தண்டின் திருப்ப திசைகள் விரிவாக ஆய்வு செய்யப்பட்டன. பால்க் விரிகுடாவில் அதிக அளவில் உள்ள இவை உயிர் மூலக்கூறு வகைப்பாட்டில் மூலமாக, தைரோசிபஸ் ரமோசஸ்தான் என்று மேலும் உறுதிசெய்யப்படுகிறது. தைரோசிபஸ் ரமோசஸில் சிற்றினத்தை கண்டறியக்கூடிய 18S, 16S ரிபோசோம் ஆர்என்ஏ மூலக்கூறு வரிசைகள் பகுப்பாய்வு செய்யப்பட்டு, NCBI இல் இதே போன்ற உயிரினங்களுடன் ஒப்பிடுகையில், 18 எஸ் ஆர்என்ஏ முடிவுகள் டி. ரமோசஸ் என்பதை நிரூபிக்கும்படியாக அமைந்தது. மேலும் சுவாரஸ்யமாக, டி..ரமோசஸ் 16 எஸ் ஆர்ஆர்என்ஏ அதே இனத்தின் (டி. ∴ப்ரூட்டிகோசஸ் மற்றும் டி. பெடோட்டி) பிற சிற்றினங்களுடன் ஒத்தவையாக உள்ளது. மேலும் mtCOI வகைப்பாட்டில் மற்ற வகை வேறுபட்ட ஹைட்ராய்டு சிற்றினங்களுடன் ஒத்தவையாக உள்ளது.. இந்த தரவு மோனோபிலெடிக் அல்லாத நிலையில் இனம்கண்டறிதலில் முன்னேற்றத்தை மேம்படுத்தக்கூடும்.

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Author contribution: GA & RR designed the experiments and analyzed the data; GA performed the sampling; KK & GA associated the experiments; GA, KK & RR wrote the paper.

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INTRODUCTION

Palk Bay on the southeastern coast of India covers ≈296km of coastline and up to 15m depth range considered as a backbone of productivity which supports a wide variety of fauna and flora. Palk Bay is known for its rich marine biodiversity which comprises: 302 marine algae, 51 Foraminifera, 12 tintinnids, 143 flora, 275 sponges, 123 non-coral coelenterates, 128 stony corals, 100 Polyzoa, 75 Polychaeta, 651 Crustacea, 733 Mollusca, 274 Echinodermata, 66 Prochordata, 580 fishes, five turtles, 61 birds, and 11 mammals (Kasim 2015). Palk Bay has a sandy rubble bottom, a shelf region that has a maximum temperature range of 26–28°C, and consists of intense upwelling regions (Kumaraguru et al. 2008). The class Hydrozoa has the largest number of species under the phylum Cnidaria. They are renowned for familiar forms of benthic, pelagic, and combined life cycle stages (Bouillon et al. 2006). Their biomass and life cycle stages are the indicator for food abundance and upwelling regions in the water column (Boero et al. 2008). These omnipresent voracious carnivore hydrozoans are one of the common bio-fouling components. These predators consume larvae of fishes, crustaceans, plankton, and benthic organisms, whereas some hydrozoan species directly consume dissolved organic matter and nutrients (Collins et al. 2006; Di Camillo et al. 2017). These voracious benthic feeders are involved as members in the energy transformation cycle, in the upwelling regions. It is considered so based on their mass and richness (Orejas et al. 2000). *Thyroscyphus ramosus* is one of the widely reported species in the Caribbean region (Germerden-Hoogeven Van 1965; Galea 2008) and regions of southern and western Atlantic coast (Allman 1888; Vervoort 1959; Winston 1982, 2009; Migotto et al. 1993), Mexican Gulf (Calder & Cairns 2009), Brazil (Shimabukuro & Marques 2006), South Africa (Warren 1907), and the Indian Ocean (Leloup 1932). The diversity of the genus *Thyroscyphus* were previously reported from the subtidal zone, at 1m depth (Kelmo & Vargas 2002) and in Cuba the species was reported to a maximum of 183–457 m depth (Nutting 1915). This species is associated with many biotic and abiotic forms and acts as a host for many organisms like other hydroids and sponges. The size ranges from 3cm to 25cm (Kelmo & Vargas 2002) during all the seasons in the breakwater region (Winston 1982). The distribution and composition of marine species, extending their geographical locations based on the suitable climate and environmental changes to survive and maintain their live forms (Hughes et al. 2000). Most research contributions were focused on commercially valuable groups rather than the inconspicuous non-commercial value benthic communities (González-Duarte et al. 2014).

In the marine ecosystem, the morphological similarities of the species and confusions in identification are resolved through DNA barcoding (Moura et al. 2008). This hampering was resolved with genetic analysis (Trivedi et al. 2016). Several gene regions, such as 16S, 18S, 28S, mtCOI and internal transcribed spacer 1 (ITS1), however, were employed to reveal their taxonomic relationships (Schierwater & Ender 2000; Collins et al. 2005; Govindarajan et al. 2006; Schuchert 2014). Mammen (1963, 1965a,b) contributed taxonomic information on c. 126 species of hydroids from southern India. Among hydroids, the genus *Thyroscyphus* is a large fleshy benthic hydroid colony that is easily visible underwater. F.H. Gravely (1927) recorded *Thyroscyphus junces* from the Pamban bridge and chank bed area. Hora (1925) collected three smaller colonies of *Thyroscyphus ramosus* (3cm size) from Shingle Island, Gulf of Mannar. Till date, this is the only known record of this genus from India. In this present study, year round abundance of *Thyroscyphus ramosus* at Rameshwaram coast, Palk Bay, Gulf of Mannar region is documented. The cryptic behavior, distribution information, ecology, habitat, and phylogenetic relationships of the hydroid species are still lacking, particularly in India. The main objective of this study is to re-describe the species and conduct a preliminary assessment of their phylogenetic relationships using morphological observations, 18S rRNA, 16S rRNA, and mtCOI gene of this species.

MATERIAL AND METHODS

Hydroid specimens were collected at Olakuda lighthouse area, Rameshwaram coast, Palk Bay (9.320188°N 79.340040° E) Gulf of Mannar region, Tamil Nadu, India, from September 2016 to September 2017 by snorkeling from shoreline up to 5m depth and as bycatch obtained from crab nets operated at 5–15 m (Figure 1). The collected hydroid specimen colonies were photographed before fixing in 4% neutralized formaldehyde solution to observe the color and morphological traits to avoid post preservation changes (Hissmann 2005; Di Camillo et al. 2010). Part of the whole colony was preserved in 99% ethanol for genetic studies (Nikulina et al. 2013; Maggioni et al. 2016). The diagrammatic details of the colony were obtained using a light microscope and morphological traits were also examined using ΣIGMA-Zeiss-Scanning Electron

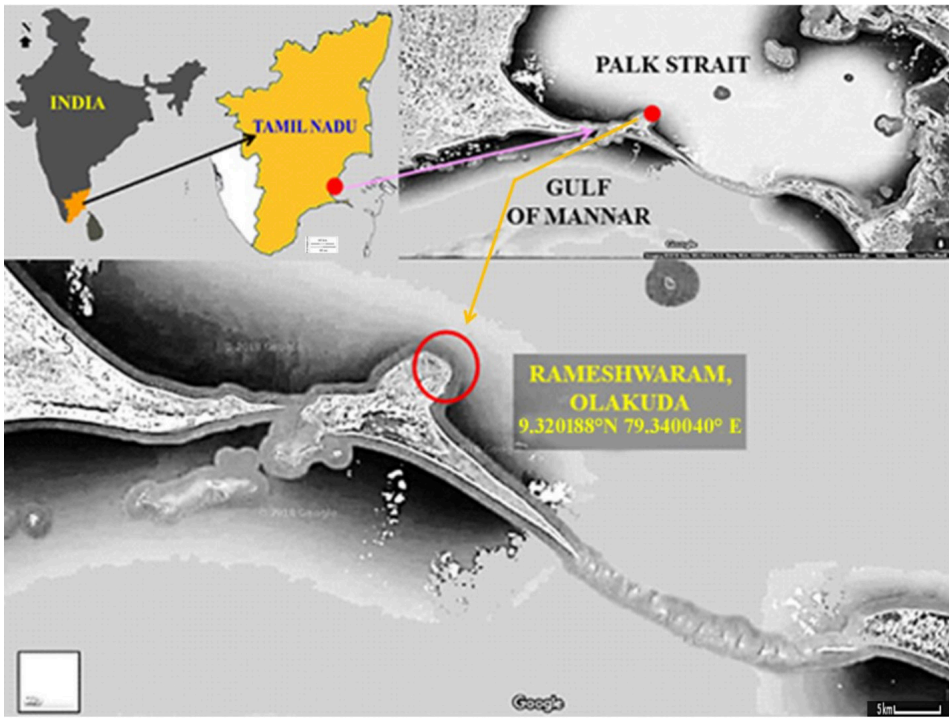

Figure 1. Sampling sites, location of the studied Rameshwaram lighthouse, Palk Bay (Gulf of Mannar, Tamil Nadu, India).

Microscopy.

Samples were identified using pictorial keys (Allman 1877; Winston 1982; Shimabukuro & Marques 2006; Calder & Cairns 2009), and online identification/ literature available in the WoRMS database (Schuchert 2018). Voucher specimen samples were submitted at the museum in the marine science department, Bharathidasan University, Marine Genomics and Barcoding Lab (MGBL) and obtained the specimen code (DMS-RR-HTR1-GoM-2016). The colonies were examined for the presence of gonophores in order to evaluate the period of sexual reproduction. The specimens were fixed with seawater and glutaraldehyde buffer for scanning electron microscopic (SEM) investigation (Di Camillo et al. 2012).

Sequencing genetic regions

The total genomic DNA was extracted in 99% ethanol preserved hydrozoa sample, following a modified protocol (Sambrook et al. 1989) from the ethanol-fixed specimen, by CAGL extraction protocol using Qiagen kit (Mandal et al. 2014). 0.7% agarose gel along with 1Kb DNA ladder was used to assess the quality of obtained DNA and their quality was estimated using a Biophotometer (Eppendorf). Universal Forward & Reverse primers, amplification of 16SrRNA gene 18SrRNA gene and COI gene were carried out and 2% agarose gel along with 100bp DNA ladder were used to confirm the PCR-generated amplicons. The amplified product was subjected to purification using the GeneJET PCR purification kit (Thermo Scientific, EU-Lithuania) in order to remove the primer-dimer and other contaminations. The acquired PCR products were subjected to sequencing using universal primers. For partial 16S rRNA (Forward primer: 5'- CGCCTGTTTATCAAAAACAT-3' and Reverse primer: 5'- GGTTTGAACTCAGATCATGT-3'), for partial 18S rRNA (Forward primer: 5'- CAGCAGCCGCGGTAATTCC-3' and Reverse primer: 5'- CCCGTGTTGAGTCAAATTAAGC -3'), for partial COI gene (Forward primer: 5'- GGTCAACAAATCATAAAGATATTGG -3' and Reverse primer: 5'- TAAACTTCAGGGTGACCAAAAAATCA -3') in forward and reverse directions using Genetic Analyzer 3500 using CAGL standardized protocol for genetic analysis of the hydrozoa species (Mandal et al. 2014). We prepared the dataset from submitted sequences in NCBI and similar sequence from NCBI-BLAST (Basic Local Alignment Searching Tool). The multiple sequence alignment was performed using Clustal X 2.0 and sequence-based evolutionary tree was performed using MEGA 7 (Tamura et al. 2013) for the estimation of genetic variations among the obtained clades of the separate molecular locus.

RESULTS AND DISCUSSION

Kingdom Animalia
Phylum Cnidaria Verrill, 1865
Class Hydrozoa Owen, 1843
Subclass Hydroidolina Collins, 2000
Order Leptothecata Cornelius, 1992
Superfamily Sertularioidea Lamouroux, 1812
Family Thyroscyphidae Stechow, 1920
Genus Thyroscyphus Allman, 1877
***Thyroscyphus ramosus* Allman, 1877**

Species natural history

The colony is transparent, pale yellow in color, smooth outer wall reaches a maximum height from hydrorhyza to tip of hydrocaulus 43.5cm without gonotheca and 24cm with gonophore. Stolen are webbed and entwined tightly with the substrates. Among the total 13 hydrorhyza two are infertile hydrorhyza (Figure 2A). Alternate Polysiphonic hydrocaulus from the hydrorhyza divided with regular intervals after every two hydrothecal pedicle internodes with a slight bent on the left and right alternative of oblique nodes (Figure 2B). Branches 8–34 with length variations were noted, smaller in upper and lower, larger branch in the middle of hydrorhyza. The branch length 3.2cm to a maximum of 8.4cm. The straight basal bottom becomes slender and crooked. Length of unfertile colony tube 1.4cm (Figure 2F). In a fertile colony after 1.8cm the distal apophysis with pedicellate hydrotheca observed distal alternate sides of entire hydrorhyza with regular distance. The supporting apophysis wider. Pedicle spirally twisted alternately (right pedicle twisted clockwise, left pedicles twisted anti-clockwise) ridged and shorter carrying hydrotheca at the upper end of the thick annulus (Figure 2D). Pedicle and hydrotheca joints distinctive (Figure 2C). Hydrotheca base larger than pedicle and cylindrical bottom and the top oblique have thick marginal ring and above the margin four blended cusps (Figure 2E). The lower side of hydrotheca distally straight and aboral side

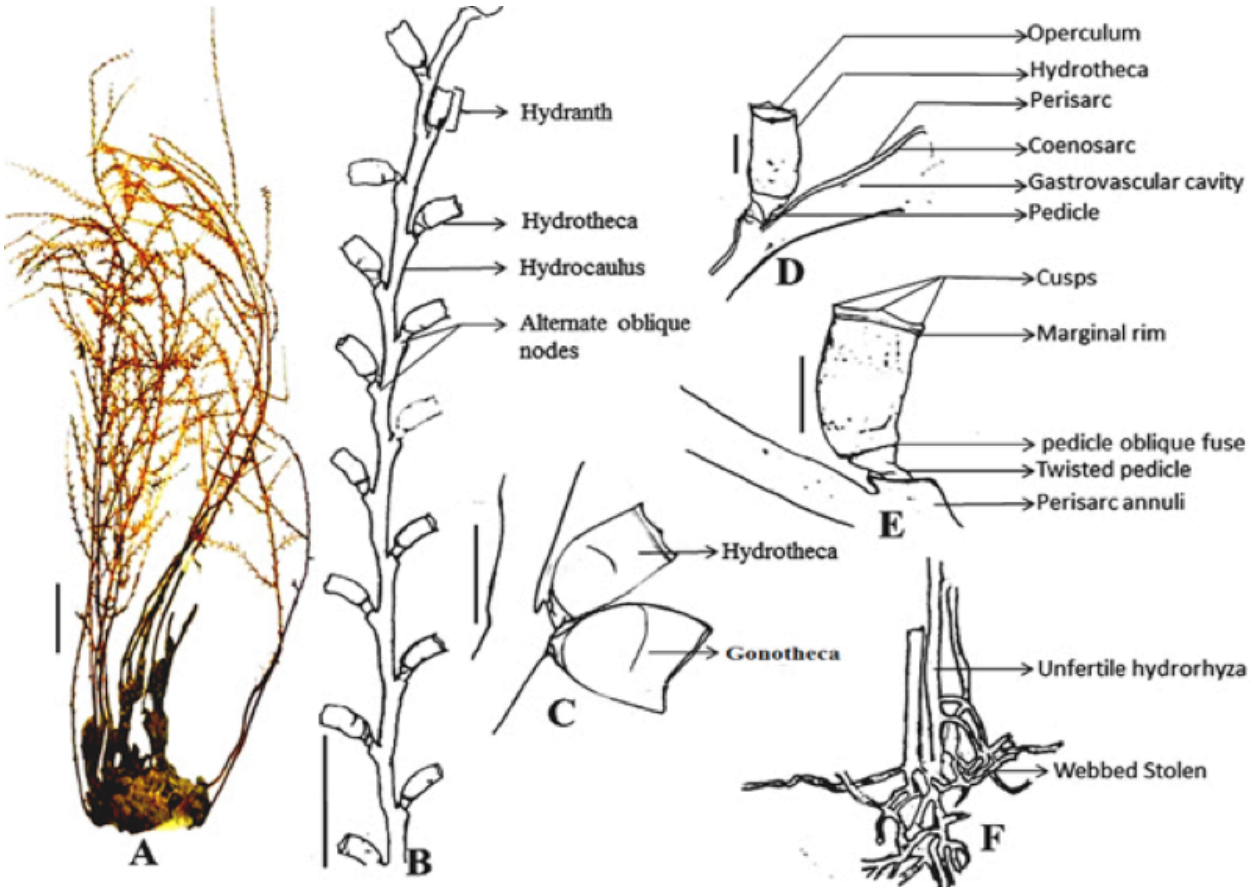

Figure 2. A—*Thyroscyphus ramosus*, specimen arise from webbed stolen with 13 hydrorhyza, 2 unfertiled included, branches upward, alternate, DMS-HA-Tr-Hap-01 | B—Hydrocaulus with15 hydrotheca | C—Arrangement of the hydrotheca and gonotheca on the pedicle stem | D—Parts of hydranth and hydracaulus | E— Cusps on margins of hydrotheca and the twisted pedicle | F—Unfertile hydrorhyza araising from the stolen. Scale bars: A—2 cm | B, D, E—0.3mm | C—0.5mm | F—1cm.

Image 1. A—*Thyroscyphus ramosus*. Colony arises on subtidal reef | B—Hydrocaulus of hydrorhyza with hydrotheca and gonotheca | C—Hydrocaulus with four alternate branched hydrotheca | D—Twisted pedicle with hydrotheca on perisarc annuli | E—hydrotheca and gonotheca arrangement, marginal rim, marginal cusps | F—Webbed stolen with unfertile tubular hydrorhyza. Scale bars: (B) 0.558mm; (C, F) 0.5mm; (D) 100mm; (E) 0.153mm.

slightly convex, basal wall thick, annulus and concave on pedicle joint. Hydrotheca asymmetrical, alternate, thick and oblique wall, and gonotheca rise beneath. Gonotheca conical shaped, situated beneath hydrotheca or on stem, larger and thin perisarc than hydrotheca. Gonothecal pedicle is shorter than hydrothecal pedicle, annulus thicker on the joint to gonothecal base. The gonothecal rim is thick and oblique marginal equidistant on opening. Some are conspicuously funnel-shaped. Measurements of hydrocaulus length between hydranths 1.156–2.983 mm of internode 225µm diameter, at node 356µm, 0–4 pedicel annulations. Hydrotheca length maximum 578µm, marginal cusp height 38–56 µm apophysis length 180–257 µm diameter, 369µm at rim maximal diameter. Gonotheca maximum 643µm length, 475µm on mouth, wider on middle 597µm maximal diameter, marginal ring 26µm height, pedicle 71µm on the aboral side (Image 1). The SEM images show the specimen characteristics of the skeleton and their actual thickness and the parts were clear in the image (Image 2).

The species were collected and described 91 years ago, from Shingle Island, Gulf of Mannar, India by Hora (1925). Morphology was distinguished by four cusps on the hydrotheca marginal ring with a single operculum. Length of the colony 3m to 24cm, with and without gonotheca was recorded. In this present study, the maximum of 43.5cm without gonophore and 24cm with

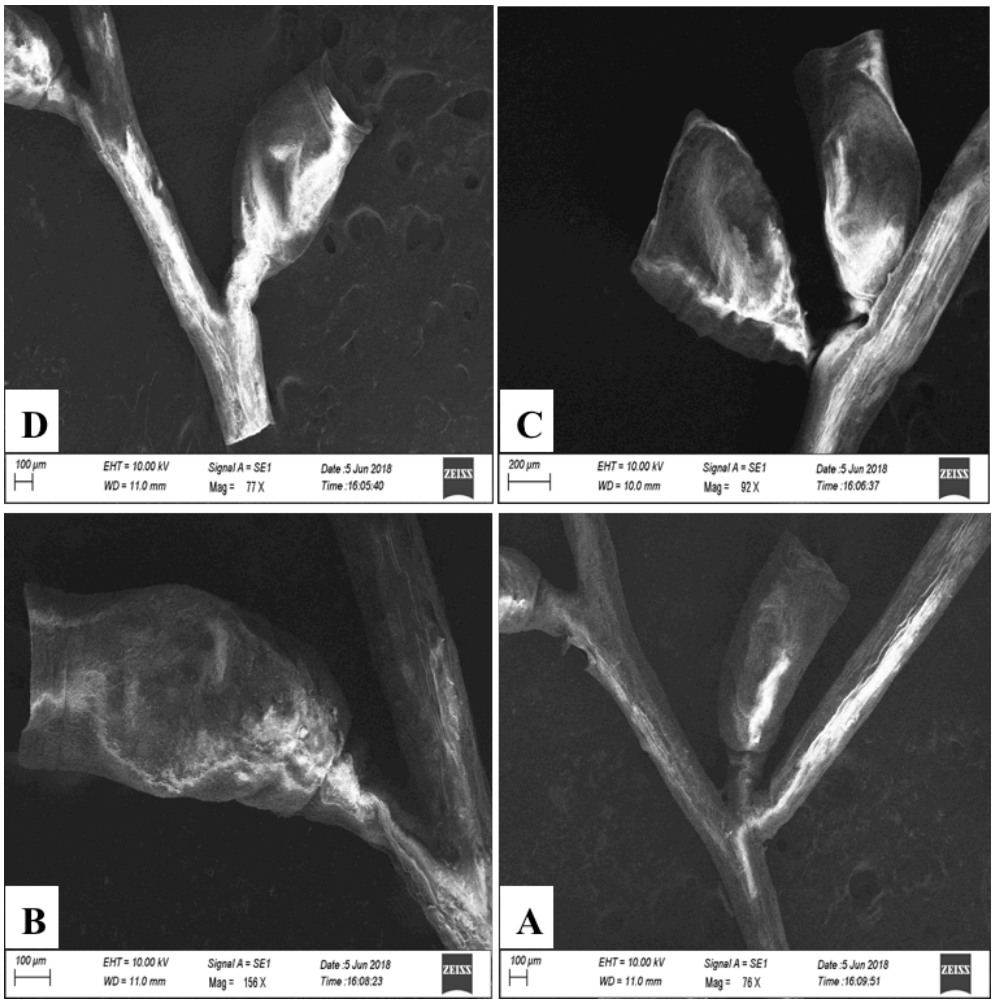

Image 2. A—Lateral view of hydrotheca marginal ring and cusps with widen hydrorhyza | B—Lateral view of one gonotheca on lower side of hydrotheca | C—Detail of hydrotheca apex, operculum, twisted pedicle | D—Internal projections of perisarc located between the branched hydrocaulus. Scale bars: (A, C, D) 100µm; (B) 200µm.

gonophore collected. In the earlier studies of the species from Shingle Island, Gulf of Mannar only 3cm, without gonophore (Leloup 1932; Migotto & Vervoort 1996) was recorded. After Winston's (1982), observation at Fort Pierce, Florida, North Beach breakwater, the year-round abundance of this species was recorded only in Palk Bay, Olakuda lighthouse region.

Ecology

The colonies occur in areas with strong current. This species grows on substratum such as sponges, shells of bivalves, on the sides of coral rock, and the sea surface covered with sandy rubbles also in vertical walls and surf zones. Occurs in shallow areas to a maximum depth of 457m.

Phylogenetic analysis (Graphical representation)

We constructed the phylogenetic tree using the neighbor-joining algorithm with 1,000 bootstrap replicates to identify the origin and replication of *Thyroscyphus ramosus* for 18S rRNA, 16S rRNA and mtCOI gene (Saitou & Nei 1987). The sequence-based evolutionary tree was constructed using MEGA 7.0, (Kumar et al. 2016) with bootstrap values of >50% numbered at the nodes. For the targeted sequence of *T. ramosus* 18S rRNA, 16S rRNA, species sequence from genus *Halecium* was used as outgroup and for the mtCOI gene *Scopalina ruetzleri* UCMPWC992 was used as the out-group due to the unavailability of sequence from the genus Halecium.

From the result of 18S rRNA gene-based tree was separated into two major clades from the out-group lineage of *Halecium labrosum* MHNG INVE29030. Our

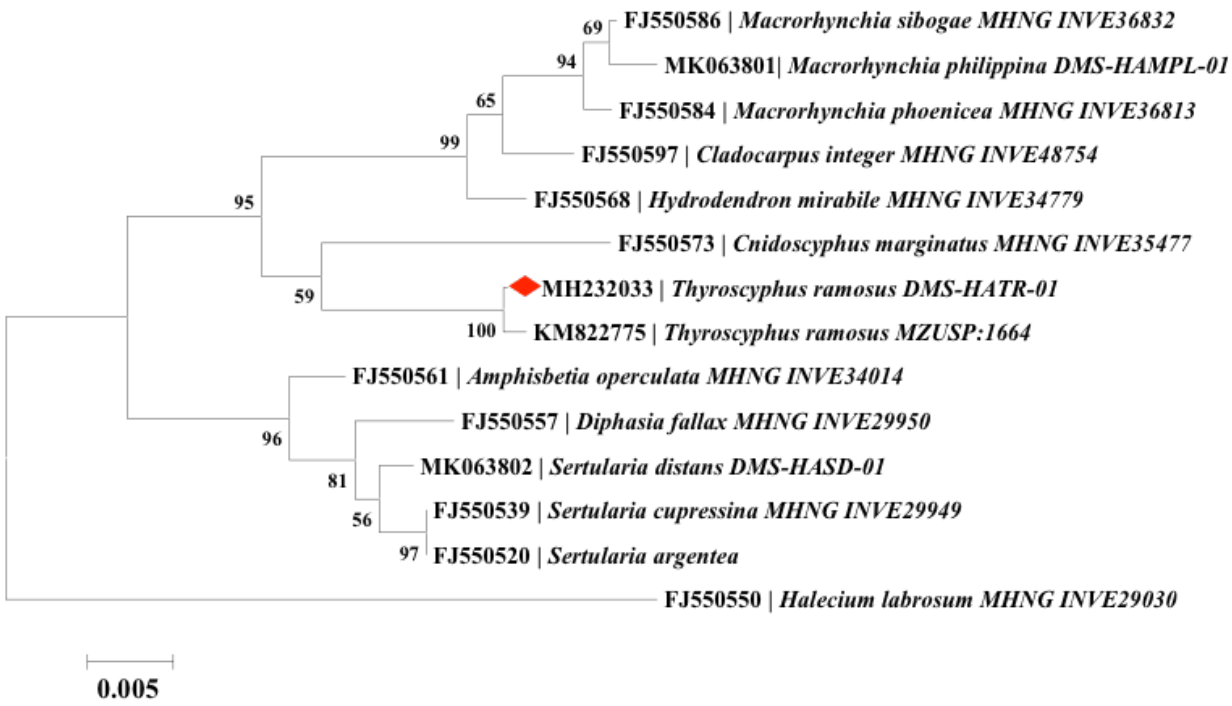

Figure 3. Two dimensional graphical representation of 18S rRNA based phylogenetic tree of *Thyroscyphus ramosus* (Red colour diamond indicates our target species), Numbers at nodes are bootstrap value >50% (*Halecium labrosum* MHNG INVE29030 used as an out group). Bar-0.005 substitutions per nucleotide position.

target species *Thyroscyphus ramosus* DMS-HATR-01 is highly supported with maximum bootstrap value to another specimen of the same species *Thyroscyphus ramosus* MZUSP:1664. The closely related second clade was formed with *Cnidoscyphus marginatus* MHNG INVE35477, which genus was accepted as *Thyroscyphus marginatus* (Allman 1877). Other minor supported clades of the *Hydrodendron mirabile* MHNG INVE34779, *Cladocarpus integer* MHNG INVE48754, *Macrorhynchia phoenicea* MHNG INVE36813, *Macrorhynchia philippina* DMS-HAMPL-01 and *Macrorhynchia sibogae* MHNG INVE36832, species of superfamily Plumularioidea. Second major clade consists of *Amphisbetia operculata* MHNG INVE34014, *Diphasiafallax* MHNG INVE29950, *Sertularia distans* DMS-HASD-01, *Sertularia cupressina* MHNG INVE29949, and *Sertularia argentea* are grouped with each other (Figure 3).

The result of the 16S rRNA gene-based tree was separated into two major clades from the out-group lineage of *Halecium mediterraneum* DNA122. The targeted species clade of *Thyroscyphus ramosus* DMS-HATR-02 highly supported with another specimen of the same genus *T. bedoti* MAL09-048, *T. fruticosus* DNA1250, *T. marginatus* bth.15.89 and *T. fruticosus* REU13-002 with maximum bootstrap value. Another major clade consists of *Sertularella ellisii* DNA1237, *S. mediterranea* MHNG INVE32948, *S. polyzonias* DNA1236, *S. ellisii* MHNG INVE32156, *S. africana* MHNG INVE34017, *S. gayi, S. simplex* MHNG-HYD-DNA1135, *S. sanmatiasensis, S. rugosa* MHNG INVE29032. Interestingly the same species of other strain *Thyroscyphus fruticosus* REU13-002 was In the closest clade and also in the nearest common ancestral clade, similar to the clades of *Sertularella ellisii* DNA1237 and *S. ellisii* MHNG INVE3215 may be originated from various species of *Sertularella* genus (Figure 4).

The result of mtCOI gene-based tree was separated into many sub-clades. The target species *Thyroscyphus ramosus* DMS-HA-Tr-Hap-01 was formed from the separate sub-clade from the same genus of the other species. The *Nanomiacara* Naca53 clade form as the ancestral for all above-mentioned sequences *and the Scopalina ruetzleri* UCMPWC992 act as an out-group for the constructed phylogenetic tree (Figure 5). This is the first report from an Asian country on 16S rRNA analysis and *mt*CO1 gene sequence of *Thyroscyphus ramosus* in the biological database. So, the identified phylogenetic neighbor organisms may act as a reference to our target organism. In future, the reported sequences may use as a reference data to our target species.

Table 1. Pairwise genetic distance was computed for 18S rRNA gene based phylogenetic related species of Thyroscyphus ramosus.

Organism	Access no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
*Thyroscyphus ramosus**	MH232033														
Thyroscyphus ramosus	KM822775	0.002													
Hydrodendron mirabile	FJ550568	0.026	0.027												
Cnidoscyphus marginatus	FJ550573	0.027	0.029	0.043											
Cladocarpus integer	FJ550597	0.028	0.029	0.008	0.041										
Macrorhynchia sibogae	FJ550586	0.033	0.033	0.014	0.042	0.013									
Macrorhynchia phoenicea	FJ550584	0.033	0.033	0.014	0.039	0.010	0.003								
Macrorhynchia philippina	MK063801	0.033	0.033	0.014	0.044	0.013	0.003	0.006							
Sertularia distans	MKo63802	0.037	0.039	0.037	0.046	0.042	0.044	0.044	0.048						
Diphasia fallax	FJ550557	0.038	0.039	0.044	0.046	0.046	0.049	0.049	0.053	0.010					
Amphisbetia operculata	FJ550561	0.039	0.041	0.039	0.044	0.041	0.042	0.042	0.046	0.010	0.014				
Sertularia cupressina	FJ550539	0.039	0.041	0.039	0.044	0.044	0.046	0.046	0.049	0.005	0.010	0.011			
Sertularia argentea	FJ550520	0.039	0.041	0.039	0.044	0.044	0.046	0.046	0.049	0.005	0.010	0.011	0.000		
Halopteris carinata	KT722401	0.039	0.037	0.034	0.049	0.039	0.036	0.039	0.039	0.041	0.046	0.036	0.042	0.042	
Halecium labrosum	FJ550550	0.072	0.072	0.067	0.070	0.072	0.070	0.074	0.074	0.063	0.062	0.055	0.065	0.065	0.062

*Target species

Table 2. Pairwise genetic distance was computed for 16S rRNA gene based phylogenetic related species of *Thyroscyphus ramosus*.

Organism	Access no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
*Thyroscyphus ramosus**	MH392732														
Thyroscyphus bedoti	MH108450	0.008													
Thyroscyphus fruticosus	MG811643	0.015	0.019												
Thyroscyphus fruticosus	MG108467	0.098	0.096	0.091											
Sertularella gayi	AM888340	0.116	0.114	0.116	0.127										
Sertularella ellisii	MG811636	0.120	0.120	0.123	0.124	0.041									
Sertularella polyzonias	MG811635	0.132	0.129	0.134	0.136	0.037	0.019								
Sertularella simplex	KX355446	0.125	0.123	0.129	0.131	0.023	0.029	0.035							
Sertularella sanmatiasensis	FN424141	0.125	0.123	0.130	0.144	0.039	0.039	0.045	0.031						
Sertularellala genoides	FJ550478	0.122	0.120	0.127	0.131	0.037	0.017	0.021	0.017	0.039					
Sertularella africana	FJ550490	0.134	0.132	0.138	0.128	0.039	0.033	0.035	0.021	0.047	0.031				
Sertularella mediterranea	FJ550479	0.124	0.122	0.127	0.135	0.039	0.017	0.023	0.031	0.043	0.021	0.029			
Thyroscyphus marginatus	MH361368	0.118	0.114	0.116	0.117	0.131	0.131	0.143	0.126	0.122	0.135	0.138	0.140		
Sertularella rugosa	AY787906	0.125	0.122	0.129	0.138	0.037	0.045	0.045	0.031	0.027	0.039	0.039	0.045	0.122	
Halecium mediterraneum	MG811603	0.147	0.149	0.154	0.147	0.108	0.097	0.104	0.104	0.108	0.101	0.100	0.108	0.161	0.112

*Target species

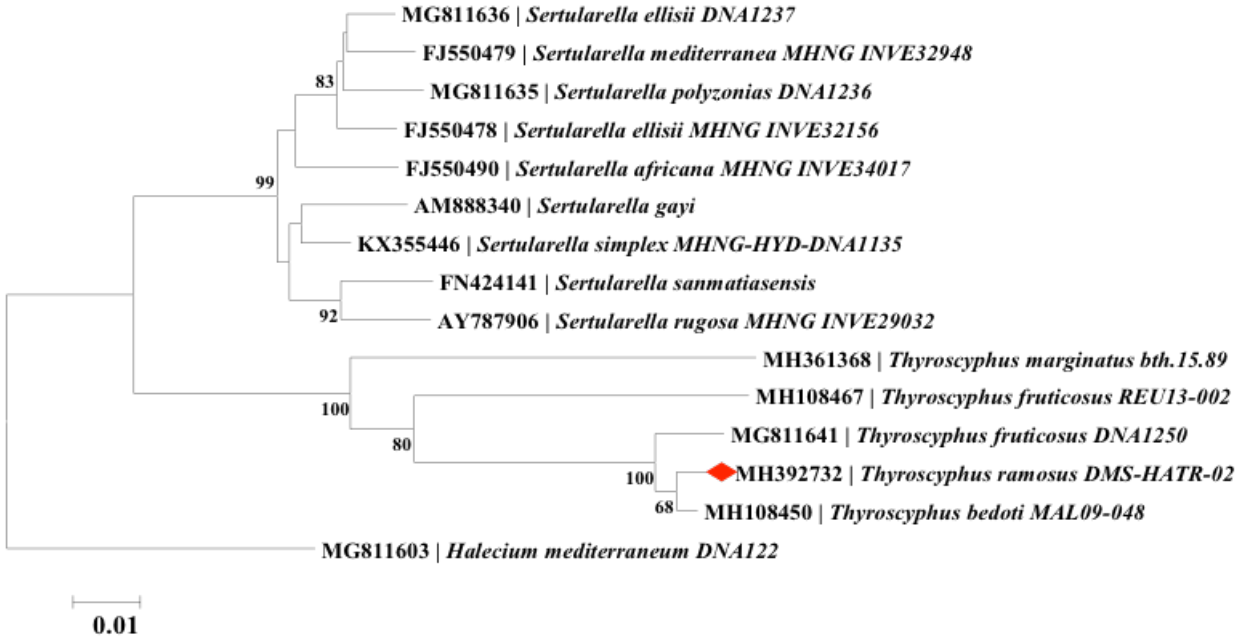

Figure 4. Two dimensional graphical representation of 16S rRNA based phylogenetic tree of *Thyroscyphus ramosus* (Red colour diamond indicates our target species). Numbers at nodes are bootstrap value >50% (*Halecium mediterraneum* DNA122). Bar- 0.01 substitutions per nucleotide position.

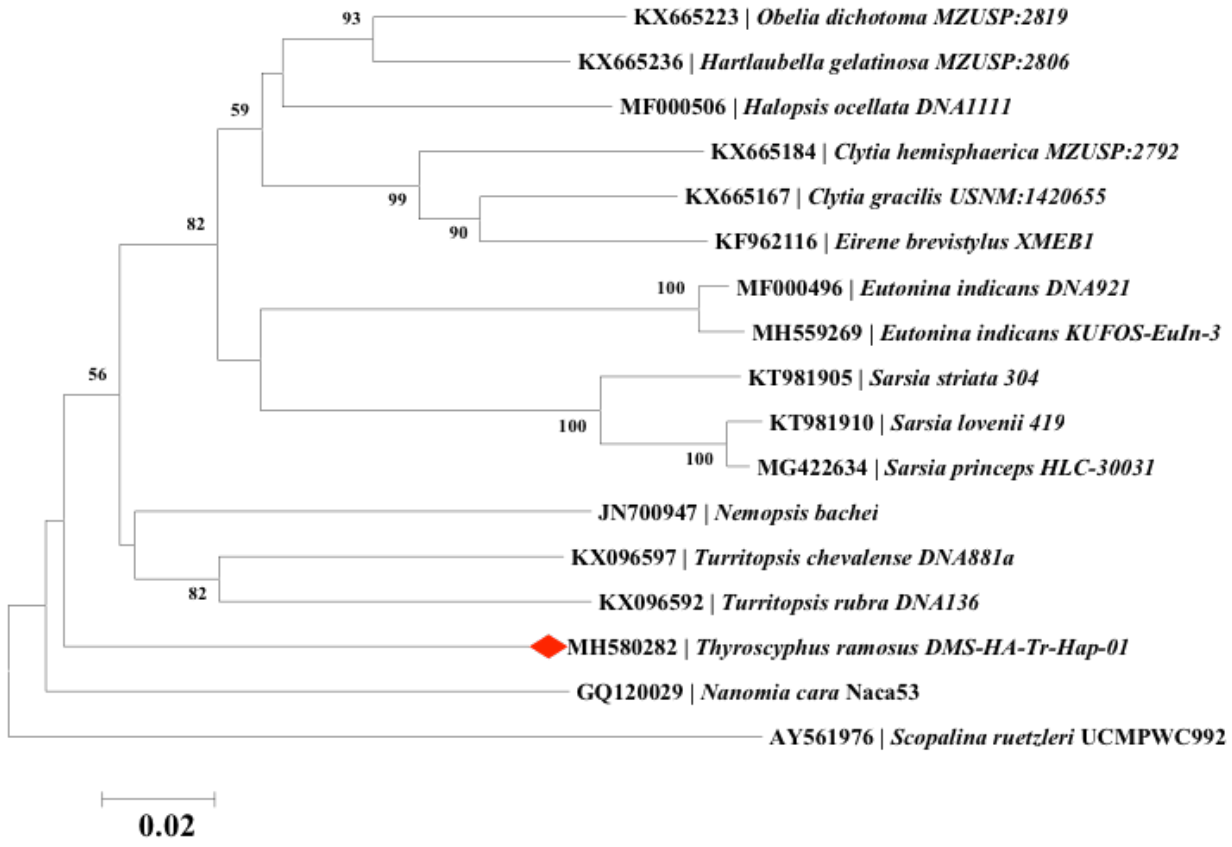

Figure 5. Two dimensional graphical representation of *mt*COI gene based phylogenetic tree of *Thyroscyphus ramosus* (Red colour diamond indicates our target species). Numbers at nodes are bootstrap value >50% (*Scopalina ruetzleri* UCMPWC992). Bar- 0.02 substitutions per nucleotide position.

Table 3. Pairwise genetic distance was computed for mtCOI gene based phylogenetic related species of Thyroscyphus ramosus.

Organism	Access no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Nemopsis bachei	JN700947																	
Sarsia striata	KT981905	0.172																
Turritopsis chevalense	KX096597	0.180	0.192															
Obelia dichotoma	KX665223	0.165	0.159	0.180														
Hartlaubella gelatinosa	KX665236	0.196	0.185	0.153	0.168													
Sarsia lovenii	KT981910	0.176	0.177	0.174	0.166	0.080												
Dendrogramma	KU716054	0.188	0.209	0.053	0.176	0.163	0.176											
Nanomiacara	GQ120029	0.199	0.195	0.178	0.189	0.168	0.172	0.172										
Halopsis ocellata	MF000506	0.182	0.191	0.230	0.181	0.192	0.177	0.232	0.187									
Clytia gracilis	KX665167	0.198	0.186	0.165	0.173	0.114	0.115	0.163	0.146	0.184								
Eutonina indicans	MF000496	0.206	0.186	0.196	0.196	0.122	0.112	0.196	0.179	0.195	0.143							
Sarsia princeps	MG422634	0.201	0.189	0.164	0.182	0.158	0.142	0.177	0.182	0.227	0.153	0.167						
Scopalina ruetzleri	AY561976	0.186	0.204	0.052	0.172	0.161	0.174	0.010	0.174	0.230	0.157	0.192	0.176					
Eirene brevistylus	KF962116	0.223	0.236	0.267	0.232	0.249	0.214	0.272	0.260	0.234	0.223	0.241	0.265	0.269				
Eutonina indicans	MH559269	0.208	0.185	0.189	0.204	0.136	0.124	0.198	0.168	0.204	0.151	0.075	0.163	0.198	0.257			
Nemopsis bachei	JN700947	0.203	0.184	0.166	0.182	0.162	0.148	0.178	0.188	0.234	0.155	0.171	0.013	0.178	0.269	0.167		
Sarsia striata	KT981905	0.206	0.184	0.206	0.194	0.149	0.134	0.194	0.180	0.189	0.142	0.100	0.167	0.196	0.250	0.098	0.165	
Turritopsis chevalense	KX096597	0.190	0.160	0.188	0.127	0.168	0.161	0.192	0.173	0.182	0.163	0.176	0.189	0.190	0.254	0.179	0.198	0.182

*Target species

Pairwise genetic distance (statistical representation)

We inferred our result with the second approach using pairwise distance (statistical data). From the result of genetic diversity of 18S rRNA, 16S rRNA and mtCOI gene were identified in the pairwise distance range between (0.0–0.074) in 18S rRNA (shown in Table 1). It reveals that no phylogenetic variation may occur in the 18S rRNA gene whereas, 16S rRNA gene, the distance arises in between the range of (0.008–0.154) and for mtCOI gene (0.052–0.272) (as shown in Tables 2 & 3). This slight genetic variation exposed in both 16S rRNA and the mtCOI gene. Even if the genes and species are different, no higher genetic variation originated from our results; this is due to the similarity between the sequence and its family.

CONCLUSION

The region in Palk Bay supports the highly diverse and abundant benthic Algal Hydroid *T. ramosus*. In places like Fort Pierce, Florida, North Beach breakwater, the species are observed year-round due to favorable environmental conditions. The abundant distribution is due to complex reasons such as nutrient availability, littoral topography and suitable conditions for their production and survival. To preserve biodiversity of the benthic indicator species, stringent environmental management practices have to be implemented in this area.

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