

## New host and geographical record of *Eudactylina pusilla* Cressey, 1967 from Indian waters with DNA barcodes

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### Manuscript history

Received 11 January 2019 | Revised 14 June 2019 | Accepted 15 June 2019 | Published online 20 June 2019

### Citation

Pradeep HD, Venu S, Kumar RR, Sumitha G, Bineesh KK and Farejiya MK (2019) New host and geographical record of *Eudactylina pusilla* Cressey, 1967 from Indian waters with DNA barcodes. *Journal of Fisheries* 7(2): 700–705.

### Abstract

The present paper reports the first record of the parasite *Eudactylina pusilla* Cressey, 1967 from the gills of the pelagic thresher shark, *Alopias pelagicus* Nakamura, 1935 collected during a multifilament longline operation at a depth of 762 m from Indian EEZ around Andaman Islands. The occurrence of this copepod gill parasite on *A. pelagicus* in the Indian waters constitutes new host record and extends the parasite's known geographical distribution, thus contributing to the knowledge of biodiversity of the parasitic copepods in Indian waters. Molecular marker based taxonomical annotation using Mitochondrial 18S r DNA sequencing also confirmed the identity of the *E. pusilla* specimen.

**Keywords:** First record; *Eudactylina pusilla*; pelagic thresher shark; Siphonostomatoida; gill parasites

## 1 | INTRODUCTION

Andaman and Nicobar Islands is one of the largest oceanic archipelago systems and is located between 6–14° north latitude and 92–94° east longitude in southern reaches of the Indian Ocean. It forms about 28% of the Indian Exclusive Economic Zone (EEZ, 0.6 million km<sup>2</sup>) (Anon 2013). Parasites are usually found in wild as well as cultured marine fishes externally as well as internally (skin, nostrils, fins, gills, flesh, intestinal, rectal etc.) and

the gills are found to be most favourite site for attachment of many parasites (Nowak 2007; Montesa *et al.* 2017). Among marine parasites 25% are crustaceans (Erias *et al.* 2000). These parasites damage the gill by feeding on the delicate tissue of the lamellae or the blood circulating within the lamellae, leading to the loss of respiratory surface area (Pillai 1985; Morales-Serna *et al.* 2014). There are several studies on fish parasites from the coast of mainland India (e.g. Pillai 1985; Ravichandran *et al.* 2009; Helna *et al.* 2012; Aneesh *et al.* 2013; Ramesh Ku-

mar and Ravichandran (2013). However, detailed knowledge of parasites and the fish infested by them in the Andaman Sea is very limited to only a few studies reporting fishes infested mainly with isopods, copepods, monogenes, trematode, cestodes and nematodes (Watchariya *et al.* 2009; Jithin *et al.* 2016; Pradeep *et al.* 2016–2019; Nashad *et al.* 2018).

Elasmobranchs are widely distributed in tropical and subtropical seas, in coastal areas, estuaries, usually near the bottom of the shallow freshwater creeks and coastal lagoons (Compagno 1984). Sharks are apex predators (Randhawa and Poulin 2010) and provide habitat for a variety of parasitic fauna (Caria 1990; Caira and Healy 2004; Caira *et al.* 2005; Randhawa and Poulin 2010; Palm 2011). The pelagic thresher sharks (*Alopias pelagicus* Nakamura, 1935) are large lamniform sharks of the family Alopiidae. They are found in all temperate and tropical oceans of the world. This species has been listed as Vulnerable to Extinction by the (IUCN) World conservation union (Reardon *et al.* 2009). Watchariya *et al.* (2009) reported six new parasite species on the shark gills in the Andaman Sea.

Genus *Eudactylina*, Van Beneden, 1853 (Siphonostomatoidea: Eudactylinidae) consists of 34 species (Deets 1994) and is exclusively reported as parasites of the gills of elasmobranchs (Boxshall and Halsey 2004; Izawa 2011; Walter and Boxshall 2018). *Eudactylina pusilla* Cressey, 1967 has a cylindrical body with chelate second antennae to hold on to the host tissue. *Eudactylina pusilla* had previously been reported from Madagascar and west coast of Florida (Cressey 1967, 1970) near Sarasota as well as from South African waters infecting the gills of the tiger shark, *Galeocerdo cuvier* (Dippenaar *et al.* 2009). Salmon (2015) has reported *Eudactylina* spp. from the gills of angel sharks, *Squatina* spp. from northeastern Gulf of Mexico.

Literature on parasite infestation from Indian coast indicates that parasites greatly infect the body surface and the gill surface of elasmobranchs. There are reports of *Kroyeria echinata* on *Sphyrna zygaena* (Rangnekar 1956); *K. sphyrnae* on *S. zygaena* and *Carcharhinus acronotus* (Rangnekar 1957) from Kerala and Mumbai coasts. Pillai (1968) reported infestation of *K. minuta* on *Scoliodon sorrakowah* from Kerala coast and on *Mobula diabolus* from the Cape Comorin, the southern tip of India. Similarly, *Eudactylina olivieri* was found on *M. diabolus* from Cape Comorin; *E. alata* from *Rhynchobatus* spp.; *E. lancifera* from *Pristis* spp. and *Rhynchobatus* spp.; *Eudactylinopsis curvatus* from *Pristis* spp. along southwest coast of India (Pillai 1985). Nashad *et al.* (2018) reported *Nemesis aggregatus* Cressey, 1967 from the gills of *A. pelagicus* from Andaman and Nicobar waters, India.

The present study reports the first record of the *E. pusilla*

Cressey, 1967 with molecular confirmation from the Indian EEZ with *A. pelagicus* being its new host species.

## 2 | METHODOLOGY

During a regular exploratory survey conducted by MFV Blue Marlin in February 2016, a total of 49 (27 females and 22 males) of *E. pusilla* of sizes ranging from 4.1 to 4.8 mm (excluding egg sacs in females) were collected from the 2nd and 3rd gills of a male *A. pelagicus* (total length 265 cm weighing 40 kg). *Alopias pelagicus* was caught as bycatch by multifilament tuna longline at 11°54' north latitude and 93°14' east longitude at a depth of 762 m (Figure 1). The gills with parasites were dipped in freshwater for 5 minutes and the detached parasites were collected carefully using forceps (Francis-Floyd and Floyd 2011). The specimens still attached to the gills were collected using forceps, taking utmost care not to break any of the appendages. Specimens were washed and photographed with stereo zoom microscope (LEICA M 205, DFC 500). A few were fixed and stored in 95% ethanol at –20 °C temperature for DNA isolation. *Eudactylina pusilla* species identification was done based on the morphological features following Cressey (1967, 1970), Pillai (1985) and Deets (1994).

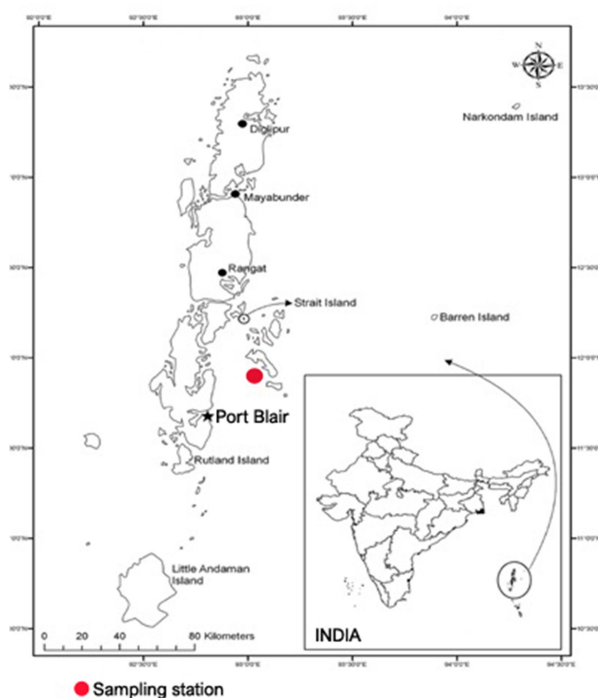


FIGURE 1 Map showing the sampling station

The genomic DNA was extracted using DNeasy Blood & Tissue kits (Qiagen, Inc) following manufacturer's protocol and 18S rDNA was PCR amplified using primers 18Sf (5'-TACCTGGTTGATCCTGCCAG-3') and 1282r (5'-TCAC-TCCACCAACTAAGAACGGC-3') (Huys *et al.* 2007). Thermal cycling parameters in PRIMA-96HIMEDIA PCR included

the following: an initial denaturation at 95°C (3 min.), followed by 50 cycles of 95°C (1 min), 45–55°C annealing temperature (1 min) and 72°C (1 min). A final extension at 72°C (5 min) to complete elongation was then performed before finally decreasing to 4°C. The PCR products were electrophoresed on 1.5% agarose gel, stained with ethidium bromide ( $10 \mu\text{g ml}^{-1}$ ) to ensure that a fragment of the correct size had been amplified. The gel was visualized on a Gel documentation system (Bio-Rad, USA). The purified PCR Products were labelled using Big Dye Terminator V.3.1 cycle sequencing kit (Applied Biosystems Inc.) and sequenced bi-directionally using ABI 3730 capillary sequencer using the primers 18Sf and 1282r. Partial sequences were generated using ABI 3730 sequencer and were submitted to the GenBank (KY420135) on 03-JAN-2017. MEGA 6 software (Tamura and Nei 1993; Tamura *et al.* 2013) was used for creating a phylogenetic tree. The voucher specimens (MUS.FSI.PB/EBP/04/2016/ 1-4) are preserved in the museum of the zonal base of Fishery Survey of India, Port Blair.

### 3 | RESULTS

**Material:** MUS.FSI.PB/EBP/04/2016/1-4; *E. pusilla* were found in 2nd and 3<sup>rd</sup> gill filaments. Significantly, majority of total copepods were attached to gill lamellae in positions perpendicular to or facing the putative flow of water over the gills (Figure 2C).

#### 3.1 | Classification

Order: Siphonostomatoida Thorell, 1859  
Family: Eudactylinidae Wilson CB, 1932  
Genus: *Eudactylina* Van Beneden, 1853  
*Eudactylina pusilla* Cressey, 1967

#### 3.2 | Description of the species *Eudactylina pusilla* Cressey, 1967 from Andaman waters

Characteristic feature of *E. pusilla* (Figure 2) is that, it has the large blunt (nearly amorphous) denticulated setae on the caudal rami and the unusually sharp terminal spines of the segments on the modified exopod of leg 2.

**Female:** The female (Figure 2A) has a triangular Cephalothorax which is anteriorly trilobed, antero-lateral with lobes, and postero-laterally bulged. The second, the third and the fourth segments are broad. The fifth segment is shorter and narrower than the fourth. Genital segment is large, roughly rounded and much narrower than the fifth segment. Abdomen is small and two segmented with the first segment broader than second. The caudal ramus is longer than wide, bearing three terminal large blunt setae (the two lateral most with a row of fine denticles), on lateral slender seta plus one dorsomedial slender seta; ventral surface with two posteriorly directed tiny triangular cuticular flaps.



**FIGURE 2** A, female *Eudactylina pusilla* (dorsal view); B, male *E. pusilla* attached to a female and C, *E. pusilla* attached to gill lamella of *Alopias pelagicus*

Antennule is rather stout and has comparatively short spines. The third segment is with proximal outer large curved spine. Antenna is stout, the second segment with a conical inner process carrying a setule, the fourth segment with two small and one large spine. Outer lobe has prominent spines. Distal spines are long and barbed. Maxilla is stout, the second segment prominently spiny. Basal segment of maxilliped is broader than the length and prolong into an apically hollowed digiti form process. Distal

segment is curved with four stout spines and a sub-apical cup like expansion.

The first four pairs of legs are biramous and trimerite. The first leg has three jointed rami, smaller exopod and endopod with long barbed apical spine. In the second leg, endopod is short, exopod modified and the terminal segment is of unusual shape, the lateral displacement of the three setae are found terminally, basal segment much longer than the rest of the limb, distal inner part expanded and distal segment possess four spines. Rami of the third and the fourth legs sub-similar and three jointed. The exopod with strong claws and endopod has stout apical spine. The fifth leg is basally bulged.

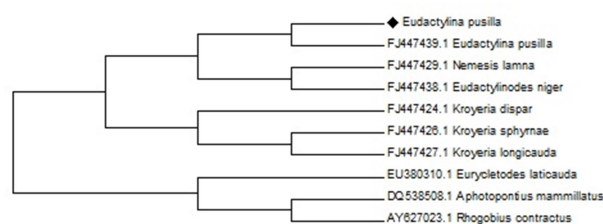
**Male:** The male has long and slender body without the dorsum being spiny or chitinised. Genital segment is large. Abdomen is large with three to four-segments. Caudal rami has plumose setae. Maxilla has slender distal segment. Maxilliped is not chelated. Exopod of legs, one to four are three-jointed, with plumose setae and endopods having one or two segments. Males are different from females, as they are smaller in size and possess small sub-chelate rather than large chelate maxillipeds in females (Kabata 1979). A male attached to a female (Figure 2B).

**Phylogenetic analysis:** Additional sequences of species of Eudactylinidae were downloaded from the NCBI database for analysis to create phylogenetic tree (Figure 3) using MEGA 6 software. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei 1993). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates is collapsed. Initial tree(s) for the heuristic search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analysis involved 10 nucleotide sequences. There were a total of 789 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.* 2013).

#### 4 | DISCUSSION

Taxonomic studies on the Siphonostomatoids also provide information about their morphology, biology, habitat preference and host association, as well as the distribution of species and their associated host taxa. Molecular analysis, especially sequencing, provides additional information on the existence of cryptic species and population structure. Reported declines in abundance of piscivorous fish species, for example, as a result of fishing pressure, with possible synergistic effects of environmental changes, and also the reported potential effects of anthropogenic and climate change on the broader coastal

biota, may result in the loss of many Siphonostomatoid species before they can be described (Dippenaar 2016).



**FIGURE 3** Molecular phylogenetic analysis by Maximum Likelihood method (◆Andaman Isolate)

*Eudactylina pusilla* was previously reported from KwaZulu-Natal Coast of South Africa from the gill filaments of *Galeocerdo cuvier* (Dippenaar *et al.* 2009); Lebepe and Dippenaar (2013) also reported *E. oliveri* from *Mobula kuhlii*, *M. eregoodootenkee* and *Manta alfredi*. The present study reports *E. pusilla* infesting the gills of the *A. pelagicus* caught from Andaman waters adding to the diversity of the parasite and reporting a new host species along with molecular studies. A few available literature on fish parasites from Andaman Sea indicated that fishes are mostly infested with isopods belonging to family Cytothoidae; copepods, family: Lernanthropidae, Bomolochidae Eudactylinidae, Caligidae, Pandaridae and Pseudocycnidae; monogenes, family: Hexabothriidae, Capsalidae; trematodes belonging to family Hirudinellidae; cestodes, family: Tentaculariidae, Pseudonybeliniidae; and nematodes belonging to family Anisakidae (Watchariya *et al.* 2009; Jithin *et al.* 2016; Pradeep *et al.* 2016, 2017a, 2017b, 2018, 2019; Nashad *et al.* 2018).

The partial sequence of mitochondrial 18S rDNA generated 789 nucleotide base pairs. Pair-wise genetic distance values were estimated based on 18S rDNA sequences using MEGA 6 software. Neighbour Joining (NJ) tree was created to provide a graphic representation of the patterning of divergences. A comparison of the DNA barcode of Andaman specimen showed 99% similarity with a query coverage of 100% to that of *Eudactylina pusilla* which was reported to be a parasite on *Galeocerdo cuvier* (GenBank: FJ447439), followed by 98% similarity to that of *Nemesis lamna* reported on *Carcharodon carcharias* (GenBank: FJ447429) by Dippenaar (2009). Though the partial rDNA sequence showed 98% similarity to that of *N. lamna* the morphological features matched *E. pusilla* which confirmed the specimen to be *Eudactylina pusilla* Cressey, 1967.

The present study confirms the range extension of the gill parasite *E. pusilla* to Indian EEZ by both morphological as well as molecular methods. It also reports *A. pelagicus* (listed as “Vulnerable” by the IUCN Red List of Threatened Species) as a new host species.

## ACKNOWLEDGEMENTS

The first author is thankful to the officers/staff of the vessel MFV Blue Marlin for their kind cooperation in collecting of samples on board the vessel. The authors are thankful to Mr Nashad M of Port Blair Base of Fishery Survey of India and Mr. Solly Solomon of Mormugao Zonal Base of Fishery Survey of India for their help in editing the figures and valuable suggestions for improving the molecular biology part. Further, the authors are thankful to the anonymous reviewers for the betterment of the manuscript.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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