

Barnacle infestation of two sea snakes *Hydrophis schistosus* and *Hydrophis curtus* in the Bay of Bengal

ANWESHAN PATRA^{1*}, ARDHENDU DAS MAHAPATRA², PRINCIA DSOUZA³, D. ANNADURAI¹ & SUDIPTA KUMAR GHORAI²

¹Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai 608502, India

²Coastal Ecology Research Laboratory, Department of Zoology, Egra S.S.B. College, Egra, Purba Medinipur, West Bengal 721429, India

³Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India

*Corresponding author e-mail: anweshan.patra@gmail.com

Barnacles (Crustacea, Isopoda, Cymothoidae) are well known as marine ectoparasites and epibionts; their hosts/substrates included a wide range of animal species including sea snakes (Jeffries & Voris, 1979; Saravanakumar et al., 2012). Barnacles have a cement gland that secretes a protein matrix for underwater adherence, a necessary component for long-term attachment. In this study, we report barnacle infestation of two sea snake species *Hydrophis schistosus* and *Hydrophis curtus* in the northern Bay of Bengal. The known distribution of these sea snakes includes the Indian Ocean, Australia's northern coast and the South China Sea (Voris, 1972; Voris et al., 1978). Both species are acknowledged as being the most abundant sea snakes along India's coast (Daniel, 2002) although, to date, barnacles have only been reported once from Indian sea snakes (Saravanakumar et al., 2012).

Sea snakes were collected from the East Medinipur coast in West Bengal, India (21° 41'45.14" N, 87° 45'05.76" E) from November 2021 to February 2022. Fishermen occasionally catch sea snakes while operating trawlers and take them to landing areas where we investigate the dead snakes for traces of external parasites. The snakes were identified by reference to Smith (1926) and Rasmussen (1993). To enable a thorough examination of the barnacles these were collected from the snakes and stored in 70% ethanol. Barnacles were identified by their morphology, using a variety of sources (Bovallius, 1887; Bowman & Tareen, 1983; Bleeker, 1857; Bruce, 1990; Schioedte & Meinert, 1881; 1884) and by mitochondrial analysis. The mitochondrial DNA was isolated from the samples using the salt extraction method (Aljanabi & Martinez, 1997). The COI mitochondrial gene was amplified via PCR using both general and degenerate primers as follows: CrustF1- TTT TCT ACA AAT CAT AAA GAC ATT GG (Costa et al., 2007) and dgHCO- TAAACTTCAGGGTGACCAAARAAYCA (Meyer, 2003). The PCR reaction had a total volume of 21 µL and contained 2 x AmpliWin PCR mix, 10 pmol/µL of forward and reverse primers and 1 µL of template DNA. Reactions were run for 35 cycles with the following parameters: 30s at 94 °C; 40 cycles of 30s at 94 °C, 90 s at 51 °C, 60 s at 72 °C; followed by a final extension of 5 min at 72 °C. The PCR products were sequenced commercially at Barcode Biosciences, Bangalore, India.



Figure 1. An acorn barnacle *Amphibalanus amphitrite* on the cloaca of the sea snake *Hydrophis schistosus*

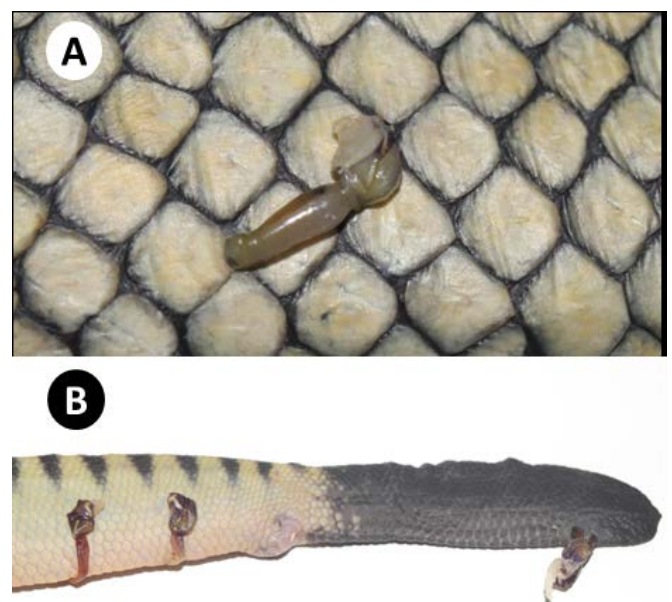


Figure 2. Small goose barnacles *Octolasmis warwickii* on sea snakes - A. *Hydrophis schistosus*, B. *Hydrophis curtus*



Figure 3. Algal growth on the skin of the sea snake *Hydrophis schistosus*

A total of 40 *H. schistosus* were examined of which 14 specimens had barnacles, and of the 26 *H. curtus* specimens 4 had barnacles. We identified two species of barnacle, the acorn barnacle *Amphibalanus amphitrite* (Fig. 1) and the small goose *Octolasmis warwickii* (Fig. 2); this is the first report of *O. warwickii* from the West Bengal coast. The identification of *O. warwickii* was confirmed for the analysis of mitochondrial DNA and sequences have been submitted to Genbank (Accession numbers PP002271 and PP349831). For *A. amphitrite*, PCR amplification did not work despite multiple attempts. So, specimens were identified only by their morphology.

Two specimens of *H. schistosus* were infested by both *A. amphitrite* and *O. warwickii*, for the rest, the barnacle infestation was restricted to a single species. In one *H. schistosus* specimen, a maximum of eight barnacles were found, of which four were *A. amphitrite* and four were *O. warwickii*. Barnacles were observed in the head to neck region (4%), midbody region (44%) and cloaca to tail region (52%). The barnacle attachment rate was higher in female sea snakes (67%) than male sea snakes (33%). Algal growth was also observed in one *H. schistosus* specimen (Fig. 3).

It is believed that certain *Octolasmis* spp may be obligate epibionts/ecoparasites on sea snakes (Jeffries & Voris, 1979). Long-term studies are needed to fully assess all the ecological variables that affect the relationship between sea snakes and barnacles.

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