

Occurrence, prevalence and molecular characterization of *Norileca indica* (Milne Edwards, 1840) (Isopoda: Cymothoidae) on bigeyescaud *Selar crumenophthalmus* (Bloch) from Andaman Islands India

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The present investigation reports the occurrence and prevalence of the parasitic cymothoid, *Norileca indica* infesting bigeyescaud, *Selar crumenophthalmus* from the Andaman Islands. Damage in the form of erosion of gill filament and necrosis in the gill chamber of the host fish, with a prevalence of 26.08% and a mean intensity of 1.5 is recorded. Molecular characterization of the mitochondrial COI and 28S rDNA genes is done to establish its phylogenetic status with its known congener. The present report is the first record of *N. indica* infesting *S. crumenophthalmus* from the Andaman Islands.

[**Keywords:** Bigeye Scud; Andaman & Nicobar Islands; Cymothoid; Isopod]

Introduction

Cymothoids are the obligate, ectoparasitic, protandrous isopods infesting a diverse array of tropical marine fishes and cause destructive activity in their hosts^{1,2}. They attach externally on the gills or in the mouth, a few burrows inside the flesh of the hosts. Some infest buccal cavity and gill, causing lesions and obstructions. The isopods infesting gills leads to callus formation on the gill arch and gill filament due to persistent irritation by the appendages of the parasites, thereby causing reduction of gill surface. Indian cymothoid fauna is still poorly known and relevant studies are scanty^{1,3}. Andaman and Nicobar Islands belonging to the union territory of India is a poorly studied area on the aspect of parasitic cymothoids. Very few reports are available on isopods fauna from these islands, which includes a record of *Norileca indica* from the buccal cavity of Scomberid fish, *Rastrelliger kanagurta*, *Joryma hilsae* from Clupeidae, *Cymothoa* sp. and *Cateossa* sp. from Carangidae and Mullidae⁴; *Ryukyua circularis* from *Amblygaster sirm*⁵. In the present contribution, the isopod, *Norileca indica* parasitizing the gill of *Selar crumenophthalmus* (Bloch, 1793) is reported from the Andaman Islands, which confirms its potential host record reported elsewhere. Its prevalence and mean intensity are reported, and the molecular characterization of its partial cytochrome oxidase unit (COI) and 28S rDNA genes were done and genetic data were submitted in the NCBI

(National Centre for Biotechnology and Information). A maximum likelihood tree using COI and 28S rDNA gene is demonstrated to show its phylogenetic relationship.

Materials and Methods

A total of 156 *S. crumenophthalmus* specimens were collected from Mohanpura fish market located in the main town of Port Blair, Andaman, during August to October, 2016. Forty-one isopods comprising 14 males and 27 females were recovered from the branchial cavity of 27 individual specimens of *S. crumenophthalmus*. The length and the width (at 4th pereonite) of the male and female isopods were measured using a digital caliper and the parasites were subsequently preserved in 90% ethanol. The parasites were identified according to the standard literature^{6,7}. Prevalence and intensity were calculated⁸. DNA was extracted from the legs of the isopod using standard protocol⁹ and polymerase chain reaction (PCR) amplification of the partial 584 bp sequence of COI gene was done using the primer sequences LCO1490 (5'-GGTCA ACAA TCATA AAGAT ATTGG-3') and HCO2198 (5'-TAAAC TTCAG GGTGA CAAA AAATC A-3')¹⁰. The amplification of partial 414 and 411 bp sequences of 28S rDNA was done using the forward IsoF (50-ACCCGCTGAATTTAAGCAT-30) and reverse IsoR (50CTCTTCAGAGTACTTTTCAAC-30) primers¹¹. PCR condition and reaction constituent follows Praveenraj et al⁵ and Qin-yi et al¹² for 28S

rDNA and COI gene respectively. The amplicon was sequenced in ABI 3500 DNA analyzer (ShrimpeX Biotech Pvt. Ltd., Chennai).

Gene sequences were aligned using the software MUSCLE¹³, and Pair-wise evolutionary distance among gene sequences was determined by the Kimura 2-parameter model¹⁴ using the software program MEGA7 (Molecular Evolutionary Genetics Analysis)¹⁵. The best fit nucleotide substitution model was selected from 24 models present in MEGA7¹⁵, based on the one with the lowest BIC scores (Bayesian Information Criterion), which are considered to best describe the substitution pattern¹⁶. The phylogenetic tree were inferred using the maximum likelihood method present in MEGA7¹⁵. Reliability of the phylogenetic tree was estimated using bootstrap values run for 1000 iterations.

Results and Discussion

In the present study, the occurrence of *N. indica* from *S. crumenophthalmus* is reported from the

Andaman Islands for the first time. A total of 14 male and 27 female *N. indica* (Fig. 1) were collected from 27 individual specimens of *S. crumenophthalmus*. Out of the 156 fishes examined 27 individuals were infested with *N. indica* with a prevalence of 26.08% and a mean intensity of 1.5. The size of female parasites (N=27) was 23.2 to 35.08 mm long (mean ± standard deviation 29.0± 1.2) and 10.37 to 15.82 mm wide at 4th pereonite (mean ± standard deviation 13.2 ± 0.5). While the male parasite (N=14) was measuring 14.0 to 19.5 mm long (mean ± standard deviation 17.0 ± 2.3) and 5.1 to 6.4 mm wide at 4th pereonite (mean ± standard deviation 5.7± 0.2). The male parasite was straight, smaller and narrower than females. The ovigerous female parasites were found positioned on the host with the ventral side facing the inner surface of the operculum and dorsal side deeply plunged in the first gill arch with the gill filaments showing considerable erosion and necrosis (Fig. 2A). The body of the female parasite was twisted to one side that is right side in the right branchial chamber



Fig. 1 — *Norileca indica* dorsal views: A. Females, B. Males

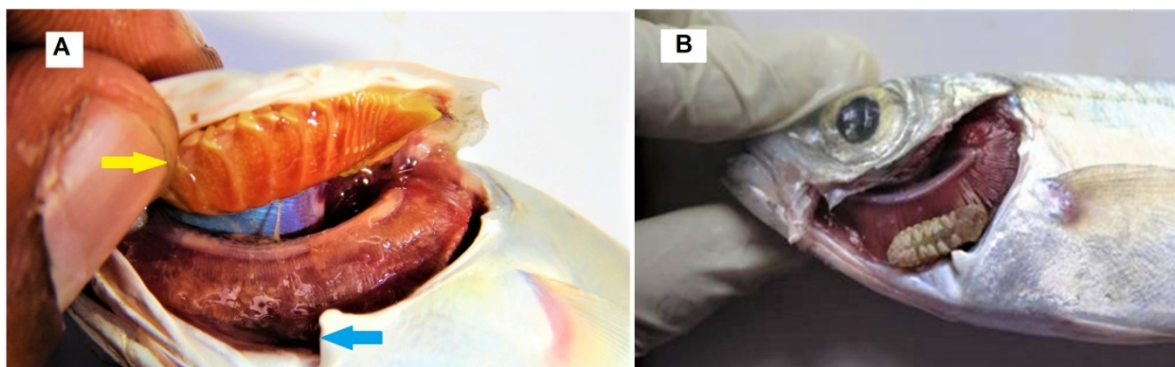


Fig. 2 — A. Female *Norileca indica* in the gill cavity of *Selar crumenophthalmus*. Yellow arrow shows the position of the head of ovigerous female attached to the inner flap of operculum and blue arrow shows the erosion and necrosis in the margins of the gill filament of 1st, 2nd and 3rd gill arch in *Selar crumenophthalmus*. B. Attachment of male *Norileca indica* over the gill filament of the host.

and *vice versa*. The male parasite on the opposite gill chamber was positioned ventrally to the inner operculum, in orientation with the head directing towards the upper half of the operculum (Fig. 2B).

PCR amplification of the partial COI gene yielded an amplicon of 584 bp and 28S rDNA gene yielded the amplicons of 411 and 414 bp. The generated sequences of *N. indica* for COI (accession number KY849589) and 28S rDNA (accession number KY963133 and KY963134) were submitted to NCBI GenBank. Model test suggested the best fit nucleotide substitution model to be the Tamura 3-parameter (TN9) with gamma distribution (+G) [AICc=3992.907, lnL= -1968.240, (+G)=0.43] for COI gene, and Kimura 2-parameter to be the best fit nucleotide substitution model [AICc=702.431, lnL= -341.023] for 28S rDNA. For COI sequences, the phylogenetic analysis suggests *Cymothoa truncata* (KT388757, KT388758) to be the close relative of *N. indica* with a raw pair-wise sequence distance of 0.40 (Fig. 3A). For 28S rDNA, with the available sequences, *Ryukyua circularis* (KX090447) was found to be the close relative of *N. indica*, with raw pair-wise sequence distance of 0.01 (Fig. 3B).

Norileca indica was first described as *Livoneca indica* (or *Lironeca indica*) from the materials from Sumatra⁶; later, it was synonymized to *Norileca indica*⁷. It is known to be widely distributed in the Indo-West Pacific¹⁷. So far, *N. indica* has been recorded from eight species of host fishes viz. *Atule mate* and *Rastrelliger kanagartha*¹⁸, *Selar crumenophthalmus*^{19,20}, *Herklotsichthys* spp.^{7,21}, *Coryphaena hippurus*²², *Alepes apercna*¹⁷, *Decapterus russelli*²³, *Secutor insidiator* and

*Nemipterus randalli*²⁴, and *Decapterus kurroides*²⁵. From Indian waters, *N. indica* was first reported from Parangipettai, east coast of India from *Rastrelliger kanagartha* with an overall prevalence of 4.5%³. Subsequently, *N. indica* was recorded in *S. crumenophthalmus* from south-west coast with overall prevalence and mean intensity of 37.94% and 1.38²⁶; *R. kanagartha* from west coast with a prevalence of 18.6%²⁷; *R. kanagartha* from Malabar coast with prevalence and mean intensity of 26.4% and 1.7²⁸; *R. kanagartha* from Andaman coast⁴; *R. kanagartha*, *S. insidiator* and *N. randalli* from north-west of Bengal with prevalence (39.03±8.04%, 40.34±1.73%, 45.43±5.68%) and mean intensity (1.13, 1.35±0.12, 1.25±0.07)²⁴; and *S. crumenophthalmus* (40.7% and 1.05) and *D. kurroides* (100% and 1.0) from Philippines²⁵. In the present investigation, *N. indica* was found in the branchial cavities of *S. crumenophthalmus*, which is very similar to the observations in previous reports, and further confirms its potential host record and distribution in the Indo-West Pacific. The female parasite's body was twisted to the left side when it occupied the left branchial cavity or to the right side when it occupied the right branchial cavity. The damage to the gills due to the attachment of ovigerous females was observed in the form of necrosis, erosion and atrophy of the 1st, 2nd and 3rd gill filaments (Fig. 2), which is corroborated with similar findings of previous authors^{7,26,28}. In the present study, individual fish was infested either by a single large female or a pair of large females, and or with small male parasite in the ratio of one per branchial cavity, and never both the sexes occupying together. The Andaman Islands share close proximity

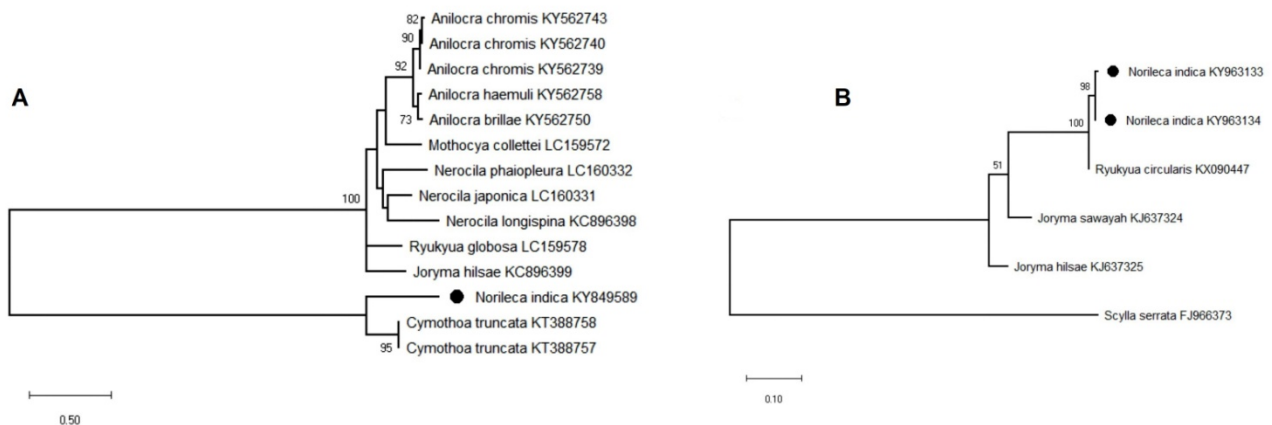


Fig. 3 — A. Maximum likelihood phylogenetic tree of fish parasitic isopods inferred from DNA sequences of cytochrome oxidase unit (COI) gene. B. Maximum likelihood phylogenetic tree of fish parasitic isopods inferred from DNA sequences of 28S rDNA gene, *Scylla serrata* is used as outgroup. (Black dots indicate the GenBank accession number generated in the present study).

to south-east Asia, especially Thailand. In the present study, a prevalence of 26.08% is recorded; which is very less when compared to the prevalence recorded from Thailand that is 70-100%²⁰. This trend could be due to the short sampling period.

Morphological identification of isopods is sometimes time-consuming and needs experience and skill, thus the generated partial COI and 28S rDNA gene sequences can serve as the reference sequence for the researchers working on parasitic isopods with molecular tools. Parasitic isopods are potential pests or disease vectors to the aquaculture industry², and very recently, Andaman and Nicobar Islands has much focus for aquaculture activities like sea farming and cage culture. Therefore, a beforehand knowledge on the fish parasitic isopods is essential for their management and remedies.

Conclusion

Studies on the parasitic isopods from Andaman and Nicobar islands are very scarce. This study constitutes the first report of *Norileca indica* as a parasite on the host fish *Selar crumenophthalmus* from the Andaman Islands. The damage caused to the host and prevalence is recorded. The molecular characterization of 28S rDNA and COI genes was carried out to generate referral barcodes, which will serve the purpose of easy identification of *N. indica* through molecular techniques. The phylogenetic studies constructed using both the genes revealed the closest congener for *N. indica*.

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References

- Trilles, J.P., Ravichandran, S. & Rameshkumar, G., A checklist of the Cymothoidae (Crustacea, Isopoda) recorded from Indian fishes, *Acta Parasitol.*, 56(4)(2011) 446-459.
- Smit, N.J., Bruce, N.L. and Hadfield, K.A., Global diversity of fish parasitic isopod crustaceans of the family Cymothoidae, *International J. Parasitol.*, 3(2)(2014) 188-197.
- Rameshkumar, G., Ravichandran, S., Sivasubramanian, K. & Trilles, J.P., New occurrence of parasitic isopods from Indian fishes, *J. Parasit. Dis.*, 37(1)(2013) 42-46.
- Jithin, K., Swapna, A., Kumar, R.R., Venu, S., Helna, A.K. & Sudha, K., Studies on crustacean parasites from commercial marine fish along the Andaman coast in comparison with Malabar coast of Kerala of Indian EEZ, *World J. Fish Mar. Sci.*, 8(1) (2016) 47-53.
- Praveenraj, J., Saravanan, K., Puneeth Kumar, P., Ravichandran, S., Baruah, A., Monlisha Devi, S., Kiruba Sankar, R. & Dam Roy, S., First record on the occurrence of *Ryukyuacircularis* (Pillai, 1954), a parasitic cymothoid (Crustacean: Isopoda) infesting the clupeid fish *Amblygaster sirm* (Walbaum) from Andaman Islands, India, *J. Parasit. Dis.*, 41(2)(2016) 593-595.
- Milne Edwards H.M. *Histoire Naturelle des Crustacés comprenant l'anatomie, la physiologie et la classification de ces animaux III* (Librairie Encyclopédique de Roret, Paris) 1840, pp. 1-605.
- Bruce, N.L., The genera *Catoessa*, *Ehhusa*, *Enispa*, *Ichthyoxenus*, *Indusa*, *Livoneca* and *Norileca* gen. (Isopoda, Cymothoidae), crustacean parasites of marine fishes, with descriptions of eastern Australian species, *Rec. Aus. Mus.*, 42(1990) 247-300.
- Margolis, L., Esch, G.W., Holmes, J.C., Kuris, A.M., & Schad, G.A., The use of ecological terms in parasitology (report of an ad hoc committee of the American society of parasitologists), *J. Parasit.*, 68 (1)(1982) 131-133.
- Bruce, L.D., Trumper, B.B., & Lightner, D.V., Methods for viral isolation and DNA extraction for a penaeid shrimp baculovirus, *J. Virol. Methods.*, 34 (1993) 245-254.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R., DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates, *Molecular Marine Biology and Biotechnology.*, 3(5)(1994) 294-299.
- Roy, P., Panda, S.P., Sethi, S.N., Rana, N., Sethi, G. & Das, B.K., Morphometric and molecular identification of Isopod parasite *Jorymahilsae* in *Pellonaditchela* (Val 1847), *J. Aqua. Trop.*, 30(1-2) (2015) 15-21.
- Qing-yi, Z., Qi-qun, C. & Wei-bing, G., Mitochondrial COI Gene Sequence Variation and Taxonomic Status of Three Macrobrachium Species, *Zool. Res.*, 30(6)(2009) 613-619.
- Edgar, R.C., MUSCLE: multiple sequence alignment with high accuracy and high throughput, *Nucleic Acids Res.*, 32(2004) 1792-1797.
- Kimura, M., A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.*, 16(1980) 111-120.
- Kumar, S., Stecher, G., & Tamura, K., MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets, *Mol. Biol. Evol.*, 33(7)(2016) 1870-1874.
- Nei, M., & Kumar, S., *Molecular Evolution and Phylogenetics* (Oxford University Press, New York) (2000).
- Trilles, J.P., Les Cymothoidae (Isopoda, Flabellifera) des côtes Françaises. III. Les Lironecinae Schiodteet Meinert, 1884. Bull du Mus Nat d'hist Nat Paris 3-serie, no. 392. *Zoologie.*, 272 (1976) 801-820.
- Avdeeve, V.V., Notes on the distribution of marine Cymothoidae (Isopoda, Crustacea) in the Australian New Zealand region, *Folia Parasitol.*, 25(1978) 281-283.
- Rockicki, J., *Lironeca indica* Edwards, 1840 (Crustacea, Isopoda) from *Selar crumenophthalmus* (Bloch), *Wiad. Parazytol.*, 38 (1982) 205-206.

- 20 Nagasawa, K., & Petchsupa, N., *Norileca indica* (Isopoda, Cymothoidae) parasitic on bigeyescaud *Selar crumenophthalmus* in Thailand, *Biogeography*, 11(2009) 131-133.
- 21 Yu, H.Y & Li, X.Z., Study on the Cymothoidae from Chinese waters, *Stud. Mar. Sin.*, 45(2003) 223-238.
- 22 Yamauchi, T., Ohtsuka, S., & Nagasawa, K., Ectoparasitic Isopod, *Norileca indica* (Crustacea, Isopoda, Cymothoidae), obtained from the stomach of *Coryphaena hippurus* (Perciformes, Coryphaenidae) in the Philippines, *Biogeography*, 7(2005) 25-27.
- 23 Ghani, N., Isopod parasites of marine fishes of marine fishes of Pakistan, *Proc. Pakistan Con. Zool.*, 23(2003) 217-221.
- 24 Behera, P.R., Gosh, S., Pattnaik, P., & Rao, M.V.H., Maiden occurrence of the isopod, *Norileca indica* (H. Milne Edwards, 1840) in pelagic and demersal finfishes of Visakhapatnam waters along north-west Bay of Bengal, *Ind. J. of Geo-Mar. Sci.*, 45 (2016) 856-862.
- 25 Cruz-Lacierda, E.R., & Nagasawa, K., First Record of *Norileca indica* (Isopoda, Cymothoidae) Parasitic on *Selar crumenophthalmus* and *Decapterus kurroides* (Perciformes, Carangidae) in the Philippines, *Comp. Parasitol.*, 84(2017) 60-63.
- 26 Neeraja, T., Gayatri, T. & Unmey, S., Occurrence of the isopod, *Norileca indica* (Isopoda: Cymothoidae) on bigeyescaud, *Selar crumenophthalmus* (Bloch) off Mumbai coast, India, *Indian J. Fish.*, 61(1)(2014) 49-56.
- 27 Rameshkumar, G., Ramesh, M., Ravichandran, S., Trilles, JP., & Subbiah, S., New record of *Norileca indica* from the west coast of India, *J. Parasit. Dis.*, 39(4)(2014) 712-715.
- 28 Panakkool-Thamban, A., AmeriKottarathil, H. and Kappalli, S., Branchialcymothoids infesting the marine food fishes of Malabar Coast, *J. Parasit. Dis.*, 40(4)(2015) 1270-1277.