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Infection parameters of *Norileca indica* and a new record of *Ceratothoa carinata* (Crustacea: Isopoda: Cymothoidae) on *Selar crumenophthalmus* (Actinopterygii: Carangiformes: Carangidae) in the waters of the Sibuyan Sea, the Philippines

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Abstract

Studies on cymothoid isopods as parasites affecting the marine fisheries and aquaculture industries are relatively scarce in the Philippines despite having detrimental impacts on their fish hosts. Parasitological examination on the bigeye scad, *Selar crumenophthalmus* (Bloch, 1793), a potential aquaculture species, in the waters of the Sibuyan Sea, Philippines was done on fish specimens collected on 21 April 2021. Out of the 88 specimens, a total of 13 big eye scads were infected with cymothoid isopod *Norileca indica* (Milne Edwards, 1840), found in the branchial cavities of the fish, resulting in a prevalence of 14.77%. A total of 20 individual isopods (13 females and seven males) were recovered, with a mean intensity of 1.53. Based on the morphological characteristics and as confirmed by the cytochrome oxidase subunit 1 (*CO1*) sequence, one host fish was also infected with *Ceratothoa carinata* (Bianconi, 1869). This appears to be the first record of *C. carinata* from the Philippines. To fully understand the implications of cymothoid parasites on the bigeye scad, further studies are recommended to account for the impacts of seasonality, reproductive stages of the host fish, and effects of abiotic factors such as water movement and depth.

Keywords

bigeye scad, carangid, *CO1*, cymothoid isopod, morphology, Panay Island, parasite infection

Introduction

The bigeye scad, *Selar crumenophthalmus* (Bloch, 1793), a potential aquaculture species, is one of the commercially important carangid fishes in the Philippines. According to the Philippine Statistics Authority (2023), the bigeye scad accounted for 6% (113 240 t) of the Philippines' overall marine fisheries production in 2022. Fishing gears such as purse seines, trawls, ring nets, drive-in nets, gill nets, hand lines, hoop nets, fish corrals, and bag nets, and even beach seines can be used to catch this species (Corpuz and Dalzell 1988). However, in the 1900s, unsustainable fishing methods caused the slow decrease of the majority of species around the world, including the bigeye scad populations (Dalzell and Peñaflor 1989; Dalzell et al. 1990). Overfishing and overexploitation have put further strain on the aquaculture industry to meet the continually expanding demand for fishery resources (Timi and Mackenzie 2015). Literature regarding the applications in aquaculture of the bigeye scad is very scarce and is only limited to rearing larvae and juveniles (Iwai et al. 1996; Welch et al. 2013; Elefante 2019). With the current advancement in aquaculture practices, the majority of systems now have access to hatchery-bred larvae, but this is not always the case. This leads some systems to rely on wild-caught fry or fish. High stocking density in the majority of aquaculture farms often leads to faster transmission of diseases and parasites from wild-caught stock that contaminates the culture system (Paperna 1987; Meyer 1991). The lack of appetite, slow growth, impaired reproduction, abnormalities in the body, a higher risk of bacterial or fungal infection in injuries caused by the parasite's attachment, and in severe cases, mortality, are only a few of the negative impacts brought on by these parasites (Meyer 1991; Smit et al. 2014).

Cymothoid isopods are parasitic in marine fishes and can infect the branchial cavity, buccal cavity, fins, and skin, or even penetrate the flesh of the host fish (Smit et al. 2014; Cruz-Lacierda and Nagasawa 2017; Aneesh et al. 2021a, 2021b). Two recorded bigeye scad parasites from the family Cymothoidae are *Norileca indica* (Milne Edwards, 1840) (see Ahmed and Khan 2012; Zubia et al. 2014; Cruz-Lacierda and Nagasawa 2017; Fafioye and Ayodele 2018) and *Ceratothoa carinata* (Bianconi, 1869) (see Martin et al. 2013; Hadfield et al. 2016). These parasites have been recorded in Australia, China, India, Indonesia, Mozambique, New Guinea, and Thailand (Martin et al. 2013; Hadfield et al. 2016; Anand Kumar et al. 2017; Seepana et al. 2021). In the Philippines, *N. indica* infecting bigeye scad was first recorded by Cruz-Lacierda and Nagasawa (2017) in the Panay Gulf and subsequently by Muji et al. (2021) in Batangas Bay. We are not aware of published reports of *C. carinata* collected within the country.

Due to the potential and economic significance of the bigeye scad in mariculture, it is essential to broaden the existing studies, particularly those on the effects of cymothoid infections in the Philippines. The study generally aimed to generate data on *N. indica* infections on the bigeye scad, caught in the Sibuyan Sea, Philippines. Specifically, the study assessed the prevalence and mean intensity of the *N. indica*. Incidentally, the study also documented the first record of *C. carinata* from the Philippines.

Materials and methods

Study area and sample collection. A total of 89 bigeye scad were purchased from commercial catches landed in two fish landing sites at northern Panay Island (Kalibo, Aklan and Tangalan, Aklan), Philippines in April 2021 (Fig. 1). Based on the reports of the fishers who operated the commercial fishing boats, these catches were fished using purse seines in the Sibuyan Sea. Fish samples were measured for total length (TL) to the nearest 1 mm, and

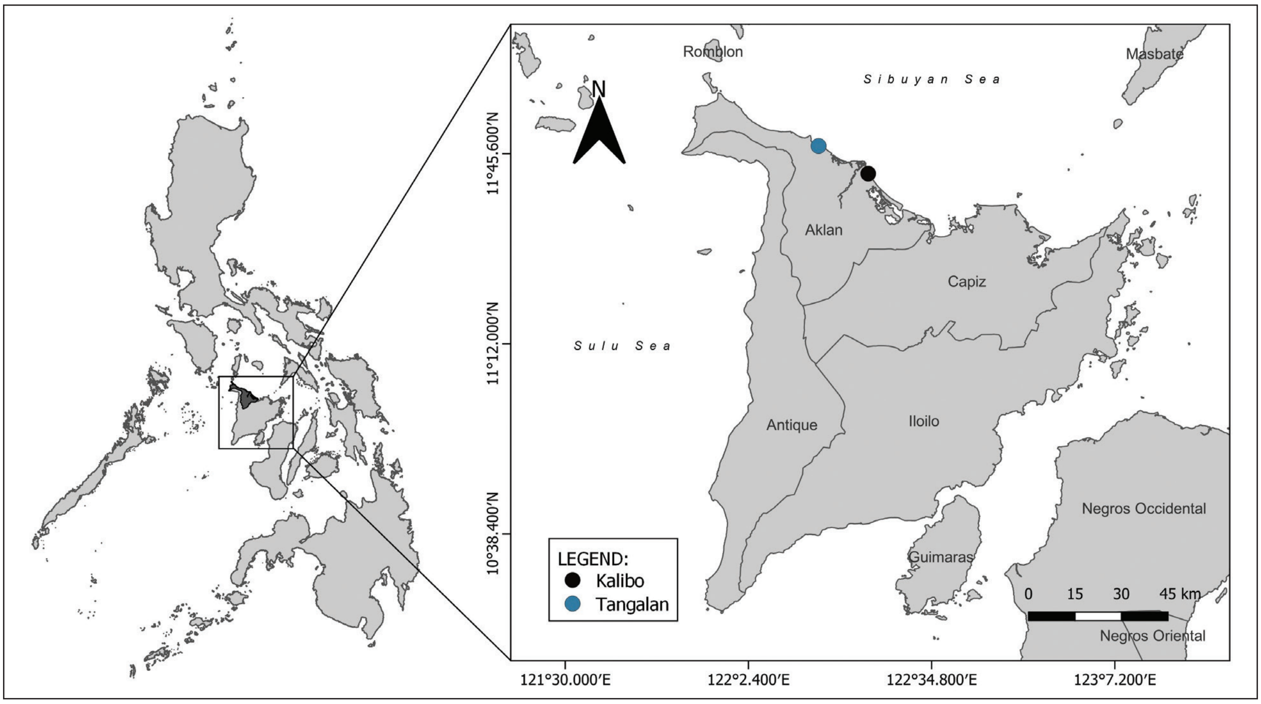

Figure 1. Map of commercial fish landing sites in northern Panay Island (Tangalan, Aklan and Kalibo, Aklan), Philippines. Fish samples were caught in the Sibuyan Sea, Philippines in April 2021.

body weight to the nearest 0.1 g. The fish samples were then examined externally, particularly in their branchial and buccal cavities for isopod parasites.

Each discovered isopod was extracted using fine forceps. Isopod samples were then measured for TL to the nearest 0.01 mm using a digital caliper, preserved in individual vials using absolute ethanol with a corresponding label, and kept in a –20°C freezer for further taxonomic and genetic identification. Morphological features included in the description were based on Martin et al. (2013), van der Wal et al. (2017), and Aneesh et al. (2022).

DNA barcoding. Genomic DNA was extracted from the appendages of females using the Wizard Genomic DNA Purification Kit (Promega) following the manufacturer's protocol. A targeted part of the mitochondrial cytochrome oxidase subunit 1 (*CO1*) gene of the specimen was amplified using the universal invertebrate primers 5′-GGTCAACAAATCATAAAGATATTGG-3′) and HC02198 (5′ TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). Pre-sequencing PCR was performed following the protocol of Aneesh et al. (2021c) with modifications. PCR reactions were performed with a total volume of 50 µL containing 25 µL of GoTaq G2 Colorless Master Mix (Promega), 2.5 µL of each 10 µM primer, 1 µL of 200 ng μL^{-1} DNA template, and 19 µL of nuclease-free water. The conditions were as follows: initial denaturation at 94°C for 5 min; followed by 35 cycles of 94°C denaturation for 30 s, annealing at 47°C for 50 s, and extension at 72°C for 2 min; and final extension of 72°C for 10 min. PCR was carried out in a T100 thermal cycler (Biorad Laboratories). The amplified 680 bp PCR products were sent to Macrogen (South Korea) for bidirectional sequencing.

The generated sequences were compared to available sequences in GenBank by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were aligned against published sequences of known cymothoid species retrieved from GenBank to determine their phylogenetic relations using the neighbor-joining method as implemented in MEGA 7 (Hall 2013). The resulting topology was assessed by bootstrapping with 1000 replications. The decapod *Penaeus merguiensis* De Man, 1888 was used as an outgroup. The sequences were also submitted to GenBank and were assigned the following accession numbers: OP808334, OP811246, and OP811247. Voucher specimens were deposited at the UPV Museum of Natural Sciences under accession numbers UPV MI 03831-03833.

Prevalence and mean intensity analyses. The prevalence and mean intensity of the isopod parasite were calculated based on Bush et al. (1997).

The prevalence is defined as the percentage of fish individuals infected with parasites of a given species related to the total number of fish examined, while the mean intensity specifies the total number of a certain parasite species per infected fish.

Results

Order Isopoda Latreille, 1817
Family Cymothoidae Leach, 1814
Genus *Norileca* Bruce, 1990

Norileca indica (Milne Edwards, 1840)

(Figs 2 and 3)

Livoneca indica.—Milne-Edwards 1840: 262.—Bleeker 1857: 21.—Schioedte and Meinert 1884: 362, pl. 15, figs 3–6.—Richardson 1910: 24.—Nierstrasz 1915: 99.—Nierstrasz 1931: 142.—Borcea 1933: 482.—Beumer et al. 1982: 33.

Lironeca indica.—Trilles 1976: 77, pl. 2, fig. 3.—Trilles 1979: 266.—Avdeev 1978: 281.—Rokicki 1982: 205, figs 1, 2.

Livoneca ornata.—Heller 1865: 145–146, pl.12, fig. 15.

Material examined. SC064 (ovigerous female): 20.75 mm TL, host *Selar crumenophthalmus*, Sibuyan Sea. SC064 (male): 12.26 mm TL, same data as preceding.

Additional material examined. Four ovigerous females with a size range of 14.72–25.44 mm TL and four males with a size range of 7.17–12.26 mm TL collected from the same host, *S. crumenophthalmus*, captured in the Sibuyan Sea. SC020 (female): 24.24 mm TL SC020 (male): 10.71 mm TL, SC030 (female): 23.34 mm TL, SC030 (male): 11.93 mm TL, SC050 (female): 25.44 mm TL, SC050 (male): 11.77 mm TL, SC086 (female): 14.72 mm TL, SC086 (male): 7.17 mm TL.

Morphological description of female. Body twisted; dorsal surface smooth and polished appearance; ventral area soft; narrowest body part at pereonite 1; widest

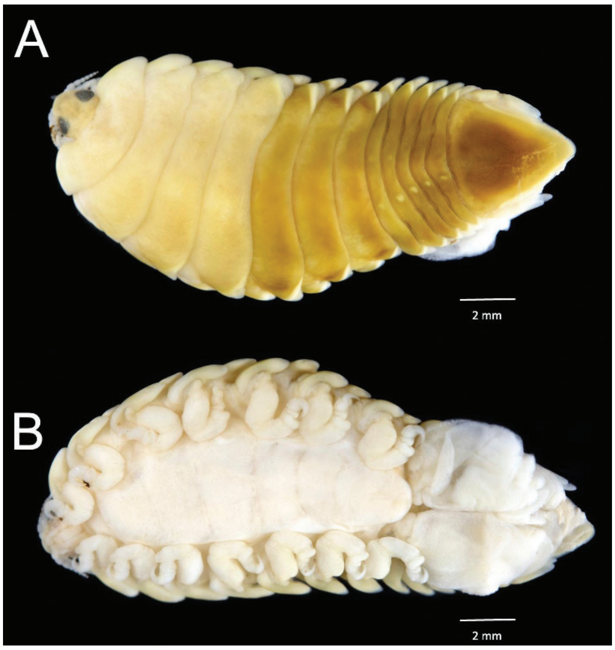

Figure 2. Ovigerous female (SC064) *Norileca indica* collected from the branchial cavity of *Selar crumenophthalmus* captured in the Sibuyan Sea, Philippines in April 2021 (**A** = dorsal view; **B** = ventral view).

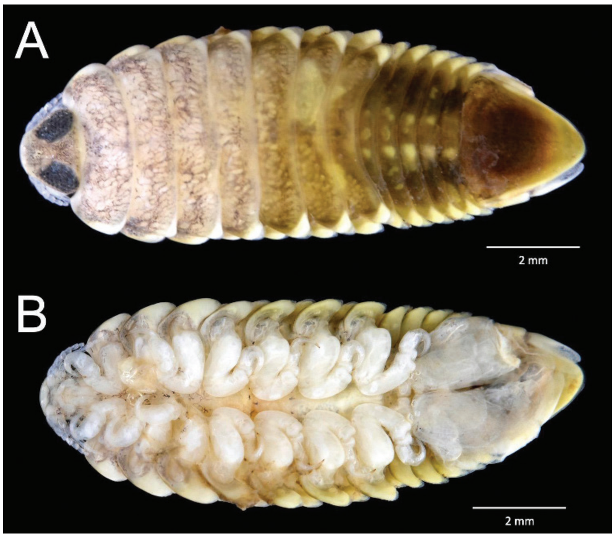

Figure 3. Male (SC064) *Norileca indica* collected from the branchial cavity of *Selar crumenophthalmus* captured in the Sibuyan Sea, Philippines in April 2021 (**A** = dorsal view; **B** = ventral view).

body part at pereonite 4. Cephalon: Triangular shape with rounded apex. Eyes: large and circular. Perionites: seven in total. Perionite 1: shortest in length. Perionites 6 and 7 narrower compared to perionites 1–5. Pleonites: narrow and positioned just behind perionite 7 overlapping with pleonite 2. Pleotelson: triangular and posteriorly pointed; dorsal surface smooth. Uropods: weak and short; visible on both sides of pleotelson in dorsal view; length not extending beyond pleotelson. Pereopods: seven pairs of soft-fleshed pereopods; pereopod 1 smallest; pereopods 2–7 gradually increasing in size. Color in preserved state: dorsal surface generally brown; anterior region light brown, transitioning to darker color towards posterior region; ventral area creamy white; pleotelson dark brown with light edges; uropods ranging from white to transparent; pereopods white.

Morphological description of male. Body size and shape: significantly smaller than females; body rather straight than twisted; dorsal surface smooth; ventral area predominantly covered with pereopods; narrowest body part at pereonite 1; widest body part at pereonite 5. Cephalon: triangular shape and rounded apex. Eyes: large and oval. Perionites: seven in total. Perionite 1 shortest. Perionites 1–4 with straight posterior margins. Perionites 5–7 with slightly concave posterior margins. Pleonites: narrow and positioned just behind perionite 7 overlapping with pleonite 1. Pleotelson: triangular and bluntly pointed; dorsal surface smooth. Uropods: weak; visible on both sides of pleotelson and nearly reaching apex of the pleotelson in dorsal view. Pereopods: seven pairs of soft-fleshed pereopods; pereopod 1 smallest; pereopods 2–4 approximately similar in size; pereopods 5–7 largest in size. Color in preserved state: dorsal surface pale brown with distinct black chromatophores; ventral area creamy white with brown coloration along edges; pleotelson dark brown with light brown edges; uropods ranging from white to transparent; pereopods white.

Order Isopoda Latreille, 1817
Family Cymothoidae Leach, 1814
Genus *Ceratothoa* Dana, 1852

Ceratothoa carinata (Bianconi, 1869)

(Fig. 4)

Cymothoa carinata.—Bianconi 1869: 210–211, pl. II, figs 2 (a–b).—Schioedte and Meinert 1883: 327–329, pl. XIII (Cym. XX) figs 1–2.—Trilles 1986: 623, tab. 1. Trilles 1994:—116–117.—Kensley 2001: 232.—Bruce 2007: 278.—Trilles 2008: 23.—Martin et al. 2013: 397–401, figs 1–3.—Nagasawa et al. 2014: 59–61, fig. 1.—Martin et al. 2015a: 266–267.

Cymothoa (*Ceratothoa*) *carinata*.—Hilgendorf 1879: 846.

Meinertia carinata.—Lanchester 1902: 378.—Stebbing 1910: 103–104.—Trilles 1972a: 1244–1245, 1256, pl. I, photos 5–7.—Trilles 1972b: 3–7, photos 1–4.—Avdeev 1979: 48, 50.

Codonophilus carinatus.—Nierstasz 1931: 132.

Ceratothoa curvicauda.—Nunomura 2006: 36–38, figs 12–13.

Ceratothoa sp. Saito 2009: 7–9, photos 1–2.

Material examined. SC005 (non-ovigerous female) extracted from the buccal cavity of the host fish: 20.30 mm TL, host *S. crumenophthalmus*, Sibuyan Sea.

Morphological description. Body shape rectangular, maintaining straight posture; longitudinal medial ridge along dorsal pereon surface present; widest part undetermined because of slight damaged of perionite 4 and 5; narrowest part observed at perionite 1. Cephalon: subtriangular in shape with rounded and broad apex. Eyes: circular, moderately small. Perionites: seven in total, becom-

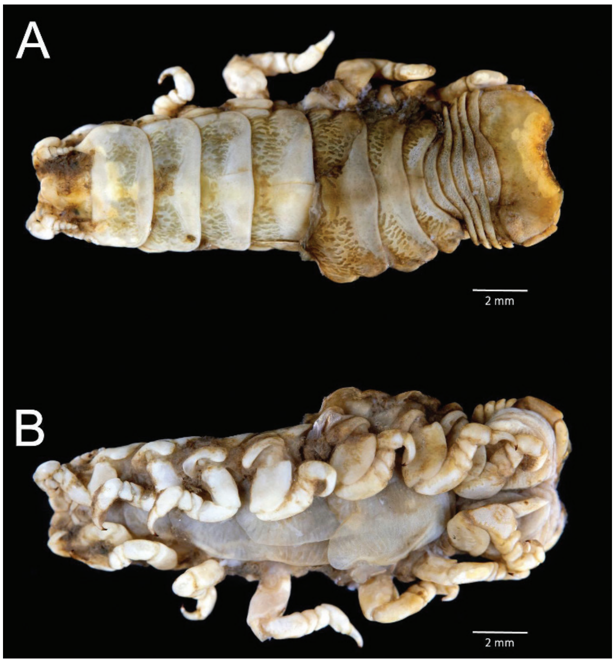

Figure 4. Non-ovigerous female (SC005) *Ceratothoa carinata* collected from the buccal cavity of *Selar crumenophthalmus* captured in the Sibuyan Sea, Philippines in April 2021 (**A** = dorsal view; **B** = ventral view).

ing narrower from perionites 5 to 7. Perionite 1: shortest; posterior margin straight; small conspicuous pointed structure extending to middle of eyes. Perionites 2–4: posterior margins straight. Perionites 5–6: posterior margins arched medially. Perionite 7: narrowest; posterior margin strongly curved medially. Pleonites: very narrow, positioned just behind perionite 7 extending to pleonite 1. Pleotelson: rather wide than long; dorsal surface with two sub-median depressions; posterior margins strongly concave. Uropods: weak; visible on both sides of pleotelson in dorsal view; similar in length to pleotelson. Pereopods: seven pairs of soft-fleshed pereopods. Pereopod 1: smallest. Pereopods 2–3: similar in size; slightly larger than previous. Pereopods 4–7: gradually increasing in size. Pereopods 5–7: largest. Color in preserved state: dorsal surface generally brown; anterior region white to brown, transitioning to darker color towards posterior region; ventral area light brown; pereopods white.

DNA barcoding. The morphological identification of the recovered parasites was confirmed by molecular analysis. Sequence analysis based on the mitochondrial *CO1* gene showed a high sequence similarity (more than 99%) of SC30 and SC064 to *N. indica*. The phylogenetic tree showing the relation of this study's specimens with other relevant cymothoid species is shown in Fig. 5. The resultant topology clearly indicates clustering of the presently reported specimens with *N. indica* KY849589.1 recovered from *S. crumenophthalmus* in the Andaman Islands, India (Praveenraj et al. 2019) and *N. indica* MF628260.1, MF628258.1, MF628259.1 from *S. crumenophthalmus* in Maputo Bay, Mozambique (van der Wal et al. 2017) supported by 99% bootstrap probability.

On the other hand, specimen SC005 showed greater than 98.00% sequence similarity with *C. carinata* confirming the morphological identification. To our knowledge, this is the first record of *C. carinata* in the Philippines. Phylogenetic analysis based on mitochondrial *CO1* genes also showed the clustering of the detected Isopoda with *C. carinata* LC724050.1, LC724049.1 recovered from *Decapterus maruadsi* (Temminck et Schlegel, 1843) in Sagami Bay, Kanagawa Japan (Fujita et al. 2023) and *C. carinata* MK652479.1 (Baillie et al. 2019) supported by 100% bootstrap probability (Fig. 5).

Prevalence and mean intensity of *Norileca indica*. In this study, 88 out of 89 bigeye scad specimens (as one fish contained *C. carinata*; specimen was not included in the analyses) of the bigeye scad collected during the month of April 2021 were examined, 13 individuals were found to have been infected with *N. indica*, leading to a prevalence of 14.77%. Of the 13 infected fish, 20 individual isopods (13 females and seven males) were extracted, resulting in a mean intensity of 1.53. All the female *N. indica* were extracted in the branchial cavities with their orientations mirroring the side of the branchial gill they attached to. Each male *N. indica* was seen along with the female isopod occupying the same gill holobranch on seven individuals of bigeye scad.

Discussion

In the presently reported study, we determined the presence of *N. indica* on the bigeye scad caught from the Sibuyan Sea in April 2021 for the first time. To date, there have only been two existing studies on the prevalence and mean intensity of *N. indica* on the bigeye scad from the Philippines. The mean intensity of *N. indica* infection in the bigeye scad

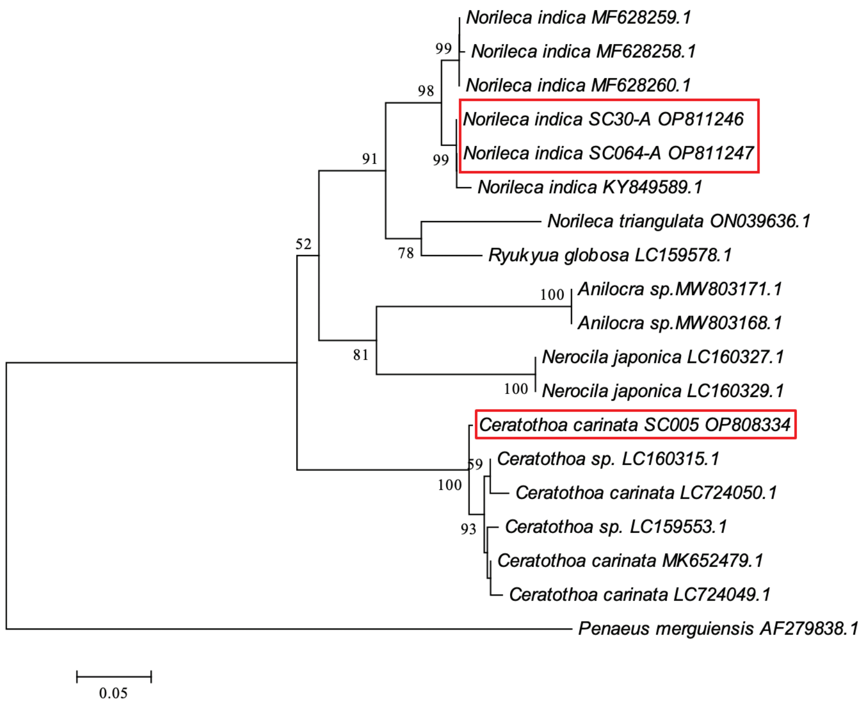

Figure 5. Neighbor-joining method used to estimate the phylogenetic relations among Cymothoidae based on mitochondrial *CO1* sequence. Bootstrap probabilities are indicated near the nodes. *Penaeus merguiensis* AF279838 was used as outgroup.

indicates the parasite load of an infected fish to be around one to two per host fish examined as also observed by Cruz-Lacierda and Nagasawa (2017) and Muji et al. (2021) in the Philippines. The presently reported study also recorded a prevalence of 14.8% which seems to be the lowest in comparison to the data recorded in the Panay Gulf (40.7%) and Batangas Bay (30%). When compared to the prevalence of *N. indica* in other southeast nations, the presently reported parameter is still quite low, especially when compared to India (Praveenraj et al. 2019; Purivirojkul and Songsuk 2020) and Thailand (Nagasawa and Petchsupa 2009) which recorded prevalence values of 21.46%–26.08% and 70%–100%, respectively. The low prevalence in this study might be due to the single sampling. Depending also on the month and year examined, parasite prevalence may vary (Cruz-Lacierda and Nagasawa 2017; Perdana et al. 2019; Jemi et al. 2020). Moreover, the prevalence of the parasite can also be attributed to the population and breeding season of the host fish (Jemi et al. 2020). Abiotic factors such as water movement and water depth may also contribute to the parasite prevalence variation (Rosa et al. 2021). To further understand the parasite–host relation in the Sibuyan Sea, monthly sampling and future studies on the bigeye scad population, reproductive biology, and analyses of associated abiotic variables are recommended.

Aside from *N. indica*, the presently reported study also collected a single specimen of another cymothoid parasite in the bigeye scad. The collected parasite was identified as *C. carinata* by its pleotelson which is rather wide than long with a concave posterior margin, subtriangular cephalon, and narrow pleonite. The identification was also confirmed by its *CO1* sequence. Similar to the report of Martin et al. (2013), no male parasites of this species were found. The hosts for this parasite have previously been identified as *S. crumenophthalmus* from Mozambique (Bianconi 1869; Hadfield et al. 2016) and Australia (Martin et al. 2013), *Decapterus macrosoma* Bleeker, 1851 from Japan (Nagasawa and Harada 2017) and India (Aneesh et al. 2022), *Decapterus maruadsi* from Japan (Nunomura 2006; Saito 2009; Nagasawa et al. 2014; Nagasawa and Harada 2016), *Decapterus maruadsi* (Temminck et Schlegel, 1844) from Japan (Nunomura 2006), *Pseudocaranx dentex* (Bloch et Schneider, 1801) from Japan (Nunomura 2006), and *Lutjanus adetii* (Castelnau, 1873) from New Caledonia (Trilles 1972a, 1972b; Martin et al. 2013). This study documents the first report of this cymothoid parasite from the Philippines.

Additionally, this study presents the first molecular characterization of cymothoid parasites infecting wild fish from the Philippine waters. DNA barcoding of *N. indica* and *C. carinata* using the sequence of the mitochondrial *CO1* gene generated a 680-bp amplicon which confirmed their morphological identification. Phylogenetic analysis of the sequences obtained in this study showed a close relation with *N. indica* isolates from India and Mozambique and *C. carinata* recovered in Japan. The use of DNA-based tools such as DNA barcoding in species identification is particularly helpful in the taxonomic studies of the family Cymothoidae as differentiating species under the family according to their morphological features appears to be challenging (Smit et al. 2014). Species identification based on the molecular structure offers multiple advantages over classical methods. However, to ensure the accuracy of the tool for future identifications, initial data on molecular characteristics submitted to public databases such as GenBank should be linked to a correctly identified specimen deposited in accessible repositories. Hence, this study utilized a combination of morphological and molecular identification methods to identify the cymothoid species recovered in *S. crumenophthalmus* from the Sibuyan Sea and to present a comprehensive initial data about the species in the region.

Conclusion

The presently reported study contributes to the limited data on parasites found in the bigeye scad from the Philippines, specifically *N. indica* and *C. carinata* as the first record of occurrence. Comprehensive studies are recommended to examine the physiological impacts of parasitism on bigeye scads at various life and reproductive stages, as well as in different seasons and fishing grounds throughout the country. Moreover, investigating mechanisms related to parasitism, host vulnerability, and immunity would be valuable, considering the potential of the bigeye scad as a species for aquaculture.

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