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Diversity and prevalence of parasitic copepods on Spanish mackerel *Scomberomorus commerson* with redescription of *Caligus infestans* from Terengganu, Malaysia

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Abstract: The Spanish mackerel *Scomberomorus commerson* is an economically important fish species in Malaysian fisheries. This study investigates the diversity, prevalence, and intensity of parasitic copepods found in Spanish mackerel from Terengganu, Malaysia with a redescription of *Caligus infestans*. Nine fish specimens were collected from three wet markets and their gill filaments were examined for the presence of parasitic copepods. The collected copepod specimens were identified to the species level, revealing three species from the family Caligidae; *Caligus asymmetricus*, *Caligus infestans* and *Caligus cybii* as well as one species from the family Pseudocycnidae, *Cybicola armatus*. Species prevalence ranged from 22% to 100%, with *C. armatus* being the most dominant parasitic copepods. The Cytochrome c oxidase subunit I (*COI*) genes of all species were sequenced, providing the first *COI* sequences for *Caligus infestans* and *C. asymmetricus*, while confirming the species identification of *C. cybii* and *C. armatus*. These findings provide baseline data on the diversity of parasitic copepods of *Scomberomorus commerson* in Malaysia, along with new locality records and DNA information for all identified parasite species.

Key words: Caligidae, Diversity, Malaysia, Pseudocycnidae, Spanish mackerel

Introduction

Scomberomorus commerson (Lacepède, 1800) (Actinopterygii; Scombriformes) also known as the Narrow-barred Spanish mackerel, is a significant marine species found in the Scombridae family (Froese & Pauly 2024). This species is recognized for its remarkable adaptability, occupying a diverse range of habitats, from the inhabiting both the deep ocean near the edges of continental shelves to shelf edges and shallow coastal waters, reef drop-offs, and lagoons (Froese & Pauly 2024). It exhibits a migratory behaviour, covering a vast distance, but also maintains resident populations in certain areas (Froese & Pauly 2024). *Scomberomorus commerson* is a crucial species for Malaysian fisheries, with an average annual catch of 4,927.6 tonnes recorded in the national fisheries statistics. Landings of this species are reported across multiple regions of Malaysia, including Perlis, Kedah, Penang, Perak, Selangor, Negeri Sembilan, Melaka, Western Johor, Kelantan, Terengganu, Pahang, Eastern Johor, Sarawak, and Sabah (Department of Fisheries Malaysia 2023).

The gills of teleost fish are an ideal habitat for various ectoparasites (Wilson & Laurent 2002). To date, four endoparasite and 21 ectoparasite species belonging to 17 families have been reported in the gills, operculum, and guts

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of *S. commerson* (Walter & Boxshall 2024). Of these, 13 species are parasitic copepods *Brachiella magna* Kabata, 1968, *Brachiella malayensis* Ohtsuka, Piasecki, Ismail & Kamarudin, 2020, *Caligus cybii* Bassett-Smith, 1898, *Caligus infestans* Heller, 1865, *Caligus pauliani* Nuñes-Ruivo & Fourmanoir, 1956, *Caligus productus* Dana, 1849–1852, *Cybicola armatus* Bassett-Smith, 1898, *Lernaeenicus alatus* Rangnekar, 1962, *Shiinoa occlusa* Kabata, 1968, *Tuxophorus cervicornis* Heegaard, 1962, *Tuxophorus cybii* Nuñes-Ruivo & Fourmanoir, 1956 and, *Unicolax ciliatus* Cressey & Cressey, 1980. Additionally, 7 parasitic monogeneans have been identified, including *Cathucotyle bilqeesae* Hadi & Bilqees, 2012, *Cathucotyle cathuaui* Lebedev, 1968, *Gotocotyla acanthura* (Parona & Perugia, 1896) Meserve, 1938, *Gotocotyla bivaginalis* (Ramalingam, 1961) Rohde, 1976, *Neothoracocotyle commersoni* Abdel Aal, Ghattas & Badawy, 2001, *Pricea multae* Chauhan, 1945, and *Pseudothoracocotyla ovalis* (Tripathi, 1956) Yamaguti, 1963) (Abdel Aal et al. 2001, Hadi & Bilqees 2012, Rothman et al. 2022, Ngo et al. 2022, Nguyen et al. 2024, Mansour et al. 2024).

Despite the importance of the species in Malaysian fisheries, studies on the diseases and parasites infecting the Scombridae family are scarce in Malaysia. To date, Ohtsuka et al. (2020) described one new species of copepod, *B. malayensis* Ohtsuka, Piaceski, Ismail & Kamarudin, 2020 recovered from the nostril of *S. commerson* from Terengganu. Thus, the present study aims to provide baseline data on the parasitic copepods infecting this fish in Malaysia. Prevalence, mean intensity, mean abundance and phylogenetic analysis of parasitic copepods infecting *S. commerson* are presented in this study. As for *C. infestans*, a thorough redescription is provided because previous works, including the most recent occurrence record by Oldewage & Van As (1989) are lacking a detailed taxonomic description with drawings being reproduced from Cressey & Cressey (1980), and lacking essential taxonomic details. This redescription seeks to rectify the insufficiencies and adhere to modern standards, while providing a more precise and thorough description of the species.

Materials and Methods

Parasite collection and identification

Fish hosts were sourced from the Kg. Lampu wet market (5°48′43.3″N, 102°32′47.7″E), LKIM Kuala Besut (5°49′50.8″N, 102°33′45.9″E), and LKIM Pulau Kambing (5°19′19.4″N, 103°07′43.7″E) in Terengganu. Specimens were purchased in July 2023. Heads of fish hosts were measured and recorded. Gills and opercula were examined, washed, and ectoparasites were extracted and counted. Ectoparasites were preserved in 95% ethanol for morphological and molecular identification. Copepods were observed using stereo (Leica EZ4E) and compound microscopes (Leica DM750 with Leica ICC50 E imager). Images were stacked in Adobe Photoshop (version 24.2.0), appendage drawings were inked digitally with Sketchbook (version 6.1.1). Prevalence, mean abundance, and mean intensity were calculated as per Bush et al. (1997), with terminology for developmental stages following Huys & Boxshall (1991), Ho & Lin (2004), and Piasecki et al. (2023). All voucher specimens are deposited in the collections of the Unisza Aquatic Biodiversity Repository, Malaysia.

Molecular identification and Deoxyribonucleic Acid (DNA) barcoding analysis

Extractions of genomic DNA from entire copepods were carried out using *FavorPrep*™ Tissue Genomic DNA Extraction Mini Kit using the Protocol for Isolation of DNA from Animal Tissue. The partial mitochondrial cytochrome c oxidase subunit I (*COI*) gene was amplified via Polymerase chain reaction (PCR) and was carried out with a total volume of 25 μL using 7.5 μL ddh2O, 0.5 μL LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G–3′) primer (10 pmol/μL), 0.5 μL HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) primer (10 pmol/μL) Folmer et al. (1994), 12.5 μL Easy myTaq DNA polymerase 2× MasterMix (Bioline) containing 60 U/μL Taq II DNA polymerase (recombinant), 400 μM dNTP mix, 3 mM $MgCl_2$,(Meridian), 4.0 μL sample DNA. The *COI* gene PCR followed these conditions: initial denaturing at 95°C for 1 min, 35 cycles with denaturing at 95°C for 1 min, annealing temperature 45°C for *C. infestans* and 54°C for *C. cybii* and *C. asymmetricus* for 1 min and elongation at 72°C for 1.30 min; followed by a final elongation at 72°C for 7 min and then dropped to 10°C. Negative controls were used to check for contamination. Confirmation of successful amplification was conducted through examination on a 2% agarose gel stained with Gel stain. The PCR products that have not been purified were sent to a private company, Apical Scientific Sdn Bhd, located in Selangor, Malaysia for purification and Sanger sequencing analysis.

Molecular identification and phylogenetic analysis

The partial mitochondrial cytochrome c oxidase subunit I (*COI*) sequences of the four species of parasitic copepods obtained from the present study were first identified using the nucleotide Basic Local Alignment Search Tool (BLAST-n) incorporated in the National Center for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). MEGA v11 software Tamura et al. (2021) was used for the sequence alignment and trimming and construction of phylogenetic tree. Maximum-Likelihood phylogenetic tree was constructed using the Tamura 3 Parameter (T92) model with Gamma distribution (+ *G*) which was selected as the best-fit model by the MEGA v11 software, using 1000 bootstraps. Two outgroups which were *Argulus* sp. (PP627426.1) and *Cymothoa indica* Fujita, 2023 (LC776736.1) were included in the phylogenetic tree analysis.

Fig. 1. A, *Caligus infestans* Heller, 1865, female; a. Light microscope image of sternal furca with ovoid accessory processed arrowed; B, *Caligus cybii* Bassett-Smith, 1898, female; C, *Caligus cybii* Bassett-Smith, 1898, male; D, *Caligus asymmetricus* Kabata, 1965, Female; E, *Caligus asymmetricus* Kabata 1965, male; F, *Cybicola armatus* Bassett-Smith, 1898, female, dorsal view; G, ventral view; H, lateral view; I, ventral view. Scale bars: 0.5 mm on A–E, 1.00 mm on F–I, 0.2 mm on a.

Results

Diversity of parasitic copepods infecting *Scomberomeros commerson*

In total, 123 parasitic copepods from four species were identified from the gills and operculum of nine *Scomberomorus commerson* purchased from wet markets in Terengganu, Malaysia. Three species are Siphonostomatoid copepods from the Caligidae family, namely *Caligus asymmetricus* Kabata, 1965 (–Kg. Lampu: 2 ♂, 6 ♀; LKIM, Kuala Besut: 1 ♀); *Caligus infestans* and *Caligus cybii* (–Kg. Lampu: 2 ♂, 5 ♀; LKIM, Pulau Kambing: 2 ♂, 12 ♀; LKIM, Kuala Besut: 4 ♂, 11 ♀) (Fig. 1). One species was from Pseudocycnidae family, *Cybicola armatus*–Kg. Lampu: 27 ♀; LKIM, Pulau Kambing: 17 ♀; LKIM, Kuala Besut: 9 ♀.

Taxonomy

Family Caligidae Burmeister, 1895
Genus *Caligus* O. F. Müller, 1785
***Caligus infestans* Heller, 1865**
(Figs. 1a, 2, 3)

Material examined. 2 ♂♂, 17 ♀♀, (UAR-CAL-2307.001-019), Kg. Lampu; 1 ♂, 2 ♀♀ (UAR-CAL-2307.020-022), LKIM, Pulau Kambing; 1 ♂ (UAR-CAL-2307.023), LKIM, Kuala Besut; all locations in Terengganu, Malaysia.

Redescription of female. Average total body length (Fig. 2A) 4.98 mm. Cephalothorax (Fig. 2A) suborbicular, 1.23 times longer than wide (2.56×2.08 mm), and 1.38 times longer than the genital complex (2.56×1.86 mm). Genital complex (Fig. 2A) is large but smaller than the cephalothorax, with a width that is 0.74 times that of the cephalothorax (2.08×1.54 mm), subrectangular shape, bearing distinct posterolateral lobes projecting toward the mid-region of the abdomen and is 1.21 times longer than wide (2.05×1.65 mm). Abdomen (Fig. 2A) indistinctly 2-segmented, with the proximal segment rectangular, narrowing anteriorly and widening posteriorly, proximal segment is 1.52 times longer than distal segment

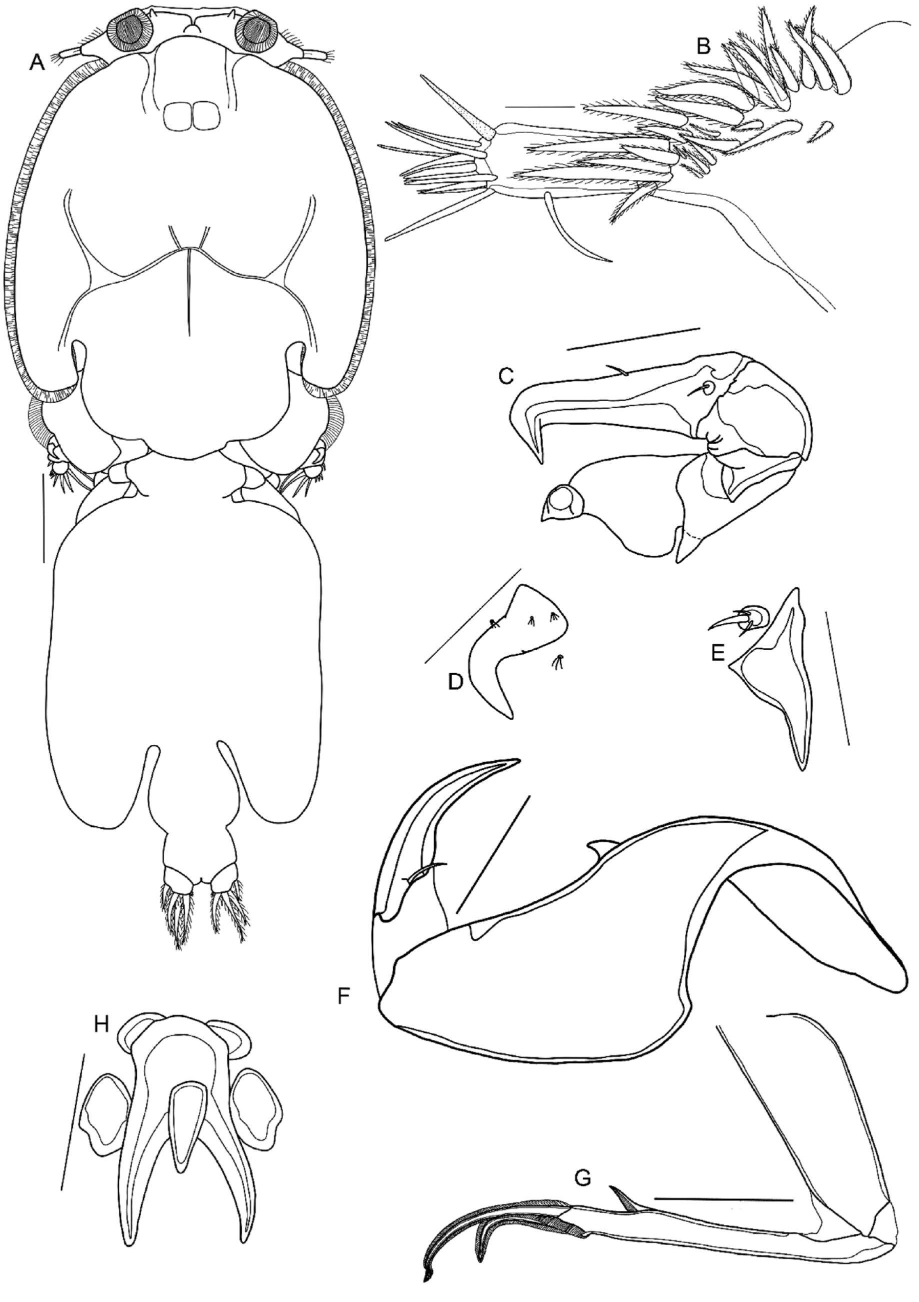

Fig. 2. *Caligus infestans* Heller, 1865, female. A, Dorsal; B, Antennule; C, Antenna; D, Postantennal process; E, Maxillule; F, Maxilliped; G, Maxilla; H, Sternal furca. Scale bars: 0.5 mm on A, 0.05 mm on B, 0.2 mm on C–H.

(0.47×0.31 mm). Caudal ramus (Fig. 2A) small, broad, being 1.64 times wider than long (0.11×0.18 mm), bearing 5 plumose setae, with the shortest innermost seta and a short outermost seta.

Antennule (Fig. 2B) 2-segmented, with 25 plumose setae on the anteroventral area of the first segment plus 2 setae located dorsally, 10 slender setae and 2 aesthetascs at the tip of distal segment, one seta positioned subapically on the distal segment. Antenna (Fig. 2C) 3-segmented; the proximal segment with pointed posterior process; middle segment subrectangular and unarmed; distal segment with a pointed, bent claw, bearing one seta in proximal and middle regions. The postantennal process (Fig. 2D) large, curved, and tapers to a bluntly pointed tip tine, resembling a hook-like or sickle-shaped structure, with 2 basal papillae bearing 3 and 4 setules respectively, and another 2 papillae, each bearing 3 setules located nearby on the sternum. Maxillule (Fig. 2E) with a dentiform process and a small basal papilla that carries 3 short setae, 2 of which are slender. Maxilliped (Fig. 2F) with robust proximal segment (corpus) bearing a medial pointed protrusion on the myxal surface; distal subchela armed with a strong, curved claw bearing a slender seta on the middle proximal area of the claw. Maxilla (Fig. 2G) 2-segmented; the proximal segment unarmed, while the slender distal segment bearing a subterminal seta with hyaline membrane along the anterior margin and 2 unequal terminal elements, each with an accessory hyaline membrane. Sternal furca (Fig. 2H) with middle area of the furca box bearing a blunt, pointed process; the tines incurved and widely divergent, along with ovoid accessory processes present on either side of the furca.

Leg 1 (Fig. 3A) biramous with slender intercoxal plate; coxa carrying a papilla bearing 2 pinnate setules on outer margin; basis bearing a plumose seta at the anterodistal corner and a short plumose seta on the posteromedial area, along with a large patch of spinules on the ventral surface; endopod vestigial process tipped with a setal vestige; exopod 2-segmented: proximal segment with a row of spinules along the posterior margin and a small spine on anterodistal corner; distal segment with 3 long plumose setae along the posterior margin, increasing in length from proximal to distal and 4 terminal elements along the distal margin: element 1 (spine) with minute serrations posteriorly, elements 2 and 3 (spines) with minute serrations anteriorly along with an accessory process, and element 4 (seta) is longer than element 1, elements 1–3 each have a pecten at their base.

Leg 2 (Fig. 3B) intercoxal plate with a narrow hyaline membrane located posteriorly; coxa with a large plumose seta on the posterodistal corner and a small setula on the proximal ventral surface; basis carrying a small seta at the anterodistal corner, with a flap of hyaline membrane on anterior margin, a slender seta in the posterior area, and a narrow hyaline membrane on the posterior margin; endopod 3-segmented: segment 1 with row of setules along the anterior margin and a plumose seta on the posterior margin; segment 2 with a double row the anterior margin and 2 plumose setae on the posterior margin along with fine setules; segment 3 with a region of denticles at the anteroproximal corner and 6 distal plumose setae; exopod with 3- segmented: segment 1 with long spine on anterodistal corner projection across ventral surface of ramus with accessory and pecten at the base of spine, with a plumose seta on posterodistal area and a row of fine setules on posteroproximal margin; segment 2 with spine on outer distal corner and a plumose seta on inner margin and a row of fine setules along the posteroproximal margin; segment 3 with 3 outer spines and 5 plumose setae, most outer spines without accessory and pecten, other 2 spines have a small hyaline membrane along the anterior margin and pinnules along the posterior side; the first and second exopodal segments bear a flap of hyaline membrane anteriorly, extending to approximately half the length of the ramus.

Leg 3 (Fig. 3C) intercoxal plate with hyaline membrane along the posterior margin, bearing plumose seta at junction. Apron with hyaline membrane on posterior margin, extending to endopod and continuing to corrugate surface on anterodistal corner. Ventral surface with dense spinules distally and small spinule cluster anteroproximally. A papilla with sensilla posterior to the cluster. Also papilla with sensilla at proximal and distal ends near posterior hyaline margin, and another papilla with sensilla near endopod base; endopod 2-segmented; first segment with fully developed velum, bearing fine setules on free margin and longest plumose seta; Distal segment with 6 plumose setae, increasing in length from outermost to innermost, second segment with fine setules; exopod 3-segmented: first segment with sickle-shaped spine, slender seta, and papilla with sensilla near exopod base on ventral surface; second segment with outer spine, fine setules on outer margin, and inner plumose seta; distal segment with 3 spiniform elements, fine setules on outer margin, and 4 plumose setae on inner margin.

Leg 4 (Fig. 3D) 3-segmented: protopod robust, with outer seta.; exopod 2-segmented, first segment subquadrate, wider than second, narrowed proximally, broadening midsection with vestigial seta posteriorly, tapering distally to outer spine with basal pecten. Second segment with 3 unequal spines distally, small outer spine, all with basal pectens.

Leg 5 (Fig. 3E) on ventral surface close to posterolateral corners with 1 papilla carrying a pinnate seta and another papilla carrying 2 pinnate setae.

Site of attachment on host: Gill wash, operculum and body surface of host.

Host: *Euthynnus alletteratus* (Rafinesque, 1810), *Galeichthys feliceps* Valenciennes, 1840, *Scomberomorus commerson*, *Scomberomorus queenslandicus* Munro, 1943 *Sphyraena barracuda* (Edwards, 1771) and *Sphyraena jello* Cuvier, 1829.

Distribution: Africa, Indonesia, India, Madagascar, Malaysia (present study), Queensland, Sri Lanka, Thai-

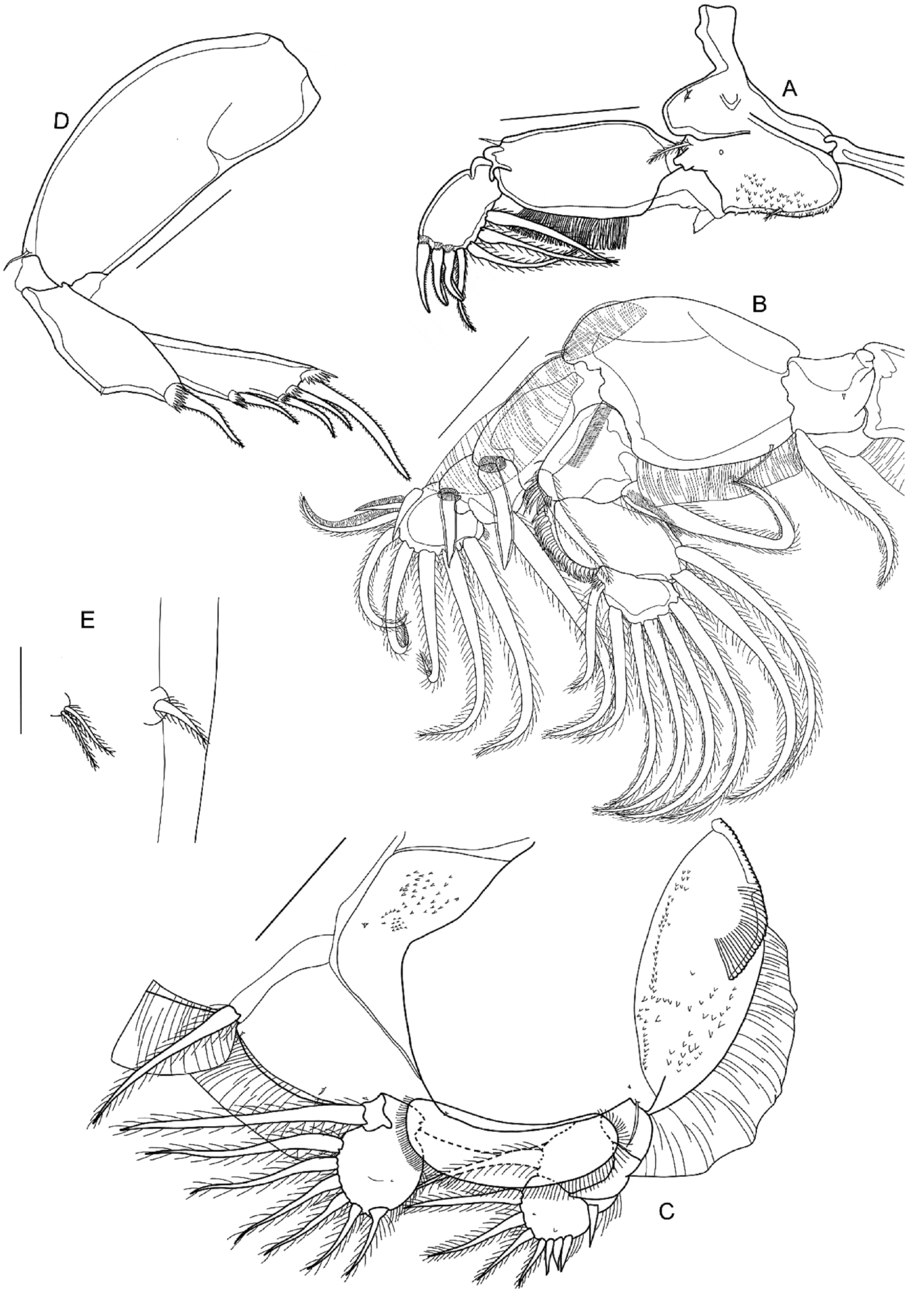

Fig. 3. *Caligus infestans* Heller, 1865, female. A, Leg 1; B, Leg 2; C, Leg 3; D, Leg 4; E, Leg 5. Scale bars: 0.2 mm on A–D, 0.05 mm on E.

land, and Yemen.

Remarks. *Caligus infestans* was first described in a German manuscript by Heller (1865). In 1956, Nuñes-Ruivo & Fourmanoir recorded *Caligus infestans* and *Caligus sphyraenae* Nuñes-Ruivo & Fourmanoir, 1956 from Madagascar, in a French manuscript (Nuñes-Ruivo & Fourmanoir 1956). They noted pronounced posterior lobes and their drawing portrayed pointed processes on the sternal furca for *C. infestans*. *Caligus sphyraenae* was later synonymized with *C. infestans* due to the lack of adult characteristics. Heegaard (1962) later described *Caligus maculatus*, a synonym for *C. infestans*, noting similarities with *C. infestans*, including a nearly square genital complex and a large open furca. Pillai (1967) recorded *C. infestans* in his reviews of parasitic copepods from Indian marine fishes, and Cressey & Cressey (1980) redescribed the species in their global survey of parasitic copepods on scombrid fishes and provided detailed drawings of *C. infestans*, but we see an opportunity to update the description to align with current standards. This species also belongs to the *bonito*-species group, but can be easily distinguished by several unique features: a prominent subquadrate abdomen with elongated posterolateral lobes extending to the middle of the abdomen (Ismail et al. 2024); sternal furca (Figs. 1a, 2H) with accessory processes on either side and along the anterior margin of the furca box, and a pointed stout structure in the middle of the furca box; and the distinctive shape of the second exopodal segment of leg 4.

Caligus asymmetricus Kabata, 1965

(Fig. 1D, E)

Material examined. 2 ♂♂, 6 ♀♀ (UAR-CAL-2307.024-031), Kg. Lampu; 1 ♀ (UAR-CAL-2307.032), LKIM, Kuala Besut; all locations in Terengganu, Malaysia. Body length of image specimen from Kg. Lampu: 3.43 mm (Fig. 1D).

Site of attachment on host: Gill wash.

Remarks. Ohtsuka & Boxshall (2019) categorized *Caligus asymmetricus* in the *Caligus bonito*-species group based on the presence of a row of denticles on the endopodal segment of leg 2. This species can be easily distinguished from other members of the *bonito* species group by its short abdomen, which is approximately as wide as it is long and less than half the length of the genital complex (Ismail et al. 2024). Boxshall (2018) reported three females and two males of *C. asymmetricu*s from *Euthynnus affinis* (Cantor, 1849), one female from *Thunnus tonggol* (Bleeker, 1851), and one female from *Auxis thazard* (Lacepède, 1800) from Australian waters. The specimen in this study shows slight differences from the Australian specimens, particularly in the relative size of the genital complex. Boxshall (2018) reported the genital complex of the Australian *C. asymmetricus* as approximately 1.2 times longer than wide, whereas the specimen in this study measures 1.03 times longer than wide (0.879×0.853 mm).

Caligus cybii Bassett-Smith, 1898

(Fig. 1B, C)

Material examined. 2 ♂♂, 5 ♀♀ (UAR-CAL-2307.033-039), Kg. Lampu; 2 ♂♂, 12 ♀♀ (UAR-CAL-2307.040-053), LKIM, Pulau Kambing; 4 ♂♂, 11 ♀♀ (UAR-CAL-2307.054-068), LKIM Kuala Besut; all locations in Terengganu, Malaysia. Body length of image specimen from LKIM Kuala Besut: 5.21 mm (Fig. 1B).

Site of attachment on host: Operculum.

Remarks. *Caligus cybii* is mostly recorded from *Scomberomorus commerson* as reported by Heegaard (1962), Kirtisinghe (1964), Pillai (1967), Cressey & Cressey (1980), Ho & Lin (2004) and Helna et al. (2018). Based on our observations, all specimens from Malaysia possess only one vestigial seta apically on the vestigial endopod of leg 1, whereas Ho and Lin (2004) reported two setules at the tip of the vestigial endopod in their specimens of *C. cybii* from Korean waters. A prominent difference observed in *C. cybii* from Terengganu is the presence of a protruding process on the posterior distal corner of the first segment of the exopod of leg 1 (Fig. 4A) which has not been previously described. *Caligus cybii* can be classified in the *diaphanus*-species group of which the main characteristic is the 3-segmented exopod on leg 4 (Boxshall 2018); compact body and the absence of a bifid cuticular rib and rosette-like array on the apron of leg 3 (Ohstuka & Boxshall 2019). However, it is interesting to note that among the *diaphanus*-species group members, *C. cybii* is the only caligid with the row of small denticles on the posterior endopodal of second segment extending to the medial region of the third segment of leg 2. Denticles on the endopod of leg 2 is the main character of the *bonito*-species group.

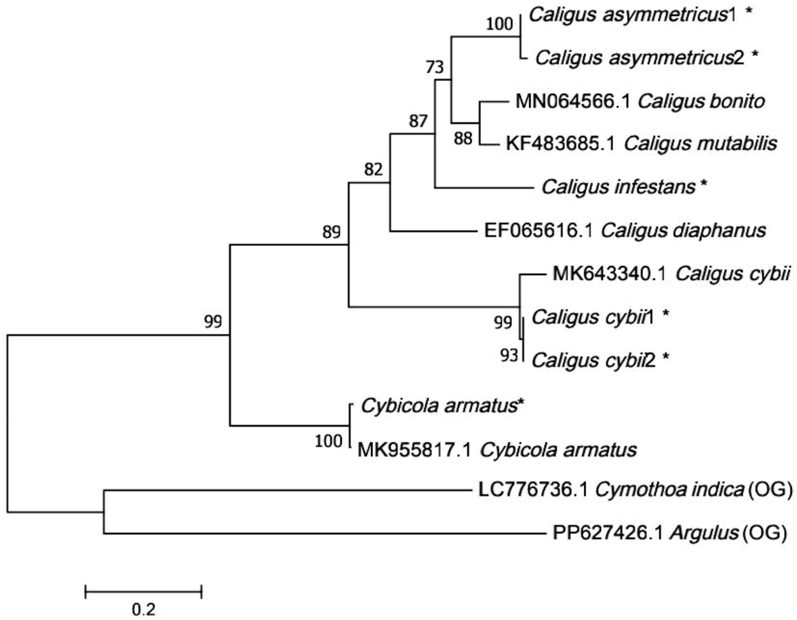

Fig. 4. The Maximum Likelihood phylogenetic tree of parasitic copepods infecting Spanish Mackerel, *Scomberomeros commersons* (Lacepede, 1800) from Malaysia. Asterisk (*) denoting *COI* sequences of parasitic copepods from this present study. OG denoting outgroup.

Family Pseudocycnidae Wilson C.B., 1922

Genus *Cybicola* Bassett-Smith, 1898

***Cybicola armatus* Bassett-Smith, 1898**

(Fig. 1F–I)

Material examined. 27 ♀♀ (UAR-PSE-2307.001-027), Kg. Lampu; 17 ♀♀ (UAR-PSE-2307.027-044), LKIM Pulau Kambing; 9 ♀♀ (UAR-PSE-2307.045-053), LKIM Kuala Besut; all locations in Terengganu, Malaysia. Body length of image specimen from Kg. Lampu: 7.70 mm (Fig. 1F).

Site of attachment on host: Operculum.

Remarks. *Cybicola armatus* is mostly found from the genus *Scomberomorus* Lacepède, 1801 primarily from *Scomberomorus guttatus* (Bloch & Schneider, 1801) (Bassett-Smith 1898; Cressey & Cressey 1980; John & Nair 1973; John & Nair 1975; John & Nair 1980; Natarajan & Nair 1972; Pillai 1964; Song & Chen 1976; Tripathi 1962). In this study, the specimen showed that the second segment and the cephalothoracic shield had equal widths (1.01 mm), whereas in Heegaard's (1962) Australian specimen, the second segment was wider than the cephalothoracic shield. Additionally, *Cybicola armatus* from *S. commerson* in Malaysia had only one lobe on the third segment, compared to the two lobes observed in the Australian specimen as described by Heegaard (1962).

Prevalence, mean intensity and mean abundance

Table 1 shows the prevalence, mean intensity and mean abundance of the four species of parasitic copepods infecting nine fish hosts in this present study. *Cybicola armatus*, which is the only parasite from Pseudocycnidae family, can be found on all examined fish hosts, contributing to the 100% prevalence of the parasites on *Scomberomorus commersons*. The number of *C. armatus* also contributed 43% (n=53) of the overall parasitic copepods found in this study.

Among the Caligidae, *Caligus cybii* exhibited the highest prevalence (77.78%), mean intensity (6.71±1.8), and the second highest mean abundance (5.22±1.8). *Caligus infestans*, with a prevalence of 66.67%, demonstrated a lower mean intensity (2.33±0.7) and mean abundance (1.56±0.7) compared to *C. cybii*. *Caligus asymmetricus* was found in only 22.22% of the hosts, the lowest prevalence among the parasites studied. Despite this, it had a relatively high mean intensity (4.50±0.9), indicating that when infections do occur, they tend to be more severe.

Molecular identification and phylogenetic analysis

Results from the BLAST-n indicate that the two *Caligus asymmetricus* sequences (PQ468448.1–645 bp) and (PQ466576.1–639 bp) and the *Caligus infestans* sequence (PQ469596.1–645 bp) from the study matched most closely the *Caligus mutabilis* Wilson, 1905 sequences deposited in the NCBI Genbank at an identical percentage of 88.63% and 86.39%, respectively where both identical percentage values are not significant to confirm their species. The *Caligus cybii* (PQ461336.1) and *Cybicola armatus* sequences (PQ468436.1) from the study had a percentage similarity of 95.49% (Query cover 98%; E-value 0) and 99.35% (Query cover 96%; E-value 0) with the deposited sequence from NCBI Genbank, thus confirming their species. The constructed Maximum Likelihood phylogenetic tree analysis of parasitic copepods identified from *Scomberomorus commerson* (Fig. 4), formed a monophyletic clade with the *C. cybii* the representative sequence from Genbank. (bootstrap 99), while *C. armatus* formed another clade with its own species representative, *C. armatus* (MK955817), also at a bootstrap value at 100, thereby further confirming the identity of these two species. Further analysis also found that there were 2 haplotypes present for *C. asymmetricus*, while *C. infestans*, *C. cybii*, *C. armatus* only had one haplotype, making the total haplotypes discovered in the study 5 haplotypes (unpublished data).

Phylogenetic analysis revealed that *C. asymmetricus* and *Caligus infestans* formed a single clade with (bootsrap 87) *C. mutabilis* (KF483685.1), the closest match found in the BLAST search. A clade that also included *Caligus bonito* (MN064566.1), and *Caligus diaphanus* (EF065616) was found with a bootsrap value of 82 which are included as the representatives of the morphologically characterized species groups. On the other hand, *C. cybii*, which belongs to the *diaphanus*-species group (Boxshall 2018), formed a separate sister group within the Caligidae family, distinct from *Caligus diaphanus*. Despite these differences, all members of the Caligidae family formed a monophyletic group in the analysis.

Discussion

The family Caligidae comprises 30 genera with 503 valid species (Walter & Boxshall 2024). Notably, the genus

Table 1. Prevalence, mean intensity and mean abundance of parasitic copepods infecting *Scomberomorus commerson* (N=9) in the present study.

Parasite	Total Parasites	Infected Samples	Prevalence (%)	Mean Intensity	Mean Abundance	Standard Error
Caligus asymmetricus	9	2	22.22	4.50	1.00	0.9
Caligus infestans	14	6	66.67	2.33	1.56	0.7
Caligus cybii	47	7	77.78	6.71	5.22	1.8
Cybicola armatus	53	9	100.00	5.89	5.89	1.0

Caligus accounts for a significant portion of this diversity, with a total of 279 species (Walter & Boxshall 2024). A total of 7 Caligidae species have been recorded infecting *Scomberomorus commerson* worldwide. In this present study, three *Caligus* were identified and we sequenced their COI genes. To date, no *COI* sequence attribute to eitherof *Caligus asymmetricus* or *C. infestans* are available on Genbank. In the BLAST-n search, the closest similarity of the *COI* sequences of both species was with *Caligus mutabilis* (with the highest hit being 86%). This similarity is below the threshold for species confirmation (Hu & Kurgan 2019). *Caligus asymmetricus*, *C. infestans* and *C. mutabilis* were morphologically classified into the *Caligus bonito*-species group based on similarities in specific morphological characteristics, particularly the presence of denticles on the second endopodal segment of leg 2 (Ismail et al. 2024, Ohtsuka & Boxshall 2019, Boxshall 2018). Thus, the monophyly of the three species with *C. bonito* is consistent with their morphological classification. The *bonito*-species group are common caligids predominantly infecting Scombridae fishes (Cressey & Cressey 1980, Ismail et al. 2024). Recently, Ismail et al. (2024) reported the first record and a new host for a member of the *bonito*-species group in Malaysia *Caligus dussumieri* Rangnekar, 1957 infecting Malabar snapper and provided the identification keys for the female specimens of the *bonito*-species group. As for *Caligus cybii*, which forms different clades with its representative *diaphanus*-group, *Caligus diaphanus* (Boxshall 2018), further analysis involving more DNA sequences of the members of this species group is necessary to clarify the evolutionary link between the caligid species included in this species group.

In the present study, although we sequenced only two specimens of *C. asymmetricus,* two COI haplotypes with 6 distinct polymorphic sites were identified, suggesting notable genetic diversity within a small sample size. *Caligus asymmetricus* has previously been recorded in India, Australia, Taiwan, and South Africa, infecting 11 species of scombrid fish (Walter & Boxshall 2024, Ismail et al. 2024). In this study, *C. asymmetricus* exhibited the lowest prevalence but a relatively high mean intensity of infection, suggesting that when infections do occur, they tend to involve multiple parasites, leading to severe infestations. The presence of such diversity, even in a limited sample, could provide important insights into the evolutionary dynamics of *C. asymmetricus* and its interactions with its hosts, making it a key target for future ecological and evolutionary research. Understanding this diversity is crucial for managing its potential impact on both wild and farmed fish populations.

All examined hosts in this present study were infected by *Cybicola armatus* which comprised almost half of the total parasites found in this present study. This species can be found on the operculum of the host. Within the genus *Cybicola*, only two valid species have been documented, with these being *Cybicola buccatus* (Wilson C.B., 1922) and *C. armatus* (Walter & Boxshall 2024). This study presents the first documented occurrence of *Cybicola* in Malaysia. This species showed a unique pattern, being present in all examined hosts. The mean intensity and mean abundance were identical (5.89±1.0), reflecting a uniform and substantial parasitic burden across the entire host population. This uniformity indicates that *C. armatus* is highly adapted to infecting *S. commerson* and may have evolved mechanisms to ensure its widespread presence. The consistent parasite load suggests a stable host-parasite relationship, possibly indicating a long-term co-evolution between *C. armatus* and *S. commerson.*

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