

# Further record of the marine fish ectoparasite *Nerocila phaiopleura* (Isopoda: Cymothoidae) from Mie Prefecture, central Japan, and an update on the distribution of the isopod in Japan

Kazuya Nagasawa<sup>1,2</sup> and Makoto Okada<sup>3</sup>

<sup>1</sup>Graduate School of Integrated Sciences for Life, Hiroshima University,  
1-4-4 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8528, Japan

<sup>2</sup>Aquaparasitology Laboratory, 365-61 Kusanagi, Shizuoka 424-0886, Japan

<sup>3</sup>Suzuka Fisheries Laboratory, Mie Prefecture Fisheries Research Institute,  
1-6277-4 Shiroko, Suzuka, Mie 510-0243, Japan

## Abstract

An ovigerous female of *Nerocila phaiopleura* Bleeker, 1857 was found on one (prevalence, 0.7%) of 141 individuals of Japanese sardine *Sardinops melanostictus* (Temminck and Schlegel, 1846) from coastal waters of the western North Pacific Ocean off Mie Prefecture, central Japan, on 6 March 2015. The female was firmly attached using its dactyli to the host's posterior body surface near the lateral line above the anal fin. A large oval-shaped wound with exposed muscles was found at the attachment site on the infected fish. Japanese sardine represents the fifth host record for *N. phaiopleura* from Mie Prefecture. In addition, based on the published information covering the period up to 2024, the distribution of *N. phaiopleura* in Japan is updated here. Most of the collection localities of the species exist in coastal Pacific waters of central and western Japan and the Seto Inland Sea. There are also two collection records of the species each from the East China Sea and the southern Sea of Japan. The species occurs in the waters affected by two warm currents, the Kuroshio, and its branch, the Tsushima Current.

## Introduction

*Nerocila phaiopleura* Bleeker, 1857 is a skin parasite of marine fishes in the southern and northern hemispheres (Suresh et al., 2024). This species was originally described by Bleeker (1857) from Indonesia

and has so far been reported from 11 countries in the Indo-West Pacific region, including Indonesia, Australia, Japan, China, Singapore, Thailand, India, Pakistan, Kuwait, South Africa (see Suresh et al., 2024 for the literature) and Malaysia (Seng and Seng, 1990). The species was also recorded from the Gulf of Thailand (reported as the Gulf of Siam, Monod, 1934) and the Bay of Bengal (Barnard, 1936).

In Japan, *N. phaiopleura* was reported for the first time in 1982 (Mitani, 1982) and has since been studied for various aspects of its biology, such as the distribution, host utilization, juveniles, and impacts on the host fish (Williams and Bunkley-Williams, 1986; Bruce and Harrison-Nelson, 1988; Hiramoto, 1996; Saito and Hayase, 2000; Nunomura, 2011; Saito et al., 2014; Nagasawa and Tensha, 2016; Hata et al., 2017; Nagasawa and Nakao, 2017; Nagasawa and Shirakashi, 2017; Nagasawa and Isozaki, 2017, 2020; Nagasawa and Kawai, 2018; Saito and Ogawa, 2019; Nagasawa, 2019; Nagasawa et al., 2019, 2020; Nagasawa and Ebisawa, 2020; Kondo et al., 2021; Saito and Okabe, 2023; Nagasawa and Suzuki, 2024). The species is not a host-specific parasite and, to date, it has been recorded from 12 species of marine fishes in seven families of two orders (Nagasawa and Isozaki, 2020; Kondo et al., 2021). Most of its records from Japan were from wild fishes but it is also known to infect farmed Pacific bluefin tuna *Thunnus orientalis* (Temminck and

---

Nagasawa, K. and M. Okada. 2024. Further record of the marine fish ectoparasite *Nerocila phaiopleura* (Isopoda: Cymothoidae) from Mie Prefecture, central Japan, and an update on the distribution of the isopod in Japan. *Nature of Kagoshima* 51: 119–124.

✉ KN: Graduate School of Integrated Sciences for Life, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8528, Japan; present address: Aquaparasitology Laboratory, 365-61 Kusanagi, Shizuoka 424-0886, Japan (e-mail: ornatus@hiroshima-u.ac.jp)

Received: 6 September 2024; published online: 10 September 2024; [https://journal.kagoshima-nature.org/archives/NK\\_051/051-024.pdf](https://journal.kagoshima-nature.org/archives/NK_051/051-024.pdf)

Schlegel, 1844) (Shirakashi, 2013; Nagasawa and Shirakashi, 2017). Although a flyingfish (called Hosotobi-uo) *Cypselurus hiraii* Abe, 1953 (Belontiiformes: Exocoetidae) was listed as one of the Japanese hosts of *N. phaiopleura* by Nunomura and Shimomura (2021), this fish species has never been reported to harbor the isopod in Japan.

Mie Prefecture is located in central Japan and faces the western North Pacific Ocean. *Nerocila phaiopleura* has been reported from four fish species in this prefecture, i.e., Japanese barracuda *Sphyræna japonica* Bloch and Schneider, 1801, Japanese jack mackerel *Trachurus japonicus* (Temminck and Schlegel, 1844), round herring *Etrumeus micropus* (Temminck and Schlegel, 1846), and gnomefish *Scombrops boops* (Houttuyn, 1782) (Nagasawa and Isozaki, 2017, 2020).

A specimen of *N. phaiopleura* was collected from Japanese sardine *Sardinops melanostictus* (Temminck and Schlegel, 1846) in Mie Prefecture in 2015 and is reported here as the fifth host record for this cymothoid from the prefecture. Moreover, the distribution of *N. phaiopleura* in Japan was previously summarized by Nagasawa et al. (2020), but it is herein updated because six papers regarding this species have since been published (Nagasawa and Isozaki, 2020; Nagasawa and Ebisawa, 2020; Kondo et al., 2021; Saito and Okabe, 2023; Nagasawa and Suzuki, 2024; this paper).

## Materials and Methods

In total, 141 individuals of Japanese sardine were collected from coastal waters of the western North Pacific Ocean (34°15'33"N, 136°37'11"E, locality 11 in Fig. 2) off Nayaura, Minami-Ise, Mie Prefecture, on 6 March 2015. These fish were transported on ice to the laboratory of Mie Prefecture Fisheries Research Institute, Hamajima, where they were measured for their body length (mm) and examined for parasitic crustaceans. One cymothoid isopod was carefully removed from a fish, fixed in 5% formalin, and then preserved in 70% ethanol. Later, at the Aquaparasitology Laboratory, Shizuoka, this isopod was observed and identified using an Olympus SZX10 stereo microscope. The specimen of *N. phaiopleura* has been deposited in the Crustacea collection of the National Museum of Nature and Science, Tsukuba, Ibaraki Prefecture, Japan (NSMT-Cr 32357). The scientific and common names of fishes mentioned in this paper follow Froese and Pauly (2024), except the scientific name of Japanese sardine, which is based on Motomura (2024).

## Results and Discussion

An ovigerous female of *N. phaiopleura* was found on one (130 mm body length) of the 141 fish examined [106–148 (mean, 129, n = 141) mm body length, 0.7% in prevalence]. The female was firmly attached using its dactyli to the host's posterior body surface near the lateral line above the anal fin, and its cephalon was oriented anteriorly (Fig. 1A, B). A large oval-shaped wound (12.5 mm long, 6.1 mm wide) with exposed muscles was found at the attachment site (Fig. 1B).

The female (Fig. 1C, D) measures 18.8 mm body length (excluding uropod rami) and 7.8 mm maximum body width. It is morphologically characterized by an elliptical, dorsoventrally flattened body, the cephalon with a broadly rounded anterior margin, pereonite 1 with a concaved anterior margin, pleonites 1 and 2 with lateral processes slightly produced, a nearly triangular pleotelson with lateral margins smoothly curved, and uropod rami slightly curved and slender. When fresh, dark chromatophores are scattered over the dorsal surface of the body. Two pale black stripes exist in parallel on the dorsal surface of pereonites 4 to 7, and two black stripes run from the lateral portions of the pleon and pleotelson to the posterior ends of uropod exopods.

These morphological features of the female correspond to the descriptions of *N. phaiopleura* from Kuwait (Bowen and Tareen, 1983), Australia (Bruce, 1987), Malaysia (Seng and Seng, 1990), India (Ravichandran et al., 2019; Suresh et al., 2024), and Japan (Saito and Hayase, 2000; Nagasawa and Tensha, 2016; Nagasawa and Shirakashi, 2017; Nagasawa et al., 2020), and the female is herein identified as this species. Although *N. phaiopleura* is not a host-specific parasite, Japanese sardine is one of the hosts frequently utilized by this parasite in Japan (Mitani, 1982; Bruce and Harrison-Nelson, 1988; Hiramoto, 1996; Saito and Hayase, 2000; Nunomura, 2011; Hata et al., 2017; Nagasawa et al., 2020; Nagasawa and Suzuki, 2024).

The wound with exposed muscles found at the attachment site of *N. phaiopleura* (Fig. 1B) is one of the typical features of injuries in the fishes infected with this parasite. In Japan, such severe wounds have been reported from Japanese sardine (Mitani, 1982; Saito and Hayase, 2000; Nagasawa and Suzuki, 2024) and other eight fish species: Japanese Spanish mackerel *Scomberomorus niphonius* (Cuvier, 1832) (Nagasawa and Tensha, 2016), Pacific bluefin tuna (Nagasawa and

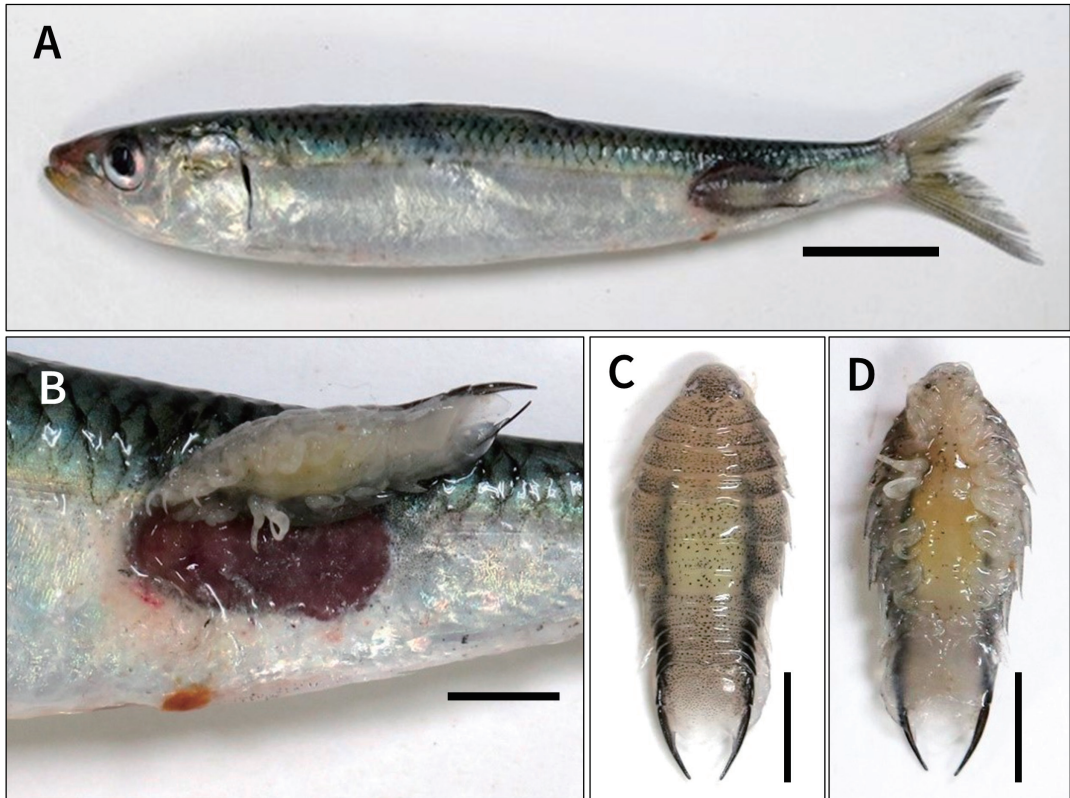


Fig. 1. Japanese sardine *Sardinops melanostictus* (130 mm body length) infected with an ovigerous female of *Nerocila phaiopleura* (18.8 mm body length, excluding uropod rami; NSMT-Cr 32357) from coastal waters of the western North Pacific Ocean off Nayaura, Minami-Ise, Mie Prefecture, central Japan, on 6 March 2015. A, an infected fish, lateral view; B, a wound with exposed muscles, lateral view; C and D, habitus of *N. phaiopleura*, dorsal and ventral views, respectively. Fresh specimen. Scale bars: A, 20 mm; B–D, 5 mm.

Shirakashi, 2017), Japanese barracuda, Japanese jack mackerel, round herring (Nagasawa and Isozaki, 2017), chub mackerel *Scomber japonicus* Houttuyn, 1782 (Nagasawa and Kawai, 2018), gnomefish (Nagasawa and Isozaki, 2020), and Kuro-mitsu *Scombrops gilberti* (Jordan and Snyder, 1901) (Kondo et al., 2021). The severe wounds are inferred to be a result from the feeding of *N. phaiopleura* on the host tissues. Slight wounds are also known from two Japanese fish species infected with *N. phaiopleura*, i.e., Japanese sardine (Mitani, 1982; Nagasawa and Suzuki, 2024) and chub mackerel (Nagasawa and Kawai, 2018; Nagasawa and Ebisawa, 2020; Saito and Okabe, 2023). Small scars are formed by insertion of the dactyli of *N. phaiopleura* in the slight wounds, which have been regarded as the early stage of injuries (Mitani, 1982; Nagasawa and Kawai, 2018; Nagasawa and Suzuki, 2024).

Previously, Nagasawa et al. (2020) showed the distribution of *N. phaiopleura* in Japan. Nevertheless, it is here updated and illustrated (Fig. 2) because the

knowledge of this species has recently increased (Nagasawa and Isozaki, 2020; Nagasawa and Ebisawa, 2020; Kondo et al., 2021; Saito and Okabe, 2023; Nagasawa and Suzuki, 2024; this paper). *Nerocila phaiopleura* has been recorded mainly from coastal Pacific waters of central and western Japan (localities 1–12, 18) and the Seto Inland Sea (localities 13–17). There is an offshore record of the species from the East China Sea (locality 19). It was also collected in the southern Sea of Japan (locality 20). There is no record of the species from the northern (i.e., Hokkaido and northern Honshu) and the southern (i.e., Okinawa) regions. While Saito and Hayase (2000) suggested to regard “*Aegathoa* sp.” reported by Nunomura (1985, 1999) as *N. phaiopleura* from Toyama Bay (locality 21), their suggestion needs to be verified.

As earlier pointed out by Nagasawa et al. (2020), the distribution of *N. phaiopleura* in Japan is affected by two warm currents, the Kuroshio, and its branch, the Tsushima Current (Fig. 2). The Kuroshio flows from the

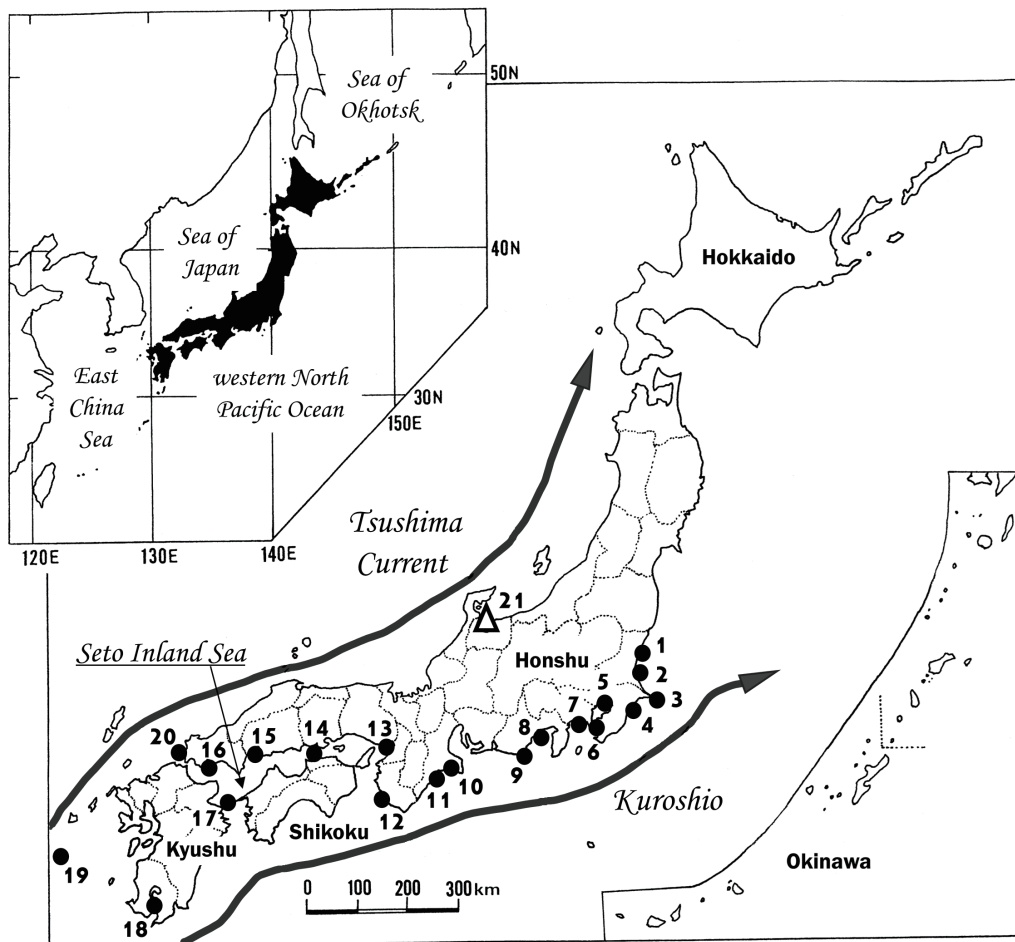


Fig. 2. Map of the Japanese Archipelago, showing the localities where *Nerocila phaiopleura* was collected between 1982 and 2024. 1, Hitachi Port (Nagasawa et al., 2020); 2, Hitachinaka (Nagasawa and Ebisawa, 2020); 3, near Cape Inubo (Nagasawa and Suzuki, 2024); 4, Kujukuri (Hiramoto, 1996); 5, Tokyo Bay (Mitani, 1982; Bruce and Harrison-Nelson, 1988; Hiramoto, 1996; Saito and Okabe, 2023); 6, Kaneda Bay and adjacent region (Mitani, 1982; Bruce and Harrison-Nelson, 1988; Saito and Hayase, 2000); 7, Sagami Bay and Katase Coast (Hata et al., 2017; Saito and Ogawa, 2019); 8, Suruga Bay (Saito and Hayase, 2000; Saito and Ogawa, 2019); 9, Omaezaki Harbor (Saito and Hayase, 2000; Nunomura, 2011); 10, Kowaura Bay (Nagasawa and Isozaki, 2017, 2020); 11, Nayaura (this paper); 12, Shirahama (Nagasawa and Shirakashi, 2013; Nagasawa et al., 2019); 13, Osaka Bay (Saito et al., 2014; Saito and Ogawa, 2019); 14, Seto Inland Sea (Hata et al., 2017); 15, Hiroshima Bay (Saito and Hayase, 2000; Nagasawa and Kawai, 2018); 16, western Seto Inland Sea (Nagasawa and Tensha, 2016); 17, Hoyo Strait (Nagasawa and Nakao, 2017); 18, Kagoshima Bay (Williams and Bunkley-Williams, 1986); 19, East China Sea (Nagasawa, 2019); 20, Hibiki-Nada Sea (Kondo et al., 2021). Locality 21 (open triangle) is Toyama Bay, where a juvenile of cymothoid ("*Aegathoa* sp.") was collected by Nunomura (1985, 1999) (see the text for identification of this juvenile). The routes of two warm currents, the Kuroshio, and its branch, the Tsushima Current, are also shown. Dotted lines indicate prefectural boundaries.

southwest to the northeast off the south coast of three major islands (Honshu, Shikoku, and Kyushu), and 13 of the 20 collection localities of the species are found in the waters along the south coast of Honshu and Kyushu. The Tsushima Current flows from the southwest to the northeast off the northwest coast of Kyushu and then off the north coast of western Honshu. The collection localities of the species in the East China Sea and the

southern Sea of Japan are found near the route of the Tsushima Current. The species was also collected at five locations in the Seto Inlands Sea, whose oceanographic conditions are largely influenced by water exchanges between this sea, the western North Pacific Ocean, and the southern Sea of Japan through three channels.

Much remains unknown on the distribution of *N. phaiopleura* in coastal waters of the Sea of Japan off

western and central Honshu (Fig. 2) and it is desirable to study the occurrence of this parasite on fishes in these waters for clarifying its distribution in Japan. Moreover, since the host specificity of *N. phaiopleura* is not strict, more work is necessary to understand its host utilization in Japanese waters.

### Acknowledgments

This study was financially supported by the Fisheries Agency of Japan for the research and assessment program of coastal fisheries resources. We thank Keiichi Kakui, Hokkaido University, Sapporo, for his assistance with literature.

### References

- Barnard, K. H. 1936. Isopods collected by the R. I. M. S. "Investigator." Records of the Indian Museum, Calcutta, 38: 147–191.
- Bleeker, P. 1857. Recherches sur les Crustacés de l'Inde Archipelagique. II. Sur les Isopodes Cymothoïdiens de l'Archipel Indien. Verhandelingen der Natuurkundige Vereeniging in Nederlandsch-Indië, 2: 20–40, 2 pls.
- Bowman, T. E. and Tareen, I. U. 1983. Cymothoidae from fishes of Kuwait (Arabian Gulf) (Crustacea: Isopoda). Smithsonian Contribution to Zoology, 382: 1–30.
- Bruce, N. L. 1987. Australian species of *Nerocila* Leach, 1818, and *Creniola* n. gen. (Isopoda: Cymothoidae), crustacean parasites of marine fishes. Records of the Australian Museum, 39: 355–412.
- Bruce, N. L. and Harrison-Nelson, E. B. 1988. New records of fish parasitic marine isopod crustaceans (Cymothoidae, subfamily Anilocrinae) from the Indo-West Pacific. Proceedings of the Biological Society of Washington, 101: 585–602.
- Froese, R. and Pauly, D. (eds.) 2024. FishBase. World Wide Web electronic publication. www.fishbase.org, version (02/2024) (17 July 2024).
- Hata, H., Sogabe, A., Tada, S., Nishimoto, R., Nakano, R., Kohya, N., Takeshima, H. and Kawanishi, R. 2017. Molecular phylogeny of obligate fish parasites of the family Cymothoidae (Isopoda, Crustacea): evolution of the attachment mode to host fish and the habitat shift from saline water to freshwater. Marine Biology, 164: 105. doi 10.1007/s00227-017-3138-5.
- Hiramoto, K. 1996. [Natural History of Japanese Sardine – Survival Strategy of “Marine Rice” –]. Chuokoronsha, Tokyo. 183 pp. (In Japanese).
- Kondo, M., Kimura, T., Yoshikawa, H. and Abe, S. 2021. New host records for *Nerocila japonica* and *N. phaiopleura* (Isopoda: Cymothoidae). Journal of National Fisheries University, 69: 103–107. (In Japanese with English abstract).
- Mitani, I. 1982. Variations of condition factor of sardine as host by *Nerocila phaeopleura* Bleeker as parasites. Bulletin of the Japanese Society of Scientific Fisheries, 48: 611–615. (In Japanese with English abstract).
- Monod, T. 1934. Isopodes marins des campagnes du “de Lanessan.” Notes de l'Institut Océanographique de l'Indochine, Saigon, 23: 1–22, 45 pls.
- Motomura, H. 2024. List of Japan's all fish species. Current standard Japanese and scientific names of all fish species recorded from Japanese waters. The Kagoshima University Museum, Kagoshima. Online ver. 26 https://www.museum.kagoshima-u.ac.jp/staff/motomura/jaf.html (18 August 2024). (In Japanese).
- Nagasawa, K. 2019. First record of *Nerocila phaiopleura* (Isopoda: Cymothoidae) in the East China Sea. Nature of Kagoshima, 46: 221–224. (In Japanese with English abstract).
- Nagasawa, K. and Ebisawa, Y. 2020. A note on *Nerocila phaiopleura* (Isopoda: Cymothoidae) parasitic on age-0 chub mackerel, *Scomber japonicus* (Perciformes: Scombridae), in coastal Pacific waters of central Japan, with a discussion on the status of coastal young chub mackerel as a major host of the isopod. Nature of Kagoshima, 47: 105–108. (In Japanese with English title).
- Nagasawa, K. and Isozaki, S. 2017. Three new host records for the marine fish ectoparasite, *Nerocila phaiopleura* (Isopoda: Cymothoidae), with a list of its known hosts. Crustacean Research, 46: 153–159.
- Nagasawa, K. and Isozaki, S. 2020. The cymothoid isopod *Nerocila phaiopleura* parasitic on gnomefish, *Scomberops boops*, in coastal Pacific waters of central Japan, with an updated list of the hosts reported from Japan. Nature of Kagoshima, 46: 525–530.
- Nagasawa, K. and Kawai, K. 2018. *Nerocila phaiopleura* (Isopoda: Cymothoidae) parasitic on chub mackerel *Scomber japonicus* in the Seto Inland Sea, western Japan, with an observation on a skin wound caused by the isopod. Cancer, 27: 83–85. (In Japanese).
- Nagasawa, K. and Nakao, H. 2017. Chub mackerel, *Scomber japonicus* (Perciformes: Scombridae), a new host record for *Nerocila phaiopleura* (Isopoda: Cymothoidae). Biosphere Science, 56: 7–11.
- Nagasawa, K. and Shirakashi, S. 2017. *Nerocila phaiopleura*, a cymothoid isopod parasitic on Pacific bluefin tuna, *Thunnus orientalis*, cultured in Japan. Crustacean Research, 46: 95–101.
- Nagasawa, K. and Suzuki, E. 2024. Infection of *Nerocila phaiopleura* (Isopoda: Cymothoidae) on Japanese sardine, *Sardinops melanostictus*, in coastal Pacific waters near Cape Inubo, central Japan, and wounds caused by this parasite on the host skin. Nature of Kagoshima, 51: 111–118. (In Japanese with English abstract).
- Nagasawa, K. and Tensha, K. 2016. *Nerocila phaiopleura* (Isopoda: Cymothoidae) parasitic on Japanese Spanish mackerel *Scomberomorus niphonius* in the Seto Inland Sea, Japan. Biogeography, 18: 71–75.
- Nagasawa, K., Shirakashi, S. and Yamamoto, S. 2019. *Nerocila phaiopleura* and *N. japonica* (Isopoda: Cymothoidae) parasitic on coastal marine fishes off Wakayama Prefecture, central Japan. Nature of Kagoshima, 46: 177–180. (In Japanese with English abstract).
- Nagasawa, K., Nitta, M., Otawa, T. and Ishikawa, T. 2020. *Nerocila phaiopleura* (Isopoda: Cymothoidae): a new record from Ibaraki Prefecture, central Japan, with a discussion of its distribution in Japanese waters. Crustacean Research, 49: 41–47.
- Nunomura, N. 1985. Marine isopod crustaceans in the coast of Toyama Bay. Memoirs of the National Science Museum, Tokyo, 18: 121–139.

- Nunomura, N. 1999. Seashore invertebrate fauna of Himi Coast, Toyama Bay 1. Animals except Mollusca. Bulletin of the Toyama Biological Society, 38: 29–39. (In Japanese with English abstract).
- Nunomura, N. 2011. Crustaceans No. 2 (Isopoda). Special Publication of the Toyama Science Museum, 24: 1–133. (In Japanese).
- Nunomura, N. and Shimomura, M. 2021. Isopoda from Japan (67) Suborder Cymothoidea, Family Cymothoidae, genera *Nerocila* and *Renocila*. Aquabiology, 43: 80–87. (In Japanese with English abstract).
- Ravichandran, S., Vigneshwaran, P. and Rameshkumar, G. 2019. A taxonomic review of the fish parasitic isopod family Cymothoidae Leach, 1818 (Crustacea: Isopoda: Cymothoidea) of India. Zootaxa, 4622: 1–99.
- Saito, N. and Hayase, Y. 2000. Note on an aegathoid stage of cymothoid isopod, *Nerocila phaiopleura* Bleeker, 1857 (Crustacea: Isopoda: Cymothoidae) stranded at Miho beach, Suruga Bay, middle of Japan. I. O. P. Diving News, 11(10): 2–6. (In Japanese with English abstract).
- Saito, N. and Ogawa, H. 2019. Growth of the aegathoid stage of *Nerocila phaiopleura* (Isopoda: Cymothoidae). Cancer, 28: 21–24. (In Japanese with English abstract).
- Saito, N. and Okabe, K. 2023. Additional record of *Nerocila phaiopleura* (Crustacea, Isopoda, Cymothoidae) infesting a chub mackerel *Scomber japonicus* (Perciformes, Scombridae) from Tokyo Bay, central Japan, Natural History Report of Kanagawa, 44: 1–4. (In Japanese with English abstract).
- Saito, N., Yamauchi, T., Ariyama, H. and Hoshino, O. 2014. Descriptions and ecological notes of free-swimming forms of cymothoid isopods (Crustacea: Peracarida) collected in two coastal waters of Japan. Crustacean Research, 43: 1–16.
- Seng, L. K. and Seng, L. T. 1990. The genus *Nerocila* (isopoda: Flabellifera) from the marine fish *Triacanthus breverostris*, Penang, Malaysia. Journal of Bioscience, 1: 87–100.
- Shirakashi, S. 2013. Parasites of cultured bluefin tuna. Japanese Journal of Veterinary Parasitology, 12: 95–104. (In Japanese with English abstract).
- Suresh, A. S., Nair, B. R. P. S., Mangalathettu, B. T. and Aneesh, P. T. 2024. Redescription and molecular characterization of the external attaching fish parasitic cymothoid, *Nerocila phaiopleura* Bleeker, 1857 (Crustacea: Isopoda) off the southwest coast of India. Acta Parasitologica. doi.org/10.1007/s11686-024-00870-7
- Williams, E. H. and Bunkley-Williams, L. 1986. The first *Anilocra* and *Pleopodias* isopods (Crustacea: Cymothoidae) parasitic on Japanese fishes, with three new species. Proceedings of the Biological Society of Washington, 99: 647–657.