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ORIGINAL ARTICLE

Simultaneous double parasitism by the parasitic cymothoids (Crustacea: Isopoda) of two genera on a single host fish *Tenualosa toli* from India

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ABSTRACT

Members of the fish parasitic isopod family Cymothoidae usually parasitize a host fish by a single species infestation. For the first time from Indian waters two species of cymothoids, the body surface infesting *Anilocra grandmaae* Aneesh, Hadfield, Smit and Kumar, 2021 and the branchial infesting *Agarna malayi* Tiwari, 1952, were reported simultaneously parasitizing the same individual toli shad, *Tenualosa toli* (Valenciennes, 1847). Each double-infested *T. toli* harboured an ovigerous female of *A. grandmaae* and an ovigerous female and male of *A. malayi*. Out of 814 host fishes collected from seven different localities, along the Kerala coast, southwest coast and Bay of Bengal coast, 113 fishes were infested with only *A. malayi* and 71 individuals were infested with only *A. grandmaae*. Interestingly, nine individuals of *T. toli* harboured both *A. malayi* and *A. grandmaae* simultaneously. Cymothoid co-occurrence is rarely reported, and this is the first report of two cymothoid species infesting a single fish host from India. Globally, it is the third record of simultaneous occurrence of two cymothoids and the first record of body surface and branchial cymothoids parasitising the same individual fish.

KEYWORDS

Agarna malayi, *Anilocra grandmaae*, Cymothoidae, double parasitism, Indian Ocean, marine fish parasite

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Introduction

Crustaceans show great diversity in body form, mode of living and habitat. Most of them are free-living and a significant proportion exhibits obligatory parasitism, infesting a diverse array of hosts, from sponges to marine mammals (Aneesh, 2014). Among these hosts, fishes are the most potential hosts for parasitic crustaceans, and the major fish parasitic crustaceans include isopods, branchiurans, cirripedes, and copepods. Even though two or more parasitic crustaceans are known to be reported from a host, usually a single fish harbours a single species of parasitic crustacean at a time. But a few reports are available on the multiple simultaneous infestations of parasitic crustaceans. Daniel and Premkumar (1967), for example, reported the simultaneous infestation of flying fish, *Hirundichthys speculiger* (Valenciennes, 1847) by a cirripede, *Conchoderma virgatum* Spengler, 1789 and a copepod, *Pennella* sp., and the incidence of multiple infestations of the Great white shark, *Carcharodon carcharias* (Linnaeus, 1758), by 5–8 different siphonostomatoid (copepod) species was also reported by Benz *et al.* (2003).

In India, the occurrence of double parasitism involving the isopod, *Nerocila phaeopleura* Bleeker, 1857 and the copepod, *Lernaeenicus sprattae* (Sowerby, 1806) was reported in anchovy fish, *Stolephorus commersonnii* Lacepède, 1803 (see Rajkumar *et al.,* 2006) and the marine fish *Hemiramphus far* (Forsskål, 1775) also showed a co-infestation by the isopod, *Mothocya plagulophora* (Haller, 1880) and the copepod, *Lernaeenicus hemirhamphi* Kirtisinghe, 1932 (see Gopalakrishnan *et al.,* 2010). Multiple simultaneous infestations were also reported in two belonid fishes: (1) the banded needle fish, *Strongylura leiura* (Bleeker, 1850) infested by one cymothoid isopod, *Mothocya renardi* (Bleeker, 1857) and three copepods, *Lernanthropus tylosuri* Richiardi,1880, *Caligodes lacinatus* Heller, 1868, and *Bomolochus bellones* Burmeister, 1833 (Aneesh *et al.,* 2013), and (2) the spot-tail needlefish, *Strongylura strongylura* (van Hasselt, 1823) infested by a cymothoid isopod, *Cymothoa frontalis* Milne Edward, 1840 and four species of copepods, *L. tylosuri, C. lacinatus, B. bellones,* and *Dermoergasilus coleus* Cressey and Collette, 1970 (Aneesh *et al.,* 2014).

Globally, few reports are available on the unusual occurrence of two species of cymothoid isopods on a single individual host fish. Recently, Welicky and Smit (2018) reported the co-infestation of the external attaching *Anilocra capensis* Leech, 1818 and the buccal-infesting *Ceratothoa africanae* Hadfield, Bruce and Smit, 2014 on the same individual hottentot sea bream *Pachymetopon blochii* (Valenciennes, 1830) from South Africa. Another two different cymothoid co-infestations were reported by Williams and Bunkley-Williams (1985) from the Caribbean. These include, the body surface infesting *Anilocra abudefdufi* Williams and Williams, 1981 and the branchial infesting *Kuna insularis* (Williams and Williams, 1985) parasitising a single individual host, the sergeant-major *Abudefduf saxatilis* (Linnaeus, 1758), and *Anilocra acanthuri* Williams and Williams, 1981 and the gill infesting *Agarna cumulus* (Haller, 1880) parasitising the doctor fish *Acanthurus chirurgus* (Bloch, 1787).

The present study reports the occurrence of two species of cymothoids, the body surface infesting *Anilocra grandmaae* Aneesh, Hadfield, Smit and Kumar, 2021 and the branchial infesting *Agarna malayi* Tiwari, 1952, simultaneously parasitizing the same individual toli shad, *Tenualosa toli* (Valenciennes, 1847) from India. To the best of our knowledge, no other cymothoid co-occurrence has previously been reported from the Indian Ocean.

Materials and Methods

The present study was conducted from January 2018 to May 2021. Based on our previous studies, the clupeid fish *T. toli*, was known to host two species of parasitic cymothoids, a gill infesting *Ag. malayi* and an external attaching *An. grandmaae* (see Aneesh *et al.,* 2018; 2021). During routine sample collection, fresh specimens of *T. toli* were collected from various fish landing centers from different localities along the Kerala coast of the Arabian Sea (Bekal, 12°24'05"N 75°00'45"E; Azhikkal, 11°56'36"N 75°18'36"E; Ayyikkara, 11°51'30"N 75°22'27"E; Perumatura, 08°37'40"N 76°47'16"E; Ponnani, 10°46'57.9"N 75°54'32"E), the southwest coast (Muttom, 08°07'48"N 77°19'12"E) and the Chennai Coast in the Bay of Bengal (Marina Beach, 13°02'57"N, 80°16'58"E). Soon after collection, the fish were

thoroughly examined (body surface, gill chamber, buccal cavity) for the presence of parasitic cymothoids. Recovered isopod specimens were preserved in 90 % ethanol. The identification of parasites was performed using a dissection microscope, according to Tiwari (1952) and Aneesh *et al.* (2018, 2021). We observed that nine fish simultaneously harbored an ovigerous female of *An. grandmaae* on the body surface and a pair of ovigerous female and male of *Ag. malayi* on either side of the gill chamber (Fig. 1; Tab. 1). The specimens were photographed using a Leica M205A dissection microscope and image capturing software (Leica Application Suit). The prevalence (P) and mean intensity (I) were calculated according to Margolis *et al.* (1982) and Bush *et al.* (1997). Sources for fish taxonomy and host nomenclature were Fish Base (Froese and Pauly, 2021) and Catalogue of Fishes (Fricke *et al.*, 2021).

Figure 1. A, Co-infestation of external attaching *Anilocra grandmaae* [arrow *Ag* (f)] and branchial attaching *Agarna malayi* [arrow *Am* (f)] infesting an individual *Tenualosa toli.* **B, C,** Ovigerous female and male of *Ag. malayi,* respectively. **D,** Ovigerous female of *An. grandmaae.*

Table 1. Parasitological indices of two species of cymothoids, the body surface infesting *Anilocra grandmaae* and the branchial infesting *Agarna malayi*, on the host fish *Tenualosa toli*.

Localities			NFO	NFI (A.m)	NFI (A.g)	NIS
Kerala coast of the Arabian Sea	1	Bekal (12°24'05"N 75°00'45"E)	84	8 (P=9.5)	6 (P=7.1)	nil
	2	Azhikkal (11°56'36"N 75°18'36"E)	110	18 (P=16.4)	11 (P=10)	2
	3	Ayyikkara (11°51'30"N 75°22'27"E)	289	39 (P=13.5)	19 (P=6.6)	6
	4	Ponnani (10°46'57.9"N 75°54'32"E)	38	8 (P=21.0)	9 (P=23.7)	1
	5	Perumatura (8°37'40"N; 76°47'16"E)	210	28 (P=13.3)	19 (P=9.1)	nil
South west coast of India	6	Muttom (8°07'48"N, 77°19'12"E)	55	7 (P=12.7)	4 (P=7.3)	nil
Bay of Bengal	7	Chennai Coast; Marina Beach (13°02'57"N 80°16'58"E)	28	5 (P=17.8)	3 (P=10.7)	nil
		Total	814	113	71	9

NFO: Number of host fish (*T. toli*) observed; NFI (A.m): number of host fish (*T. toli*) infested with *Ag. malayi*; NFI (A.g): number of host fish (*T. toli*) infested with *An. grandmaae*; NIS: number of instances of co-occurrence of *Ag. malayi* and *An. grandmaae* on an individual host fish (*T. toli*) observed; P: prevalence.

Results and Discussion

A total of 814 individuals of *T. toli* were examined from seven different localities. Of these, 113 hosts were infested with only *Ag. malayi* and another 71 individuals were infested with only *An. grandmaae*. In addition, nine individuals of *T. toli* were found to simultaneously harbour, both *Ag. malayi* and *An. grandmaae* (Fig. 1A) and these all were collected from the Malabar region of Kerala coast: two from Azhikkal, six from Ayyikkara and one from Ponnani (Tab. 1). A taxonomic summary of the recovered parasitic crustaceans is also provided below. This is the first time a marine fish host from Indian waters was observed with two species of cymothoids; the body surface infesting *An. grandmaae* and the branchial infesting *Ag. malayi* simultaneously parasitizing the same individual toli shad, *T. toli*. Each double-infested *T. toli* harbored an ovigerous female of *A. grandmaae* on the dorsal body surface, over the head or near the base of the dorsal fin, and an ovigerous female and male of *Ag. malayi* on either side of the gill chamber. The relatively large ovigerous females of *Ag. malayi* was found attached to the inner wall of the operculum, close to the postero-dorsal corner of the gill chamber, oriented upside down (Fig. 1A) while the small-sized males were found to occupy the opposite gill chamber. Cymothoid co-occurrence is rarely reported. Prior to the present observations, no temperate fish species from the Indian Ocean has been reported to be infested simultaneously by two genera of cymothoid. Globally, it is only the third record of simultaneous infestation of two cymothoids and the second record of body surface-and branchial cymothoids parasitising the same individual fish.

Systematic

Suborder Cymothoida Wägele, 1989

Superfamily Cymothooidea Leach, 1814

Family Cymothoidae Leach, 1818

Genus *Agarna* Schiödte and Meinert, 1884

Agarna Schiödte and Meinert, 1884: 329. — Barnard, 1936: 170. — Tiwari, 1952: 295–300, pl. iv, text figs. 1–2. — Pillai, 1954: 16. — Bowman and Tareen, 1983: 21, — Aneesh, 2014: 36. — Aneesh *et al.*, 2018: 3.

Type species. Agarna cumulus (Haller, 1880).

Agarna malayi Tiwari, 1952
(Fig. 1 B, C)

Agarna malayi Tiwari, 1952: 295–300, pl. iv, figs. 1–2. — Bowman and Tareen, 1983: 21. — Aneesh

et al., 2016: 1–8, fig. 1a–d. — Aneesh *et al.*, 2018: 1–22, figs. 1–14.
Indusa malayi —Pillai, 1964: 211–223, fig. 3, 7d. — Trilles, 1994: 198. — Trilles and Vala, 1975: 972.
Indusa ophueseni Pillai, 1954: 15.

Host. *Tenualosa toli* (Clupeidae), *Nematalosa nasus* (Bloch, 1795) (Clupeidae), and *Mugil ophueseni* (Bleeker, 1858) (= *Valamugil cunnesius* (Valenciennes, 1836) (Mugilidae) (Tiwari, 1952; Pillai, 1954; 1964; Aneesh *et al.*, 2016; 2018; present study).

Distribution. Kolkata (Tiwari, 1952), Travancore (Pillai, 1954; present study), Kayamkulam Lake, Kerala, southwest coast of India (Pillai, 1964; present study), Bay of Bengal and Malabar coast of Kerala, India (Aneesh *et al.*, 2016; 2018; present study).

Remarks. *Agarna malayi* was described by Tiwari (1952) based on the materials collected from *N. nasus* from Kolkata. Later, Pillai (1964) reported and redescribed based on the materials collected from *M. ophueseni* (Bleeker) off the Kerala coast. Recently the species is redescribed based on the examination of the type material and several fresh specimens collected from Kerala coast and by considering all lifecycle stages (see Aneesh *et al.*, 2018). *Agarna malayi* can be well separated from other branchial cymothoids by its largely hunched body; the shape of the cephalon and pereonite 1; body strongly recurved towards one side; cephalon roughly triangular with narrow round apex, accommodated in the deeply recessed amphicephalic process of pereonite 1; the presence of many pustules on the dorsal surface of pleotelson.

Genus *Anilocra* Leach, 1818

Anilocra Leach, 1818: 348, 350. — Desmarest, 1825: 306. — Milne-Edwards, 1840: 255. — Dana, 1853: 747. — Schioedte and Meinert, 1881: 100. — Gerstaecker, 1882: 231. — Richardson, 1905: 25. — Hale, 1926: 210. — Schultz, 1969: 153. — Kensley, 1978: 78. — Kussakin, 1979: 281. — Brusca, 1981: 140. — Brusca and Iverson, 1985: 45. — Bruce, 1987: 89. — Trilles, 1975: 303. — Trilles, 1994: 55. — Thatcher and Blumenfeldt, 2001: 270. — Welicky *et al.*, 2017: 24. — Aneesh *et al.*, 2019: 444. — Aneesh *et al.*, 2021: 323.
Canolira Leach, 1818: 350.
Epichthyes Herklots, 1870: 122.

Type species: *Anilocra physodes* (Linnaeus, 1758).

Anilocra grandmaae Aneesh, Hadfield, Smit and Kumar, 2021

(Fig. 1 D)

Anilocra grandmaae Aneesh, Hadfield, Smit and Kumar, 2021: 321–328, figs. 1–6.
Anilocra leptosoma [not *Anilocra leptosoma* Bleeker, 1857] —Aneesh *et al.*, 2017: 443–450, figs. 1–4. — Amrutha *et al.*, 2021: 95, fig. 1.

Host. *Tenualosa toli* and *Nematalosa nasus* (Clupeidae) (Aneesh *et al.*, 2019; 2021; Amrutha *et al.*, 2021; present study).

Distribution. Kerala coast; southwest coast of India; the Bay of Bengal and Malabar coast of Kerala, India (Aneesh *et al.*, 2021; present study).

Remarks. The body surface attaching cymothoid *An. grandmaae* is recently described by Aneesh *et al.* (2021) based on morphological and molecular characterization. This species was originally identified as *An. leptosoma* by Aneesh *et al.* (2019) and the re-examination of the materials suggested that it may not be the original *An. leptosoma* of Bleeker and therefore was erected as a new species. *Anilocra grandmaae*, can be identified by: body less than 4.0 times as long as wide; antennula article 3 anterodistal margin expanded, 1.2–1.4 times as wide as long; pleonite 1 visible but largely concealed by pereonite 7, lateral margin posteriorly produced; pereopods 1–4 with three prominent nodules on dactylus; endopod of pleopods 3–5 with proximomedial lobe and endopod of pleopods 3–5 with multiple folds; pleotelson ovate, lateral margins converging smoothly to a caudomedial point (Aneesh *et al.*, 2021).

Even though the simultaneous co-occurrence of 2–5 different species of parasitic crustaceans are reported from different regions, including India, the studies on co-occurrence involving two different cymothoids were very scarce (Daniel and Premkumar, 1967; Benz *et al.*, 2003; Rajkumar *et al.*, 2006; Gopalakrishnan *et al.*, 2010; Aneesh *et al.*, 2013; 2014; Welicky and Smit,

2018). Before this study, only two valid reports are available: a recent report by Welicky and Smit (2018) from South Africa and another from the Caribbean by Williams and Bunkley-Williams (1985).

Previously reported simultaneous multiple co-infestations involved one cymothoid isopod or cirriped and a copepod, or a maximum of four copepods or 2–5 copepods from different genera (Aneesh *et al.,* 2014). Interestingly, the species involved in the double, triple, or quadruple parasitism exhibit site and niche specific parasitism to avoid interspecific competition (Aneesh *et al.,* 2014). Similar observations were noted in the case of cymothoid co-infestations, which included one body surface and a branchial infesting species, or body surface and a buccal infesting species (Welicky and Smit, 2018). Similarly, the two cymothoids involved in the present study also exhibit the site and niche specific parasitization with *An. grandmaae* attached to the body surface and *Ag. malayi* settled on either side of the gill chamber (Fig. 1A).

Parasitic cymothoids exhibit a different level of oligoxenous host specificity (Smit *et al.,* 2014; Aneesh *et al.,* 2019). Most species are restricted to one, or a limited number of hosts (Smit *et al.,* 2014; Aneesh *et al.,* 2019). The toli shad, *T. toli* is the type host for *An. grandmaae* and *An. malayi* is also reported from the same host (Aneesh *et al.,* 2018; 2021). The prevalence of both species confirms their specificity towards *T. toli*. The infestation prevalence of *Ag. malayi* and *An. grandmaae* being 13.88 % (113 out of 814) and 8.72 % (71 out of 814), respectively. The prevalence of a simultaneous co-infestation is only 1.1 % (nine out of 814).

The mechanism and the reason for simultaneous co-infestation by two cymothoid genera are still unknown. Out of the seven different localities, the simultaneous co-infestation was recorded only from three nearby localities (Azhikkal, Ayyikkara, and Ponnani) along the Malabar coast, suggesting that environmental factors may have some influence on triggering the co-occurrence. Detailed studies are needed to find out the triggering mechanisms. A better understanding of the role of environmental factors and mechanisms of host-parasite interaction among cymothoids and their hosts involved in co-infestation is warranted.

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Additional Information And Declarations

Author contribution

PTA prepared the draft of the manuscript; PTA, AKH, and ABK conceived and designed the research, and critically reviewed to improve the quality of the manuscript.

Concent for publication

All authors read and approved the final manuscript.

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Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

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