New optional intermediate hosts of *Mothocya parvostis* (Isopoda: Cymothoidae): Juveniles of the cobaltcap silverside *Hypoatherina tsurugae* (Atheriniformes: Atherinidae) and the yellowfin seabream *Acanthopagrus latus* (Perciformes: Sparidae)

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Abstract

Mothocya parvostis (Isopoda: Cymothoidae) is a crustacean that infests the opercular cavities of fishes. Its main definitive host is Japanese halfbeak *Hyporhamphus sajori*. However, *M. parvostis* also infests black sea bream *Acanthopagrus schelgelii* as an optional intermediate host. Hence, to infest not only definitive hosts but also optional intermediate hosts; this is important for understanding the life history of Cymothoidae, and further information should be obtained. In this study, 20 and 144 cymothoids (mancae and juveniles, respectively) were collected from 129 cobaltcap silversides *Hypoatherina tsurugae* and 494 yellowfin seabreams *Acanthopagrus latus*. Molecular analysis of the cytochrome *c* oxidase subunit I gene and 16S rRNA genes revealed that cymothoid mancae and juveniles from the two fish species were identified to be *M. parvostis*. *Hypoatherina tsurugae* and *A. latus* juveniles were optional intermediate hosts of *M. parvostis*. *Mothocya parvostis* mancae infested juveniles of both species just after metamorphosis, grew with the host, and detached from the fish as juveniles continued growing. The parasitic status of *M. parvostis* in the three optional intermediate hosts indicated that *M. parvostis* likely reproduced from June to December, and different optional intermediate hosts were used depending on the time of year in Hiroshima Bay. Therefore, a parasitic strategy involving optional intermediate hosts might increase the infestation success of *M. parvostis* to *H. sajori*.

Keywords: Life cycle, Manca, New host record, Parasitic cymothoid, Prevalence

BACKGROUND

Cymothoidae Leach, 1818 covers 363 species and 42 genera of cosmopolitan isopod parasites (Boyko et al. 2008onwards). Their hosts include diverse taxa of fish inhabiting marine, brackish, and freshwater environments (Yamauchi 2016). These parasites attach to their hosts at four sites: opercular cavity, buccal cavity, abdominal cavity, and body surface (Smit et al. 2014). During their life cycle, free-swimming mancae (larvae) grow into juveniles and adult males on the hosts (Smit et al. 2014). Adult cymothoids change their sex from male to female (Smit et al. 2014), but cymothoids are mainly identified on the basis of the morphological characteristics of adult females; thus, morphology-based species identification is difficult, and molecular analysis is by far the only way to identify species in mancae, juveniles, and males (Fujita et al. 2021).

Belonging to Cymothoidae, *Mothocya parvostis* Bruce, 1986 infests Japanese halfbeak *Hyporhamphus sajori* (Temminck and Schlegel, 1846) as a major definitive host (Nagasawa 2020), and it shows a high prevalence of approximately 50% (Kawanishi et al. 2016; Fujita et al. 2020). It also infests the large-scale blackfish *Girella punctata* Gray, 1835 and the Japanese amberjack *Seriola quinqueradiata* Temminck and Schlegel, 1845 as definitive hosts (Bruce 1986). In Hiroshima Bay, it infests juveniles of black sea bream *Acanthopagrus schlegelii* (Bleeker, 1854) as an optional intermediate host (Fujita et al. 2020). Optional intermediate hosts are unnecessary in a parasite's normal life cycle. *Anilocra apogonae* Bruce, 1987 also has optional intermediate hosts (Fogelman and Grutter, 2008). An optional intermediate host is similar to a *paratenic host*, but parasites do not grow on paratenic hosts (Okulewicz et al. 2012). *Acanthopagrus schlegelii* juveniles are optional intermediate hosts rather than paratenic hosts because *M. parvostis* mancae grow in black sea bream juveniles (Fujita et al. 2020). In addition to *A. schlegelii*, no optional intermediate hosts of *M. parvostis* have been found.

The reproduction cycles of Cymothoidae organisms vary; *Anilocra pomacentri* Bruce, 1987 has no fixed reproduction season and reproduces throughout the year (Adlard and Lester 1995); *Mothocya epimerica* Costa, 1851 has four reproduction seasons per year (Bello et al. 1997). The reproduction cycles of *M. parvostis* are unknown, but they can also reproduce at other times of the year than Fujita et al. (2020) (from June to August); that is, juvenile fishes that appear at other times may be used as optional intermediate hosts. Thus, we focused on juveniles of the yellowfin seabream *Acanthopagrus latus* (Houttuyn, 1782), a related species of *A. schlegelii* that appears in Hiroshima Bay in seasons or periods that differ from that of *A. schelgelii. Acanthopagrus latus* is recreationally and commercially important in the Indo-West Pacific region as *A. schlegelii* (Iwatsuki 2013). One of the most significant ecological differences between *A. latus* and *A. schlegelii* is their spawning season. In particular, *A. latus* spawns in autumn (Abol-Munafi and Umeda 1994; Nishida 2022), whereas *A. schlegelii* spawns in spring (Kawai et al. 2017 2020 2021). We also focused on the juveniles of cobaltcap silverside *Hypoatherina tsurugae* (Jordan and Starks, 1901) that grow in the period between the growth seasons of *A. schlegelii* and *A. latus*. In the ecosystem, *H. tsurugae* serves as a food source for various carnivorous fishes (Mori et al. 1988) and can be the subject of recreational fishing. Its spawning season in coastal Japan is from May to July, and its juveniles are collected from June to October (Mori et al. 1988).

In this study, we performed DNA analysis to identify the cymothoids infesting juveniles of *H. tsurugae* and *A. latus* as *M. parvostis*. *Mothocya parvostis* infested these two species in different seasons from its infestation in *A. schlegelii*; thus, we updated our knowledge about the optional intermediate hosts and parasitic strategies of *M. parvostis*.

MATERIALS AND METHODS

Sample Collection

A total of 129 *H. tsurugae* and 494 *A. latus* juveniles were collected from 6 July 2021 to 14 October 2021 and from 27 October 2021 to 8 January 2022. Sampling was performed using hand, surf, and casting nets on the coast of Nomijima Island, Hiroshima Bay, Seto Inland Sea, Japan. In the case of

H. tsurugae, individuals smaller than 75 mm, which corresponds to zero age (Mori et al. 1988), were considered juveniles. In the case of *A. latus*, individuals smaller than 160 mm were considered juveniles because they were qualified as immature (Hesp et al. 2004). The collected fish and cymothoids were preserved in 99.5% ethanol.

The standard length (SL) of the fish and total length (TL) of the cymothoids were measured. The life stages of the cymothoids were divided into mancae, juveniles, and adults, following Aneesh et al. (2016) and Fujita (in press). The prevalence of *H. tsurugae* and *A. latus* was calculated by dividing the number of the infested fishes by the total number of the collected fishes. The "manca-prevalence" and "juvenile-prevalence" was calculated by dividing the number of fishes infected at each respective cymothoid stage by the total number of the collected fishes of *H. tsurugae* and *A. latus*. If a single fish was infested by mancae and juveniles, it was included in the estimation of both manca- and juvenile-prevalence.

Molecular identification

A total of 20 cymothoids from *H. tsurugae* juveniles and 19 cymothoids from *A. latus* juveniles were randomly selected for molecular analyses. Total DNA was isolated from pereopods via an alkaline lysis method (Toyobo 2012).

Analyses and species identification were performed following Fujita et al. (2020). Partial cytochrome *c* oxidase subunit I gene (COI) sequences were amplified using the primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′–TAAACTTCAGGGTGACCAAAAAATCA–3′) (Folmer et al. 1994), and partial 16S rRNA sequences were amplified using the primers 16Sar (5′-CGCCTGTTTAACAAAAACAT-3′) and 16Sbr (5′-CCGGTCTGAACTCAGATCATGT-3′) (Simon et al. 1994). The total volume for each PCR was 8.1 μL, which was composed of 1 μL of DNA, 0.78 μL of ultrapure water, 4.06 μL of 2× PCR buffer, 1.62 μL of dNTP mix, 0.24 μL of each primer (10 μM solutions), and 0.16 μL of KOD FX Neo DNA polymerase (Toyobo, Osaka, Japan). The thermocycler profile of COI involved an initial denaturation at 94°C for 2 min; 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 45 s, and extension at 68°C for 30 s; and a final extension at 68°C for 7 min. The thermocycler profile of 16S rRNA consisted of an initial denaturation at 94°C for 2 min; 30 cycles of denaturation at 98°C for 10 s, annealing at 50.5°C for 30 s, and extension at 68°C; and a final extension at 68 °C for 7 min. The PCR products were sequenced via the dye terminator method by using an ABI 3130xl genetic analyser (Applied Biosystems, CA, USA).

The sequences were aligned using MUSCLE (Edgar et al. 2004), implemented in MEGA 10 (Kumar et al. 2018), trimmed, and collapsed into haplotypes. All sequences were deposited in GenBank. Additional sequences belonging to *Mothocya*, which is distributed in Japan, were downloaded from

GenBank (Supplementary Table S1). The sequences of *Anilocra* Leach, 1818, *Ceratothoa* Dana, 1852, and *Nerocila* Leach, 1818, which are relative genera of *Mothocya* within Cymothoidae (Hata et al. 2017) that inhabit the waters of Japan (Supplementary Table S1), were included to compare genetic distances within and between species. Pairwise intra- and interspecific genetic distances with the Kimura two-parameter (K2P) model (Kimura 1980) were calculated using MEGA10, and a neighbour-joining tree (Saitou and Nei 1987) was generated using COI and 16S rRNA sequences. *Ichthyoxenos japonensis* Richardson, 1913 (NC 039713 and MF419233) was also included as outgroups.

RESULTS

Cymothoids infesting *H. tsurugae*

In this study, six mancae and 14 juveniles of Cymothoidae were collected from the opercular cavities of 129 *H. tsurugae* juveniles (Figs. 1 and 2). Of the 16 infested fishes, only three were simultaneously infested by more than two cymothoid individuals. The SL of *H. tsurugae* juveniles increased with each sampling day (Fig. 3); however, the TL of cymothoids did not significantly correlate with the SL of *H. tsurugae* juveniles (Fig. 4). The prevalence of cymothoids in *H. tsurugae* increased with each sampling day and reached a maximum of 20.8% (Fig. 5). The manca-prevalence did not change significantly during the sampling season, but the juvenile-prevalence increased (Fig. 5). In the manca- and juvenile-prevalence in terms of the length range of fish, the manca-prevalence in small fish (<20 mm) was 50%; as the fish grew, it decreased, but the juvenile-prevalence increased (Fig. 7).

Cymothoids infesting *A. latus*

A total of 80 mancae and 64 juveniles of Cymothoidae were collected from the opercular cavity of 494 *H. tsurugae* juveniles (Figs. 1 and 2). Of the 138 infested fishes, only seven were simultaneously infested by more than two cymothoid individuals. The SL of *A. latus* juveniles increased with each sampling day (Fig. 3). The TL of cymothoids was significantly correlated with the SL of *A. latus* juveniles (Fig. 4). The prevalence of *A. latus* did not significantly change during the sampling period (Fig. 5). However, after mid-December, the manca-prevalence decreased, whereas the juvenile-prevalence increased, and all cymothoids identified in early January were juveniles (Fig. 5). The manca-prevalence in terms of the length range of fish was 33.3% (<10 mm); as the fish grew, it decreased, but the increased. Furthermore, almost all cymothoids infesting large *A. latus* juveniles (>20 mm) were juveniles (Fig. 6).

Molecular identification

Our alignment matrices of the COI and 16S rRNA genes consisted of 594 and 412 bp, representing seven and eight haplotypes, respectively. Our neighbour-joining tree with COI and 16S rRNA genes showed that all haplotypes detected from cymothoids infesting *H. tsurugae* and *A. latus* formed a well-supported clade, along with the sequence identified as *M. parvostis* by Hata et al. (2017) and Fujita et al. (2020) (Fig. 7).

Pairwise intra- and interspecific genetic distances with the COI and 16S rRNA haplotypes revealed intraspecific distances smaller than the interspecific distances (Tables 1 and 2), but no overlapping was observed between them. The minimum interspecific distances within *Mothocya* were 3.1% and 1.9% before and after the inclusion of haplotypes from this study, respectively. The maximum distances between our haplotypes were 1.0% (COI) and 0.8% (16SrRNA). These values were comparable with the intra- and interspecific distances of *Anilocra*, *Cerathothoa*, and *Nerocila* (Tables 1 and 2).

Discussion

Cymothoid parasites, including *M. parvostis*, have been described on the basis of the morphological traits of adult females; thus, morphological identification is almost impossible in other life stages and in males (Fujita et al. 2021). Fujita et al. (2020) morphologically identified and described adult *M. parvostis* females from *H. sajori*, a major definitive host, and deposited their COI and 16S rRNA sequences in GenBank. In the present study, cymothoids were collected from *H. tsurugae* and *A. latus*, thereby forming a clade that encompasses an existing sequence previously identified as that from *M. parvostis* (Hata et al. 2017; Fujita et al. 2020). The intraspecific genetic distances between our collections and the sequence of *M. parvostis* from GenBank were lower than the interspecific genetic distances within *Mothocya*. Thus, the cymothoid specimens collected from *H. tsurugae* and *A. latus* were identified as *M. parvostis*. *Mothocya parvostis* was previously observed in the opercular cavity of *H. sajori*, *G. punctata*, and *S. quinqueradiata* as definitive hosts (Bruce 1986) and *A. schelgelii* as an optional intermediate host (Fujita et al. 2020). Thus, *H. tsurugae* and *A. latus* are new hosts of *M. parvostis* (NEW HOST RECORDS).

In Hiroshima Bay, the spawning of *A. schelgelii*, an optional intermediate host of *M. parvostis*, peaks in early May (Kawai et al. 2017 2020 2021), and their juveniles settle on the surf zone from late June (Kawai et al. 2019). *Mothocya parvostis* mancae initially infest *A. schelgelii* juveniles after *A. schelgelii* metamorphoses and settles. Therefore, the prevalence of *M. parvostis* in *A. schelgelii* increases rapidly from late June to early August (Fujita et al. 2020). Conversely, the spawning season of *H. tsurugae* in Hiroshima Bay has not been clearly determined, but it has been estimated to start from May to July in other regions in Japan (Mori et al. 1988). In the present study, *H. tsurugae* juveniles were collected from July to October, a period consistent with the spawning season. The SL of *H. tsurugae* juveniles just after metamorphosis is approximately 20 mm (Tsukamoto and Kimura 1993), which is close to the minimum size of *H. tsurugae* juveniles collected in this study. In addition, their prevalence increased with each sampling day similar to that of *A. schelgelii*; the manca-prevalence in 10–20 mm fish was the highest. These findings suggested that *H. tsurugae* juveniles were collectively infested with *M. parvostis* mancae once metamorphosis was completed. The juvenile-prevalence was higher than manca-prevalence in July, suggesting that *M. parvostis* mancae began infesting *H. tsurugae* juveniles before July.

The spawning season of *A. latus* in Hiroshima Bay is poorly understood, but it likely occurs in autumn (from September to November) in another region in Japan (Abol-Munafi and Umeda 1994; Nishida 2022). In the present study, *A. latus* juveniles were collected from October 2021 to January 2022, a period concordant with the spawning season. The prevalence during the sampling period did not significantly change, but the manca- and juvenile-prevalence changed. The manca-prevalence was higher than juvenile-prevalence from late October to early December, but it decreased from late December and became zero in early January. The SL of *A. latus* juveniles just after they metamorphosed was approximately 10–15 mm, which was close to the minimum size of *A. latus* juveniles collected in this study (Tran et al. 2019). In addition, the manca-prevalence in fish with a size of 9–11 mm was the highest. These findings indicated that similar to *A. schlegelii* and *H. tsurugae*, *A. latus* juveniles were collectively infested by *M. parvostis* mancae after metamorphosis.

The prevalence of *M. parvostis* in *A. schelgelii* juveniles decreases in August, and infested *A. schelgelii* juveniles are rare in September (Fujita et al. 2020). Fujita et al. (2020) stated that *M. parvostis* infestation in *A. schelgelii* juveniles is temporal, and *M. parvostis* break away from *A. schelgelii* juveniles; therefore, *A. schelgelii* juveniles might be an optional intermediate host of *M. parvostis*. In *H. tsurugae* juveniles, the juvenile-prevalence increased with each sampling day although the manca-prevalence did not significantly change. In *A. latus* juveniles, the manca-prevalence was higher than juvenile-prevalence from October to early December, but it decreased from late December. Conversely, juvenile-prevalence increased. All *M. parvostis* individuals in *A. latus* were juveniles in early January. In terms of prevalence by fish SL, all parasites in small fish were mancae; in grown fish, they were gradually replaced by juveniles. In much larger fishes (*H. tsurugae*: ≥50 mm, *A. latus*: ≥20 mm), all *M. parvostis* individuals were juveniles. As the fish further grew, infestation was not observed. These suggest that *M. parvostis* manca infested small *H. tsurugae* and *A. latus* juveniles. Once *M. parvostis* grew with fish juveniles, the parasites left their hosts. The same pattern was observed in *A. schelgelii*. Therefore, *H. tsurugae* and *A. latus* were optional intermediate hosts of *M. parvostis*.

As mentioned above, the prevalence of *M. parvostis* in *A. schelgelii* juveniles rapidly increases and then decreases (Fujita et al. 2020). Similarly, the prevalence of *M. parvostis* in *H. tsurugae* juveniles increased during the sampling period. The prevalence of *M. parvostis* by fish size in *A. latus* was similar to that of *A. schelgelii* and *H. tsurugae*. However, the prevalence of *M. parvostis* in *A. latus* juveniles did not change significantly during the sampling period. Although the cause is unknown, this finding could be attributed to multiple factors, such as the density of mancae or the period when juveniles settle. Hence, the spawning ecology of *A. latus* and the dynamics of free-swimming mancae should be determined to explain why the prevalence of *M. parvostis* in *A. latus* juveniles did not significantly change.

The reproductive cycles of Cymothoidae organisms vary; for example, *A. pomacentri* has no fixed reproduction season and thus reproduces throughout the year (Adlard and Lester 1995); *M. epimerica* has four reproduction seasons per year (Bello et al. 1997). However, the reproduction cycle of *M. parvostis* is unknown, but mancae infest *A. schelgelii* juveniles from June to August (Fujita et al. 2020). *Mothocya parvostis* mancae can survive without a host for only 10–15 days (Hatai and Yasumoto 1981). Therefore the reproductive season of *M. parvostis* included at least June to August (Fujita et al. 2020). In this study, mancae infested *H. tsurugae* from July to October and *A. latus* from October to December. Hence, *M. parvostis* could reproduce from June to December. Future collections of free-swimming mancae, and eggs and mancae from the brood pouch of ovigerous females throughout the year will help clarify the reproductive cycle of *M. parvostis*.

CONCLUSIONS

As the result of this study and Fujita et al. (2020), *A. schelgelii*, *H. tsurugae*, and *A. latus* juveniles were infested with *M. parvostis* from June to August, July to October, and October to January the following year, respectively. In other words, optional intermediate hosts were available to *M. parvostis* for at least 8 months, and *M. parvostis* might infest different hosts appropriately depending on the time of year. Fujita et al. (2020) hypothesised that after detaching from *A. schelgelii* juveniles, *M. parvostis* juveniles can infest *H. sajori*. The results of present study support this hyoithesis but the hypothesis should be further studied in detail. However, if these juveniles can infest *H. sajori* after they detach from optional intermediate hosts, using an optional intermediate host may be an excellent strategy to increase the fitness of *M. parvostis*.

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Authors' contributions

HF designed and executed the experiments, collected samples, and wrote the manuscript. KK analysed *A. lutas* and edited the manuscript. DD analysed *H. tsurugae* and edited the manuscript. TU supervised and edited the manuscript. All authors have reviewed and approved the final manuscript.

Competing interests

HF, KK, DD, and TU declare that they have no conflict of interest.

Consent for publication

Not applicable.

Ethics approval consent to participate

Not applicable.

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Table 1. Genetic distances determined using the Kimura two-parameter model for the cytochrome *c* oxidase subunit I gene (COI) sequences of Cymothoidae

Comparison level	No. Taxa	Intraspecific			Interspecific		
		Min. (%)	Max. (%)	Mean (%)	Min. (%)	Max. (%)	Mean (%)
Genus							
Anilocra	2	0.000	0.349	0.202	10.959	11.187	11.028
Ceratothoa	4	0.169	2.593	1.221	18.398	31.589	29.224
Nerocila	2	0.000	1.280	0.444	27.307	28.836	27.721
Mothocya	4	0.000	1.020	0.261	3.126	13.902	11.752
Mothocya[#]	4	0.000	1.020	0.310	3.126	14.122	11.781

[#] Including haplotypes of *Mothocya parvostis* collected in the present study.

Table 2. Genetic distances obtained using the Kimura two-parameter model for the 16S rRNA gene sequences of Cymothoidae

Comparison level	No. Taxa	Intraspecific Min. (%)	Intraspecific Max. (%)	Intraspecific Mean (%)	Interspecific Min. (%)	Interspecific Max. (%)	Interspecific Mean (%)
Genus							
Anilocra	2	1.328	1.875	1.622	18.940	19.715	19.327
Ceratothoa	3	0.000	1.229	0.643	14.043	24.881	22.661
Nerocila	2	0.000	1.050	0.638	20.564	20.564	20.564
Mothocya	3	0.000	0.836	0.219	1.003	9.994	9.356
Mothocya#	3	0.000	0.836	0.251	1.003	9.994	9.435

#Including haplotypes of *Mothocya parvostis* collected in the present study.

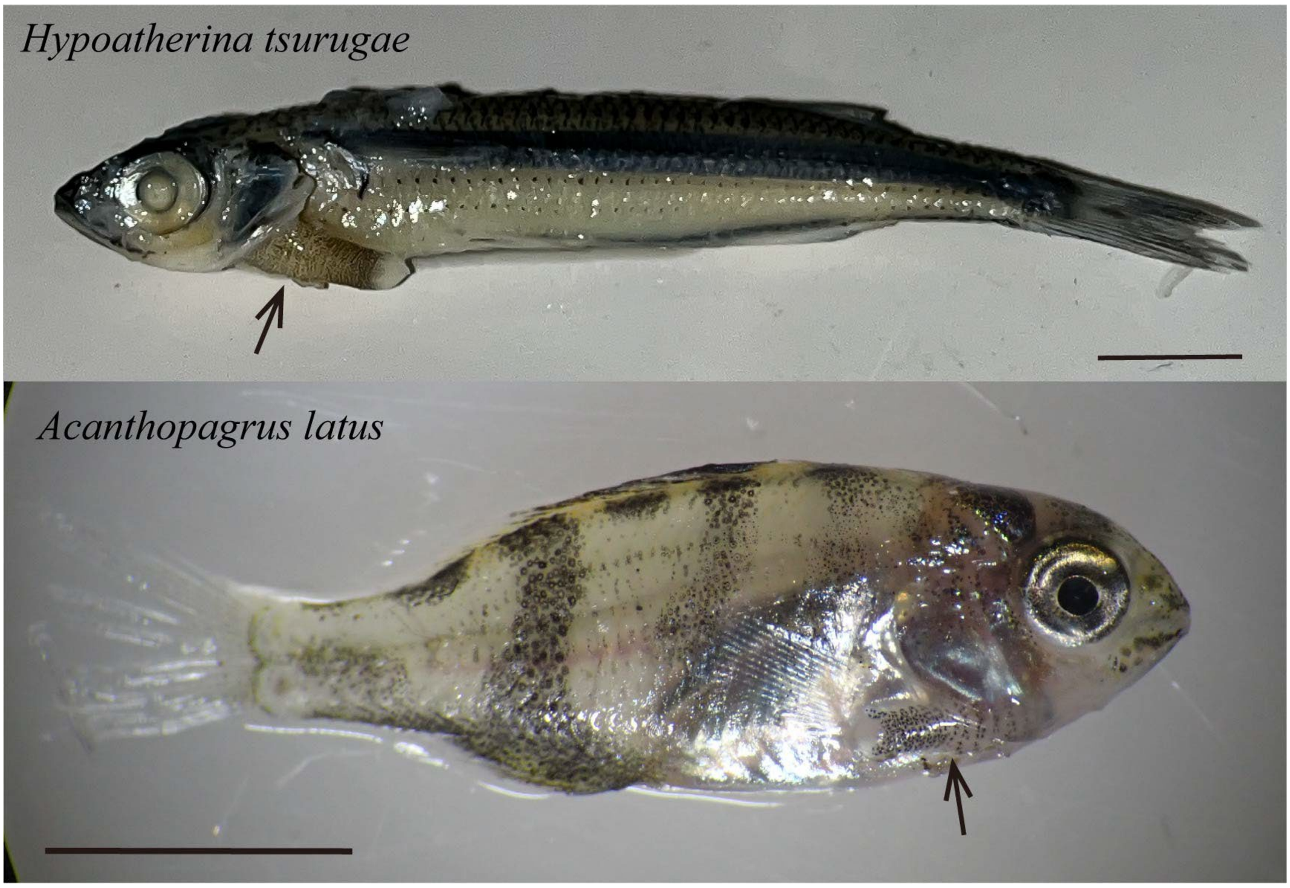

Fig. 1. Juveniles of cobaltcap silverside *Hypoatherina tsurugae* and yellowfin seabream *Acanthopagrus latus* infested with *Mothocya parvostis.* Arrows indicate *M. parvostis.* Scall bars = 5 mm.

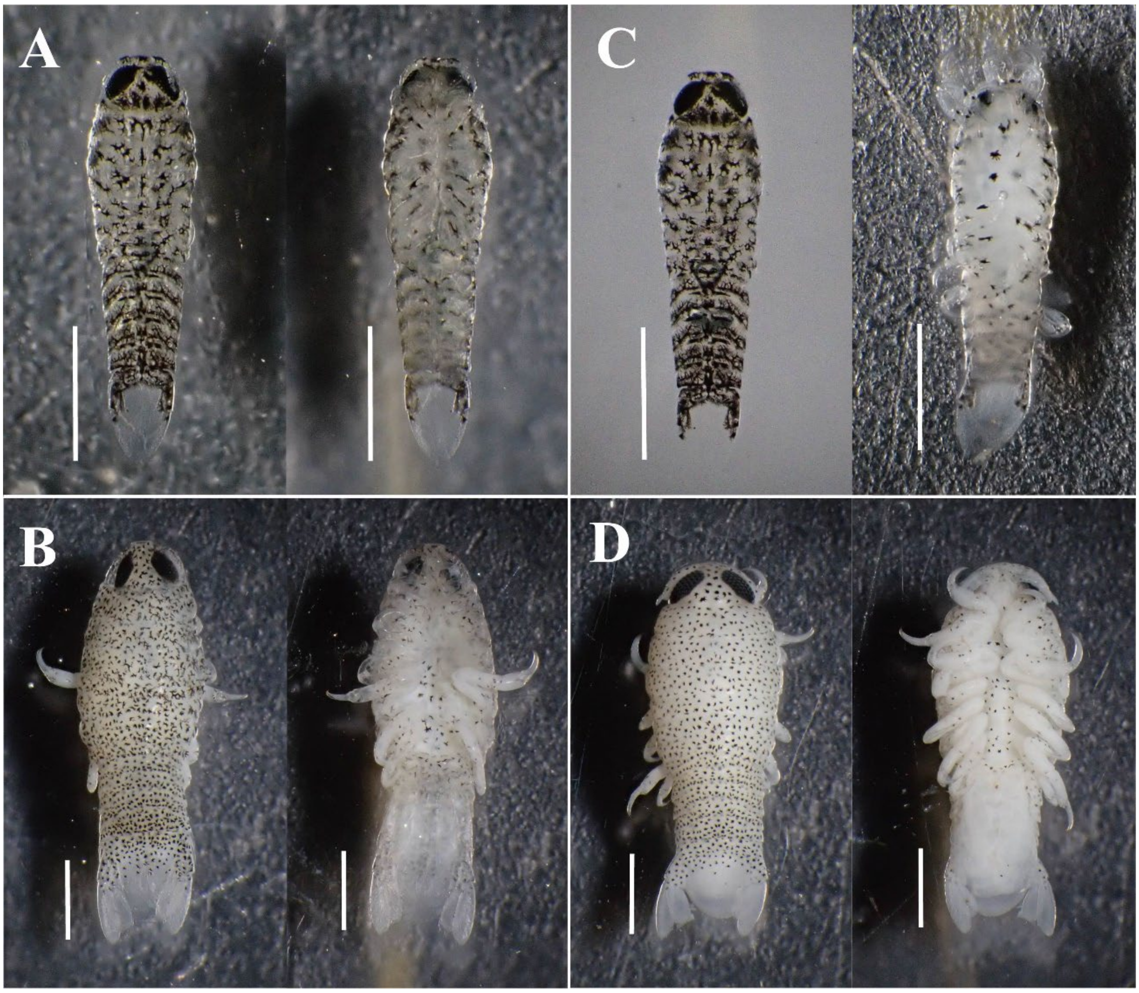

Fig. 2. Dorsal and ventral views of *Mothocya parvostis* infesting juveniles of cobaltcap silverside *Hypoatherina tsurugae* (A and B) and yellowfin seabream *Acanthopagrus latus* (C and D). A and C: mancae, B and D: *M. parvostis* juveniles. Scall bars = 1 mm.

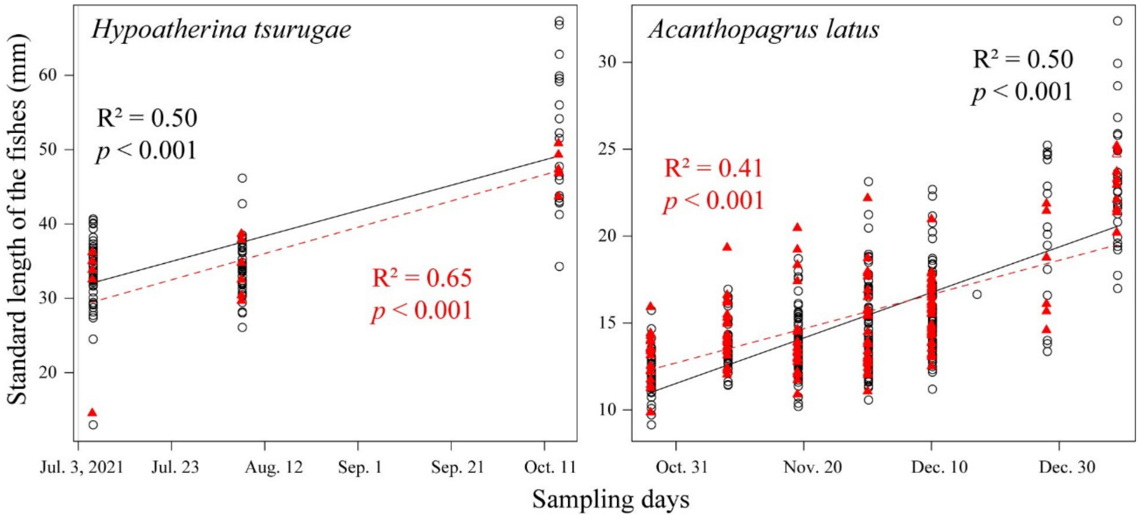

Fig. 3. Scatter plots of the standard length of non-infested and infested fish for each sampling date in cobaltcap silverside *Hypoatherina tsurugae* and yellowfin seabream *Acanthopagrus latus*. The open circles (black) indicate non-infested fish, and the closed triangles (red) correspond to infested fish. The solid lines (black) for non-infested fishes and the broken lines (red) for infested fishes are regression lines.

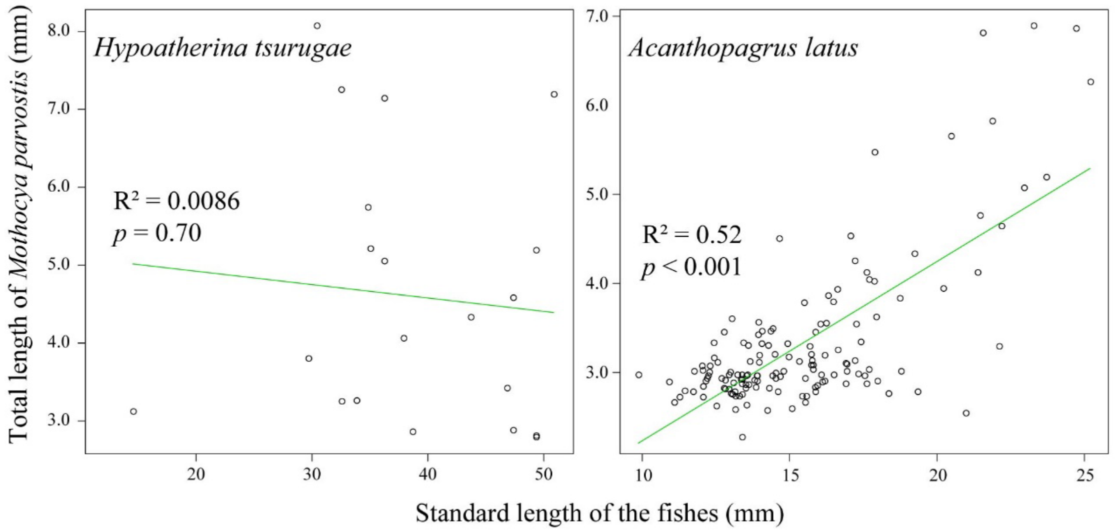

Fig. 4. Scatter plots of the standard length of fishes and the total length of *M. parvostis* in cobaltcap silverside *Hypoatherina tsurugae* and yellowfin seabream *Acanthopagrus latus*. The solid lines are regression lines.

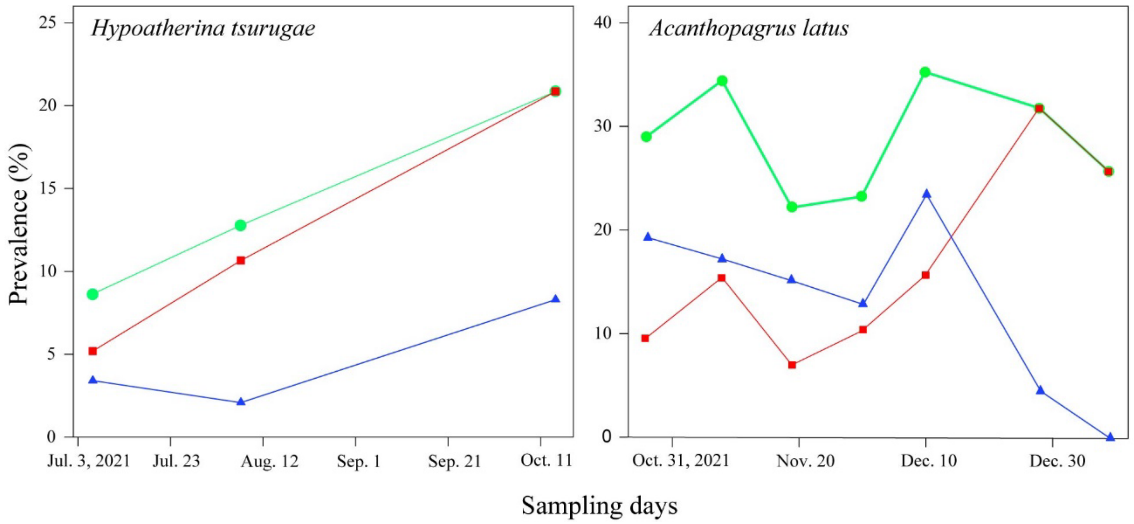

Fig. 5. Prevalence, manca-prevalence, and juvenile-prevalence for each sampling day in cobaltcap silverside *Hypoatherina tsurugae* and yellowfin seabream *Acanthopagrus latus*. Closed circles (green), closed triangles (blue), and closed squares (red) indicate the prevalence, the manca-prevalence, and the juvenile-prevalence, respectively.

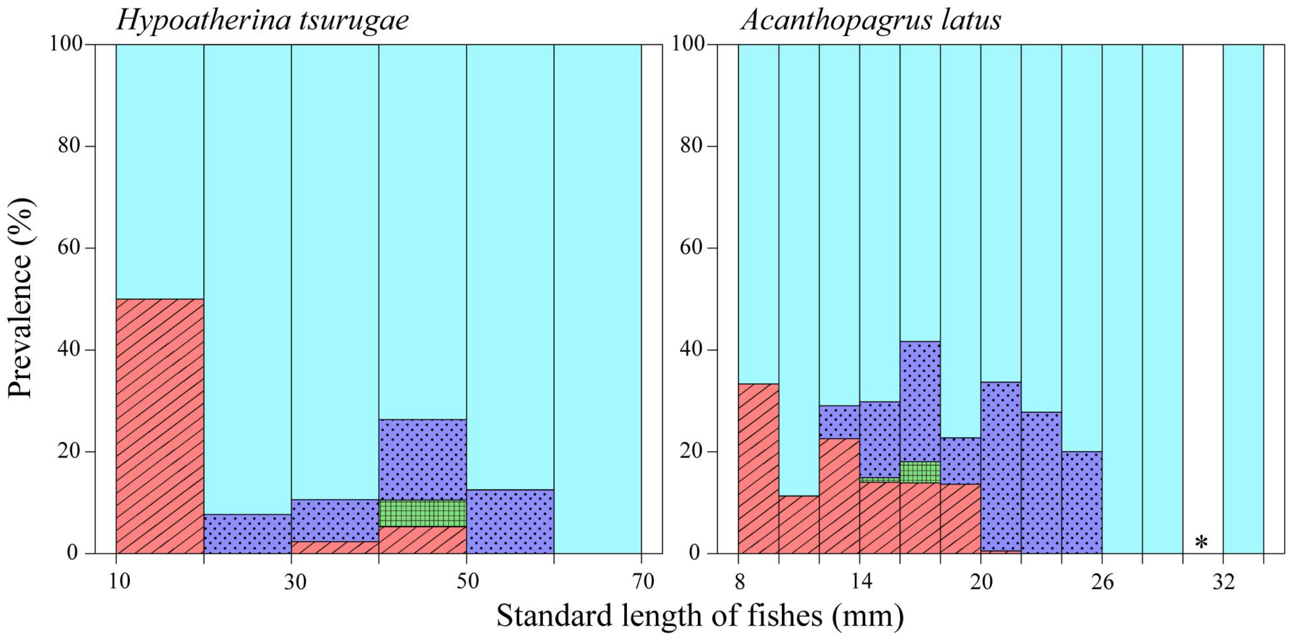

Fig. 6. Prevalence of the standard-length range of fishes in cobaltcap silverside *Hypoatherina tsurugae* and yellowfin seabream *Acanthopagrus latus*. Diagonal shading bars (red), dot bars (blue), grid bars (green), and plain bars (light blue) indicate the manca-prevalence, juvenile-prevalence, the percentage of fish parasitised by both mancae and juveniles, and non-infested fishes, respectively. The asterisk indicates no data.

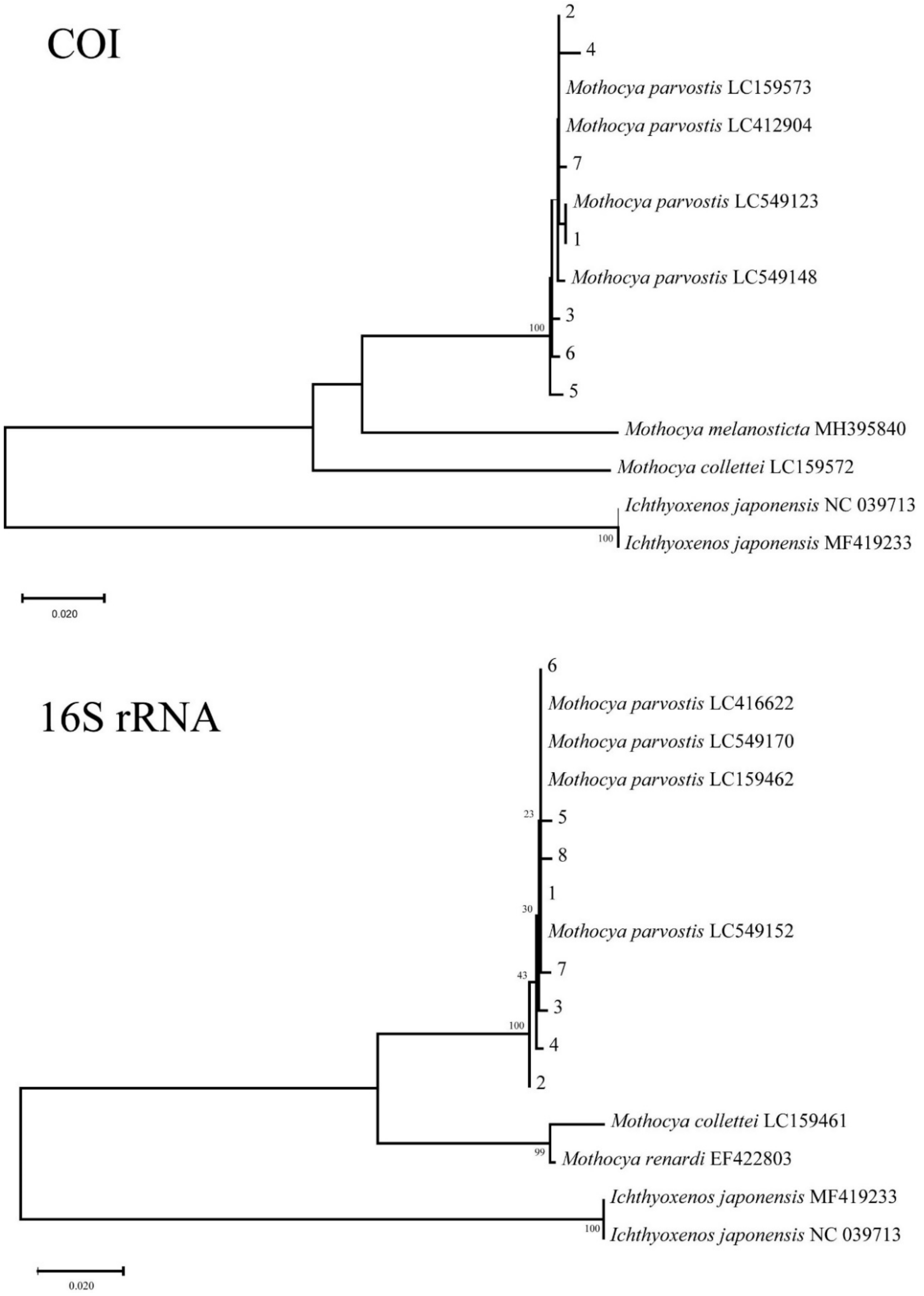

Fig. 7. Neighbour-joining trees showing seven and eight haplotypes of the cytochrome c oxidase subunit I (COI) and 16S rRNA gene infesting cobaltcap silverside *Hypoatherina tsurugae* and yellowfin seabream *Acanthopagrus latus* along with selected sequences of other cymothoids downloaded from GenBank. Bootstrap values less than 98% are not shown. The accession numbers were deposited in GenBank.

Supplementary Table S1. List of sequences from cymothoid species distributed in Japan downloaded from GenBank, with the corresponding accession numbers (Jones et al. 2008; Hata et al. 2017; Baillie et al. 2019; Fujita et al. 2020, 2021; Fujita in press; Saito and Fujita in press).

	COI	16S
Mothocya parvostis	LC159573, LC412904, LC549122, LC549123, LC549124, LC549125, LC549126, LC549127, LC549128, LC549129, LC549130, LC549131, LC549132, LC549133, LC549134, LC549135, LC549136, LC549137, LC549138, LC549139, LC549140, LC549141, LC549142, LC549143, LC549144, LC549145, LC549146, LC549147, LC549148, LC549149, LC549150, LC549151	LC159462, LC416622, LC549152, LC549153, LC549154, LC549155, LC549156, LC549157, LC549158, LC549159, LC549160, LC549161, LC549162, LC549163, LC549164, LC549165, LC549166, LC549167, LC549168, LC549169, LC549170, LC549171, LC549172, LC549173, LC549174, LC549175, LC549176, LC549177, LC549178
Mothocya renardi	MK652485, OL377828	EF422803
Mothocya collettei	LC159572	LC159461
Mothocya melanosticta	MH395840	-
Anilocra clupei	LC159540, LC160309, LC604073, LC708256	LC159426, LC604074, LC708257
Anilocra prionuri	LC159541	LC159427
Ceratothoa arimae	LC159544	LC159430
Ceratothoa carinata	MK652479	-
Ceratothoa oxyrrhynchaena	LC159545, LC159546, LC159547, LC159548, LC159549, LC159550, LC160310, LC160311, LC160312, LC626344	LC159431, LC159432, LC159433, LC159434, LC159435, LC159436, LC159437, LC160305, LC626345
Ceratothoa verrucosa	LC159556, LC159557, LC160317, LC160318	LC159444, LC159445
Nerocila japonica	LC159574, LC159575, LC160325, LC160326, LC160327, LC160328, LC160329, LC160330, LC160331, LC710516	LC159463, LC159464, LC710517
Nerocila phaiopleura	LC159576, LC160332, LC682406,	LC159465, LC682407

	MN428453,	