

Molecular identification of the aegathoid stage of *Anilocra clupei* (Isopoda: Cymothoidae) parasitizing sweeper *Pempheris* sp. (Perciformes: Pempheridae)

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In recent years, species identification by molecular analysis (DNA barcoding) has been actively carried out. This method is especially useful for taxa which cannot be morphologically identified to species. The family Cymothoidae Leach, 1818 (Isopoda), the ectoparasites of marine, brackish and freshwater fishes, all of which infest on the body surface, in the gill chamber, and in the buccal or body cavities of hosts (Brusca, 1981). Presently, they comprise 41 genera and more than 360 species (Boyko *et al.*, 2008a onwards). The cymothoids have life stages of manca, aegathoid and adult, then the adult cymothoids change sex from male to female (protandrous hermaphrodites), and their body shapes change with growth phase and sex (*cf.* Brusca, 1978). Species identification of cymothoid isopods is typically based on morphological features of mature females. Consequently, it is difficult to identify immature (including manca and aegathoid stages) and male specimens to species, because important taxonomic characteristics are lacking in these specimens. Immature cymothoids infesting the gill cavity of juvenile black sea bream *Acanthopagrus schlegelii* (Bleeker, 1854) were recorded from the Sendai Bay and Hiroshima Bay, northern and western Japan (Sekino, 1984; Saito & Yoneji, 2000). Recently, these immature cymothoids were identified as *Mothocyca parvostis* Bruce, 1986, using DNA analysis (Fujita *et al.*, 2020).

Currently, aegathoid specimens of *Anilocra* sp. (Isopoda: Cymothoidae) from juvenile sweeper *Pempheris* sp. (Perciformes: Pempheridae) (probably obliquemouth sweeper *P. japonica* Döderlein, 1883, or silver sweeper *P. schwenkii* Bleeker, 1855, of approximately 40 mm in total

length) were reported by Saito *et al.* (2018) by their morphology. However, the species of aegathoid is still unknown. Here, we re-examined the immature (aegathoid) individual of *Anilocra* sp., a museum specimen (Museum of Nature and Human Activities, Hyogo, MNHA-A2013571) of 14.5 mm in body length, collected from Yawatano (34°52'10"N, 139°06'12"E, 11 March 2018) (see Figs. 1 and 2, together with the morphological description in Saito *et al.*, 2018), and removed tissue from its left pereopods 5–7 using TNES-urea buffer (Asahida *et al.*, 1996). Template DNA was then extracted using standard phenol-chloroform methods. Partial COI and 16S rRNA genes were amplified by PCR according to Tomano *et al.* (2015) and Fujita *et al.* (2020), respectively. Dye-terminator methods were used to sequence PCR products with an ABI 3130xl Genetic Analyzer (Applied Biosystems, CA, USA); the sequences were deposited in GenBank (Table 1). BLAST searches were run on each sequence in NCBI. We established confidence values for identification with BLAST ($\geq 98\%$ similarity and an E-value = 0.0).

The genus *Anilocra* Leach, 1818 presently comprises 57 species, all of which live on the body surface of marine fishes (Boyko *et al.*, 2008b onward). Two species of *Anilocra*, *A. clupei* Williams & Bunkley-Williams, 1986 and *A. prionuri* Williams & Bunkley-Williams, 1986, have been described from Japanese waters (Williams & Bunkley-Williams, 1986; Yamauchi, 2016). For both COI and 16S rRNA sequences, *A. clupei* was $\geq 98\%$ similar to this immature specimen (Table 1). Our COI and 16S rRNA sequences showed $< 91\%$ similarity to another species, *A. prionuri* (LC159541,

Table 1. Results of NCBI database BLAST search for COI and 16S rRNA sequences for an individual of *Anilocra* sp. reported by Saito *et al.* (2018) (MNHA-A2013571).

Target region	PCR fragment length (bp)	Identification results	E-value	Percent Identity	Accession No.	Reference accession No.
COI	609	<i>Anilocra clupei</i>	0.0	100%	LC604073	LC159540
16S rRNA	432	<i>Anilocra clupei</i>	0.0	98.44%	LC604074	LC159426

LC159427). Therefore, we attribute our aegathoid specimen to *A. clupei*—a species formerly reported from two clupeid fishes, Japanese sardinella *Sardinella zunasi* (Bleeker, 1854) and round herring *Etrumeus micropus* (Temminck & Schlegel, 1846) (Williams & Bunkley-Williams, 1986; Hata *et al.*, 2017).

The present work represents the first record of *Anilocra clupei* from a non-clupeid fish. Infestation of aegathoid stage of *A. clupei* on sweeper is also the first incidence at the sampling site after the 22-years observation, and only two or three fish in the entire school of 100–200 sweeper individuals were parasitized (Saito *et al.*, 2018). All parasites were an aegathoid stage, so adult individuals were not available (Saito *et al.*, 2018). In these cases, molecular analysis is almost the only way to identify the cymothoids. The molecular analysis of immature cymothoids has enabled species identification, and contributed to our understanding of cymothoid isopod life-cycles.

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