Morphological description and molecular characterisation of a new species of *Anilocra* Leach, 1818 (Crustacea: Isopoda: Cymothoidae) from India

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**ABSTRACT**  
A fish parasitic isopod recently reported from India as *Anilocra leptosoma* Bleeker, 1857, was re-examined and morphologically compared to five closely related species: *A. capensis* Leach, 1818, *A. clupei* Williams and Bunkley-Williams, 1986, *A. leptosoma* Bleeker, 1857, *A. paulsikkeli* Welicky and Smit, 2019 and *A. pilchardi* Bariche and Trilles, 2006. This species was sequenced and compared to other known *Anilocra* species based on mitochondrial cytochrome c oxidase subunit I (COI) gene fragments. Both morphological and molecular data corroborate that the *Anilocra* species parasitising the clupeid fish *Tenualosa toli* (Valenciennes, 1847) from India should be recognised as a new species, and we describe *Anilocra grandmae* n. sp. based on the holotype and paratype females. The key characters of *A. grandmae* n. sp. include the body being 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 concealed by pleonite 7 and lateral margin posteriorly produced; pleotelson ovate, with lateral margins converging smoothly to a caudomedial point.

1. Introduction

*Anilocra* Leach, 1818, the most specious genus of body surface attaching cymothoids, currently includes 56 valid species. Leach (1818) established the genus with the description of three species (*Anilocra capensis* Leach, 1818; *Anilocra cuvieri* Leach, 1818; and *Anilocra mediterranea* Leach, 1818). Trilles (1975) synonymised *A. cuvieri* with *A. physodes* (Linnaeus, 1758) and, after 161 years, the type species for the genus (*A. cuvieri*) was designated by Kussakin (1979). Subsequently, *A. mediterranea* has also been synonymised with *A. physodes* (see Ellis 1981). A detailed generic diagnosis of *Anilocra* was provided by Bruce (1987).

Thus far, only two species of *Anilocra* have been reported from India, namely *Anilocra dimidiatia* Bleeker, 1857 originally described from Malaysia and later found on the Indian oil sardine, *Sardinella longiceps* Valenciennes, 1847 and *Anilocra leptosoma* Bleeker, 1857 originally from Java and subsequently collected in India from the Toli shad, *Tenualosa toli* (Valenciennes, 1847) (see Aneesh et al., 2019). Recently Welicky and Smit (2019), based on the drawings by Aneesh et al. (2019), suggested that the *A. leptosoma* from India may not be the original *A. leptosoma* as it differs from the *A. leptosoma* lectotype drawings by Bruce (1987) and that it shows some similarities with *A. capensis* parasitising the Hottentot seabream, *Pachymetopon blochii* (Valenciennes, 1830) from South Africa. Therefore, specimens of the aforementioned *Anilocra* sp. reported from India as *A. leptosoma* were re-examined. This study thus aimed to (1) use both morphological and molecular information based on fresh material collected from the Toli shad to determine the correct identity of the species identified by Aneesh et al. (2019) as *A. leptosoma* and (2) to provide a detailed morphological comparison of this Indian *Anilocra* sp. to some of the closely related species within the genus (such as *A. capensis*, *Anilocra clupei* Williams and Bunkley-Williams, 1986, *Anilocra paulsikkeli* Welicky and Smit, 2019 and *Anilocra pilchardi* Bariche and Trilles, 2006).

2. Materials and methods

2.1. Sampling sites

Fresh specimens of *Anilocra* were collected from the host fish *Tenualosa toli* from several fish landing centres in India (Fig. 1). These centres are from different localities along the Kerala coast of the Arabian Sea (Ayyikkara, 11°51′30″N, 75°22′27″E; Azhikkal, 11°56′36″N,
2.2. Parasite identification

The collected cymothoids were preserved in 95% ethanol for DNA studies and the remaining specimens were processed following the techniques described in Aneesh et al. (2019, 2020). One ovigerous female was designated as the holotype and one paratype was minimally dissected to conserve the specimens (the dissected appendages were kept in separate vials along with the said specimen). Methods for dissection, mounting, and drawings of appendages were according to the techniques described in Aneesh et al. (2019). The drawings of the observed mouthparts and appendages were performed using a Nikon SMZ1500 Stereo Zoom Microscope and a Nikon Eclipse80i Compound Microscope, both equipped with drawing tubes, following techniques from Hadfield and Smit (2020). Drawings were digital inked using Adobe Illustrator and a WACOM CTL-472/K0-c drawing pad. Descriptions of the species were made with the aid of the taxonomy software package DELTA (Descriptive Language for Taxonomy) (see Coleman et al., 2016), adapting a general Cymothoidea character data set originally developed by Hadfield et al. (2013). The specimens were microphotographed using the multi-focusing stereomicroscope Leica-M205A and image capturing software (Leica Application Suit).

Fish taxonomy and host nomenclature follow FishBase (Froese and Pauly, 2021) and Fricke et al. (2021). The lectotype and several fresh specimens of A. capensis, holotype and paratype of A. paulsikkeli were also examined. The non-type females of Anilocra sp. were re-examined from the Indian Museum. The holotype and paratypes are deposited in Western Ghats Field Research Centre of Zoological Survey of India, Kozhikode, India (ZSI/WGRC).

2.3. Molecular analysis

Genomic DNA was extracted from a pereopod and pleopod of two specimens following the protocol for animal tissue extraction of the NucleoSpin® Tissue Genomic DNA Tissue Kit (Macherey-Nagel, Düren, Germany). A targeted part of the mitochondrial cytochrome c oxidase subunit I (COI) gene (approximately 680 bp) of these specimens was subjected to PCR amplification with the aid of a ProFlex™ thermal cycler (Applied Biosystems by Life Technologies). and universal invertebrate primers LCO1490 (5′-GTGACTTAAAGATATTGGTTA-3′) and HC02198 (5′-TAAAATCTCAGGTTGACAAAAATCA-3′) (Folmer et al., 1994). PCR reactions were performed with volumes of 25 μl, using 12.5 μl Thermo Scientific DreamTaq PCR master mix, 1.25 μl of each primer, 7 μl of PCR-grade nuclease-free water and 3 μl of DNA. Conditions for the PCR were as follows: initial denaturation at 94 °C for 5 min; followed by 35 cycles of a 94 °C denaturation for 30 s, annealing at 47 °C for 30 s with an end extension at 72 °C for 2 min; and ending with a final extension of 72 °C for 10 min. PCR products were sequenced in both directions by a commercial sequencing company, Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa. The bioinformatics software platform, Geneious R7.1.3 (Biomatters, Auckland, New Zealand; Kearse et al., 2012), was used to assemble the sequences.

Comparative sequences of Anilocra species from GenBank were downloaded and aligned to one sequence from the current study. These sequences included: LC159542 (Anilocra sp. 1, from Japan); KYS62744 (Anilocra brillae); MK450445 (Anilocra capensis); LC159540 (Anilocra clupei); KY562753 (Anilocra haemuli); MK652476 (Anilocra physodes); LC159541 (Anilocra prionuri). Nucleotide genetic divergence in percentage (p-distance) and base-pair differences among the different species were determined using MEGA7.
partially fused to lateral lobe; coxae 5 straight or smoothly curved, rostrum folded back and lying between external-attaching cymothoid genera by: cephalon posterior margin short; mandible palp article 3 shorter than article 2; maxilla medial lobe antennula bases; antennula shorter than antenna, antennula articles 4–8 endopods with weak lobes, pleopods hardly visible (vs pleopod 5 with prominent folded fleshy lobes; pleopods clearly visible in dorsal view in Anilocra) (see Bruce et al., 2019).

Anilocra grandmaae n. sp. Anilocra leptosoma—Aneesh et al., (2019): 443–450, Figs. 1–4.

All material examined: 28 ♂♀, all from the body surface of Tenualosa toli collected from various fish landing centres such as Azhikkal (3 ♂♀), Ayikkara (2 ♂♀), Bekal (5 ♂♀), Perumatta (5 ♂♀) and Ponnani (8 ♂♀) (Kerala coast); Muttom (2 ♂♀) (south west coast); and Marina Beach (3 ♂♀) (Bay of Bengal), India.

Holotype: 1 ♂ (31.0 mm TL; 10.0 mm W), on the base of the dorsal fin of Tenualosa toli from Ponnani, Kerala coast, India, August 2018, coll. PT Aneesh (ZSI/WGRC/IR. INV./12328).

Paratypes: 1 ♂ partially dissected (34.0 mm TL; 10.2 mm W), ZSI/WGRC/IR. INV./12329; 1 ♀ (30.0 mm TL; 10.0 mm W), ZSI/WGRC/IR. INV./12330; 1 ♂ (37.0 mm TL; 11.0 mm W), ZSI/WGRC/IR. INV./14611, all with same information as holotype. 1 ♀ (35.0 mm TL; 11.0 mm W) from Perumatta, Kerala April 2019, coll. PT Aneesh (ZSI/WGRC/IR. INV./14612); 1 ♀ (35.0 mm TL; 11.0 mm W) from Perumatta, November 2019, coll. PT Aneesh (ZSI/WGRC/IR. INV./14613); 1 ♀ (31.0 mm TL; 10.0 mm W) from Ayikkara, November 2019, coll. PT Aneesh (ZSI/WGRC/IR. INV./14614); 1 ♀ (28.0 mm TL; 8.5 mm W) from Muttom, Tamil Nadu January 2020, coll. PT Aneesh (ZSI/WGRC/IR. INV./14615).

Additional material: All from the body surface of Tenualosa toli, Ayikkara, Malabar coast of the Arabian Sea, India, March 2017, coll. PT Aneesh and AK Helna; 2 ♂♀ [1 ovig. (24.0 mm; 7.0 mm W) and 1 non-ovig. (24.0 mm; 7.2 mm W)]; 1 ♀ ovig. (33.0 mm; 10.0 mm W).

Representative DNA sequences: Two newly generated mitochondrial cytochrome c oxidase subunit I (COI) partial sequences of A. grandmaae have been submitted to NCBI GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) with the following accession numbers: MW803168 (Isolate 1: 681 bp long); and MW803171 (Isolate 2: 682bp long).

ZooBank registration: The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:AF44C1C8-2518-495A-B5C3-E78CE1FD415C. The LSID for the new name Anilocra grandmaae n. sp. is urn:lsid:zoobank.org:act:205E3E1D-AB85-48B6-A2A7-02B82829F3369.

Etymology: The species name is derived from ‘Grandma’ in honour of the recently deceased grandmother of PTA, as a tribute to her memory. She always supported him in pursuing his education as well as his research career. This species is dedicated to all grandmothers.
3.1.2. Description

Female (Figs. 2–6): Body elongate, dorsal surfaces smooth, strongly arched longitudinally or with medial longitudinal ridge present, 3.1–3.4 times as long as greatest width, widest at pereonite 6, most narrow at pereonite 1, body lateral margins convex. Cephalon 1.4–1.9 times wider than long, visible from dorsal view, sub triangular; frontal margin gradually tapering anterior to eyes. Rostrum narrowly rounded, domed and broad; folded back and lying between antennula bases ventrally. Eyes oval with distinct margins or relatively well-developed, one eye 0.2 times width of cephalon. 0.5 times as the length of the cephalon. Each eye made up of ~ 5–6 transverse rows of ommatidia, each row with ~18–22 ommatidia. Coxae not visible in dorsal view. Coxae of pereonite 2 more or less equal length to respective pereonite. Coxa 3 equal or slightly shorter than pereonite 3. Coxae 4–6 half the length of corresponding pereonite. Pereonite 1 smooth, anterior border straight, anterolateral angle narrowly rounded, anterolateral margins extend to base of eyes. Posterior margins of pereonites smooth and straight. Pereonite 6 longest and widest, 5 shorter than 6, 7 slightly shorter than 5 and longer than pereonite 4, pereonite 1 and 4 subequal in length. Posterolateral margin of pereonites 4–7 obtusely rounded. Pereonite 7 partially overlapping pleonite 1. Pleonites posterior margin smooth, medially produced; pleonite 1 largely concealed by pereonite 7, slightly visible in dorsal view. Pleonite 2 not overlapped by pereonite 7; posterolateral angles rounded. Pleonites 1–5 progressively getting smaller in width; pleonite 5 narrowest, posterior margin produced medially. Pleotelson 1.1–1.3 times

Fig. 3. Anilocra grandmae n. sp. ovigerous female, holotype (34.0 mm; ZSL/WGRC/IR. INV./12328) female. (A) Dorsal view. (B) Ventral view. (C) Lateral view. (D) Cephalon dorsal. (E). Cephalon ventral. (F) Pereopod 1., (G) Pereopod 4. (H) Pereopod 7, (I). Pleotelson and uropods.
as long as anterior width, dorsal surface with two sub-medial depressions, lateral margins convex, not folded. Posterior margin converging to caudomedial point. Anterodorsal depression and medial longitudinal ridge are weakly developed.

**Antennula** approximately the same size as antenna, length shorter than antenna, extending slightly beyond midpoint of eye, bases moderately separated. Antennula consisting of 8 articles; article 3 anterodistal angle strongly produced; 1.2–1.3 times as wide as long; with no setae. **Antenna** with 10 articles; extending to middle or posterior of pereonite 2; article terminating without setae. **Mandibular molar** present, ending in an acute and slightly curved incisor processes. Mandible palp article 3 shorter than article 2; article 3 with 11–14 serrate setae, terminal setae are longer. **Maxillula** simple with four terminal robust setae; two setae are slightly larger than others. **Maxilla** medial lobe partly fused to lateral lobe; median lobe with three recurved robust setae, lateral margin with one recurved robust seta. **Maxilliped** without oostegial lobe in non-ovigerous female; article 3 with three terminal recurved spines. In ovigerous female, maxilliped article 1 modified into large, tri-lobed, oostegial lobes.

**Pereopods** 1–4 short with three nodules on dactylus (prominent in pereopods 2–4), anterior nodule large; distal side gently inclined, proximal side steep. Pereopods 1–3 distal margin of merus with one small spine; 6–7 distolateral margin of carpus and lateral surface of propodus with many minute spines. **Pereopods** generally gradually increasing in size, pereopod 7 longer than other pereopods.

**Pleopods** visible in dorsal view, exopod larger than endopod. **Proximomedial lobe** present in endopod of pleopods 3–5. **Pleopods** 1–2 endopod without proximomedial lobe, pleopod 3 proximomedial lobe moderately coiled, pleopod 4–5 proximomedial lobe well coiled. Pleopods 1–2 endopod without folds; pleopod 3–5 endopods with folds. Pleopod 3 endopod with three folds, pleopod 4 with three to four small folds, pleopod 5 multiple folded. Peduncle lobes absent. **Uropod** as long as pleotelson, peduncle 0.5 times longer than rami, peduncle lateral margin with a single seta; rami reaches up to the pleotelson apex, marginal setae absent, apices rounded. **Endopod** apically rounded, partially hidden behind the pleotelson, lateral margin weakly convex, medial margin weakly convex. **Exopod** slightly shorter than endopod, apically rounded, lateral margin weakly convex, medial margin straight,

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**Fig. 4.** *Anilocra grandmaae* n. sp. ovigerous female paratypes. (A–B) Dorsal and ventral view of ovigerous female (35.0 mm; ZSI/WGRC/IR. INV./14612). (C–D) Dorsal and ventral view of non-ovigerous female (28.0 mm; ZSI/WGRC/IR. INV./14615).

**Fig. 5.** *Anilocra grandmaae* n. sp. ovigerous female (34.0 mm; ZSI/WGRC/IR. INV./12329). (A). Cephalon frontal view. (B). Pleotelson and uropods.
Fig. 6. *Anilocra grandmaae* n. sp., ovigerous female paratype (34.0 mm; ZSI/WGRC/IR. INV./12329). (A) Antennula. (B) Antenna. (C) Mandible. (D) Maxilla. (E) Maxillula. (F) Maxilliped. (G) Distal segment of maxilliped palp. (H) Pereopod 2. (I) Pereopod 3. (J) Pereopod 5. (K) Pereopod 6. (L) Brood pouch. (M–Q), Pleopod 1–4. (R) Uropod.

Table 1
Nucleotide genetic divergence among COI sequences of *Anilocra grandmaae* n. sp. and *Anilocra* spp. available in GenBank. Values below the diagonal are expressed in percentage (p-distance) while values above the diagonal represent number of differences in nucleotides.

|   | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1 | MW803168 *Anilocra grandmaae* n. sp. | 46  | 71  | 75  | 72  | 78  | 61  | 147 | 152 |
| 2 | LC159540 *Anilocra clupei*           | 7   | 66  | 75  | 76  | 76  | 55  | 142 | 156 |
| 3 | LC159541 *Anilocra prionuri*         | 11  | 10  | 58  | 51  | 51  | 54  | 150 | 161 |
| 4 | KY562743 *Anilocra chromis*          | 12  | 12  | 9   | 47  | 48  | 62  | 151 | 158 |
| 5 | KY562744 *Anilocra brillae*          | 12  | 11  | 8   | 3   | 21  | 53  | 155 | 156 |
| 6 | KY562753 *Anilocra haemuli*          | 12  | 12  | 8   | 8   | 21  | 53  | 155 | 156 |
| 7 | LC159542 *Anilocra sp. 1*            | 9   | 9   | 10  | 8   | 9   | 153 | 157 | 157 |
| 8 | MK652476 *Anilocra physodes*         | 23  | 22  | 23  | 24  | 25  | 24  | 24  | 93  |
| 9 | MK450445 *Anilocra capensis*         | 24  | 24  | 25  | 25  | 25  | 24  | 93  | 14  |
Table 2

Inter-specific morphological character comparison between Anilocra grandmae n. sp. and closely related species collated from original descriptions and, where applicable, redescritions (see Leach, 1818; Bleeker, 1857; Williams and Bunkley-Williams, 1986; Bariche and Trilles, 2006; Welicky and Smit, 2019).

| Characters                        | A. grandmae n. sp. | A. capensis | A. clupei | A. leptosoma | A. paulsikkeli | A. pilchardi |
|-----------------------------------|--------------------|-------------|-----------|--------------|----------------|-------------|
| Antennula                         | 8 articles         | 8 articles  | 8 articles| 8 articles   | 9 articles     | 8 articles   |
| Antenna                           | 10 articles        | 10 articles | 10 articles| 10 articles  | 9 articles     | 10 articles  |
| Spines on pereopods               | Porepods 6–7 with  | Porepods 3, 7| Porepods 7 with | Absent       | Porepods 6–7   | Few (6–10)   |
| many (more than 30)              | Porepods 3, 7      | with few (7–16)| with few (9–12)| with few (7–10)| larger spines on | larger spines on |
| tiny spines                      | larger spines      | spines      | spines    | spines       | pereopods 5    | pereopods 5   |
| Pleopod folds                     | Pleopods 3–5       | Only pleopod 5| Pleopods 3–5| Pleopods 3–5| Pleopods 3–5   | Pleopods 4–5  |
| Proximo- medial lobe of pleopod   | Present in endopod of pleopod 3–5. | – | – | – | – | – |
| endopod                          |                     |             |           |             |               |             |
| Pleotelson                        | Widest at median point, narrow anteriorly and posteriorly. | Narrow | Narrow | Concave to median point | Posteriorly wider | Lateral margins nearly straight, strongly turned up; posterior margin scarcely bisinuate with broad caudomedial lobe |
| Uropod length                     | As long as pleotelson | 1.4 times longer than pleotelson | 1.3 times longer than pleotelson | As long as pleotelson | 1.1 times longer than pleotelson | Extending beyond posterior of pleotelson |
| Uropod rami                       | Subequal           | Exopod 1.3 times longer than endopod | Subequal | Subequal | Subequal | Subequal |
| Frons Antenna article 3           | Truncate           | Truncate/rounded | Truncate | Pointed | Pointed | Sub-truncate |
| Pleonot 1                         | Small              | Normal      | Normal    | Small      | Small      | Normal      |
| Posterior margin of pleonoties    | Straight           | Rounded     | Straight  | Straight   | Straight   | Posteriorlateral margins smoothly rounded |
| Nodules on pereopod dactylus      | 1–4                | Absent      | 1–4       | 2, 4       | 1–4       | 1–4         |

This cymothoid is the first Anilocra species described from India and parasitises the clupeid fish Tengola asa. This species was originally identified as A. leptosoma by Anesh et al. (2019), however, as previously mentioned, Welicky and Smit (2019) noted that the species illustrated by Anesh et al. (2019) did not conform to the redescription of the lectotype of A. leptosoma by Bruce (1987) and shared characteristics with A. capensis. Following morphological comparisons (see Table 2), this species is here confirmed to be a new species, Anilocra grandmae n. sp. and the redescription of A. leptosoma of Aneesh et al. (2019) should be disregarded. When comparing A. grandmae to A. capensis, the following suit of characters can be noted: only pleopod 5 has folds in A. capensis (vs pleopods 3–5 with folds in A. grandmae n. sp.); uropods 1.35 times longer than pleotelson (vs uropods as long as pleotelson in A. grandmae n. sp.); uropod rami exopod 1.3 times longer than endopod (vs sub equal in A. grandmae n. sp.); pereopod dactylus without nodules (vs dactylus of pereopods 1–4 with prominent nodules). Furthermore, the molecular analysis showed 152 base pair differences and only 76% similarity (Table 1).

Even though the new species is closely related to the other slender-bodied species from the “alloceraea” group such as A. clupei, A. leptosoma, A. paulsikkeli and A. pilchardi, it can be readily separated by the combinations of characters. Anilocra grandmae n. sp. can be differentiated from A. pilchardi by: pereopods 6 and 7 with more than 30 tiny spines in A. grandmae n. sp. (vs few (6–10) bigger spines on pereopods 5–7 in A. pilchardi); pleopod 3–5 with folds and proximomedial lobe (vs folds and proximomedial lobe in pleopod 4–5 in A. pilchardi); pleotelson widest at median point, narrow anteriorly and posteriorly in A. grandmae n. sp. (vs pleotelson lateral margins nearly straight, strongly turned up; posterior margin scarcely bisinuate with broad caudomedial lobe in A. pilchardi); uropod as long as pleotelson in A. grandmae n. sp. (vs uropod extending beyond posterior of pleotelson in A. pilchardi).

Similarly, the new species can also be well separated from A. paulsikkeli and A. leptosoma by pereopods 6 and 7 with more than 30 tiny spines in A. grandmae n. sp. (vs few large spines in A. paulsikkeli and spines absent in A. leptosoma); rostrum truncate in A. grandmae n. sp. (vs pointed in both A. paulsikkeli and A. leptosoma); antenna article 3 expanded in A. grandmae n. sp. (vs very sharp in A. paulsikkeli and not expanded in A. leptosoma); pleotelson widest at median point, narrow anteriorly and posteriorly in A. grandmae n. sp. (vs posteriorly wider in A. paulsikkeli and pleotelson concave to median point in A. leptosoma).

Even though A. grandmae n. sp. is phylogenetically closer to A. clupei in the molecular analysis, both species can be well separated by: pleopod 3–5 with folds and proximomedial lobe in A. grandmae n. sp.
The comparison of genetic distances among _A. grandmae_ n. sp. and the available _Anilocra_ species on GenBank showed values higher than what is normally found within a genus. Nonetheless, this is still consistent with the findings of _Welicky and Smit_ (2019). Additional sequences (COI and additional genes) of _Anilocra_ spp. would provide more information on genetic distances among species that could be linked with their morphological aspects. The use of integrative taxonomy for _Anilocra_ can aid a better circumscription of species, as in the case of the present study, enabling to draw more conclusions on the actual diversity and distribution of the genus.

Declaration of competing interest

I can confirm that all the authors have read the manuscript and accept responsibility for its contents and agree to conform to the 'Ethics in Publication' documents. All authors also do not have competing interest to declare.

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