Redescription and molecular characterisation of the fish parasitic isopod *Norileca indica* (Milne Edwards, 1840) (Crustacea: Isopoda: Cymothoidae) with a key to the genus

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*Norileca indica* (Milne Edwards, 1840) is fully redescribed based on ovigerous females collected from Maputo Bay, Mozambique, from the branchial cavity of the fish host *Seler crumenophthalmus* Bloch, 1793. An identification key to the species of *Norileca* Bruce, 1990 is given. Furthermore, a fragment of the mitochondrial cytochrome oxidase I (COI) gene from *N. indica* was sequenced for the first time. This is the first molecular characterisation of a species of *Norileca*.

**Keywords:** branchial cavity, cymothoid, genetic characterisation, *Norileca, Seler crumenophthalmus*, taxonomy

**Introduction**

Species of *Norileca* Bruce, 1990 inhabit the branchial cavity of fish hosts and are commonly recorded from pelagic fishes (Rameshkumar et al. 2015). There are three known species: *Norileca borealis* Javed & Yaseen, 1999, *N. triangulata* Richardson, 1910 and *N. indica* (Milne Edwards, 1840). *Norileca borealis* was originally described from the northern Arabian sea (Javed and Yaseen 1999), parasitising the Indian mackerel, *Rastrelliger kanagurta* Cuvier, 1817. No other recordings of this species have been reported since its original description. *Norileca triangulata* was first recorded from Tanimidao Island, the Philippines, without mention of a fish host (Richardson 1910). Specimens of *N. triangulata* have since been recorded from Australia (from Cape York, Great Barrier Reef and south-eastern Queensland), from the branchial cavity of the saillfin flyingfish, *Parexocoetus brachypterus* Richardson, 1846 and *Sardinella gibbosa* Bleeker, 1849 (Bruce 1990). More recent recordings are from the Parangipettai coastal waters on the south-east coast of India, from the goldstripe sardinella, *Sardinella gibbosa* Bleeker, 1849 (Rameshkumar and Ravichandran 2015). *Norileca indica* was originally described by Milne Edwards (1840) as *Livoneca indica*. It was later redescribed by Bruce (1990) and transferred to the genus *Norileca*.

As part of a larger project on the global diversity of cymothoids, *N. indica* specimens were collected from Maputo Bay, Mozambique, and provided the opportunity to complete a redescription of this species based on ovigerous females. In addition, this paper also presents a detailed redescription of the male, as well as the first molecular characterisation of this species and genus, using the mitochondrial cytochrome oxidase I (COI) gene.

**Methods**

*Norileca indica* specimens were collected during November 2013 from the bigeye scad, *Selar crumenophthalmus* Bloch 1793 by local subsistence fishermen in Maputo Bay, Mozambique. Isopods were analysed following the techniques of Hadfield et al. (2010, 2013). Species descriptions were made with the aid of the taxonomy software package DELTA (Descriptive Language for Taxonomy) (see Coleman et al. 2010), following a general Cymothoidae character data set originally developed by Hadfield et al. (2013) and recently updated (Hadfield et al. 2016). Ratios and measurements for the description were made using the maximum values at the middle of the specific measured article, and all proportional measurements were rounded to one decimal place. Isopod classification follows Brandt and Poore (2003) and host nomenclature follows that of FishBase (Froese and Pauly 2017) and Catalog of Fishes (Eschmeyer et al. 2017).

Genomic DNA was extracted from isopod pereopods and pleopods following the protocol for animal tissue extraction of the GeneJET™ Genomic Extraction Kit (Thermo Scientific, Waltham, MA, USA). A targeted part of the mitochondrial cytochrome oxidase I (COI) gene (approximately 680 bp) of these specimens was subjected to PCR amplification with the aid of a Bio-Rad C1000 Touch™ Thermal Cycler and universal invertebrate primers LCO1490 (5’-GGTCAACAATATCATTTAGATATTGG-3’) and HCO2198 (5’-TAACTTCAGGGTGACCAAAAAATCA-3’) (Folmer et al. 1994). The PCR protocol followed that of Ketmaier et al. (2008). PCR products were sequenced in both directions by Inqaba Biotechnical Industries.
peduncles (Bruce 1990). It can be distinguished from other cymothoid genera by pleonite 1 being the widest of the pleonites, as well as its weakly twisted body shape (Hadfield 2012).

Key to the species of *Norileca*

1. Pleonite 5 narrower than pleonite 1; uropods almost reaching posterior margin of pleotelson; pleotelson approximately 0.9 times as long as wide ........................2
   Pleonite 5 and pleonite 1 subequal; uropods two-thirds the length of pleotelson; pleotelson approximately 1.0–1.2 times as long as wide .......................... *N. indica*

2. Body twisted to the side; maxilla medial lobe with 1 robust seta and lateral lobe with 4 robust setae ............ *N. borealis*
   Body nearly straight; maxilla medial lobe with 2 robust setae and lateral lobe with 2 robust setae .......................... *N. triangulata*

**Type material**: Holotype held at the Museum Nationale d’Histore Naturelle, Paris (MNHN-IU-2007-4159).

**Type locality**: Sumatra Island, Indonesia (Milne Edwards 1840).

**Type host**: No type host recorded.

**Material examined**
Three ovigerous ♀ (33.0 mm TL, 16 mm W; 30.0 mm TL, 17 mm W; 26.0 mm TL, 13 mm W) and 1 ♂ (11.0 mm TL; 3.0 mm W), Maputo Bay, Mozambique, South Africa, November 2013, from bigeye scad *Selar crumenophthalmus* (Bloch, 1793), coll. Wynand Vlok (SAMC-A089028).

**Remarks**
*Norileca* shares several characters with *Livoneca* Leach, 1818. Both genera are similar with regard to their pereopod morphology, all of which are robust and lacking an expanded carina on the base of the pereopods; the cephalon posterior margin is trilobed; and the pleon is not immersed in the pereon with pleonites 1–5 becoming progressively narrower. *Norileca* can be distinguished from *Livoneca* in having a weakly trilobed cephalon (vs strongly trilobed in *Livoneca*) and pleonites 1–3 lateral margins which are not bilobed. *Norileca* also has an expanded mandible palp article 3, pereopods 3–4 without folds on endopods, as well as an absence of branched pleopod endopods, as well as an absence of branchiated pleopod which are not bilobed.
Two ovigerous ♀ (29.0–35.0 mm TL; 15.0–19.0 mm W) and 2 non-ovigerous ♀ (28.0–30.0 mm TL; 14.0–18.0 mm W), Maputo Bay, Mozambique, South Africa, November 2013, from bigeye scad *Selar crumenophthalmus* (Bloch, 1793), coll. Wynand Vlok (in the collection of the authors at NWU).

**Ovigerous female**
(Figures 1–4)

Length 33.0 mm, width 16.0 mm.

*Figure 1: Norileca indica* (Milne Edwards, 1840) ovigerous female (33.0 mm TL, 16.0 mm W) (SAMC-A089028). (a) Dorsal body, (b) lateral body, (c) dorsal view of pleotelson with uropods, (d) dorsal view of cephalon and pereonite 1

**Body** twisted to the right side, 2.2 times as long as greatest width, dorsal surfaces smooth and polished in appearance, widest at pereonite 4, most narrow at pereonite 1. Pereonite lateral margins posteriorly protruding. *Cephalon* 1.1 times longer than wide, visible from dorsal view, triangular. *Frontal margin* thickened and ventrally folded. *Eyes* oval with distinct margins, one eye 0.3 times the width of the cephalon, 0.3 times the length of the cephalon. *Pereonite 1* smooth, with anterior border indented and anterolateral angle weakly produced, extending to middle of cephalon. *Coxae 2–3*
wide with posteroventral angles rounded; coxae 4–7 acute, posteriorly pointed, not extending past pereonite margin. Pereonites 6 and 7 narrower than pereonites 1–5. Pleon with pleonite 1 slightly wider than other pleonites, visible in dorsal view; pleonites posterior margin not smooth, medially produced. Pleonite 2 partially overlapped by pereonite 7 posterolateral margin; posterolateral angles of pleonite 2 rounded. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 free, not overlapped by lateral margins of pleonite 4. Pleotelson as long as anterior width; dorsal surface smooth; lateral margins weakly convex, posteriorly narrow; posterior margin converging to caudomedial point.

Antennula consists of 8 articles; peduncle articles 1 and 2 distinct and articulated; article 2 1.1 times as long as article 1; article 3 1.6 times as long as wide, 0.5 times as long as combined lengths of articles 1 and 2; flagellum with 5 articles, extending to posterior margin of eye with tufts of simple setae on articles 3–6 and 8. Antenna consists of 9 articles; peduncle article 3 1.0 times as long as article 2; article 4 2.2 times as long as wide, 1.4 times as long as article 3; article 5 twice as long as wide, 0.7 times as long as article 4. Antenna flagellum with 6 articles, terminal article with 1–5 short simple setae, extending to anterior margin of pereonite 1. Mandibular molar process present, with no simple setae; mandible

Figure 2: Norileca indica (Milne Edwards, 1840) ovigerous female (33.0 mm TL, 16.0 mm W) (SAMC-A089028). (a) Antennula, (b) antenna, (c) mandible, (d) maxilliped, (e) tip of maxillule, (f) tip of maxilliped article 3, (g) maxilla
palp article 2 and 3 without setae. Maxillule simple with 4 terminal robust setae. Maxilla medial lobe partly fused to lateral lobe; medial lobe with 2 recurved robust setae, lateral lobe with 1 large recurved robust setae. Maxilliped palp article 2 without setae; article 3 with 4 recurved robust setae.

Pereopod 1 basis 1.8 times as long as greatest width; ischium 0.7 times as long as basis; merus proximal margin with bulbous protrusion; carpus with straight proximal margin; propodus 1.1 times as long as wide; dactylus slender, 3.8 as long as propodus, 3.8 times as long as basal width. Pereopods 3–6 similar to pereopod 2, gradually increasing in size towards posterior, all without setae. Pereopod 7 basis 0.6 times as long as greatest width; ischium 0.8 as long as basis, without protrusions; merus proximal margin with large bulbous protrusion; 0.4 times as long as wide, 0.3 as long as ischium; carpus 0.4 times as long as wide, 0.2 as long as ischium, with slight bulbous protrusion; propodus as long as wide, 0.4 as long as ischium; dactylus slender, 1.9 as long as propodus, 2.7 times as long as basal width.

Pleopods without setae, exopod larger than endopod. Pleopod 1 exopod 1.1 times as long as wide, lateral margin weakly convex, distally broadly rounded, medial margin strongly convex; endopod 1.2 times as long as wide, lateral margin convex, distally broadly rounded, medial margin straight; peduncle 0.3 times as wide as long. Pleopods 2–5 similar to pleopod 1. Pleopods 3–5 with fleshy folds present, increasing in size from pleopod 3–5. Peduncle lobes present, increasing in size from pleopod 1–5.

Figure 3: Norileca indica (Milne Edwards, 1840) ovigerous female (33.0 mm TL, 16.0 mm W) (SAMC-A089028). (a) Ventral cephalon, (b) oostegites, (c) pereopod 1, (d) pereopod 7
Figure 4: *Norileca indica* (Milne Edwards, 1840) ovigerous female (33.0 mm TL, 16.0 mm W) (SAMC-A089028). (a) Pleopod 1 dorsal view, (b) pleopod 2 dorsal view, (c) pleopod 3 dorsal view, (d) pleopod 4 dorsal view, (e) pleopod 5 dorsal view, (f) pleopod 1 ventral view, (g) pleopod 2 ventral view, (h) pleopod 3 ventral view, (i) pleopod 4 ventral view, (j) pleopod 5 ventral view
Uropod more than half the length of pleotelson; peduncle 0.7 times longer than rami, lateral margin without setae; rami not extending beyond pleotelson, marginal setae absent, apices narrowly rounded. Endopod 2.3 times as long as greatest width, without setae. Exopod not extending to end of endopod, 3 times as long as greatest width, without setae.

Male
(Figures 5–7)

Length 11.0 mm, width 3.0 mm. Body straight, not twisted, 2.7 times as long as greatest width, widest at pereonite 5, most narrow at pereonite 1, pereonite lateral margins mostly posteriorly ovate. Cephalon 0.79 times longer than wide, visible from dorsal view, triangular, not immersed in pereonite 1. Frontal margin thickened, ventrally folded. Eyes oval with distinct margins; one eye 0.6 times length of cephalon. Pereonite 1 smooth, anterior border indented; anterolateral angle weakly produced, extending past the posterior margin of eyes. Posterior margins of pereonites smooth and straight. Coxae 2–3 wide; with posteroventral angles rounded; 4–7 acute, posteriorly pointed; not extending past pereonite margin. Pereonites 6 and 7 becoming more progressively rounded posteriorly. Pleon with pleonite 1 largely concealed by pereonite 7; pleonites posterior margin smooth, mostly concave. Pleonite 2 not overlapped by pereonite 7; posterolateral angles of pleonite 2 rounded. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 free, not overlapped by lateral margins of pleonite 4. Pleotelson as long as anterior width; dorsal surface smooth; lateral margins weakly convex, posterior margin converging to caudomedial point.

Antennula more stout than antenna, longer than antenna; consists of 8 articles; peduncle articles 1 and 2 distinct and articulated; article 2 as long as article 1; article 3 1.4 times as long as wide, 0.5 times as long as combined lengths of articles 1 and 2; flagellum with 5 articles, extending to anterior of pereonite 1, with tufts of setae on articles 3–8. Antenna consists of 9 articles; peduncle article 3 1.1 times as long as article 2; article 4 1.5 times as long as wide,

Figure 5: Norileca indica (Milne Edwards, 1840) male (11.0 mm TL, 3.0 mm W) (SAMC-A089028). (a) Dorsal body, (b) lateral body, (c) dorsal view of cephalon and pereonite 1, (d) dorsal view of pleotelson with uropods, (e) penes
Figure 6: *Norileca indica* (Milne Edwards, 1840) male (11.0 mm TL, 3.0 mm W) (SAMC-A089028). (a) Antenna, (b) antennula, (c) pereopod 1, (d) pereopod 7, (e) mandibular palp, (f) maxilliped, (g) tip of maxillule, (h) maxilla
1.3 times as long as article 3; article 5 1.6 times as long as wide, as long as article 4. Antenna flagellum with 7 articles, terminal article terminating in 1–5 short simple setae, extending to anterior margin of pereonite 1. Mandibular molar process present; palp article 2 with 3 distolateral setae, article 3 with 6 simple setae. Maxillule simple with 4 terminal robust setae. Maxilla medial lobe not fused to lateral lobe; medial lobe with 2 recurved robust setae, lateral lobe with 1 large recurved robust setae. Maxilliped consists of 3 articles; palp article 2 without setae; article 3 with 4 recurved robust setae.

**Pereopod 1** basis 1.7 times as long as greatest width; ischiurn 0.6 times as long as basis; merus proximal margin with slight bulbous protrusion; carpus with rounded proximal margin; propodus 1.3 times as long as wide; dactylus slender, 1.8 as long as propodus, 3.8 times as long as basal width. **Pereopod 7** basis 1.6 times as long as greatest width; ischiurn 0.9 as long as basis, without protrusions; merus proximal margin with slight bulbous protrusion, 0.6 times as long as wide, 0.3 as long as ischiurn; carpus 0.6 times as long as wide, 0.2 as long as ischiurn, without bulbous protrusion; propodus 1.4 times as long as wide, 0.5 as long as ischiurn; dactylus slender, 1.7 as long as propodus, 3.6 times as long as basal width.

**Pleopod 1** exopod longer than endopod. **Pleopod 1** exopod 1.3 times as long as wide, lateral margin weakly convex, distally narrowly rounded, medial margin weakly oblique; endopod 1.6 times as long as wide, lateral margin slightly straight, distally narrowly rounded, medial margin straight; peduncle 0.3 times as wide as long, without retinaculae. **Pleopod 2** appendix masculina with parallel margins, 0.8 times as long as endopod, distally acute. **Pleopod 5** with fleshy folds present. Peduncle lobes present, increasing in size from pleopod 1 to 5.

**Uropod** same length as pleotelson, peduncle 0.8 times longer than rami, peduncle lateral margin without setae; rami extending to pleotelson apex, marginal setae absent, apices narrowly rounded. 2.5 times as long as greatest width, without setae. **Exopod** 2.4 times as long as greatest width, without setae.

**Penes** prominent, 2.3 times as long as basal width, tubercules connecting at base.

**Distribution**

Off the Zambezi estuary, Mozambique and Tanjona Vilanandro, north-western coast of Madagascar (previously Cape Saint André) (Rokicki 1982); Mayotte Island (Trilles 1976); Pakistan (Behera et al. 2016); India (Rameshkumar et al. 2013b, 2015) as well as India eastern coast and Indian eastern coast and Gulf, Province of Iloilo, the Philippines (Cruz-Lacierda and Mariveles and Luzon Islands (Schioedte and Meinert 1884; Trilles 1976; Yamauchi et al. 2005) obtained N. indica from the stomach of the common dolphinfish, Coryphaena hippurus Linnaeus, 1758 where the natural host would have been eaten by the dolphinfish.

Behera et al. (2016) recorded N. indica from Randall’s threadfin bream, Nemipterus randalli Russell, 1986, which is a doubtful host record. It is the first and only host record from the family Nemipteridae and a photograph provided by Behera et al. (2016: see fig. 5c) does not represent N. indica.

**Molecular characterisation**

Two ovigerous females were sequenced (forward and reverse). Ovigerous female (30.0 mm TL, 17 mm W) produced a 686 bp contig of the COI gene (accession number: MF628258), and ovigerous female (26.0 mm TL, 13 mm W) produced a 679 bp contig of the COI gene (accession numbers: MF628259 and MF628260), respectively. These COI gene sequences are the first sequences to be generated for N. indica and the genus Norileca, therefore species validity and generic placement based on molecular evidence could not be done.

**Remarks**

Norileca indica attaches to the ventral part of the host branchial cavity, with the cephalon to the anterior end of the host, and with its ventral side (abdomen/brood pouch) directed outwards, facing the branchial operculum (Bruce 1990; Neeraja et al. 2014; Rameshkumar et al. 2015; Behera et al. 2016). Its asymmetrical body is twisted to the left when it occupies the right branchial cavity and
Figure 7: *Norileca indica* (Milne Edwards, 1840) male (11.0 mm TL, 3.0 mm W) (SAMC-A089028). (a) Pleopod 1 dorsal view, (b) pleopod 2 dorsal view, (c) pleopod 3 dorsal view, (d) pleopod 4 dorsal view, (e) pleopod 5 dorsal view, (f) pleopod 1 ventral view, (g) pleopod 2 ventral view, (h) pleopod 3 ventral view, (i) pleopod 4 ventral view, (j) pleopod 5 ventral view
twisted to the right when it occupies the left branchial cavity (Nagasawa and Petchsupa 2009; Neeraja et al. 2014). Norileca indica can be recognised by its twisted body, with a 2.1–2.5 length-to-width body ratio and the oval eyes have distinct margins. The pleotelson of N. indica is approximately as long as it is wide and the uropods are two-thirds the length of the pleotelson length. Furthermore, pleonite 5 is about as wide as pleonite 1.

Males are similar in appearance to females but smaller in size and with a straight body shape. Ovigerous females differ from non-ovigerous females in having a larger body length-to-width ratio as they become wider as eggs develop in the broodpouch. Both Rokicki (1982) and Bruce (1990) reported that N. indica is ventrally positioned in the host gill cavity with the cephalon facing the anterior end of the host and the abdomen outwards toward the operculum (in a lateral position). Four of the currently collected specimens were also ventrally positioned; however, two were positioned with the dorsal surface to the operculum.

Norileca indica can be distinguished from N. triangulata by being larger than the latter, with its body twisted to the side, a straight sided pleon, smaller eyes, as well as shorter uropods and a shorter mandible palp article 3 (Bruce 1990). It differs from N. borealis by having shorter uropods, larger eyes, and a larger length-to-width ratio of the pleotelson. Pleonite 1 and 5 of N. indica are more equal in width than that of N. borealis where pleonite 5 is narrower than pleonite 1. In addition, N. borealis has a medial lobe with 4 recurved robust setae (two recurved robust setae on N. indica), and article 3 of the maxilliped with three recurved robust setae (4 recurved robust setae on N. indica) (Javed and Yasmeen 1999). Even though N. borealis and N. triangulata are more similar to each other than to N. indica, they can be distinguished from each other by body shape, ventral margin of the cephalon, as well as pleon and pleopod morphology.

Since the redescription of N. indica in 1990, records of this species from the Indian subcontinent have mainly been made due to the collection of its fish hosts for subsistence and commercial use by local fishermen. Many of its host species, including Seler crumenophthalmus and Decapterus kurroides, are considered to be commercially important fish species (Argente et al. 2014; Cruz-Lacierda and Nagasawa 2017). Other publications from this region provided new host or locality information on N. indica as well as some ecological data including prevalence, mean intensity and abundance (Neeraja et al. 2014; Rameshkumar et al. 2015; Behera et al. 2016; Jithin et al. 2016). Despite this species being frequently collected, limited work has been done recently on the morphology and taxonomy of this species.

Discussion

The redescription of N. indica by Bruce (1990) was based on non-ovigerous females. Here we present the first detailed redescription of an ovigerous female specimen. Ovigerous females display diagnostic characteristics and structures that may not be present or as well developed in non-ovigerous females and males.

Cymothoid isopods are protandrous hermaphrodites, making them difficult to identify during different developmental stages. As part of the female development, the pleotelson becomes wider and other structures (such as the gonopod, eye- and uropod perimeter, and the first antenna) become shorter in length (Cock and Munguia 2015). Males tend to be morphologically similar to one another; therefore, accurate and comprehensive descriptions of males are essential to ensure species identification can potentially be made in the absence of ovigerous females.

Norileca indica is widely distributed and morphological analyses combined with molecular analyses will provide a better understanding of this species. These analyses will confirm species identity, even during the natatory stage of development (see Jones et al. 2008). It is essential to combine genetic characterisation of a species with an accompanying description of that species based on the same material in order to verify the identification of the species. Only when the identification of the species is accurate can phylogenetic analysis be effective, providing useful information without having the confusion of misidentified species as is currently the problem with cymothoids.

The use of combined molecular and morphological data for phylogenetics is becoming more prominent within taxonomic publications. This combination improves the resolution, internal support and overall quality of phylogenetic studies (Caddick et al. 2002; Scotland et al. 2003). In many instances, there exists a lack of either morphological or molecular data (Giribet et al. 2001). This is also the case with N. indica, as no other publication is available that provides a comprehensive data set of both morphological and molecular results of this species. The use of molecular techniques seems to eliminate morphological bias as well as over- and under-estimations of biodiversity that is occasionally associated with traditional morphological analyses (Lefébure et al. 2006).

The first molecular characterisation of N. indica presented here contributes to the limited pool of molecular information of the Cymothoidae (currently only 28 of the 385 known and accepted cymothoid species have been sequenced), while also providing a Norileca COI sequence that can be used for cymothoid studies as well as species identifications.

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